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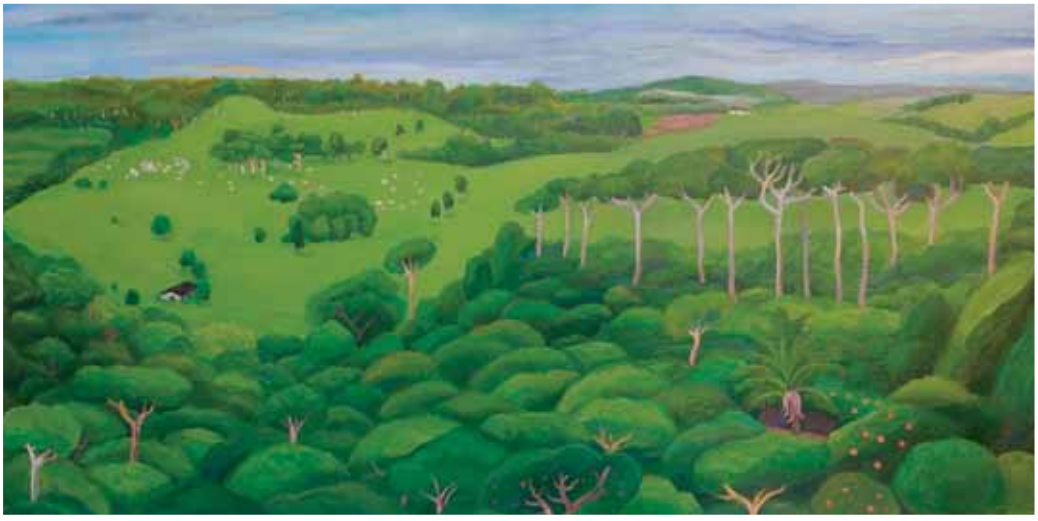

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La Tapa (Ver p xx)
Los palos rosas, 2015
Daniela Kantor

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REUNIÓN CONJUNTA SAIC SAI SAFIS 2018

LXIII REUNIÓN ANUAL DE LA

SOCIEDAD ARGENTINA DE INVESTIGACIÓN CLÍNICA (SAIC)

LXVI REUNIÓN ANUAL DE LA

SOCIEDAD ARGENTINA DE INMUNOLOGÍA (SAI)

REUNIÓN ANUAL DE LA

SOCIEDAD ARGENTINA DE FISIOLOGÍA (SAFIS)

CON LA PARTICIPACIÓN DE

SOCIEDAD ARGENTINA DE VIROLOGÍA (SAV)

ASOCIACIÓN ARGENTINA DE NANOMEDICINAS (NANOMED-ar)

14-17 de noviembre de 2018
Hotel 13 de Julio – Mar del Plata

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Claudia Pérez Leirós
Pablo Baldi
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JOINT MEETING SAIC SAI SAFIS 2018

LXIII ANNUAL MEETING OF

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November 14-17, 2018
Hotel 13 de Julio – Mar del Plata

RESPONSIBLE EDITORS

Claudia Pérez Leirós
Pablo Baldi
Alberto Crottogini

LA TAPA

Los palos rosas, 2015

Daniela Kantor

Técnica: Acrílico sobre bastidor. Medidas: 35 x 70 cm

Daniela Kantor es diseñadora gráfica (FADU-UBA), historietista, ilustradora y pintora. Desde 2014 es docente en la materia Ilustración, cátedra Roldán, FADU, y da talleres para niños (Filbita 2017, taller de comics librerías Matilda-Tigre, taller de historietas CCK, etc.) Estudió con el maestro Alberto Breccia dibujo de historieta y con Carlos Gorriarena realizó el Curso de color. Asistió al Taller de acuarela y pastel de Carlos Nine y realizó clínicas de pintura con Mariano Sapia y Tulio de Sagastizábal. Además de ilustrar muchos libros para niños y adolescentes (Editoriales Troquel, Abran Cancha, Puerto de Palos, Santillana, etc.), es parte de la revista de historietas El tripero, publica en revistas (Barcelona, Zona de obras, Crisis, suplemento Ñ, entre otras). Publicó su primera novela gráfica: Mujer primeriza (2014). Su proyecto de segundo libro de historietas Naturalella obtuvo la primera mención del Premio Nueva Historieta Argentina (2016) y fue publicado en parte en Dis-tinta, el compilado de Liniers y Martín Pérez (Ed. Sudamericana, 2016). Expone sus pinturas desde 2003; recientemente exhibió en Cic.edu.ar

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PALABRAS DE BIENVENIDA

Estimados colegas y amigos,

Nos complace darles la bienvenida a la Reunión Conjunta SAIC SAI SAFIS 2018 de la **Sociedad Argentina de Investigación Clínica (SAIC),** la **Sociedad Argentina de Inmunología (SAI)** y la **Sociedad Argentina de Fisiología (SAFIS),** que este año también cuenta con la participación de la **Sociedad Argentina de Virología (SAV)** y la **Asociación Argentina de Nanomedicinas (NANOMED-ar).**

El Programa Científico es abarcador y cubre los aspectos más sobresalientes e innovadores de las diferentes disciplinas. Contamos con la presencia de investigadores argentinos y extranjeros de la mayor jerarquía internacional que expondrán los avances de su trabajo en conferencias y simposios. Además, se han inscripto más de 760 trabajos de estudiantes de doctorado, becarios, investigadores, médicos residentes y otros profesionales del ámbito de la Salud con los últimos resultados de sus investigaciones, los que serán expuestos en forma de comunicaciones orales y pósters. Se han seleccionado algunos de estos trabajos para su presentación en simposios para favorecer el intercambio con los pares extranjeros. Asimismo, Jurados de expertos han pre-seleccionado trabajos para competir por distintos premios: se otorgarán los Premios León Cherny al mejor trabajo multidisciplinario, Honorio Bigand al mejor proyecto presentado por investigadores jóvenes, Eduardo Soto al mejor trabajo en Neurociencias, Irene Faryna de Raveglia en Oncología, Leonardo Satz en Inmunología, SAFIS Jóvenes Investigadores en Fisiología, Camilión de Hurtado en Fisiopatología Cardiovascular y César Milstein en Enfermedad de Chagas. Se otorgará un premio de la American Society for Microbiologists en el área de Infectología y Menciones a los mejores pósters por áreas de la SAIC. Estos premios constituyen un estímulo para los grupos de investigación argentinos que mejoran la calidad de sus trabajos año tras año y se otorgan merced al generoso aporte de las fundaciones Cherny, Bigand, de la Dra Pasquini, de la Familia Camilión de Hurtado y de las empresas ETC Internacional y Novartis Argentina SA. Habrá también minicursos, encuentros con expertos y exposición comercial.

El principal objetivo de esta Reunión Conjunta es ofrecer a los asistentes el marco académico propicio para alentar la interacción entre científicos argentinos y con pares extranjeros que investigan las bases moleculares y bioquímicas de las enfermedades humanas. Nuestras sociedades reúnen a investigadores y académicos de las distintas ramas de la Biomedicina, con un importante enfoque en la medicina traslacional. Desde la organización alentamos la discusión y formación científica en un clima de intercambio cordial y multidisciplinario.

Aprovechamos la oportunidad para agradecer a las comisiones directivas de las sociedades participantes quienes, en un año de crecientes complicaciones económicas y de funcionamiento, han trabajado con enorme dedicación y responsabilidad para el éxito de esta Reunión. Nuestro agradecimiento a las instituciones oficiales y no gubernamentales que apoyaron la organización de este evento a través de subsidios u otros aportes; a las empresas y entidades que auspiciaron y acompañan con su presencia este Congreso; a las empresas organizadoras y a la gerencia del Hotel 13 de Julio por su amabilidad y profesionalismo.

Esperamos que disfruten de este encuentro en sus aspectos científicos y académicos como también en salidas sociales aprovechando las instalaciones turísticas de esta espléndida ciudad de Mar del Plata.

Dra. Claudia Pérez Leirós	**Dr. Pablo Baldi**	**Dr. Alberto Crottogini**
Presidente SAIC	Presidente SAI	Presidente SAFIS

WELCOME WORDS

We are pleased to welcome you to the SAIC SAI SAFIS 2018 Joint Meeting, organized by Sociedad Argentina de Investigación Clínica (SAIC), Sociedad Argentina de Inmunología (SAI) and Sociedad Argentina de Fisiología (SAFIS), with the participation of Sociedad Argentina de Virología (SAV) and Asociación Argentina de Nanomedicinas (NANOMED-ar).

The scientific program is comprehensive, spanning the most glowing and innovative aspects of the diverse fields. Outstanding international experts from Argentina and from abroad will discuss their recent advances in the setting of conferences and symposia. In addition, PhD and postdoctoral fellows, young investigators, resident physicians and other health professionals will address the recent results of their research in over 760 communications during poster and oral sessions. A number of these works have been selected for presentation in symposia, in order to foster interactions of their authors with foreign colleagues. Likewise, expert juries have pre-selected communications to compete for the following awards: The León Cherny Award to the best multidisciplinary research, The Honorio Bigand Award to the best project presented by young investigators, The Eduardo Soto Award to the best research in Neuroscience, The Irene Faryna de Raveglia Award in Oncology, The Leonardo Satz Award in Immunology, The SAFIS Young Investigators in Physiology Award, The Camilión de Hurtado Award in Cardiovascular Pathophysiology and The César Milstein Award in Chagas Disease. A Prize in the field of Infectology from The American Society for Microbioloy, as well as Mentions from SAIC to the best posters, will also be awarded. These awards convey a motivation to the Argentine research groups that progressively improve the quality of their investigations, and are granted thanks to the generosity of the Cherny and Bigand Foundations, Dr. Pasqualini, the Camilión de Hurtado Family and the companies ETC Internacional y Novartis Argentina SA. Minicourses, Meeting with the Expert Sessions and a commercial exhibit will also take place during the Joint Meeting.

The main goal of this Joint Meeting is providing the attendees with an appropriate academic framework to encourage interactions between Argentine scientists and colleagues from abroad who investigate the molecular and biochemical bases of human ailments. The members from our societies are investigators and academics from diverse biomedical areas with a strong focus in translational medicine. From the Organizing Committee, we firmly encourage scientific discussion and training in an atmosphere of warm, multidisciplinary interaction.

We take advantage of this opportunity to thank the Boards of the participating Societies which, in a year of increasing economic and managing complications have worked with enormous commitment and responsibility for the success of this Meeting. Our gratitude, as well, to the official and private institutions that supported the organization of this event with grants or other financial contributions; to the sponsoring and organizing companies and entities; and to the staff of 13 de Julio Hotel for their kindness and professionalism.

We wholeheartedly hope that you enjoy this Meeting in its scientific, academic and social aspects, while profiting the attractions of this beautiful, splendid Mar del Plata.

Dr. Claudia Pérez Leirós
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REGENERATING CNS MYELIN - FROM MECHANISMS TO EXPERIMENTAL MEDICINE

Robin J.M. Franklin

Wellcome Trust-MRC Cambridge Stem Cell Institute, University of Cambridge, United Kingdom

Remyelination, the process by which new myelin sheaths are restored to demyelinated axons, represents one of the most compelling examples of adult multipotent stem cells contributing to regeneration of the injured CNS. This process can occur with remarkable efficiency in multiple sclerosis (MS), and in experimental models, revealing an impressive ability of the adult CNS to repair itself. However, the inconsistency of remyelination in MS, and the loss of axonal integrity that results from its failure, makes enhancement of remyelination an important therapeutic objective. There is now compelling evidence that ageing is the major contributor to the declining efficiency of remyelination and that this is largely due to a failure of stem cell differentiation. This talk will cover some of our recent studies on how ageing effects many aspects of CNS remyelination, including the divergent properties of CNS progenitors of different developmental origin and how changes in the mechanical properties of the ageing brain change the properties of CNS progenitors.

EMBO Keynote Lecture

MAINTENANCE AND REACTIVATION OF IMMUNOLOGICAL MEMORY

Andreas Radbruch

Deutsches Rheumaforschungszentrum Berlin, a Leibniz institute, and Charité University Medicine Berlin; radbruch@drfz.de

Recent observations have fundamentally challenged the classical view that immunological memory is maintained by coherent populations of circulating and proliferating immune memory cells. Distinct populations of memory T lymphocytes and memory plasma cells residing in epithelial tissues and in the bone marrow have been described. They provide first-line protection and long term memory to prevailing antigenic challenges of the environment. We have now also identified memory B lymphocytes of the bone marrow as a population distinct from their splenic counterparts in terms of repertoire and phenotype. Immune memory cells of the bone marrow are individually docking onto stromal cells, implying that stromal cells determine the capacity of immunological memory. There they rest in terms of mobility and activity. These resident memory lymphocytes apparently are not maintained by (homeostatic) proliferation. As we could show for memory plasma cells, their survival is dependent on cell contact to the stromal cell, inducing PI3K signaling, and on the cytokines April or BAFF from their environment, inducing NFkB signaling. In synergy, both signaling pathways in memory plasma cells upregulate expression of the vital transcription factor IRF4 and prevent caspase-induced apoptosis. Memory T and B lymphocytes are maintained by PI3K signalling as well in the bone, suggesting that stromal cells play a pivotal role for the persistence of immunological memory, by preventing apoptosis of the memory cells through contact-dependent PI3K signaling. In secondary immune reactions, resident quiescent T and B lymphocytes obviously have to be mobilized from their memory niches. We could show for resident CD4+ memory T lymphocytes that this mobilization leads (a) to the formation of “Immune clusters” in the bone marrow, resulting in amplification of the specific memory lymphocytes, and (b) to the emigration of specific resident memory T lymphocytes into the blood, and their participation in the secondary immune reaction.

SAIC CONFERENCE ‘ALBERTO TAQUINI’

SIGNALING NEW THERAPEUTIC APPROACHES IN HEPATOCELLULAR CHOLESTASIS

Marcelo G. Roma

IFISE-CONICET, Universidad Nacional de Rosario

Hepatocellular cholestasis is associated with a functional failure in the capability of hepatocytes to produce bile. It is often due to a functional impairment in the main transporters involved in the canalicular efflux of solutes acting as driving force for bile flow generation (*e.g.*, bile salts and gluthation, transported via Bsep and Mrp2, respec-

tively). Functional impairment of hepatocellular transporters is often associated with hormonal and inflammatory causes, with estrogens and cytokines being prototypical pathogenic factors, respectively. Hepatocellular cholestasis is nowadays understood as an unbalance of signaling pathways inducing changes in nuclear receptors and protein-kinase-signal cascades that regulate transcriptionally and postranscriptionally these transporters, respectively. A plethora of the so-called 'orphan nuclear receptors' have been recently identified to regulate the expression of hepatocellular transporters, including FXR (a Bsep regulator) and PXR/CAR (Mrp2 regulators), among the more important ones. They spontaneously orchestrate an adaptive response under cholestatic conditions when activated by retained endogenous ligands. When this defense is overwhelmed by the cholestatic insult thus making the disease apparent, it can be therapeutically reinforced by the use of more potent nuclear receptor ligands; this opens the possibility to design tailor-made therapeutic strategies based upon the use of an adequate arrange of these ligands. Protein-kinase-signal cascades prompting canalicular transporter internalization and further degradation have also been actively identified by our group, such as PKC/p38^{MAPK}-mediated endocytic internalization and PI3K/Akt/Erk1/2-mediated intracellular arrest of the endocytosed transporters. We have successfully used inhibitors of these 'cholestatic' signal tranduction pathways to counteract both endocytic internalization of canalicular transporters and the associated secretory dysfunction. All these overwhelming advances in our understanding of physiopathology of cholestasis have opened the possibility to envisage new and exciting therapeutic alternatives in cholestasis, able not only to restore the expression of key transported affected in cholestasis, but also to afford its appropriate functional localization.

SAFIS CONFERENCE

BILIRUBIN: FROM A WASTE COMPOUND TO A MESSENGER MOLECULE

Claudio Tiribelli
Scientific Director, Italian Liver Foundation-AREA Science Park, Basovizza, Trieste, Italia

Bilirubin possesses multiple biological actions with interaction in a complex network of enzymatic and signaling pathways. Unconjugated bilirubin (UCB) is known to be one of the most potent endogenous antioxidant substances. While hyperbilirubinemia has long been recognized as an ominous sign of liver dysfunction, recent data strongly indicate that mildly elevated bilirubin (BLB) levels can be protective against an array of diseases associated with increased oxidative stress. These clinical observations are supported by new discoveries relating to the role of BLB in immunosuppression and inhibition of protein phosphorylation, resulting in the modulation of intracellular signaling pathways in vascular biology and cancer, among others. Collectively, the evidence suggests that targeting BLB metabolism could be considered a potential therapeutic approach to ameliorate a variety of conditions. The fact that the liver is the main organ controlling the bioavailability of bilirubin emphasizes the central role of this organ in human health.

SAI CONFERENCE

MYCOBACTERIUM TUBERCULOSIS: NUTRITIONAL IMMUNITY LINKED TO HOST MACROPHAGE ONTOGENY

David G. Russell
Cornell University, Ithaca, NY, USA.

Mycobacterium tuberculosis (Mtb) is currently the greatest single cause of death from infectious disease. Unfortunately, our progress towards development of a vaccine is hampered greatly by our limited understanding of immune-mediated control. The infection cycle is maintained predominantly by intramacrophage bacilli suggesting that this is the key unit of infection. While much previous attention has been focused on macrophage-mediated killing of Mtb we are starting to believe that immune-dependent, nutritional restriction of bacterial growth may represent the more significant means of limiting disease progression.

To understand how infection by Mtb is modulated by host cell phenotype we characterized those host phagocytes that controlled or supported bacterial growth during early infection, focusing on the ontologically-distinct alveolar macrophage (AM) and interstitial macrophage (IM) lineages. Using fluorescent Mtb reporter strains we found that bacilli in AM exhibited lower stress and higher bacterial replication than those in IM. Interestingly, depletion of AM reduced bacterial burden, while depletion of IM increased bacterial burden. Transcriptomic analysis revealed that IM were glycolytically-active, whereas AM were committed to fatty acid oxidation. Intoxication of infected mice with the glycolytic inhibitor, 2-deoxyglucose, decreased the number of IMs yet increased the bacterial burden in the lung. Furthermore, in *in vitro* macrophage infections, 2-deoxyglucose treatment increased bacterial

growth while the fatty acid oxidation inhibitor Etomoxir constrained bacterial growth. The data reveal the tight relationship between host and bacterial metabolism. We demonstrate that different macrophage lineages respond divergently to Mtb infection, with IM exhibiting nutritional restriction and controlling bacterial growth, and AM representing a more nutritionally-permissive environment. Moreover, in an empirical, high throughput chemical screen against intra-macrophage Mtb we identified novel inhibitors of bacterial enzymes involved in the degradation of host-derived cholesterol as a point of vulnerability. Together, these data stress the significance of the metabolic interface between host and pathogen.

SAFIS CONFERENCE 'DR. HORACIO CINGOLANI'

RYANODINE RECEPTOR: THE EPICENTER OF CARDIAC DYSFUNCTION

Martín Vila-Petroff
Centro de Investigaciones Cardiovasculares Dr. Horacio Cingolani-CONICET-UNLP

Ryanodine receptor type 2 (RyR2) is a homotetrameric protein complex that regulates Ca2+ release from the sarcoplasmic reticulum (SR) into the cytosol of cardiac myocytes. RyR2 are involved in the process of excitation contraction coupling, which links sarcolemmal depolarization with the rise of cellular Ca2+ transients and subsequent contraction. RyR2 activity is influenced by the integrated effects of associated co-proteins, ions, and post-translational modifications. In healthy muscle, RyR2 are regulated to support cellular function. However, a pathological increase in the degree of post-translational modifications of the RyR2 channel impairs their ability to properly deactivate, leading to Ca2+ leak from the SR resulting in a spectrum of Ca2+-dependent pathologies that include cardiac systolic and diastolic dysfunction, arrhythmias, and structural remodeling. Our investigation, over the last decade has focused on CaMKII, a kinase whose activity is triggered by an increase in intracellular Ca2+. Although ongoing investigation has provided evidence for multiple targets for CaMKII, RyR2 seem to be one of particular functional importance. Our research highlights the critical importance of CaMKII-dependent RyR2 phosphorylation in the pathogenesis of cardiac pathologies of different etiology, including ischemia and reperfusion injury, heart failure, contractile dysfunction associated with sepsis, arrhythmias, apoptosis and digitalis toxicity. Thus, CaMKII inhibition emerges as a potential strategy to prevent cardiac disease. However, the ubiquitous nature of CaMKII expression and its effects on different targets precludes its inhibition as a therapeutic tool. The involvement of RyR2 in the development of cardiac disease allows us to postulate an alternative therapeutic approach, which is to directly target RyR2 function.

SAI CONFERENCE 'LEONARDO SATZ'

RELATIONSHIP BETWEEN PULMONARY VIRAL INFECTIONS AND ASTHMA DEVELOPMENT

Nicholas Lukacs, PhD.
Mary H. Weiser Food Allergy Center, University of Michigan Medical School, Ann Arbor, USA.

The developing immune response in infants is central to establishing a balanced system that reacts appropriately to infectious stimuli but does not induce altered disease states with potential long term sequelae. Respiratory syncytial virus (RSV) is often the first clinically relevant pathogen encountered in life and appears to alter the immune system, affecting future responses, such as those leading to childhood allergies and asthma. Other early life processes also contribute to the development of immune responses including assembly of the microbiome that has a particularly important role early in life. Our studies have explored the role of the developing microbiome on the maturation of immune responses and identified that both are altered significantly after a pulmonary RSV infection. Our data show that early neonatal infection with RSV alters immune responses both locally in the lung as well as systemically for weeks/months after the infection suggesting a prolonged altered innate immune phenotype, including the development of allergic diseases. These latter data reflect clinical findings where neonates have increased risk of severe RSV infection and those most severely infected have an increased incidence to develop allergic disease during childhood. In our ongoing studies results indicate that these altered phenotypes can be mitigated by maternal factors that include both *in utero* and post-natal metabolic control of immune development dependent upon the maternal microbiome. Our data also highlight that the change in immune function is associated with major shifts in the neonatal RSV-induced bacterial community composition of the gut and changes in plasma metabolite profiles that are directly altered by modifying the mother's microbiome before and during pregnancy. Together, these studies demonstrate that the influence of microbiome and associated metabolites are critical for the development of an appropriate non-pathogenic immune response that can be primarily influenced by maternal and environmental factors.

SAIC CONFERENCE

TOWARDS PROGRAMMABLE AND INTELLIGENT NANOMATERIALS

Galo J. A. A. Soler-Illia
Instituto de Nanosistemas, Universidad Nacional de Gral San Martín (UNSAM). DQIAyQF, FCEN, UBA - CONICET

In the last decade, a significant advance took place in materials chemistry. Two key factors of this progress are the development of reproducible nanomaterials synthesis, and the control of self-assembly processes. The combination of these powerful concepts leads to produce multiscale materials with hierarchical architectures, which mimick the complexity of those found in Nature. Mesoporous materials (MM) with high surface area and controlled mesopore diameter (2-50 nm) are an example of these complex materials. The pore systems can be "decorated" with organic, biological or nanoscale functions. This field evolved from the mere production of high surface area matrices to programmable nanosystems, in which confinement effects, responsivity, or collaborative functionality can be imparted into the structure through the control of positional chemistry of different chemical building blocks. The richness of this emergent field will be presented by discussing the design pathways to MM with finely tuned pore size, connectivity or wall nature. Mesopores can be then modified by molecular species, biomolecules or polymers, leading to hybrid MTF with an amazing variety of chemical behaviors. An exquisite tuning of the properties can be achieved by combining synthetic and characterization tools with theoretical models and simulations, essential to understand the complexity of the synthesis paths and the final properties. This in-depth knowledge is key to ultimate nanosystems design. The combination and feedback of synthesis, characterization and modeling leads to pre-designed nanosystems with complex structures and functional location. Confinement, interactions and localized reactivity can be used as topological tools for building nanosystems able to host different chemical or biochemical groups with well-defined positioning. These concepts permit to build tunable catalysts, enzyme cascade hosts, intelligent bioscaffolds, remotely activated nanoparticles, chemical-to-optical transducers or perm-selective membranes. A potentially infinite variety of nanosystems with externally controllable behavior is at our disposal, opening the path to design intelligent matter.

SAI CONFERENCE

THE COMPLEMENT SYSTEM: FROM ANCIENT DEFENSE TO TRANSLATIONAL MEDICINE

Peter F. Zipfel
Department Infection Biology, Leibniz Institute for Natural Product Research and Infection Biology, Friedrich-Schiller University, Jena, Germany

Complement as a basic part of the immune network controls tissue homeostasis. Activated complement initiates inflammation and coordinates both innate and adaptive immune responses. Furthermore, this central cascade interacts with other tissue networks, like the coagulation system and coordinates numerous humoral and cellular immune responses.

By protective immune surveillance complement recognizes and eliminates infectious microbes. However microbial pathogens have developed specific means to interfere and block complement recognition. In addition complement directs inflammatory silent removal of altered self-particles or induces moderately inflammatory responses. Inappropriate removal of modified self-material results in accumulation of debris, inflammation and auto-immune diseases.During the last years understanding on the role of complement in the clearance of modified self-material has significantly increased. In addition genetic and functional approaches demonstrated the role of complement in numerous human diseases including kidney and retinal disorders. Furthermore approval of complement targeting inhibitors for the glomerular disease hemolytic uremic syndrome and paroxysmal nocturnal hemoglobinuria has highlighted that complement targeting is an option for therapy. At present a large list of new complement inhibitors is developed and new inhibitors are evaluated in clinical trials. This opens new avenues for treatment of complement mediated human disorders.

The understanding of the role of complement in disease pathology combined with the approval of complement inhibitors for therapy has advanced the understanding of this important homeotic system and new treatment options for other diseases will appear in the near future.

The lecture will focus on the role of complement regulation by Factor H and the Factor H related proteins in the glomerular diseases Hemolytic uremic syndrome and C3 Glomerulopathy and will highlight how genetic alterations increased the understanding of disease pathology and opened new ways for therapy.

SAFIS CONFERENCE

'GAS CHANNELS'

Walter Boron

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The traditional view had been that gases move through membranes by dissolving in the lipid phase. Our group then discovered the first CO_2-impermeable membranes (gastric-glands apical membranes) and the first membrane protein (aquaporin-1, AQP1) permeable to a gas (CO_2). Using pH measurements, we and others then described the CO_2 and NH_3 permeabilities of several AQPs and rhesus (Rh) proteins. We described the first examples of blockade of gas transport, and of gas selectivity (particularly striking among the AQPs). Preliminary work now suggests that we have identified the first examples of channels permeable to O_2 and to N_2. For O_2, we use stopped-flow absorbance spectroscopy to monitor the deoxygenation of hemoglobin (Hb) in mouse RBCs exposed to an extracellular O_2 scavenger. We find that the amino-reactive agent DIDS or the sulfhydryl reagent pCMBS substantially slow deoxygenation, as do the knockouts of AQP1, RhAG, or both. Mathematical modeling suggests that AQP1/Rh accounts for ~60% of O_2 permeability, with pCMBS blocking an additional ~30%. Our approach for N_2 has been to inject a *Xenopus* oocyte (which normally sinks) with a bubble of air (which makes it float). Applying pressure (P_{Clamp}) to the air phase compresses the bubble, raising density and causing the oocyte to achieve neutral buoyancy at a fixed distance below the surface. As gas (mainly N_2) diffuses from bubble to cytosol to extracellular fluid, we maintain neutral buoyancy by reducing P_{Clamp}. The heterologous expression of certain AQPs or Rh proteins speeds the time course of the P_{Clamp} decay, suggesting that these proteins behave as N_2 channels. We suggest that gas channels play important physiological roles in cells with high gas-transport rates, that the manipulation of such channels could be useful in certain pathological states, and that one should be able to design channels with specific gas-permeability properties.

co-authors: Dale Huffman, Fraser J. Moss, Rossana Occhipinti, Brian Zeise, and Pan Zhao.

SAIC CONFERENCE 'ALFREDO LANARI'

NEW STRATEGIES TO PREVENT PREGNANCY COMPLICATIONS

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The aim of our work is the study of certain complications of pregnancy and delivery. Preterm birth (PTB), which occurs before 37 weeks gestation, is one of the most frequent obstetrical complications in humans. Several studies regarding the risk factors, causes and treatments for this syndrome support that "not one answer fits all". However, it is becoming increasingly clear that one of the major risk factors is inflammation and/or infection in the fetoplacental unit. We have developed a mouse model of inflammation-associated preterm delivery induced by lipopolysaccharide (LPS) that resembles the clinical presentation in humans. This model allowed us to demonstrate that melatonin, a very safe compound for human use, may be considered as a novel tocolytic agent, with the additional capacity of protecting fetuses from inflammation-induced alterations during pregnancy. Environmental enrichment (EE) refers to the exposure of laboratory animals to housing conditions that offer an enhanced stimulation of sensory, cognitive and motor systems, in comparison to standard housing conditions. We have evaluated the effect of EE housing as a potential treatment for reducing PTB rate. Our results showed a highly protective effect of EE against an immune challenge during pregnancy. Miscarriage refers to the loss of the pregnancy before 20 weeks of gestation, without outside intervention. It affects up to 20 percent of recognized pregnancies. Spontaneous and cytokine-boosted abortion rates have been linked to LPS exposure. We have developed a murine model to study the mechanisms of LPS-induced embryonic resorption (ER). With this model, we have studied the pathophysiological mechanisms by which the endocannabinoid system participates in the triggering of ER and the fundamental protective role of the progesterone in the reversal of this process. Deepening our knowledge on the mechanisms of pathologic processes leading to early miscarriage and PTB will allow specific and suitable interventions for their prevention.

SAI CONFERENCE

UV RADIATION, VITAMIN D AND IMMUNITY

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Exposure to UV radiation (UVR) stimulates a systemic immunosuppression, even at sub-erythemal doses. Exposure to UVR, particularly UVB, activates anti-inflammatory and immunosuppressive pathways that help to modulate skin diseases (psoriasis, atopic dermatitis) and regulate some infection and vaccination outcomes. For systemic diseases such as multiple sclerosis, type 1 diabetes, asthma, schizophrenia, autism and cardiovascular disease, any positive consequences of UVR exposure are more speculative but reduced UVR exposure is a risk factor for the development of these inflammatory conditions, particularly those initiated early in life. The contribution of UVR-induced vitamin D3 is an important question for design of anti-inflammatory therapeutics. Vitamin D is made by absorption of UVB photons by 7-dehydocholersterol in skin cells, with the active mediator produced by further hydroxylation in the liver and kidneys, as well as in peripheral tissues. 1,25 dihydroxy-vitamin D3 is essential for bone health but there is currently less evidence that it is the UVR-induced mediator responsible for all the immunoregulatory properties described above for UVR exposure. These conclusions have come from trials of vitamin D supplementation for inflammatory conditions. Other studies in mice and humans, including the use of narrowband UVB of 311-312 nm with reduced erythemal and carcinogenic properties, have provided evidence for mediators other than vitamin D in UVR-induced immunoregulation. These mediators include UVR-induced nitric oxide, cis-urocanic acid, melatonin, neuropeptides and antimicrobial peptides. Murine studies and human trials of narrowband UVB also suggest UVR-induction of regulatory cells independently of the actions of vitamin D. In mice, repeated suberythemal UVR exposure and diets containing different levels of vitamin D have independent effects on the gut microbiome which in turn may regulate immune cell development. In summary, UVR stimulates the induction of regulatory cells and immunoregulatory mediators by both vitamin D-dependent and -independent pathways.

SAI CONFERENCE

REGULATION OF INNATE-LIKE B-1 CELL RESPONSES IN INFECTION

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B cell responses to pathogens are complex and multi-layered and are facilitated by innate-like B-1 as well as conventional B-2 cells. Together these two distinct B cell subsets facilitate both, immediate-early as well as long-lasting immunity. Preexisting antibodies, either natural or infection-induced, provide an early layer of immune protection, effectively reducing initial pathogen dissemination. Preexisting natural IgM is generated mostly by the B-1 cells, fetal/neonatal-derived B cells whose BCR repertoire is shaped by positive selection for self-reactivity. The self-reactive antibodies that are produced by B-1 cells, even in the complete absence of microbial stimulation, were demonstrated to be critical for tissue homeostasis, conventional B cell development and early immune defense. Paradoxically, the presence of natural self-reactive antibodies reduced autoimmune antibody production. Natural IgM production seems to be regulated at least in part by tonic BCR-signaling strengths. But B-1 cells also rapidly respond to pathogen invasion, driven by their responses to innate signals. What regulates some B-1 cells to respond to infection, while others remain engaged in producing natural IgM, remains unknown. Seemingly contradictory findings exist about whether and how the presence of CD5+ B-1a and CD5- B-1b cells may contribute to these distinct functions. Data will be presented suggesting that CD5 expression by B-1 cells reflects their function and level of responsiveness to BCR signaling, rather than a biological fixed immune phenotype that identifies discrete B-1 cell subsets. Thus, CD5 does not irreversibly mark distinct B cell subsets.

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SAIC-SAV SYMPOSIUM: VIRAL INFECTIONS UPDATE

HIV RESERVOIRS: WHY THE INFECTION CANNOT BE ERADICATED?

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Although antiretroviral therapy (ART) allows sustained viral suppression (VS) in blood plasma, the ability to eradicate human immunodeficiency virus type 1 (HIV-1) has not been yet achieved. The principal obstacle to viral eradication is the quick establishment of HIV-1 in its target cells, mainly in resting memory CD4 + T cells, at the earliest phases of primary infection. The half-life of these resting cells can reach up to several years and contribute to the persistence of the infection, being recognized as the principal HIV-1 reservoir. Estimation of the reservoir size could be important to determine patient response and avoid the possibility of plasma viral load (pVL) rebound. An effective biomarker to measure reservoir size and residual ongoing viral replication has not been established; however, cell-associated HIV-1 DNA (CA-HIV-DNA) and 2-long terminal repeat (2-LTR) circles levels, are commonly used for these purposes.

We determined the decay rate of HIV-1 DNA reservoir in vertically infected children throughout long-term sustained viral suppression (VS) and how it was affected by the time of infection at VS .CA-HIV-DNA in peripheral blood mononuclear cells had a significant decay during the first two years of VS, and subsequently reached a plateau The 2-LTR circles frequency decayed significantly, from 82.9% at pre-VS to 37.5% and 28.1% at 2 and 4 years of VS, respectively. Our results highlight that achieving VS during the first 18 months of infection limit the establishment of HIV-1 reservoirs, reinforcing the clinical benefit of very early effective therapy in children.

IMMUNE RESPONSE IN THE PATHOGENESIS OF CHC: T CELL POPULATIONS AND CYTOKINE MILIEU IN LIVER AND PERIPHERAL BLOOD

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Introduction: In Chronic Hepatitis C (CHC) the immune system is involved in liver damage; but, the role of each immune cell is unknown. We aimed to evaluate T cell populations and cytokine milieu in liver and peripheral blood (PB) to elucidate the immune system role in CHC liver disease.

Methods: Liver biopsies and concomitant PB samples from 48 untreated adult CHC patients were analyzed. CTL (CD8), Th (CD4), Th17 (IL17A/CD4-IL17A), Treg (Foxp3/CD4-CD25hi-Foxp3) and Th1 (Tbet/CD4-IFNg) cell frequencies were evaluated by immunohistochemistry on formalin-fixed biopsies and by flow cytometry in PB. TGFβ, IFNg, IL6, IL1β, IL8, IL10, IL23, IL21 levels were evaluated by RT-qPCR in fresh liver and by CBA in plasma. PB samples from uninfected donors were included. Results were related to hepatitis and fibrosis severity.

Results: Liver infiltrates showed Th predominance, high Treg and Th1 but low Th17 frequency. Th17 cells and Th17/Treg ratio showed fibrosis association (both p=0.04). TGFb ($p<0.05$, r=0.49), IL8 ($p<0.01$; r=0.49) and IL6 ($p<0.05$, r=-0.43) displayed correlations with Th17 frequency. While TGFβ, IL23, IL1β were associated with hepatitis severity (all $p<0.05$), IL8 was associated with advanced fibrosis (p=0.004). IL10 correlated to IL6, IL21 and IL23 (all $p<0.05$; r=0.43, r=0.66, r=0.39, respectively) and was higher in severe hepatitis cases.

The PB lymphocyte profile in CHC patients was similar to donors, but cytokines pattern showed higher levels in patients, being IL6 and TGFβ significantly elevated (p=0.03; p=0.04).

Conclusion: The liver immune microenvironment in CHC depicted a complex cytokine milieu that allows the Th17 and Treg interplay. Although Treg was not directly involved in liver damage, high IL10 levels might reflect a different Treg activation status throughout disease progression. Th17 and IL8 might have a key role in fibrogenesis. While CHC peripheral lymphocyte frequency showed no alterations, the cytokine profile delineated an activated scenario.

ELIMINATION AND CONTROL OF MEASLES IN ARGENTINA IN A GLOBAL CONTEXT

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Measles (M) is a highly contagious and serious disease caused by a paramyxovirus. Before 1980, an estimated 2.6 million deaths occurred each year. M is still one of the leading causes of death among young children worldwide, despite the availability of a safe and effective vaccine. Approximately 134,200 deaths due to M occurred in 2015. The WHO Directing Council established in 1994 the elimination goal for 2000 and in 2001, the Initiative to ensure that no child dies because of M. Among the goals proposed for 2020 are reducing Measles deaths by 95% and achieving the elimination of M in at least 5 WHO regions. The WHO estimates that systematic vaccination against M prevented 17.1 million deaths worldwide between 2000 and 2014, with a decrease of 79% in deaths. In 2016, PAHO / WHO determined that America had eliminated the endemic transmission of M but in 2016 Europe reported 3553 cases with vaccination coverage less than 95%. The last endemic case of M in Argentina was reported in 2000 and endemic transmission was stopped in 2002, without deaths since 1998. Since 2002, there have been 26 documented imported cases of M or associated with importation, 22 between 2010 and 2018. In the current situation of low endemicity, new challenges are presented: cases are sporadic; it is difficult to detect them, obtain the appropriate samples and epidemiological information. The performance of the serological assays is getting lower forcing genomic detection assays into the diagnosis. Sequencing is essential to establish and confirm transmission chains, explain import phenomena or post-vacunal cases. In populations highly vaccinated with low endemicity, the absence of natural "booster" by exposure to the virus will lead antibodies to decline over time and vaccinated people may be reinfected. Improving clinical notification, laboratory confirmation and gathering epidemiological information is essential.

SAIC SYMPOSIUM: METABOLISM AND ITS IMPACT ON DISEASE OUTCOME

MOLECULAR MECHANISMS INVOLVED IN PROSTATE TUMOR GROWTH AND PROGRESSION ASSOCIATED TO METABOLIC SYNDROME. GENES AND MIRNAS RELATED TO CTBP1 PATHWAY.

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Prostate cancer (PCa) is the second commonest diagnosed malignancy and the fifth leading cause of cancer mortality in men. Metabolic Syndrome (MeS) is linked to increased PCa risk and aggressiveness by unknown mechanisms. C-terminal binding protein 1 (CTBP1) is a transcriptional co-repressor of tumor suppressor genes that is activated by low NAD+/NADH ratio. Our group established a MeS-like/PCa mice model that identified CTBP1 as a novel link associating both diseases. We found that CTBP1 is overexpressed in high grade human PCa, and its depletion decreased PCa growth in MeS mice. To understand the molecular mechanism underlying the link between MeS and PCa mediated by CTBP1, we investigated PCa development and progression in MeS mice models. We identified an mRNAs and miRNAs profile regulated by CTBP1 that is associated to PCa development and progression in xenografts generated in MeS mice using expression microarrays and bioinformatic analysis. CTBP1 diminished the prostate cellular adhesion and altered the cellular morphology, which induced mesenchymal phenotype and filopodia number in a mechanism mediated by the hsa-miR-196b-5p. Moreover, CTBP1 depletion in primary tumors significantly decreased circulating tumor cells and spontaneous metastasis with an increment of hsa-miR-30b-5p plasma circulating miRNA in MeS mice. Finally, MeS increased hypertrophy, hyperplasia, inflammation and mRNA/miRNA expression of adipose tissue which induced CTBP1 expression and PCa cell proliferation. Our studies uncover the role of CTBP1/MeS in PCa development and progression. Targeting of CTBP1 expression might be considered for PCa management and therapy in the subset of patients with MeS.

DIET AND THE BRAIN. A VIEW FROM BASIC BIOMEDICINE.

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In the last few years, increased attention has been put in understanding the modulatory role of diet on the central nervous system. On the one hand, epidemiological evidence suggests that consumption of "western diets"

(high intake of fat, carbohydrates and industrially processed food) constitutes a risk factor for neurodegenerative and neurovascular disorders. On the other hand, evidence shows that dietary restriction (DR) ameliorates the impact of age-associated diseases, such as Alzheimer's (AD). However, underlying mechanisms are not clear yet and could involve the modulation of multiple biological pathways. Our objective is to describe and understand histological, biochemical and molecular consequences in the brain and associated behavioral changes in response to A) the consumption of a high-fat diet (HFD) and B) dietary restriction in animal models. Our results show that C57BL/6 mice that were fed a moderately HFD (45% of kCal from fat vs. 12% in control diet) since weaning displayed peripheral and central inflammation along with impaired insulin signaling without overweight. Cognitive deficits were found in HFD mice, concomitantly with decreased hippocampal adult neurogenesis. Using an in vitro model of fatty acid exposure on microglia we found that secreted exosomes, as a mean for intercellular communication, induced dendritic remodeling on primary hippocampal neurons. Finally, using a transgenic model of AD we studied the neuroprotective capability of periodic DR. We found that DR for 6 weeks was associated with decreased activation of hippocampal microglia, increased neurogenesis and reversal of cognitive impairment in AD mice. We studied the communication between astrocytes and microglia in vitro and found that astrocytes under nutrient restriction are able to prevent amyloid-induced microglial activation. Our results suggest that diet has a significant role on brain function and structure, with degenerative or protective effects, and that glial cells are possible effector cells and potential therapeutic targets.

FAT DIET SWEET DIET: FROM LIPIDS TO HEART, AN ENDLESS TRASLATIONAL JOURNEY

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Alterations in plasma lipoproteins levels are hallmarks of cardiovascular disease (CVD). Several co-morbidities including diabetes and obesity have been shown to increase CVD risk, in part due to changes in lipoprotein profile, characteristic of insulin resistance (IR) states. The interplay among synthesis, catabolism and structural modifications, conditions the levels and atherogenicity of lipoproteins. The increase of VLDL remnants in plasma is the first step of lipoprotein metabolism after fat-rich meal intake, as in the case of glycaemia after carbohydrate-rich meal intake. In two different models of IR in rats, we demonstrated an increased secretion of larger and TG over-enriched VLDL particles from the liver, as well as a reduction in lipoprotein lipase (LPL) activity from adipose tissue and heart. Consequently, the delayed catabolism of VLDL and its remnants increases the residence time of these lipoproteins in circulation. In parallel, we reported for the first time an increase in endothelial lipase (EL) activity in the same model, counterbalanced with decreased LPL. The enzyme contribution to the lipoprotein profile differs according to each tissue; heart enzymes are the major responsible of plasma TG behaviour, meanwhile adipose tissue EL mainly conditioned HDL levels. However, when evaluating human epicardial adipose tissue LPL activity, we observed that although it decreased with IR in coronary patients, remained increased compared to controls, supporting that not only transcriptional but also post-translational modifications are involved in the enzyme regulation. These results support our previous findings, which showed that plasma LPL activity is reduced in patients with IR and obesity, meanwhile plasma EL present increased activity in the highest obesity and IR degrees, accounting for the hypertriglyceridemia and the decreased HDL-cholesterol levels. Our studies provide new insights into the role of lipolytic enzymes and its regulation in determining lipoproteins levels and characteristics, beyond fatty acids supply to different tissues.

SELECTED ABSTRACT FOR SYMPOSIUM

CARDIAC HYPERTROPHY IN OBESITY: LEPTIN-TRH INTERACTION.

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Cardiac TRH induce left ventricular hypertrophy (LVH) and fibrosis, its inhibition prevent hypertrophy.The adiponectin leptin induces TRH in CNS. We hypothesized that in obesity, the increase of TRH induced by hyperleptinemia is responsible of the LVH, until now mostly attributed to pressure load. We studied obese Agouti mice suffering hypertension with hyperleptinemia and found LVH with increased TRH gene expression. Consequently we found higher ($p<0.05$) fibrotic and hypertrophic markers vs lean (BL/6J). As pressure could explain results we treated obese mice with diuretic (hydroclorothiazide 20 mg/kg/day) from weaning (n=9), the diuretic group was normotensive in contrast to control obese mice. Nevertheless both groups developed ($p<0.05$): LVH, higher TRH gene and elevated fibrotic and hypertrophic markers suggesting that LVH is not induced by hypertension.

In contrast to Agouti, we studied obese Ob/Ob mice lacking leptin due to a disruption in their gene. Mice are normotensive, without LVH despite their obesity. We treated 2 groups with leptin (sc. 80 ug/kg/day) or saline from weaning for 15 days. Only the group treated with leptin developed LVH (LV weight/tibia length, $p< 0.05$, n=7) vs saline, pointing out that LVH is leptin dependant. As hypothesized, in this group we found an increase ($p<0.05$) in cardiac TRH accompanied by higher expression of type III collagen suggesting that leptin-TRH interaction is required for obesity-induced LVH. To confirm cardiac cells TRH's leptin induction, cardiomyocytes derived cell line H9C2 (n=6) was stimulated with leptin (10 and100 ng/ml). TRH expression (rt-PCR) and peptide (WB) were increased ($p<0.05$) post leptin at both concentrations. Moreover we developed cardiomiocytes primary culture from neonates, in which leptin stimulus (80 ng/ml, 24 hs) increased ($p<0.05$) TRH content vs controls confirming the direct TRH induction by leptin in heart cells.
Finally, obese-induced LVH is leptin-dependent, who probably stimulates hypertrophy and fibrosis by its TRH induction

SAI SYMPOSIUM: REGULATION OF THE IMMUNE RESPONSE

REGULATORY B CELLS

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Regulatory B cells (Bregs) participate in the maintenance of immunological self-tolerance by actively suppressing self-reactive lymphocytes. Little is known, however, about the molecular mechanism of their development. Utilising a transcriptomics approach, we identified the aryl hydrocarbon receptor (AhR) as critical transcription factor for the differentiation of IL-10^{+}Bregs. Specific deletion of AhR in B cells leads to a profound reduction in the frequency of IL10+Bregs resulting in the development of exacerbated arthritis compared to the control group. In addition, whereas B regs isolated from control mice were able to suppress arthritis in recipient wild type mice, adoptive transfer of AhR deficient B cells failed to suppress disease. Finally, we show that the Ahr-IL-10 regulated transcriptome promoted Breg cell stability by inhibiting programs that induce pro-inflammatory response and that B cells lacking Ahr display a core transcriptional profile that is similar to B conventional cells.

METABOLIC REGULATION OF T CELL DIFFERENTIATION AND FUNCTION

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T lymphocyte activation is regulated by the metabolism of glucose, fatty acids and amino acids, allowing the cell to meet increased energetic and biosynthetic demands. We find that exogenous nutrient availability regulates the terminal differentiation of activated naïve CD4+ T cells into distinct effector fates. Remarkably, activation of naïve CD4+ T cells under conditions of glutamine deprivation causes them to terminally differentiate into Foxp3^{+} regulatory T cells (Treg) with potent *in vivo* suppressor function. In fact glutamine-deprived CD4+ T cells that are activated under Th1 polarizing conditions do not differentiate into Th1 cells but, instead, differentiate into Treg cells, demonstrating that glutamine deprivation attenuates a Th1 cell fate and promotes a Treg cell fate. Moreover, we will discuss recent data showing that the availability of glutaminolytic metabolites determines whether activated CD4 T lymphocytes differentiate into an effector versus Treg cell fate. Thus, nutrient availability and downstream metabolites govern the T cell differentiation program and immune responsiveness.

ENDOPLASMIC RETICULUM STRESS MEDIATOR PERK CENTRALLY REGULATES THE FUNCTION OF MYELOID-DERIVED SUPPRESSOR CELLS IN TUMORS.

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Myeloid-derived suppressor cells (**MDSCs**) play a fundamental role in the inhibition of protective immunity against tumors and represent a major limitation to cancer immunotherapies.Upon infiltration into the stressful tumor microenvironment (**TME**), MDSCs acquire a potent immunosuppressive activity.However, the central mechanisms governing this behavior remain elusive. Here, we sought to elucidate the mechanistic crosstalk between the intrinsic activation of the endoplasmic reticulum (**ER**) stress-activated PKR-like kinase (**Perk**) and the regulatory effects of MDSCs in tumors. Our results show an increased activation of the ER stress-associated mediators, including Perk, in tumor-associated MDSCs, which correlated with a heightened immunosuppressive

activity. Inhibition of ER stress activation through Tauroursodeoxycholate delayed tumor growth, rendered MDSCs incompetent to block T-cell function,and overcame tumor-induced T-cell tolerance. In agreement, conditional deletion of Perk in ER stressed-MDSCs dramatically abrogated their immunosuppressive activity, attenuated tumor progression,and enhanced effector T-cell activity in tumors. Mechanistically, Perk deletion in MDSCs impaired the activation of the anti-oxidant inducer, Nrf2, resulting in the accumulation of reactive oxygen species (**ROS**) and blunted mitochondrial function. Pharmacological activation or genetic overexpression of Nrf2 restored suppressive activity and mitochondrial activity in ER stressed Perk-null MDSCs. Interestingly, Perk-deficient MDSCs underwent release of mitochondrial DNA into the cytosol, activated the STING-IRF3 pathway, upregulated IFN type I genes, and transformed into immunogenic subsets. Together, our findings demonstrate for the first time the primary role of Perk in the regulatory activity of tumor-MDSCs and provide a strategy to functionally reprogram MDSCs within tumors into myeloid subsets that induce anti-tumor immunity and enhance the efficacy of major anti-cancer treatments.

YOUNG RESEARCHER PRESENTATION.

DAMPENING INFLAMMATION BY TYROSINE KINASE TYRO3, AXL, AND MERTK RECEPTORS AND THE IMMUNE HOMEOSTASIS IN HUMAN INFLAMMATORY DISEASES.

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Negative regulatory feedback is a critical aspect of the homeostatic immune response and disruption on this point could lead to inflammatory-based disease. The tyrosine kinase receptors TYRO3, AXL and MERTK (TAM) and their ligands Protein S (PROS1) and growth arrest-specific 6 (GAS6) are critical players in maintaining immune homeostasis by dampening inflammatory response, mediate efferocytosis and to contribute to tissue repair process. We have demonstrated that PROS1/TAM signaling functions at the interface of adaptive and innate immunity by limiting the intensity and, perhaps, the duration of the immune response. Furthermore, we have shown that TYRO3, but not AXL and MERTK, is a key regulator of Th2 response when is activated by PROS1. The main goal of our research is to reveal the participation of this axis in autoimmune and chronic inflammatory human diseased such as Multiple sclerosis (MS) and Inflammatory Bowel Disease (IBD). MS is a chronic inflammatory and autoimmune disorder affecting the central nervous system by infiltration of auto-reactive Th1/Th17 cells. Interestingly, it has been shown that patients with MS and helminthes infection exhibited the lower number of relapses and lesion activity compared with uninfected individuals with MS. We are investigating if the TAM axis is enhanced in a Th2 environment promoting the parasite-driven protection mechanism in patients with MS concomitant infected with helminthes. On the other hand, loss of T cell-derived PROS1, as well as genetic ablation of Axl and Mertk, increases the inflammatory signature of macrophages in a mouse model of IBD. In this sense, our work intends to determine the role of TAM signaling on the balance of inflammatory and tissue repair macrophage skewing in patients with Crohn's disease and Ulcerative Colitis characterized by chronic inflammation and widespread tissue damage. Our long-term goal is to contribute to new molecular and cellular mechanisms that limit chronic inflammation, autoimmune responses and favoring tissue repair properties that could lead to new pharmacological strategies for these destructive diseases.

SAIC-NANOMED-AR SYMPOSIUM I

THE FAULT IS ON MACROPHAGES, SAYS NM LA-BECK: WHY THE ANTITUMORAL NANOMEDICINES ARE BAD ANTITUMORAL AGENTS

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Nanomedicines entered the world pharmaceutical market 23 years ago. Despite of the enormous expectancies and money invested, nanomedicines still fail in being more efficient that low molecular weight antitumorals. The reason seems to lie behind the recent discovery that pegylated liposomal nanoparticles such as the Doxil/Caelyx doxorubicin formulation and certain polymeric nanoparticles, have the potential to enhance tumor growth in an immune competent murine model of cancer. Nearly 3 years before, a series of simple and elegant experiments carried out by the team conducted by NM La Beck and A.A. Gabizon, revealed that nanocarriers of structure identical to that of Doxil, increased tumor angiogenesis and suppressed the antitumor immune responses as indicated by decreased cytokine production by tumor macrophages and cytotoxic T cells, diminished tumor infiltration of tumor-specific T cells, and decreased number of dendritic cells in tumor draining lymph nodes.

Shortly after, the tumor associated macrophages (TAM), primary cells that internalized nanocarriers in the tumor microenvironment, were identified as the paradoxical inducers of tumor growth, with a specific activity triggered upon nanocarriers phagocytosis. Such important findings point the way towards developing new targeted antitumoral nanomedicines, focused on including agents capable of eliminating this specific cellular response.

BIOPOLYMER NANOCARRIERS FOR ONCOLOGICAL DRUGS: SYNTHESIS, CHARACTERIZATION AND IN VITRO AND IN VIVO EVALUATION

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Nanoparticles (NPs) are submicron size entities which can be made from a wide variety of polymers. The existing anticancer agents usually do not show selectivity between cancerous and normal cells leading to systemic toxicity and adverse effects which limits the maximum permissible dose to be applied. This is the case of tamoxifen (TMX), fundamental drug for the treatment of breast cancer conforming to the WHO. This selective estrogen receptor modulator has been the Trojan horse for the endocrine treatment of estrogen-receptor-positive breast cancer. Depending upon the dose and the concentration has several side effects as endometrial carcinoma for postmenopausal women, liver cancer, venous thrombosis, pulmonary emboli and an ocular effect includes retinopathy and corneal opacities. Another interesting drug to treat breath cancer is desmopressin (DDAVP); an innovative Peptide in Cancer Treatment. Considering biopolymers capability for high loading drugs and to modulate drug release, this work studies the physicochemical and biomedical properties of PLGA nanoparticulated systems that could carry TMX or DDAVP in order to improve its therapeutic effect. TMX and DVAP loaded PLGA NPs were fully characterized by scanning electron microscopy (SEM), thermogravimetric analysis (TGA), differential scanning calorimetry (DSC), and Fourier transform infrared spectroscopy (FTIR). Additionally, encapsulation efficiency and in vitro cell viability assay were performed. TMX and DDAVP loaded PLGA NPs were successfully obtained showing an average size around 200 nm. Morphologically, a quasi-spherical regular shape was observed for all of the PLGA NPs, which demonstrating that the selected experimental conditions for each case allow control of the formation of the polymeric particles, their dimensions and their properties. Suitable encapsulation efficiencies for both active principles were achieved whereas in vitro an in vivo tesst give promising results. The capitalization of the therapeutic effect as a result of the fulfillment of the proposed biotechnology objectives was achieved in both cases.

NANOMEDICINES FOR MACROPHAGE TARGETING ORAL DELIVERY OF ENZYMES, ANTIOXIDANT, AND ANTI-INFLAMMATORY DRUGS

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Oral delivery is preferred for local administration of intestinal therapeutics. For example, for inflammatory bowel diseases (IBD) that are characterized by chronic inflammation and epithelial injury induced by the uncontrolled activation of the mucosal immune system, the local delivery of anti-inflammatory drugs with minimal exposure of healthy or distant tissues would provide a novel treatment approach for patients with IBD. Oral delivery and macrophage targeting of enzymes, antioxidant, and anti-inflammatory drugs however, are hamper by the harsh conditions of the gastrointestinal tract. In this presentation I will describe two strategies developed by our group to overcome these limitations and improve macrophage targeting oral delivery of superoxide dismutase (SOD), dexamethasone and natural antioxidants.

POLYMERIC MICELLES FOR THE DEVELOPMENT OF NANOVACCINES FOR INFECTIOUS DISEASES AND CANCER

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The design and development of nanosystems is a promising field to improve the efficacy and safety of vaccines against infectious diseases and cancer. Due to its size, the large surface area/volume ratio and the ability to functionalize its surface and to encapsulate antigens and adjuvants, these systems have the potential of a delivery directed towards the antigen-presenting cells, where the immune response will start. Nowadays, there are several formulations of nanovaccines based on liposomes and virus-like-particles that were approved for use in humans. Non-ionic block copolymers based on polyoxyethylene (PEO) and polyoxypropylene (PPO) blocks

are the most investigated non-ionic polymers for the development of vaccine adjuvants. Above the CMC, linear PEO-PPO-PEO copolymer aggregates to form micelles with a hydrophobic core of PPO, while PEO chains form the hydrophilic shell. At a certain concentration and temperature, some of these copolymers are able to organize themselves forming gel-like structures, allowing a sustained release of drugs and proteins. The adjuvant activity of the block copolymers is influenced by PPO block size. As the size of this block increases, maximum activity is achieved. However, the adjuvant activity is also affected by the amount of PEO, being optimal when it is found in low concentrations. The selection of the copolymer type depends on the properties of the antigen, finding a greater interaction with hydrophilic soluble proteins when polymers have a higher percentage of PEO while hydrophobic proteins with transmembrane regions interact better with polymers with a higher percentage of PPO. However, it remains to deepen on the interactions of these nanosystems with immune system cells and the impact of the composition and the physicochemical properties of the systems developed in their efficacy and safety.

SAIC SYMPOSIUM: TRANSPORT AND THE BLOOD BRAIN BARRIER

ANGIOTENSIN II-INDUCED HYPERTENSION IN MICE INCREASES CA2+ ACTIVITY WITHIN ASTROCYTE MICRODOMAIN CONTRIBUTING TO ENHANCED PARENCHYMAL ARTERIOLE TONE

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The mechanisms underlying cognitive decline are unknown but evidence support hypertension as an important contributor in this process. Although focus has been placed on the effect of hypertension on vascular function, less is understood of its effects on non-vascular cells (i.e., astrocytes, microglia). Astrocytes form a functional unit (the neurovascular unit) with blood vessels, and astrocyte Ca^{2+} changes modulate cerebral blood flow. Thus, we determined whether hypertension-induced changes in parenchymal arteriole (PA) responses concomitantly altered astrocyte and microglia function. Chronic hypertension was induced in mice by 28-day angiotensin II (Ang II) infusion. Astrogliosis and microglia activation was assessed with immunolabeling against GFAP and Iba1, respectively. The functional dynamic of the neurovascular unit was assessed using a brain slice model where we measured PA tone along with Ca^{2+} events within astrocytes subcompartments (soma, processes and microdomains). In hypertensive mice, resting PA tone and myogenic responses were significantly increased. Chronic hypertension significantly increased spontaneous Ca^{2+} events within astrocyte microdomains and, in support of enhanced vessel-to-astrocyte signaling, astrocyte Ca^{2+} events induced by myogenic constrictions. The transient potential receptor vanilloid 4 (TRPV4) channel, expressed in astrocyte endfeet, respond to hemodynamic stimuli such as increased pressure/flow. Supporting a role for TRPV4 channels in aberrant astrocyte Ca^{2+} dynamics in hypertension, cortical astrocytes from hypertensive mice showed augmented TRPV4 channel currents and Ca^{2+} responses to the selective channel agonist GSK1016790A. In addition, pharmacological TRPV4 channel blockade or genetic deletion abrogated hypertension-induced increases in PA tone. An increased in reactive astrocytes and microglia activation was supported by augmented GFAP and Iba1 expression. Together, these data supports the notion that chronic hypertension not only increases PA tone but concomitantly enhances intracellular astrocyte Ca^{2+} dynamics. We hypothesize aberrant astrocyte Ca^{2+} events in microdomains constitute an early event towards the progression of neurovascular unit dysfunction and onset of cognitive decline.

BLOOD-BRAIN BARRIER BREAKDOWN IN ALZHEIMER'S DISEASE AND OTHER DEMENTIAS

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We and others have shown that chronic attenuation of the mammalian target of rapamycin (mTOR) ameliorates memory deficits and reduces the accumulation of amyloid-beta(Aβ), causally implicated in Alzheimer's (AD), in surrogate models of the disease. We showed that mTOR promotes brain microvascular dysfunction and disintegration, reducing clearance of Aβ and driving brain microvascular and parenchymal Aβ accumulation and disease progression through the inhibition of endothelium-dependent brain vascular reactivity and interneuron-dependent neurovascular coupling, both dependent on nitric oxide (NO) bioavailability. An intact blood-brain barrier (BBB) limits entry of proinflammatory and neurotoxic blood-derived factors into the brain parenchyma. The BBB is damaged early in AD, which contributes significantly to disease progression. We report that, inde-

pendent of its role in the regulation of NO bioavailability, mTOR drives blood-brain barrier breakdown in models of AD amyloidosis (hAPP-J20 and Tg2576 mice) and in a model of vascular cognitive impairment associated with atherosclerosis (LDLR-/- mice). Using an in vitro model of BBB we found that mTOR drives BBB disruption in these disease models through downregulation of specific tight junction proteins and upregulation of matrix metalloproteinase-9 (MMP-9) activity as well as circulating inflammatory factors. Together, our data establish mTOR as a critical mediator of BBB breakdown in surrogate models of AD and vascular cognitive impairment, and suggest that FDA-approved drugs such as rapamycin and rapalogs could be used for the restoration of BBB integrity early in the progression of AD and other brain disease states that have BBB breakdown and vascular dysfunction as a common etiology.

BRAIN VASCULAR ALTERATIONS IN NEUROADAPTATIVE RESPONSES: AT1 RECEPTORS AS A RELEVANT TARGET

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Amphetamine exposure is validated as a pharmacological tool to resemble several psychiatric diseases, such as the dopaminergic/glutamatergic imbalance in schizophrenia and mania. However, its effects extend beyond neurotransmission, as psychostimulant exposure has been associated to brain vascular damage and neuroinflammation. Angiotensin II AT1 receptors (AT1-R) are implicated in brain micro-vascular physiological responses; whereas their over-expression is related to inflammatory mediators release, oxidative damage, and endothelial dysfunction in pathological conditions. Using adult Wistar rats exposed to an amphetamine-induced behavioral and neurochemical sensitization protocol, pretreated with an AT1-R antagonist, we analyzed detrimental consequences involving: blood brain barrier (BBB) permeability, microvessels rearrangement, glial reactivity and behavioral performance. For these purpose astroglia and microglia activation was assessed with immunolabeling against GFAP and CD11b, respectively. The vascular alterations were analyzed using von willebrand factor for vascular rearrangement at brain level and in isolated microvessels AT1-R, lipid peroxidation and HSP70 expression were determined. The animals' performances were tested on Y-maze, Holeboard and Hot plate. The BBB permeability was analyzed using an Evans blue technique. Our results showed that amphetamine induced: -Evans blue leakage in hippocampus, -increased glial reactivity and microvessel rearrangement at prelimbic prefrontal and somatosensory cortex,- AT1-R increased expression and cellular stress in isolated microvessels, -working memory deficit and altered perception. Remarkably, our results showed prevention of amphetamine-induced working memory deficit by AT1-R blockade in line with absence of glial and vascular alterations over prelimbic prefrontal cortex. Similarly, AT1-R antagonism prevented amphetamine-induced structural modifications in somatosensory cortex along with altered thermal nociception. The neuroadaptative responses, evoked by the psychostimulant and depending on AT1-R, might resemble some features described for neurodegenerative pathologies, as they involve neuroinflammation and vascular rearrangement, with local vulnerability in cortical areas. In this way, our contribution supports the dysregulation of central Angiotensin II as trigger for neurovascular unit alterations underlying brain disorders.

SELECTED ABSTRACT FOR SYMPOSIUM

DIFFERENTIAL CORTICAL AND STRIATAL ASTROCYTES GLUTAMATE TRANSPORTERS EXPRESSION AND TNF-ALPHA RELEASE

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IMBIOMED – UBA, CONICET. CEFYBO-CONICET-UBA

Astrocytes provide metabolic and structural support to the brain and are essential partners in neurotransmission and behavior. GLT1 and GLAST glutamate transporters in astrocytes do most of the work by clearing extracellular glutamate. Their expression and activity are influenced by cytokines, growth factors and reactive oxygen species (ROS). We have previously shown that brain-derived neurotrophic factor (BDNF) reduces ROS accumulation in astrocytes and neurons induced by 3-nitropropionic acid (3-NP), a toxin that causes mitochondrial dysfunction and oxidative stress as it occurs in Huntington's disease (HD). Being the striatum more susceptible to 3-NP, here we investigated BDNF effect on glutamate transporters expression in primary cortical and striatal astrocytes. Astrocytes were incubated for 24 h with 3-NP ± BDNF. BDNF increased GLT1 expression in cortical and striatal

astrocytes per se and in the presence of 3-NP ($p<0.05$). BDNF alone increased basal GLAST expression in cortical astrocytes ($p<0.001$). To evaluate if neurons regulate GLT1 and GLAST expression, we treated astrocytes with neuronal conditioned medium (NCM) from a HD neuronal striatal cell model ST14A-Q120 (Q120), which expresses human mHtt with 120 glutamine repeats and ST14A-Q15 (Q15) which expresses normal human Htt with 15 glutamine repeats. We found that only NCM from Q120 cells decreased astrocyte viability ($p<0.001$) and also modulated GLT1 but not GLAST expression. Since 3-NP induces inflammation, we evaluated tumor necrosis factor-alpha (TNF-alpha) levels by ELISA in cortical and striatal astrocytes treated with 3-NP ± BDNF or NCM. Interestingly, in both astrocyte populations, BDNF reduced the increase in TNF-alpha levels induced by 3-NP. Moreover, production of TNF-alpha was higher in striatal than in cortical astrocytes. Also, NCM stimulated TNF-alpha release only in striatal astrocytes. A better understanding of BDNF effects on glutamate transporters modulation could provide new strategies for the treatment of neurodegenerative diseases.

SAIC SYMPOSIUM: SIGNAL TRANSDUCTION AND MOLECULAR MECHANISMS OF DISEASE

ASSEMBLY OF NEURONAL CONNECTIVITY BY NEUROTROPHIC FACTORS AND LEUCINE-RICH REPEAT TRANSMEMBRANE PROTEINS

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Proper function of the nervous system critically relies on sophisticated neuronal networks interconnected in a highly specific pattern. The architecture of these connections arises from sequential developmental steps such as axonal growth and guidance, dendrite development, target determination, synapse formation and plasticity. Leucine-rich repeat (LRR) transmembrane proteins have been directly linked to different human brain disorders, including autism, schizophrenia, obsessive-compulsive disorders, epilepsy, essential tremor, Alzheimer and Parkinson´s disease. The members of this superfamily of proteins execute their functions acting as trans-synaptic cell adhesion molecules involved in target specificity and synapse formation or working in cis as cell-intrinsic modulators of neurotrophic factor receptor trafficking and signaling. Here, we present a novel physiological contribution of the LRR transmebrane protein Lrig1 as an endogenous inhibitor of hippocampal dendrite morphogenesis and branching. Our data establish that Lrig1 is an essential molecule linking TrkB signaling to dendrite development and suggest that Lrig1 contributes to shape distinctive patterns of dendritic arborization in specific neuronal populations in response to neurotrophins. Furthermore, loss of Lrig1 led not only to morphological abnormalities, but also to social interaction deficits, highlighting the importance of this cell-intrinsic modulator for normal nervous system development and plasticity. Because several neuropsychiatric disorders are associated with altered dendrite morphology and social phenotypes, our findings raise the possibility that Lrig1 dysfunction may contribute to different neurological disorders.

IDENTIFICATION OF A CENTROSOMAL PROTEIN COMPLEX THAT ENSURES PROPER SPINDLE ORIENTATION AND LUMEN FORMATION IN 3D EPITHELIAL CELL CULTURES

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Epithelia are three-dimensional arrangements of cells organized into structures which delimit morphologically and physiologically different compartments. At the cellular level, epithelial cells are characterized by the presence of an apical pole, facing the lumen of an organ or the outer environment, and baso-lateral domains, involved in cell-extracellular matrix and cell-cell interactions. Each pole is characterized by its specific protein and lipid membrane composition, and its distinct cellular organelles organization, which ensures the vectorial absorption and secretion of proteins and other molecules. Most epithelia organize to form hollow organs with a single lumen. This organization requires the accurate three-dimensional arrangement of cell divisions, which is dependent on mitotic spindle alignment with respect to epithelia apico-basal axis. Mitotic spindle orientation is defined by signaling pathways that provide molecular links between specific spots at the cell cortex and astral microtubules, which have not been fully elucidated. AKAP350 is a centrosomal/Golgi scaffold protein, implicated in the regulation of microtubule dynamics. Using 3D epithelial cell cultures, we found that cells with decreased AKAP350 expression (AKAP350KD) formed polarized cysts with abnormal lumen morphology. Analysis of mitotic cells in AKAP350KD cysts indicated defective spindle alignment. We established that, at the spindle poles, AKAP350 interacts with EB1, a microtubule associated protein that regulates spindle orientation. Decrease of AKAP350 expression lead to a significant reduction of EB1 levels at spindle poles and astral microtubules. Conversely, overexpres-

sion of EB1 rescued the defective spindle orientation induced by deficient AKAP350 expression. The specific delocalization of the AKAP350/EB1complex from the centrosome decreased EB1 levels at astral microtubules and lead to the formation of 3D-organotypic structures which resembled AKAP350KD cysts. Altogether, these results showed that AKAP350 recruits EB1 to the spindle poles and, therefore, ensures EB1 presence at astral microtubules, uncovering interesting mechanistic data on the factors that govern spindle orientation during epithelial organogenesis.

PLURIDIMENSIONAL EFFICACY AT HISTAMINE RECEPTORS. WHEN SIMPLE ANTAGONISM IS NOT ENOUGH

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H1 and H2 histamine receptor ligands, although developed many decades ago, are still effective for the treatment of allergic and gastric acid–related conditions and rank among the most widely prescribed and over the counter-sold drugs in the world. They exert their action by antagonizing the effects of histamine over H1 and H2 histamine receptors, both belonging to the G protein coupled family of receptors (GPCRs). Advances in the comprehension of GPCRs, allow understanding the mechanisms of activation of the different events that could be mediated by a receptor, e.g. G-protein activation, desensitization, internalization and G protein independent signaling among others. Based on this, the concept of pluridimensional efficacy accounts for the different behaviors triggered by a GPCR where it is the ligand–receptor complex, and not the receptor itself, which governs the final downstream signaling outcome and the cellular response. Concerning the wide range of possible events mediated by a GPCR, a ligand may cause differential activation of some, but not all of them leading to biased agonism. In the present work, we show that several histaminergic ligands, typically used to block histamine effects, mimic histamine action concerning receptor desensitization, internalization or even signaling through a G protein independent pathway. These novel aspects of the pharmacology and molecular mechanisms of these ligands are discussed with the aim to optimize current therapies, and to avoid undesired side effects when used in standard treatments.

SELECTED ABSTRACT FOR SYMPOSIUM

INHIBITION OF TOLL-LIKE RECEPTOR 2 ACTIVITY BY THE NITRONE SPIN TRAP 5,5-DIMETHYL-1-PYRROLINE N-OXIDE (DMPO) THROUGH DIRECT INTERACTION WITH THE TOLL/INTERLEUKIN-1 RECEPTOR DOMAIN (TIR)

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Inflammatory activation of macrophages throughout Toll-like Receptors (TLRs) is a fundamental step in the development of immune response triggered by bacterial and fungal infections. TLR4 and 2 deregulated signaling has been implicated in a great number of inflammatory diseases, thus their inhibition has become a therapeutic target. Previously, we found that the nitrone spin trap 5,5-dimethyl-1-pirroline N-oxide (DMPO) dampens lipopolysaccharide (LPS)- triggered TLR4 receptor signaling in RAW264.7 cells at transcriptomic and functional levels. However its effect on TLR2 signaling remains unclear. Because TLRs 4 and 2 have structural similarities in their intracellular domain responsible for signal transduction called TIR, we hypothesize that the effects of DMPO in these two receptors were caused by direct binding of the spin trap to their TIR domain. Unfortunately TLR4 TIR domain has not been crystallized, therefore we use combined techniques of docking, molecular dynamics simulations and QTAIM (Quantum Theory of Atoms In Molecules) calculations to determine the interaction between DMPO and TLR2 TIR domain. Our results show that DMPO could bind to four specific residues in a key region implicated in signal transduction known as BB-loop. To corroborate these results we used an experimental model based on hTLR2.6-expressing HEKs cells and determine that DMPO can block zymozan-triggered-TLR2-mediated NF-kb activation. Because TLRs bind to adaptor protein MyD88 (Myeloid differentiation primary response 88) we used co-immunoprecipitation to test whether DMPO can prevent TLR2-MyD88 binding and found no effect. Taking together our results show that DMPO blocks TLR2 signaling without preventing completely the coupling of adaptor protein to its TIR domain. This can be due to DMPO disrupting proper coupling of TLRs with MyD88 by direct binding to BB-loop region responsible for signal transduction. These data encourages the use of DMPO derivatives as mechanism-based TLR inhibitors. Supported by PIP916-PICT3369 to DCR and SEGM.

SAI SYMPOSIUM: INNATE IMMUNITY

STRATEGIES BY PATHOGENIC FUNGUS C. ALBICANS TO EVADE THE HUMAN IMMUNE SYSTEM

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Upon infection the human pathogenic fungus *Candida albicans* is immediately attacked by the innate immune system. This includes the complement system but also innate immune cells like neutrophils and monocytes. Despite the strong immune response *Candida albicans* can survive in a host, especially in immunocompromised patients like those with HIV infection, with transplants or suffering from diabetes. Survival of the fungus depends on evasion mechanisms that can affect many reactions of the immune response. Like other pathogenic microbes *Candida albicans* learned to cover its surface with human regulatory molecules like complement factor H in order to protect from complement attack, or by secreting enzymes as defense strategy. However, *Candida albicans* also modulates the immune response of monocytes to also influence the developing adaptive response. A very new field in this respect especially in response to fungal infections is the generation and communication of immune cells via microvesicles. We show for the first time that Candida induces the release of microvesicles from monocytes which is enhanced when *Candida albicans* is opsonized with complement proteins. In this process soluble beta glucan of the fungus attaches to the CR3 receptor on monocytes and induces vesicle formation which in turn modulates the immune response.

UNCONVENTIONAL SECRETION OF IL-1B BY AN AUTOPHAGY-MEDIATED MECHANISM IN HUMAN NEUTROPHILS

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Neutrophils are the most numerous leukocytes in human circulation with a production that dramatically increases during inflammation or systemic infection. They represent the first line of cellular defense against bacterial and fungal infections, playing crucial roles in the response to a broad range of clinically relevant pathogens. Recently, the concept that neutrophils are solely microbicidal cells has been revised. In fact, different studies have demonstrated their ability to respond to environmental cues and undergo transcriptional reprogramming leading to de novo synthesis of cytokines. As we previously demonstrated, neutrophils secrete Interleukin-1 beta (IL-1β), a key pro-inflammatory cytokine that exerts pleiotropic effects on both the innate and adaptive immune system.
IL-1β is synthesized in the cytoplasm as a precursor, pro-IL-1β, which is proteolytically processed to acquire biological activity. We have previously demonstrated that human neutrophil IL-1β processing is dependent on caspase-1, but we also identified a potential role for the neutral proteases elastase and/or proteinase-3 in IL-1β processing. Unlike proteins endowed with the leader (N-terminal signal) peptides, IL-1β is a leaderless cytosolic protein which cannot enter the conventional secretory pathway normally operating via the endoplasmic reticulum and the Golgi apparatus. Thus, despite strenuous efforts in many laboratories, how IL-1β is secreted is still a matter of intense debate. Our recent findings revealed that an unconventional secretory autophagy mechanism is involved in IL-1β secretion in human neutrophils. We found that despite neutrophils also release pro-IL-1β, only the secretion of the mature isoform appears to be mediated by autophagy. We also determined that neutrophil serine proteases might modulate IL-1β secretion.
Considering the diverse infectious and inflammatory conditions where neutrophils infiltrate the tissues in large numbers, our findings provide potential targets to identify tools to control IL-1β-mediated inflammation in those diseases where neutrophil derived-IL-1β plays a crucial role in their pathogenesis.

NEUTROPHIL IMMUNOREGULATORY FUNTION ON γδ T CELLS: ROLE OF SERINE PROTEASES

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γδ T cells are non-conventional, innate-like T cells, characterized by a restricted TCR repertoire. They participate in protective immunity response against extracellular and intracellular pathogens, tumor surveillance, modulation of innate and adaptive immune responses, tissue healing, epithelial cell maintenance, and regulation of physiological organ function. In our laboratory, we have investigated the role of neutrophils during the activation of human blood γδ T cells through CD3 molecules. We found that the up-regulation of the activation marker CD69 expression, and the production of IFN-γ and TNF-α induced by anti-CD3 antibodies were potentiated by neutrophils. We

also showed that the inhibition of caspase-1 and neutralization of IL-18 did not affect neutrophil-mediated modulation. By contrast, the treatment with serine proteases inhibitors prevented the potentiation of γδ T cell activation induced by neutrophils. Moreover, the addition of elastase to γδ T cell culture increased their stimulation, and the treatment of neutrophils with elastase inhibitor prevented the effect of neutrophils on γδ T cell activation. Furthermore, we demonstrated that the effect of elastase on γδ T cells was mediated through the proteases-activated receptor, PAR1, since the inhibition of this receptor with a specific antagonist, RWJ56110, abrogated the effect of neutrophils on γδ T cell activation.

YOUNG RESEARCHER PRESENTATION

CRITICAL ROLE FOR SEC22B-DEPENDENT ANTIGEN CROSS-PRESENTATION IN ANTI-TUMOR IMMUNITY

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$CD8^+$ T cells mediate antigen-specific immune responses that can induce rejection of solid tumors. In this process, dendriticcells (DCs) are thought to take up tumor antigens, which are processed into peptides and loaded onto MHC-I molecules, aprocess called “cross-presentation”. Neither the actual contribution of cross-presentation to anti-tumor immune responses northe intracellular pathways involved *in vivo* are clearly established because of the lack of experimental tools to manipulate thisprocess. To develop such tools, we generated mice bearing a conditional DC-specific mutation in the sec22b gene, a criticalregulator of endoplasmic reticulum–phagosome traffic required for cross-presentation. DCs from these mice show impairedcross-presentation *ex vivo* and defective cross-priming of $CD8^+$ T cell responses *in vivo*. These mice are also defective for anti-tumorimmune responses and are resistant to treatment with anti–PD-1. We conclude that Sec22b-dependent cross-presentation in DCs is required to initiate $CD8^+$ T cell responses to dead cells and to induce effective anti-tumor immune responsesduring anti–PD-1 treatment in mice.

SAFIS SYMPOSIUM: ADVANCES IN RENAL PHYSIOLOGY AND PATHOPHYSIOLOGY

NEW BIOMARKERS OF ACUTE KIDNEY INJURY

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Acute kidney injury (AKI) is common in intensive care units. The mortality rate for hospitalized patients who develop AKI is approximately five times higher than without AKI. Despite the introduction of new therapies, the mortality associated with this pathology has improved little over the last years. The routinely available clinical parameters of kidney disease (plasma creatinine and urea) do not provide in practice either a sensitive or specific indication of renal function, and show AKI well after the injury has occurred. Early detection of this pathology could permit implementation of salvage therapies and improve patient outcomes. Over the last years, tubular proteins released during tubular insult have garnered much attention as promising AKI urine biomarkers. Neutrophil gelatinase associated lipocalin (NGAL), kidney injury molecule-1 (Kim-1) and N-acetyl-D-glucosaminidase (NAG) have demonstrated to be early predictors for diagnosis or outcome of AKI in human as well as in animal models. Related to this issue, in the last years we have been trying to validate different proteins excreted in urine as noninvasive biomarkers of AKI of different etiologies. Our group was pioneering in detecting the Organic Anion Transporter 5 (Oat5), the Sodium-Dicarboxylate Cotransporter 1 (NaDC1) and Caveolin-2 (Cav-2) in urine. The urinary excretion of these proteins has been evaluated in different experimental models of renal diseases in rats and compared with traditional parameters of renal function (plasma urea and creatinine, creatinine clearance, urinary alkaline phosphatase activity, histological lesions). The results obtained in experimental models of AKI (ischemic, obstructive and nephrotoxic-induced by methotrexate, cisplatin and mercuric chloride) allow us to propose Oat5, NaDC1 and Cav-2 as potential biomarkers of different stages of this disease. The next step would be passing from preclinical animal research to clinical trials in order to evaluate the utility of these proteins as biomarkers of AKI in humans.

BEYOND WATER TRANSPORT: ROLE OF AQP2 IN MODULATING CALCIUM SIGNALS

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There is a growing body of evidence indicating the involvement of aquaporins water channels (AQPs) in numerous cellular processes, apparently not only related to their canonical function of water permeation, with important implications in physiological and pathological processes. For instance, it has been demonstrated that AQPs have a role in cell volume regulation, migration, apoptosis, cell proliferation, angiogenesis and tumor growth, although the involved mechanisms are not well understood. Presumably, in these processes, AQPs may exert an influence in cell signaling by different mechanisms such as crosstalk with other cell membrane proteins or by forming macromolecular complexes. Several studies, including ours, reported evidence that activation of the TRPV4 non selective Ca^{2+} channel, by hypotonic stimulus is influenced by the presence of AQPs, and it is responsible for Ca^{2+} entry which, in turn, triggers cell volume regulation mechanisms. Studying the consequences of the interactions between TRPV4 and AQP2 is of particular interest in the cells of the renal collecting tubule, which under physiological conditions are exposed to sudden variations in osmolarity and flow (both stimuli involved in the activation of TRPV4). We also proposed that, secondary components of TRPV4-dependent Ca^{2+} homeostasis would also be affected by AQPs, such as ATP release, Ca^{2+} release from internal stores, and/or activation of store-operated channels (SOCs). We showed in renal cells that AQP2 is physically associated with TRPV4 and with the small-conductance potassium channel (SK3). This interaction is crucial for the activation of SK3 by TRPV4, and relevant to modulate the magnitude of store operated calcium entry (SOCE). Since it has been recently demonstrated that SOCE plays an important role in AQP2 translocation to the apical plasma membrane, this mechanism may be important for rapid adjustments in renal free water handling.

EFFECT OF FEMALE SEX HORMONES ON KIDNEY FUNCTION, BLOOD PRESSURE AND SALT SENSITIVITY

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About one-third of the world's population has abnormally high levels of blood pressure, hypertension, which is responsible for almost 50% of deaths from stroke and coronary heart disease. Salt sensitivity is a risk factor for cardiovascular morbidity and mortality and other diseases as well.

We reported a model of salt sensitive hypertension in adult ovariectomized (oVx) Wistar rats. oVx is performed at 60 days of life and rats are studied 3 months later (Di Ciano L et al Am J Physiol 2015). oVx rats are normotensive under normal salt intake (NS, 0.24% NaCl), but upon a high salt intake (HS, 1% NaCl) oVx rats developed a blood pressure profile of salt-sensitive hypertension. Hypertension is accompanied by a reduced ability to excrete sodium. Our studies on kidney molecules related to sodium balance found that the circuit Dopamine D1-like receptor, cytochrome P450 4A and Na+, K+-ATPase (D1DR - Cyp 4A – NKA, respectively) is altered by the absence of ovary hormones.

Lately, we observed that cotransporters NKCC2 and NCC are also dysregulated in oVx rats and do not properly respond to a HS intake. In oVx HS rats estrogen treatment (E2) avoided Na+ retention and hypertension. Instead, progesterone (P4) treatment did not. E2 supplementation restores the integrity of D1DR - Cyp 4A – NKA circuit, and the normal response of NKCC2 and NCC to HS intake. P4 supplementation, though partially improving D1DR - Cyp 4A – NKA circuit and NKCC2 response, fails to normalize NCC expression and sodium excretion. In oVx rats HS intake also promotes changes in the expression of proteins related to sodium transport in peripheral blood mononuclear cells, mainly peripheral lymphocytes. Therefore, sodium transport is modified at several levels of normal physiology. In this context, deranged renal function and adaptive immunity may contribute to the pathophysiology of salt sensitive hypertension.

HEAT SHOCK PROTEIN 70/CHIP/NOX4 INTERACTION IN THE ANTIOXIDATIVE EFFECT OF LOSARTAN IN PROXIMAL TUBULE CELLS FROM SPONTANEOUSLY HYPERTENSIVE RATS (SHR)

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Background: The effects of the Angiotensin II/Angiotensin II type 1 receptor (AT1R) are dependent on reactive oxygen species (ROS) production, which are stimulated by NADPH oxidase activation. ROS act as signaling molecules that mediate Angiotensin II-dependent signal transduction pathways. The Angiotensin II generated in

proximal tubules induces renal injury through NADPH oxidase-dependent ROS production. Increased oxidative stress in SHR was found to contribute to proapoptotic and profibrotic mechanisms which are critical for renal injury. Cloned from the kidney, Nox4 belongs to the Nox family of NADPH oxidases Hsp70 regulates a diverse set of signaling pathways through their interactions with proteins. CHIP is a E3 ubiquitin ligase that targets proteins for polyubiquitination and degradation.
Aim: We study whether Hsp70/CHIP contributes to the negative regulation of Nox4 after AT1R blockage. Methods/Results: Primary culture of proximal tubule epithelial cells (PTCs) from SHR and WKY were stimulated with Angiotensin II (AII) or treated with Losartan (L) or Losartan plus Angiotensin II (L+AII). Losartan decreased AT1R and Nox4 while enhancing caveolin-1 and Hsp70 protein expression in SHR PTCs. Immunoprecipitation and immunofluorescence proved interaction and colocalization of increased Hsp70/CHIP with decreased Nox4 in SHR PTCs (L) vs (All). Hsp72 knockdown resulted in enhanced Nox4 protein levels, NADPH oxidase activity and ROS generation in SHR (L) and (L+AII) revealing that Losartan was unable to abrogate aII effects on Nox4 expression and oxidative activity. Moreover, MG132 exposed PTCs (L) demonstrated blocked ubiquitinated Nox4 degradation and increased colocalization of Nox4/Ubiquitin by immunofluorescence. Conversely, Hsp72 depletion reduced Nox4/Ubiquitin colocalization causing Nox4 upregulation due to proteasomal degradation inhibition, although Losartan treatment.
Conclusion: Our study demonstrates that after AT1R blockage, Hsp70 interacts and cooperates with CHIP to regulate Nox4 ubiquitination and proteasomal degradation providing a possible explanation for the mechanism that drives the Losartan antioxidative effect.

SAIC SYMPOSIUM: NEUROPEPTIDES IN LEARNING AND THE NEUROENDOCRINE REGULATION

IDENTIFICATION OF SEX-SPECIFIC PACAP/PAC1 MICROCIRCUITS IN THE AMYGDALA THAT REGULATE FEAR LEARNING AND EXTINCTION IN MICE

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Learned fear responses to threatening situations are vital to survival. Disproportionate or inappropriate fear can, however, lead to pathological states and increased vulnerability for developing anxiety-related disorders such as post-traumatic stress disorder (PTSD). Understanding the biological mechanisms that regulate fear responses are thus critical for assessing risk, discovering biomarkers, instituting preventative measures, and designing new treatment strategies for these disorders. Several studies have now demonstrate sex-depended and sex independent linkages of the neuropeptide PACAP and its plasma membrane receptor PAC1 are to PTSD and panic disorders at both genetic and epigenetic levels. These findings complement a considerable set of prior evidence implicating PACAP/PAC1 signaling in stress and fear circuitries. Lacking, however, is any knowledge of specific PACAP/PAC1 receptor microcircuits in the fear circuitry, and how such circuits would operate to regulate various aspects of fear learning and extinction. We have thus begun to dissect at the cellular, molecular, and behavioral levels the involvement of PACAP-PAC1 signaling in regulating fear within the amygdala, a key brain region involved in fear leaning. As experimental tools to do this we have generated 1) PAC1loxP/loxP mice that allow localized elimination of PAC1 receptors to be achieved by stereotaxic delivery of adeno-associated virus (AAV2) expressing Cre recombinase and 2) PACAP-EGFP reporter mice that allow visualization of PACAP-ergic neurons and their axons at high resolution. Data obtained so far infer the existence of at least two distinct PACAP/PAC1 receptor microcircuits within the amygdala that operate in a striking sex-specific manner.

MOLECULAR MECHANISMS INVOLVED IN THE SPATIOTEMPORAL SIGNALING RESPONSE OF THE CRH SYSTEM

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The main goal of our work is the identification and characterization of cellular mechanisms and molecular components involved in signaling responses of the corticotropin-releasing hormone (CRH) system that includes the G protein-coupled receptors CRHR1 and CRHR2, and CRH and CRH-related peptides urocortins 1-3. CRH system dysregulation is causally linked to stress-related disorders: psychiatric conditions (depression, anxiety, addictions), neuroendocrinological alterations, and to the onset of neurodegenerative diseases. We investigate the spatiotemporal features of signaling responses of the CRH system by means of molecular and cell biology approaches including optical methods, in functionally relevant contexts. We demonstrated that ERK1/2 activation

downstream CRHR1 in a hippocampal neuronal context involves a first acute phase dependent on B-Raf and and protein kinase A, and a second sustained phase dependent on CRHR1 internalization. Thus, CRHR1 activates G protein–dependent and internalization-dependent signaling mechanisms.The cyclic AMP (cAMP) response downstream CRHR1 includes the atypical soluble adenylyl cyclase (sAC) besides classic transmembrane adenylyl cyclases in neuronal and endocrine cells. Only sAC activity is essential for internalization-dependent cAMP generation and sustained ERK1/2 activation responses, revealing a functional association between sAC-generated cAMP and endosome-based GPCR signaling. Similar sustained cAMP responses downstream CRHR1 are detected in established hippocampal neuronal cell lines and in ex vivo primary neuronal cultures from wild-type and conditional mice mutants. We are currently exploring other activated effectors (Akt, CREB) of the CRH system response, and extended recently our analyses to the CRHR2. A precise definition of CRH signaling mechanisms with spatial and temporal resolution will enable identification of novel targets for pharmacological intervention in neuroendocrine tissues and specific brain areas involved in CRH-related disorders. Funded by ANPCyT, CONICET and FOCEM (COF 03/11).

SELECTED ABSTRACT FOR SYMPOSIUM I

HUMANIN, A MITOCHONDRIAL-DERIVED PEPTIDE RELEASED BY ASTROCYTES, PREVENTS SYNAPSE LOSS IN HIPPOCAMPAL NEURONS.

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INBIOMED & IBCN Prof. De Robertis CONICET Facultad de Medicina UBA

Ovarian hormones are neuroprotective, in part by activating neural steroid receptors but also by regulating the release of neurotrophic factors by glial cells. After menopause, loss of ovarian hormones is often associated brain hypometabolism, synaptic failure and mitochondrial dysfunction.

Humanin (HN) is a mitochondrial-derived peptide with cytoprotective, metabolic, and anti-inflammatory effects in tissues with high metabolic rates and whose expression decreases with age. Our previous data in vivo show that HN colocalizes with astrocyte markers and its expression decreases in the hippocampus of hormone-deprived female rats. Still, little is known about ovarian hormone regulation of HN expression and release by astrocytes and the effects of this peptide on neuronal function. The aim of this study was to evaluate the effects of ovarian hormones in the expression and release of HN by astrocytes and HN action on synaptic parameters in hippocampal neurons in vitro.

To this aim, cultured astrocytes were incubated with estradiol (E, 1 nM), progesterone (P, 1 uM), E+P or vehicle and intracellular HN expression was evaluated by FACS. In parallel, cultured hippocampal neurons were exposed to glutamate in a condition that induces dendritic atrophy and reduces synapse number in the presence or absence of HN (0,01 uM) and the expression pattern of the pre-synaptic marker synaptophysin (SYN) was evaluated by immunocytochemsitry and ImageJ software.

Our results show that E+P increased HN expression per cell and HN levels in astrocyte conditioned media ($p<0.05$; Student's t test). Also, HN prevented glutamate-induced reduction in puncta number and total puncta area for SYN ($p<0.05$, ANOVA).

Our results indicate that ovarian hormones positively regulate the expression and release of an astroglial peptide likely involved in synapse maintenance. Our study could help find new therapeutic targets for interventions that may promote a healthier lifespan for post-menopausal women.

SAIC-NANOMED-AR SYMPOSIUM II

PEROXIDASE CATALYTIC ACTIVITY OF IRON OXIDE NANOPARTICLES AND ITS EFFECT ON BIOLOGICAL SYSTEMS

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Fenton's reaction is known since the late nineteenth century. This is an oxidative reaction where Fe+2 ions catalyze a peroxidase-like reaction with the production of free radicals (Reactive Oxygen Species - ROS). These radicals can cause cell death. The iron oxide nanoparticles (IONP), which have a wide range of use in medical applications (drug delivery, contrast agent, molecule separation, magnetic fluid hyperthermia, etc.), have the ability to catalyze this reaction, and therefore be facilitators of cell death. This fact can be use in cancer treatments. In this presentation will be shown results of the catalytic activity of IONP and the quantification of free radicals produced by means of electronic paramagnetic resonance spectroscopy (EPR).

NANOMEDICAL APPROACH FOR THE MANAGEMENT OF CHRONIC WOUND BIOFILM INFECTION

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Chronic wounds are those do not progress through the healing process successfully and pathophysiology is not yet completely understood. However, altered vascularisation, prolonged inflammation and the inability of immune cells to control bacterial infection are critical challenges that interfere in the physiologic healing of chronic wounds. This environment is propitious for bacterial growth and biofilm formation that further intensifies inflammation, inhibiting tissue repair. For that reason, recurrent surgical procedures (wound debridement or tissue amputation) are needed to avoid life-threatening complications in patients. The lack of antibiotic efficacy against mature biofilms is attributed to restricted drug accessibility, their predominant mechanism of action in targeting metabolically active bacteria and the increase of antibiotic resistance in biofilm cells. The cost and complexity of treating chronic wound biofilms infections remain a serious challenge that require the development of new approaches for effective anti-biofilm treatment. Antimicrobial agents delivery by lipid or polymer nanoparticles is considered a promising strategy for overcoming biofilm resistance. In this sense, lipid nanovesicles are attractive due to their biocompatibility and ability to incorporate lipophilic as well as hydrophilic drugs. Lipid nanovesicles could protect the antimicrobial agent from binding to matrix material and enzymatic inactivation, penetrate the matrix of the biofilm and remain there releasing the antimicrobial agent in high doses in the proximity of bacteria. However, the poor stability of lipid nanovesicles is a drawback for adequate biofilm eradication. We have previously reported that the presence of Halorubrum tebenquichense archaeolipid in lipid nanovesicles increased their colloidal and chemical stability. In the current work we demonstrated that archaeolipid nanovesicles that encapsulated a natural antimicrobial agent, improved anti-biofilm activity.

DESIGN, SYNTHESIS AND CHARACTERIZATION OF FUNCTIONALIZED METAL NANOPARTICLES FOR APPLICATIONS IN MELANOMA TREATMENT

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Malignant melanoma is the most deadly skin cancer, is highly metastatic and resistant to conventional therapies. Advances in nanomaterials contributed in recent years to the development of new strategies for cancer treatment. In this context, we have envisioned the use of two types of metallic nanoparticles: Magnetic nanoparticles (MNPs) and gold nanoparticles (AuNPs), both designed for the treatment of refractory melanoma. MNPs are highly biocompatible, stable and can be directed under an external magnetic field. Moreover, incubation of cells with MNPs can increase intracellular levels of reactive oxygen species (ROS). We demonstrated that the combined treatment of MNPs and ionizing radiation (IR) induces cytotoxic oxidative stress and sensitizes melanoma cells. On the other hand, targeting cancer metabolism has emerged as a promising therapeutic strategy. In this context, multi-resistant melanoma cells have increased mitochondrial respiration and high level of ROS. Disruption of this metabolism, in combination with IR, could be an effective means for this highly resistant cancer. However, mitochondrial targeting of therapeutic agents is still challenging. A way to overcome this limitation is by using nanoparticles. AuNPs are also highly biocompatible, passively accumulate in tumors by enhanced permeability and retention effect and can also be modified for selective mitochondrial active targeting. In addition, they possess unique electronic properties that make them excellent radiosensitizing agents. We demonstrated that AuNPs functionalized with the mitochondriotropic residue triphenylphosphonium, in combination with IR, radiosensitize radioresistant melanoma cells. In conclusion, our results show that these novels metallic functionalized NPs can potentially be used as radiosensitizers for refractory melanoma treatment. Due to their interesting physicochemical and biological properties, these types of NPs could be promising for the development of multifunctional platforms for cancer diagnosis and therapy.

NANOTECHNOLOGY BASED ON FLUORESCENT SILICA FOR BIOMEDICAL DIAGNOSIS

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In the last decade, the trend of biomedical and pharmacological applications of nanotechnology points to the development of integral nanosystems, not only as carriers but also as diagnostic agents. This conception emerges as theranostics. Luminescent nanomaterials based on silica find applications as theranostic agents in biomedicine. This project is devoted to the design, synthesis and physicochemical characterization of fluorescent amor-

phous silica nanoparticles. They are intended, in general, to diagnosis as platforms for flow cytometry and, in special, for the treatment of bone disease. Nanoparticulate silicon dioxide, SiO2, material known as “silica” has certain properties that make it a good candidate as a biomaterial: it has a very labile surface for functionalization, ease of synthesis and biocompatibility. The silica in the form of nanoparticles has been postulated as a bioactive material and beneficial for bone. The bone system is the target of therapeutic choice considering that all the associated pathologies (inflammatory, infectious and oncological) are diseases with high social impact and that lead to a low quality of life in patients who suffer them. In this way, the development of fluorescent silica nanoparticles as a platform for the anchoring of drugs for the treatment of bone pathologies represents a promising nanotechnological tool for the diagnosis by flow cytometry, treatment and monitoring of the evolution of the pathologies to be treated.

A MECHANICAL APPROACH TO SMALL-DIAMETER VASCULAR GRAFTS DEVELOPMENT

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Still to these days there is a strong need for small-diameter vascular grafts (SDVGs) for long-period implantation [1]. Being natural vessels a tissue that is permanently subjected to pulsatile solicitation, their substitute must mimic their characteristic mechanical response. The only synthetic vascular grafts approved as replacement are stiff and fail mainly due to reocclusion, attributed to intimal hyperplasia at the distal anastomosis. Recent studies have reported a strong correlation between graft mechanical properties and intimal hyperplasia onset and severity. In this sense, dynamic mechanical compliance mismatch between native artery and the artificial graft has been identified as a key determinant of SDVGs success [2]. Despite several studies proving this, there has been little progress in the research and development of SDVGs with biomechanical properties matching the native ones [3]. Natural vessel extracellular matrix components (elastin and collagen) are responsible for its unique mechanical response. The structure of arteries and the relationship between arterial tissue components is becoming the main subject of graft development.
Different approaches were addressed, with natural and synthetic polymers. In this presentation, the use of electrospun structures will be presented to achieve successful grafts. The mechanical behavior of synthetic grafts will be compared with the exhibited by human native vessels
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PHARMACEUTICAL NANOTECHNOLOGY: CONTRIBUTIONS IN HUMAN, ANIMAL AND ENVIRONMENTAL HEALTH

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In Argentina, the market for pharmaceutical laboratories is the US $ 1,690 million per year with an annual growth of 17%. It is a sector whose differentiation is highly dependent on the pace of innovation. In the pharmaceutical market, around 90% of new pharmaceutical patents and 40% of new drugs entering the market have formulation problems. Other problems for the appearance of new products are low bioavailability or toxicity. The development of nanopharmaceutical products has generated in the field of drug delivery new opportunities for innovation and the development of successful products. However, in our region it is a market to develop South America represents only a market share of 2% compared to the global market. PANARUM S.A.S. is a pharmaceutical nanotechnology startup that develops nanopharmaceutical products from a platform of technologies for the production of liposomes, polymeric nanoparticles, and nanocrystals of active ingredients aimed at the pharmaceutical, veterinary and related industries. In this presentation, we will share our notions of the pharmaceutical sector and the various strategies in the development of new nanopharmaceutical products and the market in the local sector.

SAI SYMPOSIUM: MUCOSAL IMMUNOLOGY

REGULATION OF ALLERGIC RESPONSES TO FOOD BY COMMENSAL BACTERIA

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Food allergies are a major public health concern and an unmet clinical need. A marked generational increase in disease prevalence has been noted in industrialized societies worldwide. We have proposed that the increasing prevalence of food allergies can be explained, in part, by alterations in the composition and function of the commensal microbiome. In support of this hypothesis we described a role for mucosa-associated bacteria in protection from allergic sensitization in mice (Proc. Natl. Acad. Sci. 2014, 111: 13145-13150). To understand how the microbiota regulates allergic disease in humans we have, in our most recent work, colonized germ free mice with bacteria from the feces of healthy or cow's milk allergic (CMA) infants. We discovered that mice colonized with CMA infants' microbiota exhibited an anaphylactic response to the cow's milk allergen -lactoglobulin, while mice colonized with healthy infants' microbiota were protected against an allergic response. Analysis of differences in composition between our human fecal donors allowed us to develop a microbiota signature that distinguishes the CMA and healthy populations in both the human donors and the colonized mice. Significant differences in the composition of this signature between the two populations were independently validated in a larger patient cohort. Moreover, analysis of gene expression in ileal intestinal epithelial cells of colonized mice identified a significant correlation between the genes associated with allergy protection and taxa from the Lachnospiraceae family (the same family identified as allergy protective in our PNAS report), supporting a causal role for specific bacterial species in protection against food allergy. These robust, pre-clinical, gnotobiotic models are an ideal system to identify key host-microbial interactions that contribute to allergic sensitization to food and will inform the development of novel microbiome-modulating therapeutics to prevent or treat food allergy.

MYELOID CELL ACTIVATION AND EFFECTOR FUNCTION DURING HELMINTH-INDUCED TYPE 2 IMMUNE RESPONSES

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We have examined macrophage and neutrophil activation in the lung after helminth infection. Using in vivo fate-mapping technologies, we find that after helminth infection monocytes recruited to the lung differentiate into alveolar macrophages and assume different functions from resident alveolar macrophages including increased expression of arginase 1 and associated increased capacity to kill parasites in lung tissues. Our findings further indicate that macrophages mediate parasite killing by depleting local arginine concentrations around the parasite. These macrophages exhibit a long lived phenotype in the infected lung and are capable of mediating accelerated resistance to helminth infection upon subsequent exposure to infection. Helminth-induced neutrophils play a critical role in the priming of anti-helminth macrophages in the lung and RNAseq analyses reveal different activation states from neutrophils activated during microbial infections, including expression of type 2 response associated genes and cell cycling genes. These studies indicate that both macrophages and neutrophils are differentially regulated during helminth infection to mediate effector functions specifically tailored to promote host protective immunity against multicellular parasites.

LOCAL PRODUCTION OF IGE IN THE GUT OF PATIENTS WITH FOOD ALLERGY

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Allergen-specific IgE antibodies are responsible for the pathogenesis of allergic diseases such as asthma, allergic rhinitis, atopic dermatitis and food allergy, and can lead to severe life-threatening anaphylaxis. It is known that in non-atopic individuals IgE synthesis is tightly regulated. Depending on the species the serum half-life of IgE is from hours to few days (the shortest of all immunoglobulin isotypes). However, there is a sustained production of IgE, and the location and pathway by which IgE is produced and regulated are poorly understood in humans. In addition, it has been unclear whether the production of IgE follows a similar pattern in mice and man. We have

studied B cells in the colonic mucosa of patients with food allergy and we have characterized active germinal centers with local production of IgE. The Th2-dominant environment of these tissues, which includes high levels of IL-33, TSLP, IL-4, IL-5 and IL-13 may be responsible for the sequential and direct isotype switches at mucosal sites. In this context, intestinal epithelial cells and endothelial cells are implicated as key cell sources of soluble factors that maintain, and likely trigger, the type-2 inflammatory process that promotes the local production of IgE.

In conclusion, we have described that the intestinal mucosa has the intrinsic capability to produce IgE. Colonic tissue of patients with food allergy has all tools for affinity maturation by somatic hypermutation, clonal expansion, and class switch recombination to IgE. Most of these pediatric patients showed negative skin tests and could have been diagnosed as non allergic or non-IgE allergic. Therefore, these findings may provide valuable tools with diagnostic and therapeutic consequences.

YOUNG RESEARCHER PRESENTATION

COULD PROBIOTICS BE PROPOSED AS AN OPTIONAL TREATMENT FOR IBD?

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Inflammatory bowel diseases (IBD) are a group of disorders, including the most conspicuous ones ulcerative colitis (UC) and Crohn´s disease (CD), which are characterized by chronic and relapsing inflammation of the gastrointestinal tract. Affected individuals suffer from debilitating symptoms with severe complications, and a higher risk of colorectal cancer. The frequency of IBD is increasing worldwide, seriously affecting health care costs and inbreeding hospital beds. Over the last decades great effort has been dedicated to understanding the pathogenesis of these inflammatory disorders. It is known that lamina propria T cells (LPTC) play a central role in these pathologies, through the secretion of pro-inflammatory cytokines along with a defective apoptosis. Although novel therapeutic options have arisen there is no effective therapy for most patients. Furthermore, responder patients may become non-responder during treatment. For these reasons there is a need for novel therapeutical strategies.

Kefir is a fermented milk with health-promoting properties that has been used as treatment for gastrointestinal disorders since ancient times. In our group we have established a method for developing microbe-specific T cell lines from LPTC of IBD patients. We showed that microorganisms from kefir (*Enterococcus durans*and *Lactobacillus kefiri*) or their conditioned media modulated anti-CD3-/anti-CD28- induced proliferation and secretion of pro-inflammatory cytokines. In addition, we found that these probiotics suppressed NFKB activation.

In conclusion, we found that probiotic strains from kefir and their metabolites can modulate pathogen-specific activated T cells from IBD patients. These findings may pave the way for novel therapeutic options for patients with IBD.

SAFIS SYMPOSIUM: THE MITOCHONDRION AS TARGET OF DISEASE AND THERAPY

MITOCHONDRIAL PROTECTION BY HYPOTHERMIA IN NEONATAL CEREBRAL HYPOXIA.

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Neonatal ischemic hypoxic encephalopathy is an important cause of neonatal mortality and long-term neurological sequelae. Cerebral palsy is the most devastating complication of the survivors. Hypothermia is the only therapeutic intervention that has been shown to be beneficial in newborns with moderate encephalopathy. There is clinical evidence of its neuroprotective effect, it decreases mortality and the sequelae. During the hypoxic event the death of some neurons takes place, and also leads to a “primary failure”, with mitochondrial dysfunction, that is recovered at least partially as long approximately 30 to 60 min. It is followed by a “latent” phase during which electroencephalogram activity is suppressed, but high energy phosphates are normal or near normal. In this phase there is a reduction in cerebral metabolism, with an increase in tissue oxygenation. Then

a third phase called “secondary failure” is established, characterized by a secondary energetic deterioration of 6 to 15 hours duration, typically associated with seizures, mitochondrial failure, and eventual increase in neuronal death. The severity of the secondary oxidative metabolism impair and mitochondrial dysfunction triggered by an initial hypoxic event is associated with an increased risk of mortality and sequelae in the neurodevelopment of these patients. Mitochondrial dysfunction is considered key in neuronal injury. We are currently evaluating the effect of hypothermia on the mitochondrial function of the brain in a porcine model of global cerebral hypoxia in newborns. Mitochondrial dysfunction was evidenced in the biopsies of the cerebral cortex, which is recovered after a first acute hypoxia, but not after the recurrence of hypoxia. Our results correlate with the histological findings and the parameters that generally control brain function in the clinical setting, near infrared spectroscopy and integrated amplitude electroencephalogram. The protective effects of global hypothermia in brain mitochondrial functions and clinical outcome are under investigation.

CFTR SIGNALING MECHANISMS AND THEIR EFFECTS ON MITOCHONDRIA

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Mutations in the *CFTR* gene cause cystic fibrosis. When the CFTR chloride channel was cloned most work focused on non-genomic effects of CFTR. We instead hypothesized that the complex cystic fibrosis (CF) phenotype should be the result of the differential regulation of a net of CFTR-dependent genes. By using differential display (DD), we demonstrated that many genes showed differential expression between CF cells and CF “corrected” cells. The first CFTR-dependent gene characterized was c-Src, which in turn up-modulated MUC1. Then, we focused in two spots that, contrary to c-Src, showed reduced expression on CF cells. Both DD spots corresponded to mitochondrial proteins, MTND4 and CISD1 (a new gene), the first associated to a reduced mitochondrial complex I (mCx-I) activity in CF cells. We then found that IL-1β produced an autocrine positive feed-back loop that increased its own expression and activity and was responsible for the mtCx-I failure and the increased oxidative stress in CF cells. For the second gene, CISD1, codified by the nuclear genome, it has been very difficult to assign a function, and many distinct functions has been attributed by other authors. Then, we hypothesized that the signal regulating the differential gene expression in CF could be the intracellular Cl^- concentration. Thus, by using DD, we found the existence of Cl^- -dependent genes, including *GRLX5* and *RPS27*, and showed that Cl^- was acting as a second-messenger for CFTR. Then, we demonstrated that increased intracellular Cl^- induced IL-1β secretion through a complex mechanism not yet completely understood, which might involve several parallel pathways. In fact, we demonstrated that Cl^- modulates IL-1β expression and secretion through a novel mechanism of NLRP3 inflammasome activation. In conclusion, Cl^-behaves as a signaling effector and proinflammatory signal, regulating the expression of specific genes, some of them involved in modulation of mitochondrial activity.

PERINATAL ASPHYXIA AND BRAIN DEVELOPMENT: MITOCHONDRIAL DAMAGE WITHOUT ANATOMICAL OR CELLULAR LOSSES

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Perinatal asphyxia remains a significant cause of neonatal mortality and is associated with long-term neurodegenerative disorders. In the present study, we evaluated cellular and subcellular damages to brain development in a model of mild perinatal asphyxia. Survival rate in the experimental group was 67%. One hour after the insult, intraperitoneally injected Evans blue could be detected in the fetuses’ brains, indicating disruption of the blood-brain barrier. Although brain mass and absolute cell numbers (neurons and non-neurons) were not reduced after perinatal asphyxia immediately and in late brain development, subcellular alterations were detected. Cortical oxygen consumption increased immediately after asphyxia, and remained high up to 7 days, returning to normal levels after 14 days. We observed an increased resistance to mitochondrial membrane permeability transition, and calcium buffering capacity in asphyxiated animals from birth to 14 days after the insult. In contrast to ex vivo data, mitochondrial oxygen consumption in primary cell cultures of neurons and astrocytes was not altered after 1% hypoxia. Taken together, our results demonstrate that although newborns were viable and apparently healthy, brain development is subcellularly altered by perinatal asphyxia. Our findings place the neonate brain mitochondria as a potential target for therapeutic protective interventions.

MITOCHONDRIAL FUNCTION IN MYOCARDIAL INJURY DUE TO ISCHEMIA-REPERFUSION AND IN CARDIOPROTECTION

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Cardiovascular diseases are the main cause of morbidity and mortality in the modern world. Among ischemic heart diseases, acute myocardial infarction is the most frequent ant it is characterized not only by cell death but also by a progressive impairment of the ventricular function. As a fine control of respiration is essential to meet energy demands of cardiomyocytes, during the development of heart disease, the loss of mitochondrial function is a key mediator of cell injury and death. Indeed, mitochondria have a critical role in the control of heart metabolism since they are the cellular source of ATP, as well as Ca2+ reservoirs and production sites of reactive O2 and nitrogen species (ROS/RNS). The interplay between mitochondria and other cellular components is considered to regulate the cellular energy levels and redox state. When ischemia takes place, reperfusion is implemented to avoid cell damage. Paradoxically, this maneuver also results in myocardial injury known as reperfusion injury. The absence of O2 during ischemia determines a state of maximum reduction of the mitochondrial respiratory chain. Upon reperfusion, the escape of electrons from the respiratory chain is accelerated, increasing ROS and RNS generation.In this setting, cellular bioenergetics result impaired and this is evidenced by a reduction in mitochondrial respiration, uncoupling and impaired ATP production; as well as by changes in redox balance. Many studies have investigated strategies to reduce ischemia/reperfusion injury through the so called cardioprotective procedures. The approaches that have been fostered include endogenous and pharmacological conditioning. The mechanism involved in the majority of cardioprotective interventions comprises the preservation of mitochondrial integrity and function, together with the maintenance of ROS within physiological levels compatible with signaling and reliable energy supply.

SAIC SYMPOSIUM: INFECTIONS AND PREGNANCY

ROLE OF PLACENTAL-DERIVED IFNB IN THE PROTECTION OF THE FETUS AND THE MOTHER AGAINST VIRAL INFECTIONS

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Viral infections during pregnancy are associated with adverse effects to the mother and the fetus.Infection with ZIKA virus (ZIKV) during the first trimester of pregnancy induces adverse fetal outcomes, including microcephaly and fetal demise. The placenta plays a critical role in the protection against viral infection through the expression of type I interferon beta (IFNβ) and its downstream signals- Interferon StimulatedGenes (ISGs). IFNβ expression and function is critical for the protection against viral infections; however, the ISGs mediating IFNβ protective effect are unknown.

We will discuss the function of placental derived IFNβ as an immune modulator providing protection against viral infections as well as regulating maternal immune responses.

DIFFERENTIAL LOCAL PLACENTAL INNATE IMMUNE RESPONSE AGAINST TWO PROTOZOAN PARASITES: TRYPANOSOMA CRUZI AND TOXOPLASMA GONDII

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Congenital transmission of the zoonotic parasites, Trypanosoma cruzi (T. cruzi) and Toxoplasma gondii (T. gondii), is a relevant public health problem. Although the majority of infected healthy individuals have no symptoms, in immunocompromised or congenitally infected individuals, the parasites can cause severe disease or even death. Congenital transmission of pathogens is the consequence of complex interactions among the parasite, maternal and fetal/newborn immune responses, and placental factors, with the placenta the least-studied component of this “trilogy”. Interestingly, the congenital transmission rates for T. cruzi are low in contrast to the transmission rates for T. gondii, which are high. During congenital transmission, the parasites must cross the placental barrier where the trophoblast, a continuous renewing epithelium, is the first tissue to have contact with the parasite. Importantly, the epithelial turnover is considered part of the innate immune system since pathogens, prior to cell invasion, must attach to the surface of cells. The trophoblast turnover involves cellular

processes such as proliferation, differentiation and apoptotic cell death, all of them are induced by the parasite. Here, we show the current evidence about the trophoblast epithelial turnover as a local placental innate immune response against T. cruzi but not against T. gondii. On the other hand, the trophoblast expresses all of the mammalian Toll like receptors (TLRs). Both parasites are recognized by TLR-2, TLR-4, TLR-7 and TLR-9. Here we show that in ex vivo infected human chorionic villi explants T. cruzi and T. gondii induce a differential TLR and cytokine profile. Moreover, our results show that T. cruzi infection is related to TLR-2 expression and activation contrarily to T. gondii, whose infection is mediated by TLR-4 and TLR-9. We conclude, that the local placental innate immune response is effective against T. cruzi but not against T. gondii.

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SHIGA TOXIN PRODUCING ESCHERICHIA COLI INFECTIONS, INSIGHTS INTO POSSIBLE COMPLICATIONS DURING PREGNANCY.

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Infections during pregnancy are associated with adverse outcomes including miscarriage, premature rupture of membranes, preterm birth, growth restriction and stillbirth. Shiga toxin producing *Escherichia coli* (STEC) is a group of gastrointestinal bacteria that causes diarrhea, hemorrhagic colitis, and can develop a systemic complication known as hemolytic uremic syndrome (HUS). The main virulence factor of STEC is Shiga toxin (Stx) which crosses the intestinal barrier, reaches the bloodstream and damages the target cells through binding the globotriaosylceramide (Gb3) receptor. Among the various Stx subtypes, Stx1 and Stx2 are of eminent clinical importance in human infections being Stx2 associated with more severe cases than Stx1. STEC infections affect mainly young children, although the large HUS outbreak with a Stx2-producing enteroaggregative *E. coli* strain in Europe in 2011 involved more adults than children, and women were overrepresented. We propose that symptomatic or asymptomatic STEC infections during pregnancy may cause maternal or fetal damage mediated by Stx2. Ours studies in rats showed that Stx2 binds the utero-placental unit and causes adverse pregnancy outcomes. Stx2 during early pregnancy induces decidual necrosis, promotes a pro-inflammatory environment that together with high hypoxia levels leads to pregnancy loss. In addition, we demonstrated that an active immunity against Stx2 protects the mother and fetus from Stx2 cytotoxicity. On the other hand, Stx2 during late pregnancy induces placental abruption, intrauterine hemorrhage and premature delivery of dead fetuses. In vitro studies involving human trophoblast cells indicate that Stx2 produces loss of cell viability, including apoptosis, and impairs trophoblast cell migration and invasion. Additionally, Stx2 modulates MMP2 activity suggesting that metalloproteinases can be affected by the toxin. Recent studies suggest that nitric oxide may be related to damages induced by Stx2. Although nowadays there are not reports indicating that Stx2 affect pregnancy in humans, our data suggest that Stx2 may generate complications during gestation.

SELECTED ABSTRACT FOR SYMPOSIUM

CHARACTERIZATION OF RECENTLY TRANSMITTED HIV-1 ENVELOPES CHILDREN INFECTED BY VERTICAL TRANSMISSION

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INTRODUCTION: HIV-1 infection in a new host results from the transmission of a reduced group of cuasispecies. In adults, transmission of HIV-1 events are initiated from a single variant in 80% of the cases but characterization of early transmitted variants in children is limited to few studies. This transmission is associated with the prevalence of strains that use CCR5 co-receptor (R5) to infect the target cells but previous studies have found the presence of 24% of variants that use the CXCR4 co-receptor (X4) in children younger than 3 months. Our objective is to characterize the co-receptor usage and genetic diversity of the early transmitted HIV-1 viruses by a high sensibility sequencing technology in a group of recently infected children.

MATERIALS AND METHODS: Plasma samples were obtained from 7 HIV-1 vertically infected infants with a median of age of 2 months (IQR: 1,5-2,5). A 380bp fragment of HIV-1 env gene was amplified and sequenced by MiSeq (Illumina) with 15.000-20.000 reads per sample. Co-receptor usage was predicted with Geno2pheno tool. Diversity of HIV-1 quasiespecies was calculated with SAVAGE software. Quasispecies with a frequency >10% were considered HIV-1 linages.

RESULTS: A single HIV-1 linage was found in 4/7 (57%)

infants. All of them showed co-existence of X4 and R5-using strains, with predominance of X4 (1 case) or R5 (3 cases). The remaining 3 cases showed more than one lineage. However, all viral sequences were found to use exclusively the CCR5 co-receptor.
CONCLUSION: Single HIV-1 lineages are responsible for productive infection in 57% of children. X4-using strains were present either as majority or minority variants in 4/7 infants. These characteristics may impact early disease progression and response to first line therapy in children and interestingly suggest that high genetic diversity of envelopes does not condition the use of a single co-receptor of viral entry.

SAIC SYMPOSIUM: ENDOCRINOLOGY

TARGETING THE ALTERED METABOLIC PHENOTYPE OF THE PERITONEAL CELLS IN WOMEN WITH ENDOMETRIOSIS TO TREAT CONDITION-ASSOCIATED PELVIC PAIN

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Endometriosis is a chronic and incurable condition associated with debilitating pain and subfertility that affects approximately 176 million women worldwide. It is a complex, and heterogeneous disorder of unknown aetiology defined by the presence of endometrial-like tissue (lesions) outside the uterus. Despite an estimated prevalence in women that mirrors that of diabetes, Crohn's disease, and rheumatoid arthritis, the full socioeconomic impact of endometriosis is considerably under-estimated. Treatment options are inadequate and largely confined to surgical ablation/excision of the lesions (a procedure that is associated with high recurrence rates) and ovarian suppressive drugs (which have important side-effects). Although benign, endometriosis shares cancer-like features and has a mutation profile (in deep infiltrating lesions) reported to be similar to that of ovarian cancer. This lecture will demonstrate how our understanding of the process of development and disease progression in cancer can inform the underlying aetiology of endometriosis and help to identify novel treatments.

HUMAN ADRENAL CORTEX: EPIGENETICS AND POSTNATAL FUNCTIONAL ZONATION

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The human adrenal cortex, involved in adaptive responses to stress, fluid homeostasis, and secondary sexual characteristics, arises from a tightly regulated development of a zone and cell type-specific secretory pattern. However, the molecular mechanisms governing adrenal functional zonation, particularly postnatal zona reticularis (ZR) development, which produce adrenal androgens in a lifetime-specific manner, remain poorly understood. In humans, adrenarche is the consequence of a process of postnatal organogenesis in which a continuous layer of reticularis cells develops and thickens forming the ZR at around 6 to 8 years of age. The main route of androgen synthesis by ZR cells is the classical Δ5 pathway from 17OH-pregnenolone to DHEA and is directly correlated with a specific downregulation of HSD3B2 expression in these cells. Moreover, 17OH-progesterone, adrenal precursor of cortisol synthesis, could also be converted to dihydrotestosterone via an alternative, so-called "backdoor pathway". This backdoor pathway for androgen synthesis was identified as a dominant pathway in humans harboring steroid biosynthetic defects in which adrenal 17OH-progesterone accumulates. However, its relevance to human adrenal normal physiology is largely unknown. We described that the postnatal normal human adrenal cortex would express the enzymes to complete all the steps in the backdoor pathway to DHT. Furthermore, our data gave first hints that intraadrenal backdoor pathway metabolites would have physiological significance as paracrine / autocrine regulators of adrenocortical function by modulating cortisol synthesis by inhibiting CYP17A1 activity. Emerging evidence points to epigenetics as another regulatory layer that could contribute to the modulation of both local adrenal zonation and systemic metabolic signals. We showed that DNA methylation is not involved in specific down-regulation of HSD3B2, NR4A1 and RARB genes in androgen-secreting cells of human adrenal cortex. Finally, our results open promising paths to deepen our understanding of mechanisms governing the fascinating and intriguing zonation and homeostasis of human adrenal cortex. The role of the backdoor pathway should be determined. We hypothesized that specific miRNAs may be involved in postnatal human adrenal cortex zonation by contributing to the ZR-specific down-regulation of adrenal HSD3B2 expression. The discovery of novel players controlling these processes would help to elucidate the causes and outcome of premature adrenarche, hyperandrogenic disorders, adrenal insufficiency, and adrenocortical cancer.

THE PITUITARY TGFB FAMILY, NOVEL TARGET FOR THE TREATMENT OF PITUITARY TUMORS.

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Pituitary tumors are commonly benign adenomas, accounting for 10-15% of all intracranial neoplasms. Among functioning pituitary tumors, prolactinomas are the most frequently observed in the clinic (about 40%). Despite the universal use of dopamine agonists with high efficiency in reducing prolactin levels and decreasing tumor size, there is a subset of prolactinomas (between 15 and 20%) that do not respond to this treatment, named dopamine agonist resistant prolactinomas. This group represents a major challenge for clinical management, because up to date, no alternative treatments have been found. Patients need radiotherapy and probably a transeptosfenoidal surgery, with the risks and cost it implies. On the other hand sex differences in the prevalence of prolactinoma have been described in humans and in mice models. The prevalence is higher in fertile women, and in several mice models females but not males, develop prolactinomas. Even though ovarian hormones are involved, other ovarian factors (inhibin?) are also involved.

We postulate that the inhibitory action of TGFβ1 and activin on lactotroph proliferation and PRL synthesis represent targets for alternative treatments.

We demonstrated that i- Pituitary TGFβ1 activity and activin expression are reduced in prolactinomas vs normal pituitaries; ii- in vivo treatments that improve pituitary TGFβ1 activity are able to reduce the hyperprolactinemia, and counteract the tumor growth; iii- activation of Gi protein-coupled receptors (e.g.: dopamine type II receptor, membrane progesterone receptor α) lead to an increase in pituitary TGFβ1 activity decreasing prolactin secretion; iv- TGFβ1 activity and activin expression are higher in male pituitaries (protective factors?); v- Ovariectomy in mice models recovers pituitary TGFβ1 activity and activin expression avoiding prolactinoma development.

These findings open new possible therapies in treatment for prolactinomas, especially in those that are resistant to dopaminergic drugs.

SELECTED ABSTRACT FOR SYMPOSIUM

IN VIVO AND IN VITRO INHIBITORY EFFECT OF DEXAMETHASONE ON THE THERMOGENIC PROCESS OF BEIGE ADIPOCYTES.

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Glucocorticoids (GCs) modulate the biology and function of white and brown adipose tissue (AT); however, GCs effect on beige adipocytes activation remains unknown. We studied dexamethasone (DXM) effects on the thermogenic activity of beige adipocytes from Retroperitoneal AT (RPAT). Male rats were divided into four groups: control (CTR) and DXM injected (sc 0,03mg/Kg/d for 7 days, DXM) and housed at room temperature, and CTR and DXM housed under cold stimulus (4°C for 7 days, CTR-C and DXM-C, respectively). RPAT pads were dissected, weighted and processed for UCP-1 and PGC1α quantification (RT-PCR). Both, DXM and cold exposure decreased RPAT mass/100 gr body weight ($P<0.05$). RPAT from DXM-C showed reduced levels of UCP-1 ($p<0.01$, vs CTR-C) and a tendency toward lower levels of PGC1α gene expression. We also studied the effect of DXM in vitro on adipocyte precursor cells. Stromal vascular fraction cells were isolated from RPAT from control male rats and cultured up to confluence. Cells were then incubated or not with 0,25μM DXM for 48hs (DXM and CTR cells, respectively). Thereafter, cells were induced to differentiate with a pro-beige cocktail. On day 8 after differentiation, a subset of CTR and DXM cells were incubated in basal condition or with forskolin (10μM, FSK) for 4hs (CTR-FSK and DXM-FSK, respectively). Cells were then processed to quantify UCP-1 and PGC1α mRNAs. We found that DXM decreased UCP-1 expression in FSK stimulated adipocytes ($p<0.0001$, vs. CTR-FSK). Additionally, and following a similar protocol, we evaluated DXM effect on trans-differentiation, by incubating or not with 0,25μM DXM the last 48hs of culture. Again, we found that DXM decreased UCP-1 mRNA levels in DXM-FSK adipocytes ($p<0.0001$, vs. CTR-FSK). Overall, DXM inhibited the thermogenic program in RPAT beige adipocytes, in vivo and in vitro after cold and FSK stimulation, respectively, by regulating UCP-1 gene expression. (PICT2015-2352)

SAI SYMPOSIUM: NEUROIMMUNOLOGY

IL-33/ST2 PATHWAY IN CEREBRAL MALARIA AND INDUCED COGNITION DEFECTS

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Cerebral malaria (CM) is a severe complication of *Plasmodium falciparum* infection associated with a high mortality rate and long-term neurocognitive impairment in survivors. In mice, *Plasmodium berghei* ANKA (*Pb*A)-induced cerebral malaria reproduces several of these features, it is $CD8^+$T-cell mediated, and influenced by T_H1/T_H2 balance. IL-33, a cytokine highly expressed in the central nervous system, is overexpressed in brain undergoing CM. While wild-type mice succumb to CM within 10 days with brain microvascular obstruction and hemorrhages, micedeficientfor IL-33 receptor ST2 survive with no neurological sign, preserved cerebral microcirculation, reduced brain inflammation and T cell sequestration.

In wild-type mice *Pb*A-infection induces short term and spatial memory defects, prior to blood brain barrier (BBB) disruption, together with increased IL-33 expression by oligodendrocytes. By contrast, ST2-deficient mice do not develop *Pb*A-induced cognitive defects, with reduced neuroinflammation and absence of neurogenesis defects in the hippocampus.

Thus IL-33/ST2 orchestrates CNS microglia and oligodendrocytes responses at an early stage of *Pb*A-infection, with an IL-1β / IL-33 amplification loop, responsible for an exacerbated neuroinflammation context and associated neurological and cognitive defects.

USE OF DISTINCT EFFECTOR MECHANISMS BY THE IMMUNE SYSTEM TO CONTROL DIFFERENT LIFE CYCLE STAGES OF A SINGLE PATHOGEN, TOXOPLASMA GONDII, IN THE BRAIN

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Toxoplasma gondii, an obligate intracellular protozoan parasite, is one of the pathogens that can establish a chronic infection in the brain. The parasite proliferates as tachyzoites during the acute stage, and thereafter converts to the cysts in various organs, especially in the brain to establish the chronic infection. IFN-g is required to control the proliferation of tachyzoites during the acute stage of infection. This cytokine is also critical for preventing reactivation of the chronic infection, which is initiated by rupture of cysts, followed by proliferation of tachyzoites. Of interest, our studies revealed that in addition to T cells, innate immune cells other than NK cells need to produce IFN-g to prevent reactivation of cerebral *T. gondii* infection. We discovered that brain-resident cells are the critical innate IFN-g producer to cooperate with T cells for preventing reactivation of the infection. In contrast to the protective immunity against the tachyzoite stage, our studies uncovered that the immune system employs the perforin-mediated activity of $CD8^+$ T cells to target the tissue cysts of *T. gondii* in the brain, and that the perforin-mediated attack by $CD8^+$ T cells is associated with an accumulation of phagocytes into the cysts. Dense granule protein 6 of the parasite was identified as a key target of the $CD8^+$ T cells to initiate the anti-cyst immune process. The immune system efficiently utilizes two distinct effector mechanisms to control *T. gondii* in the brain depending on the life cycle stages of the pathogen, IFN-g against the fast-growing acute stage form and perforin-mediated cytotoxic activity against latent chronic stage form.

BACTERIAL INFLAMMATORY ACTIVATION OF MICROGLIA KILLS NEURONS BY PHAGOPTOSIS

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Central nervous system invasion by bacteria of the genus *Brucella* results in an inflammatory disorder called neurobrucellosis. *B. abortus* infects microglia, eliciting their activation and production of pro-inflammatory mediators. Evidence of neurological involvement occurs to varying degrees in nervous systems of patients with neurobrucellosis. With the aim of determining the putative mechanisms involved in this phenomenon we established murine primary cultures of neurons and microglia to demonstrate that, due to *B. abortus* infection, microglial primary phagocytosis (phagoptosis) actively induces neuronal death, without inducing neuronal apoptosis. This phenomenon was due to microglia-TLR2 activation by *Brucella* lipoproteins. *B. abortus*-activated microglia secrete nitric oxide (NO) and increase their phagocytic ability. NO induced the exposure of eat-me signal on neurons (phosphatidylserine, PS). Blocking PS-binding protein milk fat globule epidermal growth factor-8 (MFG-E8) interaction, or microglial vitronectin receptor-MFG-E8 interaction was sufficient to prevent neuronal loss without inhibiting microglia activation. Furthermore, inhibition of UDP/P2Y6 purinergic signaling also prevents phagop-

tosis of viable neurons by *B. abortus*-activated microglia. Hence, our results demonstrate a novel form of inflammatory neurodegeneration for a bacterial infection, where inflammation cause exposure of eat-me signal on neurons, leading to their death through primary phagocytosis. These results describe part of the mechanisms whereby *B. abortus* could induce neuronal death during neurobrucellosis.

YOUNG RESEARCHER PRESENTATION

PI3K INHIBITORS PREVENT TLR2-MEDIATED MYELOID LEUKOCYTE RECRUITMENT, PROINFLAMMATORY GENE EXPRESSION AND THE PRESENCE OF LC3B+ PUNCTA IN THE CNS.

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Acute brain injury leads to the recruitment and activation of immune cells including resident microglia and infiltrating peripheral myeloid cells, which contribute to the inflammatory response involved in neuronal damage. Here, we reported that TLR2 stimulation by peptidoglycan (PGN) from Staphylococcus aureus, in vitro and in vivo, induced microglial cell activation followed by autophagy induction. Furthermore, we evaluated if phosphatidyl-inositol3 kinase (PI3K) pharmacological inhibitors LY294200 and 3-methyladenine (3-MA) can modulate the innate immune response to PGN in the central nervous system. We found that injection of PGN into the mouse brain parenchyma (caudate putamen) triggered an inflammatory reaction, which involved activation of microglial cells, recruitment of infiltrating myeloid cells to the injection site, production of pro-inflammatory mediators, and neuronal injury. In addition, we observed the accumulation of LC3B + CD45 + cells and colocalization of LC3B and lysosomal-associated membrane protein 1 in brain cells. Besides, we found that pharmacological inhibitors of PI3K, including the classical autophagy inhibitor 3- MA, reduced the recruitment of myeloid cells, microglial cell activation, and neurotoxicity induced by brain PGN injection. Collectively, our results suggest that PI3K pathways and autophagic response may participate in the PGN-induced microglial activation and myeloid cells recruitment to the brain. Thus, inhibition of these pathways could be therapeutically targeted to control acute brain inflammatory conditions.

SAFIS SYMPOSIUM: MEMORY NEUROPHYSIOLOGY

FORGETTING TO REMEMBER: BIOLOGICAL MECHANISMS OF ADAPTIVE FORGETTING

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Neurobiological research on memory has focused on the mechanisms underlying memory storage, with less attention to forgetting. However, forgetting is a ubiquitous phenomenon that is actively promoted in many species. How and whether active forgetting mechanisms are driven by adaptive behavior is unknown. Here we show that control processes that enable flexible behavior in mammals promote active forgetting of traces that hinder retrieval of memories needed to guide behavior. We found that when rats retrieved their prior experience with an object to guide new exploration, it significantly reduced their later recognition of other objects previously encountered in that environment. Consistent with similar findings in humans, this retrieval-induced forgetting was competition-dependent, cue-independent, long-lasting, and reliant on control processes mediated by the prefrontal cortex: Silencing medial prefrontal cortex with muscimol selectively abolished the forgetting effect. cFos imaging revealed that prefrontal control demands declined over repeated retrievals as competing memories were forgotten, revealing a key adaptive benefit of forgetting. Occurring in 88% of the 63 rats studied, this finding establishes an unusually robust model of how active forgetting harmonizes the mnemonic ecosystem with behavioral demands, and permits isolation of its circuit, cellular and molecular mechanisms.

THE HYPOTHESIS OF BEHAVIORAL TAGGING IN THE FORMATION OF LONG-TERM MEMORIES

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Memories are experience-dependent internal representations of the world that can last from short periods of

time to a whole life. The formation of long-term memories relies on several biochemical changes, which inducing modifications in the synaptic efficiency change the way the neurons communicate each other.Interestingly, the formation of a lasting memory does not entirely depend on learning itself; different events occurring before or after a particular experience can affect its processing, impairing, improving, or even inducing lasting memories. The Behavioral Tagging hypothesis postulates that the formation of lasting memories relyon at least two parallel processes: the setting of a learning tag that determines which memory could be stored and were; and the synthesis of plasticity-related proteins,which once captured at tagged sites will allow the consolidation of a memory for long periods of time. Therefore a weak learning, only able to induce transient forms of memories but also capable of setting a learning tag, could be benefited from the proteins synthesized by a different strong event, processed in the same areas, by using them to consolidate its own lasting memory.Here, I describe the postulates of the Behavioral Tagging hypothesis, and revise several experiments that we performed from rodents to humans in order to discuss its implications on learning and memory processing.

PRENATAL ETHANOL EXPERIENCES: ESTABLISHMENT OF MEMORIES THAT IMPACT UPON LATER BEHAVIORAL AND PHYSIOLOGICAL RESPONSES TO THE DRUG

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Moderate ethanol doses recruit sensory capabilities of the near term fetus. When ethanol accumulates in the amniotic fluid, altricial mammals process its chemosensory attributes. This early experience results in later recognition of the odor and taste of the drug. Yet, the acquisition of ethanol-related memories is far more complex than a mere sensory familiarization effect. Different physiological effects of the drug modulate these memories via associative learning processes. The unborn organism is capable of acquiring conditioned responses mediated by the drug´s central reinforcing effects. Rat fetuses, during stages analogous to the 2nd and 3rd human gestational trimesters, associate ethanol´s sensory cues with such positive reinforcing effects; a phenomenon that promotes heightened ethanol affinity. The early motivational effects of the drug coexist with other physiological consequences of the state of intoxication; particularly with a significant respiratory depression. Recent studies show that sequential exposure to subteratogenic doses, sensitize the organism to both, the reinforcing and respiratory depressant effects of ethanol. This sensitization favors associative learning mechanisms that will later impact upon ethanol preference and respiratory neuroplasticity. In human neonates we have conducted different studies aimed at determining ethanol olfactory preferences as a function of maternal ingestion of the drug during gestation. The studies also analyzed concomitant physiological disruptions caused by re-exposure to the drug´s odor. Newborns representative of mothers that frequently consumed ethanol during pregnancy exhibited appetitive facial expressions when stimulated with ethanol odor. These babies also showed significant breathing depressions when stimulated with minimal amounts of ethanol odor. When combining the results obtained in preclinical and clinical studies, it is clear that early exposure to the drug, not sufficient to result in Fetal Alcohol Syndrome, still endangers the organism in terms of the establishment of ethanol affinity as well as in terms of a relatively severe alteration of the respiratory network.

WRITE AND REWRITE MEMORIES: THE ROLE OF THE RECONSOLIDATION FUNCTION IN MEMORY UPDATING

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The ability to make predictions based on stored information is a general coding strategy. Would this capacity imply an important flexibility to rewrite memories as a consequence of a permanent changing world? In our field it is widely accepted that after acquisition, a short-term memory requires a consolidation process, which turns this labile trace into a stable and long lasting one. Accepting the flexibility we can postulate that memories are dynamic rather than static and, after being consolidated, they can be modified through further experience. In the last years, it has been shown that acquired memories can become active and update its content or strength by the labilization-reconsolidation process. More specifically, inactive memories can be reactivated through the presentation of cues (reminders) already existent at the time of acquisition. This results in memory reactivation (labilization), followed by a process of re-stabilization (reconsolidation). Using a behavioural approach, I am going to show you our results, supporting the reconsolidation functions in a declarative memory in Humans. Then, our results in the searching of the neural footprints associated with the strengthening function with functional magnetic resonance imaging (fMRI). Finally, I want to analyze not only the future avenues whereas the reconsolidation

process could open a novel therapeutic window to modify dysfunctional memories and/or to improve memories of everyday life, but also as a mechanism for maintenance of some psychopathologies such as anxiety disorders.

SAIC SYMPOSIUM: MOLECULAR DIAGNOSIS IN ONCOLOGY

NGS IN BREAST AND COLORECTAL CANCER. NEW CHALLENGES FOR THE PHYSICIANS TO UPDATE THE DAILY PRACTICE

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Rapid and sophisticated improvements in molecular analysis have allowed us to sequence whole human genomes as well as cancer genomes, and the findings suggest that we may be approaching the ability to individualize the diagnosis and treatment of cancer. This paradigmatic shift in approach will require clinicians and researchers to overcome several challenges including the huge spectrum of tumor types within a given cancer. Colorectal cancer is the third most common cancer in the world and the second leading cause of cancer-related death in the western world. Meanwhile breast cancer is the first cause of death in women cancer around the world. From 5 to 10 percent of them all are hereditary. The most important step leading to the diagnosis of hereditary cancer syndrome is the compilation of a thorough family history of cancer. After, molecular genetic testing may then provide verification of the diagnosis, when a germ-line pathogenic mutation is present in the family. Next-generation sequencing (NGS) allows for simultaneous sequencing of multiple cancer susceptibility genes and, for an individual, may be more efficient and less expensive than sequential testing. NGS systems use massively parallel sequencing to generate hundreds of millions of short (36- to 150-bp) DNA reads that can be aligned to the human genome.

The application of NGS to breast and colorectal cancer has been associated with tremendous advances and promises for increasing the understanding of the disease. However, there still remain many unanswered questions, such as for example the role of structural changes of tumor genomes in cancer progression and treatment response/resistance. There are different exciting possibilities for integrating NGS in clinical practice. Targeted genetic sequencing could for example help to detect mutations in genes of therapeutic importance. The hope is that NGS data will shorten the road to personalized medicine, in which treatments and therapies are tailored to target the unique spectrum of mutations that define individual tumors.

LIQUID BIOPSY: MONITORING CANCER PROGRESSION AND TREATMENT IN THE BLOOD.

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Liquid biopsy emerged as a rapid, cost-effective and noninvasive method to assess tumor genetic characteristics at different time points during the course of disease. The detection of circulating DNA fragments carrying tumor-specific genetic alterations, known as circulating tumor DNA (ctDNA), in the plasma of cancer patients has been successfully used to monitor treatment response, evaluate the presence of residual disease after potentially curative treatment and monitor tumor recurrence. Liquid biopsies offer the possibility to monitor tumor dynamics with high sensitivity and specificity. During this talk it will be discussed the potential utility of liquid biopsy to monitor treatment response and detect acquired resistance in metastatic non-small-cell lung cancer patients treated with tyrosine kinase inhibitors and metastatic RE-positive breast cancer patients treated with endocrine therapy.

NEXT GENERATION SEQUENCING-BASED MUTATIONAL PROFILE OF PEDIATRIC AML PATIENTS: PRELIMINARY RESULTS

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Introduction

Acute myeloid leukemia (AML) is a heterogeneous disease associated with poor outcome. Recurrent chromosomal abnormalities and response to treatment are used to stratify patients in risk groups. Next-generation sequencing (NGS) has been used to demonstrate the presence of alterations in several genes. The definition of a mutational profile in AML could be useful for improving risk group definition. Aims: 1-to describe the incidence of mutations in AML, 2-to correlate the mutations with genetic features, 3-to evaluate their prognostic impact.

Patients and Methods

133 DNA samples from non-APL AML patients were sequenced on a Miseq platform, using a customized amplicon-based NGS panel. A total of 172,360 bp of DNA regions were covered with 1,207 amplicons(mean coverage:1,308). Sequences obtained were analyzed by in-house bioinformatics-pipeline.

Results

At least one pathogenic variant was detected in 84/133 analyzed cases. Overall, 218 mutations were found in 84 cases. The most frequently mutated genes: FLT3-ITD(10.5%), BCOR(8.3%), KMT2A(8.3%), RB1(8.3%), BCORL1(6.8%), and in the group of normal karyotype: FLT3-ITD(27.3%,p=0.0155), CEBPA(18.2%,p=0.0010), NPM1(27.3%, p<0.00001) and WT1(18.2%,p=0.0010). TET2 mutation predominated in the low-risk group(p=0.0401). Complete remission was achieved in 91% of cases (median follow-up:19 months). LFSp(SE) was 61(5)% for the whole population. The LFSp(SE) of patients with at least one variant was 60(7)%, and without variants LFSp(SE):63(8)(p=0.8446). In the normal karyotype, the genotype NPM1/CEBPA-mutated plus FLT3/WT1-wild-type disclosed a LFSp(SE):100(0)% while remaining patients LFSp(SE):56(13)%(p=0.166). Within the low-risk group, mutational status of 14 selected genes identified a group of patients with lower LFSp [48(18)% vs 84(11)%,p=0.0341].

ConclusionsWe describe the incidence of mutations in pediatric AML by NGS. TET2 mutations were associated with low-risk cases. There was a higher incidence of mutations in FLT3-ITD, CEBPA, NPM1 and WT1 within the normal karyotype group. The genotype NPM1/ CEBPA-mutated plus FLT3/WT1-wild-type showed a trend to a better LFSp. In the low-risk group, mutational profile of 14 selected genes with adverse prognosis allowed identification of patients with poorer outcome. Our results suggest that profiling of genetic alterations could be useful as a clinical tool to improve risk-stratification of AML patients in our setting.

SELECTED ABSTRACT FOR SYMPOSIUM

DIFFERENTIAL GALECTIN-1 BINDING PATTERNS IN HER2+ HUMAN BREAST CANCER CELL LINES DICTATE CANCER STEM CELL-LIKE PHENOTYPE AND MODULATE SENSITIVITY TO TRASTUZUMAB.

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IBYME CONICET

Galectins decode glycan-containing information in a number of cell receptors adjusting signaling thresholds and modulating cellular functions. In particular, galectin-1 (Gal1), binds to terminal N-acetyllactosamine residues on glycosylated proteins in the absence of α2-6 sialic acid (SA) capping (Gal1 permissive glycophenotype) promoting tumor immune suppression and sustaining aberrant angiogenesis. Here, we aim to explore the glycosylation signature of HER2+ breast cancer cells to investigate if Gal1 is involved in resistance to trastuzumab (TZ) targeted therapy. Previously, we have demonstrated that TZ sensitive (TZS) BT-474 and SK-BR-3 cell lines exhibited a Gal-1 restrictive glycophenotype (high α2,6 SA capping) compared to TZ resistant (TZR) JIMT-1 cell line. In accordance with the glycophenotype, TZR cell line bound and expressed higher levels of Gal1 when compared to TZS cell lines. Interestingly, knocking down Gal1 sensitized TZR cell line to TZ challenge (p<0,05). Cancer stem cells (CSC) represent a subpopulation of tumor cells with capacity of self-renewal and differentiation to drive initiation, growth and metastasis. Moreover, breast CSC (CD44+/CD24/low/ALDH1+) exhibit resistance to anti-cancer treatments promoting tumor recurrence. Herein, exposure of TZR JIMT-1 cell line to Gal-1, increase (p<0,0001) CD44+/CD24low/Aldh1hi population and, also increasing EMT and differentiation markers. In addition, in vitro migration (p<0,05) and mamosphere formation (p<0,001) were induced upon Gal1 treatment. Importantly, Gal1-induced CSC phenotype was reversed by the addition of an anti-Gal-1 antibody. Furthermore, these results were supported by analysis of public databases arrays (GSE62327) showing that patients who presented complete response to TZ exhibited higher levels of ST6GAL1, lower CD24 (p<0,01) expression and higher Aldh1 (p<0,01) reinforcing our hypothesis from a clinical standpoint. In summary, our study demonstrates that individual HER2+ human breast cancer cells display particular “glycosylation signatures” which in association with Gal1 expression and binding patterns, promote an increase in breast CSC favoring resistance to anti-HER2 targeted therapy and metastasis.

SAIC – ISNIM SYMPOSIUM: IMMUNE-NEUROENDOCRINE REGULATION

NEUROIMMUNENDOCRINE REPROGRAMMING IN HEALTHY ADOLESCENTS FOLLOWING CHILDHOOD MALTREATMENT

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Introduction: Early life stress (ELS) exposure, including physical, sexual and psychological abuse, as well as physical or emotional neglect, is associated to long-term effects on mental health. The individual's earlier years of life are characterized by rapid neurobiological and psychological development; therefore, ELS is considered an important risk factor for psychological impairment and increased vulnerability to mood disorders. Here, we investigated the effects of ELS on endocrine and immune pathways in healthy adolescents without psychopathology. Methods: Thirty adolescents with history of childhood maltreatment and twenty-seven adolescents without ELS history were recruited. Blood and hair samples were obtained from all participants. Lymphocytes were isolated and stimulated in vitro to evaluate lymphocyte subsets, Th1/Th2/Th17 cytokines, mitogen-activated protein kinase (MAPK) and nuclear factor kappa B (NF-kB) signaling pathways as well as lymphocyte sensitivity to dexamethasone by flow cytometry. Brain-derived neurotrophic factor (BDNF) and hair cortisol were assessed with enzyme-linked immunosorbent assays (ELISAs). Results: Adolescents with history of ELS had increased percentages of T-cell activation markers (CD3+CD4+CD25+ and CD3+CD69+) and senescent T cells (CD8+CD28- and CD4+CD28-) as well as decreased percentages of NK (CD3-CD56+) and NK T cells (CD3+CD56+). Following stimulation, lymphocytes of ELS+ adolescents produced significantly more IL-2, IL-4, IFN-gamma and IL-17 and engaged more MAPK ERK and NF-kB signaling. In addition, ELS was associated with higher hair cortisol levels in parallel to increased lymphocyte resistance to dexamethasone as well as low plasma BDNF levels, reflecting the chronic stress effects on neuroendocrine parameters. Conclusion: These data indicate the presence of immune activation and pro-inflammatory profile in healthy adolescents exposed to ELS, which could contribute to increased vulnerability of trauma-related psychopathology in adolescence and adult life. The underlying mechanisms of this impairment may include the enhanced activation of both MAPK and NF-kB signaling in parallel to partial resistance to glucocorticoids.

MELATONIN AS AN OCULAR MODULATOR OF INFLAMMATORY DISEASES

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Uveitis and optic neuritis are prevalent ocular inflammatory diseases, and highly damaging ocular conditions. Uveitis comprises different forms of ocular inflammation affecting the uveal tract (iris, ciliary body, and choroid), and adjacent ocular structures. Optic neuritis is a neuropathy, which affects mostly young adults and children, and provokes primary inflammation, demyelination, and axonal injury in the optic nerve, that leads to retinal ganglion cell and vision loss. No matter uveitis and optic neuritis are eye morbidity and visual disability main causes, the complexity of the biochemical and immunological mechanisms implicated in their genesis and development are elusive. Still, several lines of evidence support that both uveitic and optic neuritis damage are due to cytokines release by infiltrated leukocytes and other inflammatory mediators, like arachidonic acid metabolites, reactive oxygen species, and nitric oxide, among many others. Both diseases are currently treated with corticosteroids, but they do not have adequate efficacy and are often associated with severe side effects. Thus, uveitis and optic neuritis remain a challenging field to ophthalmologists and a significant public health concern. Melatonin, an ubiquitous molecule that has been localized in plants and animals, is a key regulator of the circadian physiology, and also participates in the regulation of very diverse physiological processes, such as sleep, immune and vascular response, and reproduction, among many others. It has been demonstrated that melatonin provides powerful retinal protection against oxidative stress. Moreover, melatonin is an efficient antioxidant and antinitridergic, and has the ability to reduce prostaglandin and tumor necrosis factor levels both in the retina and optic nerve. Since melatonin protects ocular tissues against inflammation, it could be a potentially useful anti-inflammatory therapy in ophthalmology. Therefore, the aim of this work was analyzing the effects of the treatment with melatonin on experimental models of uveitis and optic neuritis.

MODULATION OF INNATE IMMUNITY BY AUTOPHAGY: THE IMPACT ON NEUROINFLAMMATION AND LEUKEMIA CELL BIOLOGY

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Autophagy plays a crucial role in neurodegenerative diseases, although the precise mechanisms underlying these processes are poorly understood and little is known about the effects of the autophagic process and its regulation in microglial cells (MC). In addition, autophagy has dual role in cancer depending of the type of tumor and this response can promote cell survival or cell death, as well. Our laboratory was focused in studying the role of autophagy in the regulation of neuroinflammation and leukemia cell biology. We evaluated the effects of autophagy on the production of pro-inflammatory mediators by alpha-synuclein (α-syn)-stimulated

MC, and on neuronal viability in a co-culture system. In addition, we studied the autophagy dynamics in MC after α-syn stimulation. We found that autophagy induction in MC before exogenous α-syn stimulation, downregulated IL1β, IL-6, TNF-α and NO production. Furthermore, we showed by time-lapse experiments with BV2 GFP-LC3 microglial cells that LC3B is attracted by lysosomes containing α-syn fibrils and we demonstrated by live-CLEM imaging that α-syn is targeted by autophagic vesicles. On the other hand, autophagy inhibition led to α-syn-stimulated MC death, indicating a protective role for autophagy during this process. In another set of experiments we observed that stimulation of TLR2 in MC induced cell activation, autophagy and autophagy-dependent cell death in vitro and in vivo. Now, preliminary results indicate that TLR2 stimulation in Chronic Lymphocytic Leukemia (CLL) cells induces increased levels of LC3B II and modulated Fludarabine-induced apoptosis. Overall, our results suggest that autophagy may play a role in the modulation of neuroinflammation and leukemia cell death.

REDISTRIBUTING ENERGY BETWEEN THE BRAIN AND THE IMMUNE SYSTEM: THE CONTRIBUTION OF IL-1

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The human brain consumes about 20 % of the energy available in the body, which mainly derives from glucose oxidation. The metabolic activity of the brain is remarkably constant. On the other side, glucose uptake markedly increases in activated immune cells, which under basal conditions already consume 15-20 % of the glucose available, indicating a deviation of glucose supply upon stimulation of the immune system. Interestingly, glucose incorporation by both neural and immune cells is largely independent of insulin. Interleukin-1 (IL-1) is a cytokine produced by many different cell types. Besides its important role in immune and inflammatory responses, it can affect several neuroendocrine mechanisms. Among them, we have shown that IL-1 induces a profound, long-lasting, and insulin-independent hypoglycemia in mice, which is caused by central and peripheral actions of the cytokine. This effect is even more pronounced in insulin-resistant diabetic obese animals. Furthermore, despite hypoglycemia and in contrast to insulin, IL-1 increases whole brain metabolism. We have also recently reported that in vitro stimulated neurons and astrocytes can produce enough IL-1 to increase glucose incorporation in these cells. Our previous studies showed that IL-1 is produced in the brain during increased synaptic activity, such as long-term potentiation (LTP), and during learning a task, processes that require large amounts of energy. Brain-borne IL-1 is essential for LTP maintenance and for learning. We have also shown that intracerebroventricular administration of IL-1 results in the induction of IL-1 and other cytokines in the spleen, indicating a possible link between central and peripheral processes. Based on these results, our hypothesis is that IL-1 can facilitate glucose transport and increase energetic metabolism in immune and brain cells, thus contributing to regulate glucose distribution according to certain priorities during highly demanding immune and neural processes. De-regulation of IL-1 effects at central or peripheral levels could contribute to processes leading to obesity and insulin resistance, and to neuropsychiatric pathologies associated with metabolic alterations.

SAI SYMPOSIUM: AUTOIMMUNITY

TOWARDS A CURE FOR SYSTEMIC LUPUS ERYTHEMATOSUS – TARGETING PATHOGENIC MEMORY PLASMA CELLS

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From the “immunereset” therapy, an ablation of the immune system and its regeneration from progenitor cells, also termed “autologous stem cell transplantation” (ASCT), we have learned that in more than 60% of patients with chronic inflammatory diseases, in particular those with Systemic Lupus Erythematosous (SLE), the immune system not only has initiated the disease, but it also drives it in the chronic phase. More precisely, it is apparently imprinted, pathogenic immune cells which drive the disease, since the regenerated, naive immune system of most immunresetted patients is tolerant and does not induce relapses of the disease. We had shown earlier, that longlived “memory” plasma cells, maintained in the bone marrow, in inflamed tissues and in secondary lymphoid organs comprise a separate entitiy of immunological memory and are responsible for sustained secretion of protective antibodies. We had defined memory plasma cells secreting pathogenic autoantibodies as a new target of therapy, an unmet medical need, because they are refractory to conventional immunosuppressive

strategies. Pathogenic memory plasma cells can induce lupus-like nephritis on their own, as we could demonstrate by transfer of plasmablasts of NZB/W mice, a model for SLE, into $Rag^{-/-}$ mice. Generic ablation of memory plasma cells as such, using the proteasome-inhibitor bortezomib, has yielded impressive results in the NZB/W model of lupus and lately also in patients with systemic lupus erythematosus. However, pathogenic plasma cells do regenerate in these patients, unlike the immunresetted patients, suggesting that plasma cell ablation has to be combined with therapeutic strategies preventing their regeneration. To target pathogenic plasma cells of a defined specificity selectively, we have now developed (auto)antigen-specific affinity matrices for plasma cells: (Auto)antigen-specific plasma cells are targeted by their own secreted (auto)antibodies, bound to the autoantigen-affinity matrix on their surface, for lysis by complement. We have demonstrated efficacy of this approach *in vitro*, and now, for the first time also *in vivo*, ablating selectively plasma cells of a given specificity from spleen and bone marrow of mice. Whether this approach can be tailored for SLE, remains to be shown, but for the first time it offers an option for the selective elimination of the pathogenic plasma cells causing the disease.

SPECIALIZATION OF REGULATORY T CELLS IN TH1 RESPONSES

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CD4+Foxp3+ Treg cells are essential for maintaining self-tolerance and preventing excessive immune responses that cause autoimmunity. In the context of Th1 immune responses, co-expression of the Th1 transcription factor T-bet with Foxp3 is essential for Treg cells to control Th1 responses. T-bet-dependent expression of CXCR3 directs Treg cells to the site of inflammation. However, the suppressive mediators enabling effective control of Th1 responses at this site are unknown. We determined the signature of CXCR3+ Treg cells arising in Th1 settings and defined universal features of Treg cells in this context using multiple Th1-dominated infection models. Our analysis defined a set of Th1-specific co-inhibitory receptors and cytotoxic molecules that are specifically expressed in Treg cells during Th1 immune responses in mice and humans. Among these, we identified the novel co-inhibitory receptor CD85k as a functional predictor for Treg-mediated suppression specifically of Th1 responses, which could be explored therapeutically for selective immune suppression in autoimmunity.

CAUSES AND CONSEQUENCES OF CHRONIC INFLAMMATION OF THE PROSTATE: FROM HUMAN DISEASE TO ANIMAL MODELS AND VICEVERSA

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The prostate is the target of disorders that affect men of all ages. Pathologies range from infection, to chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS), to benign hyperplasia and cancer. CP/CPPS is a prevalent disease affecting men younger than 50 years, and characterized by pelvic pain and chronic inflammation of the prostate of unknown etiology. Autoimmune responses against prostate antigens were revealed in CP/CPPS patients. In fact, self-reactivity of Th1 cells to prostate and seminal plasma proteins were detected in a considerable proportion of patients in the absence of infection, suggesting a Th1 autoimmune response as the underlying disease mechanism.These autoimmune responses were associated with elevated levels of inflammatory cytokines and chemokines, and leukocyte subpopulations in semen. Interestingly, striking alterations in the semen quality of these patients were also reported.

Besides, rodent models of experimental autoimmune prostatitis (EAP) have allowed an important advance in the understanding of human disease.Different susceptibilities to the induction of EAP were observed among mouse strains. NOD mice were the most susceptible to the induction of EAP when compared to C57BL/6 or BALB/c mice. After immunization, NOD mice developed specific Th1/Th17 cell-mediated responses that caused severe histopathology in the prostate and the induction of chronic pelvic pain.In fact, INFγ has shown to be critical to confer pathogenic T cells the capability of homing to the prostate, where they induced leukocyte recruitment and also pelvic pain development. Besides, Treg have been shown to be critical during the inductive phase of EAP. Indeed, Treg-depletion made resistant mice vulnerable to EAP development by significantly increasing pathogenic Th1 cells.

Altogether, our results support the notion that an autoimmune response to prostate antigens may be the underlying cause of CP/CPPS. Th1 cells induce local inflammation and pelvic pain development, thus emerging as a putative therapeutic target in CP/CPPS patients.

SAIC-SAI SYMPOSIUM: IMMUNOLOGY OF REPRODUCTION

INFLAMMATION AND IMPLANTATION: AN EVOLUTIONARY NEED FOR THE SUCCESS OF PREGNANCY

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Approximately half of all human embryo implantations result in failed pregnancy. Multiple factors may contribute to this failure, including genetic or metabolic abnormalities of the embryo. However, many of these spontaneous early abortion cases are attributed to poor uterine receptivity and abnormal immune responses. Although many fertility disorders have been overcome by a variety of assisted reproductive techniques, implantation remains the rate-limiting step for the success of the in vitro fertilization (IVF) treatments.

Pregnancy has been considered as an anti-inflammatory condition where inflammation and the presence of maternal immune cells represent an adverse response to the embryo with detrimental consequences for the pregnancy. However, we, as well as others, have demonstrated that inflammation and the immune cells associated with the inflammatory process are necessary for the success of pregnancy. While for many years pregnancy has been considered a single immunologic event; in reality it can be divided in at least three immunologic phases characterized by distinct biological processes: implantation, plantation and parturition.

During implantation, the inflammatory process, and the maternal immune cells present at the endometrium, play a critical role in the preparation of the surface epithelium of the uterus by enhancing uterine receptivity as well as in the process of tissue repair and removal of cellular debris; two important aspects for normal placentation and induction of tolerance to paternal antigens. We will discuss the evolutionary need for inflammation during implantation and the role of the endometrial stroma in promoting the inflammatory process necessary for trophoblast migration.

IMMUNE NETWORK DURING PREGNANCY

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Already at early pregnancy stages, active tolerance needs to be generated towards paternally derived structures present not only at fetal-maternal interface but also in the maternal body. At the same time, the mother needs to maintain immunity against pathogens. Incomplete immune tolerance to paternal antigens can lead to pregnancy complications and prematurity. Subclinical infections that jeopardize the fine-tuned immune balance between tolerance and immunity might danger the pregnancy and at worst result in intrauterine fetal death. The importance of immune cells and in particular of regulatory T cells and IL-10 secreting B cells in the maintenance of the immune balance will be discussed on the basis of data obtained from experimental mouse models as well as with the help of samples from patients undergoing pregnancy complications vs normal pregnancies.

CONTROL OF THE INFLAMMATORY RESPONSE DURING THE IMPLANTATION PERIOD: ASSOCIATION WITH THE RETICULAR STRESS AND THE UNFOLDED PROTEIN RESPONSE

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Embryo implantation in humans requires the generation of a sterile inflammatory response for blastocyst invasion; however, it is still unclear how it is induced. During decidualization, endometrial stromal cells suffer reticular stress (RS) and trigger unfolded protein response (UPR), allowing them to expand their endoplasmic reticulum and the production of immunomodulators. This physiological RS generates the activation of sensing proteins, which induces kinase/Rnase-TXNIP expression, activating the inflammasome. This multiprotein system allows caspase-1 activation, which catalyzes the cleavage of inactive pro-form IL-1b to secretory mature IL-1b associated with proimplantatory effects. However, the sterile inflammatory response should be later controlled in favor of a tolerogenic microenvironment to sustain pregnancy. Therefore we wondered if the decidualization program induces a sterile inflammatory response through the induction of RS/UPR, and how it is regulated. We used different approaches as in vitro models of decidualization and implantation, with different cell lines, as well as in vivo murine models. In vitro results were also validated in endometrial biopsies from fertile women, with recurrent spontaneous abortions (RSA) or with in vitro fertilization failures (RIF). Briefly, the obtained results suggest that human decidualization process is accompanied by a physiological RS/UPR, which induces a physiological and sterile inflammatory response associated with an increase of

IL-1b production. These processes were differentially affected in RSA and RIF patients in comparison with fertile women, suggesting their relevance in reproductive pathologies. In addition, the decidualization process involves the modulation of different mediators such as the secretion of the vasoactive intestinal peptide (VIP). Using two approaches: VIP(+/-) and Foxp3-knock-in-GFP mouse females either pregnant or in estrous we demonstrated that this neuropeptide is a key regulator of the immune-tolerance capable of inducing Tregs and favoring the selective recruitment of maternal Tregs to the uterus contributing to successful implantation and the maintenance of pregnancy.

SELECTED ABSTRACT FOR SYMPOSIUM

RELEVANCE OF THE CYSTEIN-RICH SECRETORY PROTEIN (CRISP) FAMILY FOR MALE FERTILITY

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Cysteine-Rich Secretory Protein (CRISP) 1, 2, 3 and 4 are mainly expressed in the male reproductive tract and have key roles in mammalian fertilization. In spite of this, single mutant mice for these proteins are fertile suggesting the existence of compensatory mechanisms between homologous CRISP members. Based on this, the aim of the present work was to study the functional relevance of CRISP proteins for fertility by carrying out a functional characterization of mice lacking the four members of the family. For this purpose, we used quadruple knockout (QKO) mice generated in our laboratory by the novel CRISPR/Cas9 technique. Fertility was tested by caging male mice with control females for 5 days and it was determined by the number of born pups 21 days after observation of copulatory plug. Results showed that fertility of QKO mice was severely impaired compared to controls ($p<0,0001$). Consistent with the observed fertility rates and with the reported role of CRISP proteins in fertilization, the percentage of fertilized eggs recovered from the ampulla 10-15 h after mating was significantly lower than control ($p<0,0001$). Finally, to study the mechanisms underlying these *in vivo* defects, sperm fertilizing ability was analyzed *in vitro* using oocytes surrounded by both the *cumulus oophorus* and the *zona pellucida* (ZP), just by the ZP or denuded of both coats. Results revealed that QKO sperm were completely unable to penetrate and fertilize oocytes under all the analyzed conditions. Together, these observations indicate the key role of CRISP proteins for male fertility and the existence of compensation within the CRISP family, providing important information for the diagnosis and treatment of infertility as well as for future male contraceptive development.

SAIC SYMPOSIUM: NANOTECHNOLOGY IN ONCOLOGY

THERAPY, DIAGNOSTIC AND THERANOSTICS OF CHRONIC DISEASES BY USING NANOPLATTFORMS

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The advent of nanotechnology has radically changed the way we diagnose, image and treat diseases, with novel nanoplatforms capable clinically important functions, including detecting cancer at its earliest stages and location, as well as delivering anticancer therapeutics specifically to tumor cells. The nanotechnology approach to chronic diseases has focused on three main avenues: early detection; imaging for diagnostics or assessment of targeted delivery.

Also multifunctional therapeutics are of interest, whereby nanoplattforms are loaded with multiple functional moieties capable of selective targeting, imaging and delivery of specific drugs to malignant cells or toxic aggregates (1,2,3,4). In relation with this is possible to mention the so called theranostics which consist in the diagnostic and treatment of pathologies in a unique procedure (5).

In the talk will be discussed the potential use of different nanomaterials multifunctionalized with different biomolecules for cancer, cardiovascular diseases and Alzheimer's disease theranostics, diagnostic in vitro for the ultrasensible detection of biomarkers and nanoplattorms for drug delivery and treatment (6,7). The state of the art of clinical applications of nanomaterials in diagnostic and treatment will be commented.

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REDEFINING ESTROGEN RECEPTOR SIGNALING: OPPORTUNITIES FOR THE TREATMENT OF ENDOCRINE RESISTANT BREAST CANCER IN THE CONTEXT OF NANOTECHNOLOGY

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Seventy five percent of breast tumors are estrogen receptor alpha (ER) positive and tamoxifen is the main treatment for these patients. However, approximately one third of patients will eventually suffer a recurrence. We previously showed that fibronectin (FN), through its interaction with 1 integrin, induces tamoxifen resistance. Moreover, in human breast tumor tissues, cumulative data shows an inverse correlation between FN levels and response to
endocrine treatment. Thus, we asked the question of whether ER signaling could, in some way, be coupled to 1 integrin. Our work shows that estrogens induce endocytosis in breast cancer cells and that endocytic trafficking modulates the steady state levels of ER. This regulation is determined by the interaction of FN with 1 integrin. Super resolution microscopy and biochemical approaches revealed that ER and 1 integrin co-localize at the cell surface and in endosomes, and follow the same degradation dynamics. Inhibition of endocytosis by both biochemical and genetic approaches leads to a complete inhibition of ER's transcriptional
activity suggesting that membrane ER, contrary to what is thought, would play a key role in the regulation of transcription. Most importantly, presence of ER+ endosomes were found in normal and malignant human breast tissues. Based on these observations we designed a multifunctional polymeric nanoparticle that carries tamoxifen and is functionalized with iRGD, a peptide shown to interfere with binding of 1 integrin to FN. Our results show that this strategy leads to a reversal of FN's protective effect and that in vivo, specific homing to the tumor site is achieved. Redefining ER signaling will contribute to a better understanding of factors that lead to breast cancer progression and will aid in the identification of novel therapeutic approaches.

SELECTED ABSTRACT FOR SYMPOSIUM I

THE USE OF MICROFLUIDIC DEVICE TO ANALYSE BLADDER CANCER STEM CELLS.

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UTN, Facultad de Ingenieria, Univ South Florida, Miilipore Sigma Corp, Instituto de Oncologia Angel H. Roffo

Introduction: Lab on a Chip (LOC) culture systems are presented as a useful tool that allows the growth of tumor cells, their identification and isolation using specific markers. This type of device has the additional advantage of using low amounts of cell and reagents. Cancer stem cells (CSCs), a small heterogeneous population of cancer cells, associated with metastases and tumor recurrences. They can be identified by their growth independent of anchorage and by markers such as Oct4 and CD44, among others. The objective of this study was to analyze in a LOC device, using an invasive bladder cancer cell line (MB49-I), the growth of spheres, to identify CD44 expression and to capture differential CSC through functionalization of LOC surface with CD44 antibody. Results: Plating increasing number of cells (8, 16 and 32cell/µl) we determined 32cell/µl as the optimal number of cells to seed, resulting in a 70% of sphere forming efficiency after 14 days. The resulted spheres expressed CD44, observed directly inside of the microdevice (immunofluorescence). Furthermore, the surface of the microdevice was successfully functionalized with CD44 antibody using silane, enabling to capture CSC and to identify cells by this specific surface marker. In addition, we observed that the volume of reagents used in the microdevice resulted in 29 times lower compared to traditional plates of cell culture. Conclusion: LOC devices are a promising tools since using a minimum amount of reagents and samples, allow growth, identification and isolation of cancer cells. This microdevice promises to be considered as a novel technology to be used as a complement or replacement of traditional studies in the culture of tumors and could be an alternative in the future to isolate circulating CSCs.

SELECTED ABSTRACT FOR SYMPOSIUM II

HMOX-1 DEFICIENCY IMPAIRS BONE TURN OVER AND REMODELING. MOLECULAR IMPLICATIONS FOR PROSTATE CANCER BONE METASTASIS.

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Bone is frequently the unique site of prostate cancer (PCa) progression. Upon metastases, tumor cells interact with the bone microenvironment interrupting the tissue balance. Heme oxygenase-1 (HO-1) appears as a potential target in PCa maintaining the cellular homeostasis. Our hypothesis is that HO-1 modulation in bone cells is implicated in the capacity of PCa cells to metastasize to bone. We aimed at: assessing the physiological impact of Hmox-1 gene Knock-out (KO) on bone metabolism in vivo; determining the transcriptional landscape of genes involved in tumorigenesis and bone remodeling growing in co-culture systems of PCa cells with primary mouse osteoblasts (PMOs) isolated from BALB/c Hmox-1 +/+, +/- and -/- mice and analyzing whole secretomes from co-cultures of PCa cells with bone progenitor cells to evaluate key osteogenic factors favoring the aggressive phenotype in PCa. Histomorphometric analysis of bones from transgenic KO Hmox-1 mice exhibited significant decrease in bone density with reduced bone remodeling parameters. Correlation between the expression of Hmox-1 and Runx2, Col1a1, Mcsf1 and Opg (RT-qPCR) was observed in PMOs. FACS studies showed two populations of PMOs with different ROS-levels. The high ROS-levels population was increased in PMOs Het compared with WT, but was strong reduced in PMOs KO, showing restrained ROS tolerance in KO cells. PMOs gene expression was altered by co-culture with PC3 cells showing a more osteoclastic profile. Moreover, HO-1 induction in PCa cells growing in co-culture with bone progenitor cells (MC3T3, RAW 264.7) reflected a significant modulation of key bone markers (PTHrP, OPG and RANK) associated with proliferation and differentiation. Secretome analysis (ESI MS/MS) of conditioned media form these co-cultures revealed markers for PCa Neuro-Endocrine differentiation as compared to secretomes form controls. In summary, we showcase that the extent of HO-1 expression intervenes in bone remodeling and disrupts the communication between bone and prostate tumor cells.

SAI SYMPOSIUM: TUMOR IMMUNOLOGY

MANIPULATING INFLAMMATION TO RAISE CANCER IMMUNOGENICITY

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Immunotherapy constitutes the most promising pan-cancer treatment approach since the development of the first chemotherapies. Unprecedented outcomes continue to be observed in multiple cancer types including malignancies once thought treatment refractory. Responses, especially complete and durable, are nevertheless only observed in a limited fraction of patients underscoring the need for basic research to elucidate the basis for these remarkable but rare outcomes. Our group at the Cancer Research UK Manchester Institute investigates the signals and pathways that regulate the establishment of tumour microenvironments that support or restrain cancer progression spontaneously or following treatment. Combining the use of genetically engineered pre-clinical cancer models with the analysis of samples from cancer patients we have recently identified NK cells as key drivers of cancer-inhibitory inflammation. In cancer models rendered immunogenic by genetic ablation of the cyclooxygenase (COX)-2 pathway, NK cells were essential for initiating an inflammatory response that preceded and stimulated conventional type 1 dendritic cells and cytotoxic T cell-dependent tumor growth control. Similarly, analysis of cancer datasets suggested that the COX-2 pathway regulates the cellular and molecular inflammatory profile of human cancers across various malignancies. A gene signature that integrates COX-2-induced tumor-promoting factors and NK cell-driven anti-tumor mediators discriminated cancer biopsies with divergent immune cell composition. Moreover, this signature predicted overall patient survival and response to PD-1/PD-L1 blockade. Thus, our findings establish NK cells as central mediators of spontaneous and therapy-induced T cell cancer immunity and suggest the COX-2 pathway and its associated signature as a powerful indicator of patient outcome and response to therapy.

MODULATING THE GUT MICROBIOME TO ENHANCE RESPONSES TO IMMUNE CHECKPOINT BLOCKADE

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Recent evidence suggests that the gut microbiome has profound influences on the host immune system, including anti-tumor immunity. Specifically, pre-clinical studies suggest that the gut microbiome strongly influences response to immune checkpoint blockade. These findings were recently validated in several patient cohorts – demonstrating differences in the diversity and composition of the gut microbiome of responders (Rs) versus non-responders (NRs) to anti-PD-1 therapy in patients with metastatic melanoma as well as other cancer types. In our cohort of patients with metastatic melanoma treated with anti-PD-1 therapy, Rs exhibited higher

diversity of bacteria in the gut microbiome as well as a higher abundance of bacteria such as *Ruminococcaceae, Clostridiales*, and *Faecalibacterium.* In contrast, NRs had a low diversity of gut bacteria and a higher abundance of Bacteroidales. Importantly, this was associated with differences in progression-free survival (PFS) on anti-PD-1 therapy. Mechanistic studies were performed, demonstrating that germ-free mice receiving fecal microbiota transplant (FMT) from Rs have delayed tumor growth and enhanced response to anti-PD-L1 therapy as compared to FMT from NRs. Together, these findings suggest that the gut microbiome may impact anti-tumor immunity and responses to immune checkpoint blockade and, further, that modulation of the gut microbiome, via FMT or designer consortia or dietary and lifestyle change may enhance responses to therapy and potentially limit toxicities. These findings also underscore the notion that to improve cancer therapy, we must seek to completely understand the complex interplay of host and tumor genetics, the host immune system, and the all commensal organisms.

TARGETING TORID-1 ENHANCES ANTITUMOR IMMUNITY AND AUGMENTS THE EFFICACY OF IMMUNE CHECKPOINT BLOCKERS BY UNLEASHING INFLAMMASOME ACTIVATION.

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Although immune checkpoint blockers have yielded significant clinical benefits in patients with different malignancies, the efficacy of these therapies is still limited prompting the identification of novel immunotherapeutic targets. Here we show that disruption of of TOlerance-Related and InduceD cation transporter-1 (Torid-1/Tmem176b), contributes to CD8+ T-cell mediated tumor rejection by unleashing NLPR3 inflammasome activation. Lack of Torid-1 enhances the antitumor activity of anti-CTLA-4 antibodies through mechanisms involving caspase-1/ IL-1 activation. Accordingly, patients responding to checkpoint blockade therapies display an activated inflammasome signature. Finally, we identify BayK8644 as a potent Torid-1 inhibitor that promotes CD8+ T-cell-mediated tumor rejection and reinforces the antitumor activity of both anti-CTLA-4 and anti-PD-1 antibodies. Thus, pharmacologic de-repression of the NLRP3 inflammasome by targeting Torid-1 may enhance the therapeutic efficacy of immune checkpoint blockers.

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SAIC - LEÓN CHERNY AWARD

INHIBITION OF THE INTEGRIN ALPHA-V BETA-3 REVERTS THE PARADOXICAL EFFECT OF LEVOTHYROXINE REPLACEMENT DURING BEXAROTENE THERAPY IN CUTANEOUS T-CELL LYMPHOMA (CTCL)

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Bexarotene (Bex), a RXR agonist used for CTCL treatment, causes hypothyroidism in more than 90% of patients, thus requiring the concomitant administration of levothyroxine (T4). We found that physiological levels of thyroid hormones (TH) contribute to the malignant phenotype of CTCL by activating TH membrane receptor (mTR), the integrin αVβ3. Using RNA-sequencing and qPCR assays in Bex-treated HuT78 CTCL cells we found that Bex induces transcriptional and biological changes related to decreased cell proliferation and chemotaxis, as well as increased proliferation and interferon response. Lack of TH supplementation increased apoptosis and decreased proliferation of CTCL cells in vitro. Also, we studied the impact of T4 addition to Bex treatment (BexT4+) in mice bearing a syngeneic TCL solid tumor and found that Bex decreased tumor growth ($p<0.001$ vs Vehicle), being this effect even higher in the absence of T4 ($p<0.05$ vs Bex). However, Bex alone decreased the anti-lymphoma immunity, as shown by a decrease of activated CD8 + T-cells and of IFNγ and TNFα production ($p<0.05$ vs BexT4+), thus indicating that T4 replacement is necessary to avoid a negative immune microenvironment. Since T4 activates both the ubiquitous TH nuclear receptor and the more restricted integrin αVβ3 that is overexpressed in CTCL cells, we investigated if the mTR inhibition would impair the pro-survival effect of TH and its role in Bex treatment in vivo. We demonstrated that genetic and pharmacologic inhibition of the integrin αVβ3 with cilengitide resulted in improved bexarotene-induced effects on apoptosis, cell proliferation and render significantly smaller tumors ($p0.001$ vs Vehicle and $p<0.05$ vs BexT4+), while maintaining the lymphoma immunity. Our results provide a mechanistic rationale for evaluating the addition cilengitide to therapeutic regimens that are based on Bex and T4 supplementation.

EPIGENETIC ALTERATIONS ARE A HALLMARK OF HEPATOCELLULAR CARCINOMA AND IDENTIFY POTENTIAL THERAPEUTIC TARGETS.

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Hepatocellular carcinoma (HCC) is the second leading cause of cancer-related death worldwide and has no curative options for advanced disease. Recently, a causal link has been established between deregulation of epigenetic modifiers (EM) which catalyze post-translational modification of histones and the hepatocarcinogenesis. Our aim is to investigate EM alterations in HCC and to preclinically assess the therapeutic potential of their inhibition. To this end, the Cancer Genome Atlas dataset of HCC patients (n=365) was analyzed. We found that 75% of patients have at least one EM (n=90) mutated. In addition, 43% of the EM are up-regulated when compared tumor vs. non-tumoral tissues. Kaplan Meier analysis showed that high expression of 12 EM correlates with a poor prognosis in HCC patients. Then, in vitro assays showed that epigenetic inhibitors that target bromodomain (JQ-1), methyltransferases (BIX-1294 and LLY-507) and Jumonji (JmjC) demethylases (JIB-04, GSK-J4, SD-70 and ML-324) reduced human HCC cell survival, cell cycle arrest and cell death. Even more, the pan-inhibitor of JmjC JIB-04 showed antitumoral effect in mice bearing orthotopic HCC. In addition, we performed RNA-Seq analysis of HuH7 cells treated with JIB-04, GSK-J4 or SD-70 to identify the mechanisms involved in their antitumoral effect. We found that JmjC inhibitors induce a similar gene expression program related with cell proliferation, and cell death induction on HuH7 cells. Even more, we found that several genes depleted by JmjC inhibitors are highly expressed in tumor vs non-tumor tissues and that their high expression correlates with a poor prognosis in HCC patients. Finally, we identified a 5 gene signature (CENPA, KIF20A, PLK1, NCAPG and CTH) that could be used for prognosis prediction and potentially defines group of patients that could be benefited by a therapy based on JmjC inhibitors. Our results indicate that EM are interesting targets for therapeutic strategies against HCC.

WHY IS THE MACULA PARTICULARLY SUSCEPTIBLE TO NON-EXUDATIVE AGE-RELATED MACULAR DEGENERATION? LESSONS FROM THE MOUSE

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Non-exudative age-related macular degeneration (NE-AMD) represents the leading cause of blindness in the elderly. The macular retinal pigment epithelium (RPE) lies in a high oxidative environment because its high metabolic demand, mitochondria concentration, reactive oxygen species levels, and macular blood flow. It has been suggested that oxidative stress-induced damage to the RPE plays a key role in NE-AMD pathogenesis. The fact that the disease limits to the macular region raises the question as to why this area is particularly susceptible. We have developed a NE-AMD model induced by superior cervical ganglionectomy (SCGx) in C57BL/6J mice, which reproduces the disease hallmarks exclusively circumscribed to the temporal region of the RPE/outer retina. The aim of this work was analyzing RPE regional differences that could explain AMD localized susceptibility. Adult male C57Bl/6J mice were used. Histological, ultrastructural and biochemical parameters were studied. Lower melanin content, thicker basal infoldings, higher mitochondrial mass, and higher levels of antioxidant enzymes, were found in the temporal RPE compared with the nasal region (*P < 0.05 vs. nasal RPE, by Student´s t-test). Moreover, SCGx induced a decrease in the antioxidant system, and in mitochondria mass, as well as an increase in mitochondria superoxide, lipid peroxidation products, nuclear factor erythroid 2–related factor (Nrf2) and heme oxygenase-1 levels, and in the occurrence of damaged mitochondria exclusively at the temporal RPE (**P < 0.01 vs. nasal RPE from sham-treated eyes; a: P < 0.01 vs. temporal RPE from sham-treated eyes, by Tukey´s test (F=4.53)). These findings suggest that despite the well-known differences between the human and mouse retina, it might not be NE-AMD pathophysiology which conditions the localization of the disease, but the macular RPE histologic and metabolic specific attributes that make it more susceptible to choroid alterations leading initially to a localized RPE dysfunction/damage, and secondarily to macular degeneration.

A CONTINUOUS EXPOSURE TO URBAN AIR POLLUTION AGGRAVATES MYOCARDIAL INFARCTION IN MICE: THE ROLE OF LUNG INFLAMMATION AND IMPAIRED CARDIAC MITOCHONDRIAL FUNCTION

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Air pollution exposure is associated with increased mortality due to myocardial infarction (MI). It has been suggested that breathing polluted air leads to pulmonary and systemic inflammation, which might affect MI progression. To address this hypothesis, we evaluated the contribution of a continuous exposure to air pollution over experimental MI in mice, with particular focus over lung inflammation and cardiac mitochondrial function. Male eight-week-old BALB/c mice were exposed to filtered air (FA, control) or urban air (UA) inside whole-body inhalation chambers located in a highly populated area of Buenos Aires City for up to 16 weeks. After 8 weeks, mice breathing UA showed a 56% increase in total leucocyte count in bronchoalveolar lavage (BAL) samples (FA: 1.0±0.2 x10^5 cells, p<0.05), and a 104% increase in BAL protein concentration (FA: 0.30±0.04 mg/mL, p<0.05). Both BAL leucocyte count and protein concentration were still significantly increased after 12 weeks, together with a 3-fold increase in MCP-1 levels. Consistently, lung histology showed inflammatory leukocyte recruitment, edema, and thickening of the alveolar wall. Lung oxidative stress might precede inflammation, as increased phospholipid oxidation and decreased SOD activity were observed after 4 and 8 weeks, followed by a later increase in NADPH oxidase activity. Moreover, BAL analysis by flow cytometry showed increased alveolar macrophage activation and nitric oxide production in exposed mice after 12 weeks. In this group, a significant increase in TNF-α and IL-6 plasma levels were also observed. At this time point, UA exposure lead to a 53% increase in ischemia/reperfusion injury (FA: 43±4 % risk area, p<0.01). Mechanistically, UA-exposed mice showed impaired cardiac mitochondrial function, characterized by decreased active state respiration, inner mem-

brane depolarization, increased oxidants production, and decreased ATP production rate. Taken together, our data highlights the importance of considering environmental factors in the development of cardiovascular diseases in urban areas.

LONG NON-CODING RNAS FROM TELOMERES RESPOND TO OXIDATIVE STRESS AND EPITHELIAL-MESENQUIMAL TRANSITION (EMT)

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Long non-coding RNAs transcribed from telomeres, known as TERRA (telomeric repeat-containing RNA), are associated with telomere and genome stability. TERRA expression is elevated in human cancer tissues, however little is known about their function. Oxidative stress damages biomolecules and activates signaling cascades involved in cell proliferation, apoptosis, and metastasis. Since telomeres are prone to oxidative damage leading to their dysfunction, our objective is to characterize TERRA expression in oxidative stress, the mechanisms involved and its relevance in EMT. H2O2 induces TERRA expression in HEK-293T cells, and is prevented by antioxidant treatment. It was reported ROS are increased in brown (BAT) but not in white adipose tissue of mice exposed to cold. Importantly, we found increased TERRAs only in BAT of mice exposed to cold. In HEK-293T cells exposed to H2O2, ChIP shows that chromatin landscape is modified favoring telomere transcription. TERRAs interact with HP1α/γ, both proteins found recruited to subtelomeres. Since HP1γ interacts with transcriptional machinery, TERRAs may stimulate their own expression by recruiting HP1γ to subtelomeres. TERRA induction is lost 1-2h after removal of H2O2 from culture medium, suggesting they have protective functions. This is supported by rapid increase of TERRA upon a second H2O2 challenge. PKA inhibitor H89 blocks the increase of TERRA induced by H2O2, suggesting that PKA controls TERRA induction. Treatment of cells with drugs that disturb cytoskeleton integrity or growing cells on surfaces of different stiffness that generate differential cytoskeleton tension also modifies TERRA levels. In fact, cytoskeleton rearrangements of EMT-transformed NMuMG cells associate with higher TERRAs and lack response to H2O2, mimicking what occurs in T47D breast cancer cells that exhibit telomere dysfunction evidenced by confocal microscopy. In summary, we show that TERRAs are induced in response to oxidative stress and may be induced during EMT, being potentially novel early markers of cancer progression.

SAIC - IRENE FARYNA DE RAVEGLIA AWARD

ADIPOSE TISSUE FROM METABOLIC SYNDROME MICE INDUCES AN ABERRANT MIRNAS AND GENE EXPRESSION PROFILE CRITICAL IN PROSTATE CANCER DEVELOPMENT

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Metabolic syndrome (MeS) is a pathophysiological disorder that increases prostate cancer (PCa) risk and aggressiveness. White Adipose Tissue (WAT) is an active organ that contributes to PCa development by affecting the circulating levels of pro-inflammatory cytokines and hormones. WAT is also the main source of circulating miRNAs which can be absorbed by other cell types, where they regulate gene expression. Moreover, MeS is not only associated with an increase in the amount of exosomes derived from WAT, but also with a change in the miRNAs content of those exosomes, which may be related to the metabolic complications of this disease. Our hypothesis is that circulating miRNAs released by WAT from MeS mice interact with PCa cells, inducing their proliferation. To assess this hypothesis, MeS was induced in C57BL/6J male mice by chronically feeding animals with high fat diet (HFD). After 15 weeks, TRAMP-C1 PCa murine cells were injected *s.c.* on HFD and control diet fed mice. MeS induced tumor growth and the expression of Ctbp1, Fabp4, IL-6, miR-221-3p, miR-143-3p, miR-146a-5p, miR-27a-3p and miR-138-5p in the allografts compared to control animals. In addition, WAT from MeS mice showed an induction in the expression of IL-6, Tnf-α, Mcp1, Fabp4, Pparγ, miR-221-3p, miR-27a-3p, miR-34a-5p, miR-155-5p, miR-138-5p, miR-146a-5p and repression of Ctbp1, Ctbp2, Cyclin D1, miR-196a-5p and miR-143-3p compared to WAT from control mice. Moreover, WAT from MeS mice induced the proliferation and expression of Ctbp1, IL-6, miR-221-3p, miR-27a-3p, miR-138-3p, miR-34a-3p and miR-143-3p on TRAMP-C1 cells in co-cultures. Interestingly, miRNAs were isolated from the co-culture medium and hybridized with miRNAs expression microarrays, identifying several miRNAs released by the interaction between WAT-MeS mice/tumor cells compared to WAT-control mice/tumor cells. In summary, MeS induces an aberrant miRNA and gene expression profile in WAT and tumors critical in prostate tumor growth.

INTEGRATIVE TISSUE PROTEOMICS DISSECTS CLEAR AND DISTINCT PROTEOMES IN PROSTATE CANCER AIDING IN THE DISCOVERY OF NOVEL RISK STRATIFICATION BIOMARKERS.

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Formalin-fixed, paraffin-embedded (FFPE) tissues are highly valuable resources for translational proteomics studies. Although it is well known that prostate cancer (PCa) is a progressive disease involving multiple gene alterations, little is known at the proteome level. The biopsy Gleason score (BGS) is used as an aid for physicians to evaluate the prognosis of men with PCa using samples from prostate tissues. However, BGS can fail to clearly distinguish between some Gleason grades, leading to incorrect treatment of PCa patients. To identify potential PCa protein biomarkers for risk stratification, we carried out an in-depth proteomics analysis (ESI-MS/MS) using human PCa and Benign Prostatic Hyperplasia (BPH) FFPE tissues. We have explored the high-throughput data in two different ways.

First, we identified differentially expressed proteins between PCa and BPH samples. We filtered the proteins based on spectral counts, resulting in the selection of 11 candidates. To assess the clinical significance of these peptides we performed an integrative bioinformatics analysis using public database repositories (Oncomine, cBioportal, TCGA, GEO, 29 datasets, n=3794). We identified YWHAZ, a novel androgen receptor (AR) co-activator, to be strongly associated with poor prognosis across different PCa datasets. Overall and relapse-free survival were significant shorter when comparing patients with high *vs.* low expression for this gene (Hazard Ratio >1, P<0.05). Strikingly, when performing a multivariate analysis, YWHAZ displayed high significant correlation with poor patient prognosis, independent from BGS.

In parallel, to contextualize the proteomics data, we moved away from conventional analytical approaches and evaluated differential protein complexes (CORUM) calculating PCa and BPH patient proteomics signature profiles (PSPs). Of note, the top ranked cluster upregulated in PCa was the MLL complex (p-value= 4.37x10-5), a protein complex that acts as a co-activator of the androgen receptor (AR). In summary, targeting AR co-activator complexes may represent novel therapeutic avenues for castrate resistant prostate cancer.

CONTRIBUTION OF GALECTIN-1 TO HEPATOCELLULAR CARCINOMA CELL DRUG RESISTANCE

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Hepatocellular carcinoma (HCC) is characterized by a high resistance to chemotherapy. P-glycoprotein (P-gp) is an ATP-dependent drug efflux pump. Its overexpression in HCC is associated with a decrease in intracellular drug concentration, leading to chemotherapeutic failure. Galectin-1 (Gal1), a β-galactoside-binding protein, is overexpressed in HCC and it is related to tumor aggressiveness. Recent studies have shown that Gal1 may have a role in HCC chemoresistance. Nevertheless the mechanisms underlying this effect remain unclear.

Our aim was to investigate the molecular basis of Gal1-mediated chemoresistance in HCC cells.

We stably transfected human HCC HepG2 cells to overexpress (HepG2Gal1) or silence (shRNAs) Gal1 expression, with the corresponding control cells.

By inoculating cells into NSG mice we observed a decrease in the volume of control-derived tumors treated with doxorubicin (DOX,4.5 mg/kg i.v., once a week for 3 weeks) compared with HepG2Gal1-derived treated tumors (0.43±0.03cm3 vs 1.39±0.38cm3, p<0.05). By comparison of half maximal inhibitory concentration values in cell viability experiments (MTT), we observed that Gal1 overexpression significantly protects HepG2 cells from DOX (1.31μM vs control cells:0.81μM) and sorafenib (32.25μM vs control cells:13.36μM) exposure, while silencing Gal1 sensitizes cells to drug citotoxic effect.

By fluorescence techniques we found a decrease in intracellular DOX concentration in HepG2Gal1 cells versus HepG2 cells (3.09±0.47 vs 4.47±0.28 pmol/μgr total protein, 6h-treatment, p<0.01). Gal1 knockdown induced the opposite effect (p<0.01). Gal1 overexpression increased Pgp protein levels in a PI3K-dependent manner (immunoblotting, p<0.05). Co-incubation of HepG2Gal1 cells with 2μM DOX and 20μM verapamil-a Pgp inhibitor-diminished cell viability compared with cells incubated only with DOX (p<0.05). Similar results were obtained by siRNA-mediated P-gp knockdown (p<0.05).

Thus, Gal1 protects HCC HepG2 cells from DOX- and sorafenib-induced cell death. Also, Gal1-overexpressing cells accumulate less intracellular DOX. Moreover, P-gp is involved in Gal1-induced resistance to DOX.

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AMNIOTIC MEMBRANE CONDITIONED MEDIUM INHIBITS TUMORAL CELLS PROLIFERATION AND MODULATES RELATED MICRORNAS EXPRESSION IN HEPATOCARCINOMA MODELS IN VITRO.

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The stem cells, and particularly the placental stem cells, have called the focus of attention for their therapeutic potential to treat different diseases, including cancer. There is plenty evidence about the anti-tumoral effects of the human amniotic membrane given by their antiproliferative, antiangiogenic and proapoptotic properties. The amnion and its cells both secrete unknown factors and physically interact with tumor cells. Liver cancer is the fifth cause of cancer in the world, with a poor prognosis and survival. Alternative treatments to radio- or chemotherapy have been searched. We have previously demonstrated that the amniotic membrane conditioned medium (AM-CM) inhibits DNA synthesis and viability in hepatocarcinoma cells. In the present work we aimed to evaluate the antiproliferative properties of the AM-CM in hepatocarcinoma cells. We have analyzed the expression of key cell cycle proteins (cyclin D1, p53, p21, MDM-2) by Western blot and qRT-PCR, in HepG2 cells treated with AM-CM. In addition, we have analyzed the regulation of miR-15a, miR-210, miR-206 and miR-145 expression (pro and antiOncomiRs involved in hepatocarcinoma physiology) by qRT-PCR. We found that AM-CM reduced (5 ± 0.5 fold) the expression of both Cyclin D1 mRNA and protein. We observed that this conditioned medium was able to promote the expression (2.2 ± 0.3 fold) of p53 and (4 ± 0.05 fold) p21 mRNA and proteins, leading cells to growth arrest. Moreover, AM-CM induced an increase (3 ± 0.3 fold) in nuclear p21 expression comparing with the cytoplasmic one, observed by immunofluorescence assay. As p53 levels were increased, MDM-2 expression was downregulated (3.1 ± 0.2 fold), as expected. Interestingly, HepG2 and Huh-7 treatment with AM-CM produced an upregulation of antiOncomiRs 15a and 210, and a downregulation of the proOncomiRs 206 and 145. We provide new evidence about the promising novel applications of the human amniotic membrane in liver cancer.

FGF2 INDUCES BREAST CANCER GROWTH THROUGH LIGAND-INDEPENDENT ACTIVATION AND RECRUITMENT OF ERA AND PRBΔ4 ISOFORM TO MYC REGULATORY SEQUENCES

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We have demonstrated that progesterone receptor (PR) activation is involved in fibroblast growth factor 2 (FGF2)-induced cell proliferation and tumor growth in experimental breast cancer models, and that estrogen receptor (ERα) and PR interact after progestin priming. However, it still remains unknown whether ERα and PR interact after hormone receptor ligand-independent activation. The aim of this study was to determine if ERα, in addition to PR, was also involved in FGF2-induced tumor progression and to identify interactors at hormone receptor binding sites in the MYC promoter after FGF2 stimulation. FGF2 increased ERα and PR ($p<0.01$) phosphorylation and nuclear co-localization of both receptors ($p<0.001$) in human MCF-7 and T47D breast cancer cells. We focused on the enhancer and proximal regions of MYC promoter using luciferase constructs, ChIP and DNA pull-down following LC-MS/MS assays. FGF2/estrogens/progestins had similar effects activating gene expression. FGF2 increased ERα and PR recruitment to both MYC enhancer and proximal regions ($p<0.001$), while ICI182780 or mifepristone inhibited FGF2-induced gene transcription ($p<0.001$). The proteomic studies revealed that FGF2 induced the recruitment of several interactors at both MYC enhancer and proximal promoters, being ERα-dependent and ERα-independent, respectively. Moreover, we identified additional PR isoforms besides PRA and PRB, which have been previously described only at the mRNA level: PRBΔ4, PRM and PRC. FGF2, but not progestins, activated PRBΔ4 isoform rendering a proliferative advantage to PRBΔ4 expressing cells. Interestingly, PRBΔ4 is related with worse prognosis in luminal breast cancer patients suggesting a role in the onset of endocrine resistance. MYC inhibitors decreased FGF2-induced cell proliferation (T47D cells, $p<0.001$) and tumor growth (C4-HI tumor, $p<0.001$). We conclude that FGF2 exerts estrogenic-like effects inducing interactions between ERα and classic/novel PR isoforms placing MYC as a promising therapeutic target after endocrine therapy failure in breast cancer.

SAIC – YOUNG INVESTIGATORS BIGAND AWARD

DEVELOPMENT OF METABOLIC ALTERATIONS BY GESTATIONAL STRESS

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Prenatal exposure to stress may program the fetal HPA axis, leading to altered metabolism in later life, associated with obesity and diabetes. This could be worsen if the individual doesn't eat healthy. We studied the effect of prenatal stress (PS) on metabolism and the impact of high fat diet (HFD). Pregnant BALB/c and C57BL/6J female mice were stressed during the last week of pregnancy. Offspring were fed with HFD or a standard diet (SD). We observed that PS only: increased cholesterol (CHOL) and triglycerides (TRI) levels in BALB/c males ($p<0.001$ vs NPS+SD), C57BL/6J males showed alterations in the insulin sensitivity test (IST). When we added the HFD influence: PS+HFD males of both strains and BALB/c females had a higher body weight than NPS+HFD and PS+SD groups ($p<0.001$). In the IST, C57BL/6J mice showed alterations due to PS together with HFD. Females BALB/c PS+HFD had increased TRI and CHOL ($p<0.001$vs NPS+HFD, $p<0.05$ vs PS+SD). Plasmatic TRI were also increased in C57BL/6J males ($p<0.001$ vs NPS+HFD and PS+SD). The gene expression in adipose tissue showed a decrease in ADIPO in BALB/c and in females C57BL/6J of PS+SD ($p<0.01$ vs NPS+SD). FOXO1 increased in BALB/c PS+SD females ($p<0.05$ vs NPS+SD), while SIRT1 decreased in BALB/c PS+SD males ($p<0.001$ vs NPS+SD). BALB/c males were more sensitive to developing metabolic alterations due to PS, BALB/c females PS showed an increase in body weight and plasma TRI. Males and females C57BL/6J developed metabolic alterations due to PS only after HFD feeding. In both strains females showed greater resistance to PS. The decrease in the expression of ADIPO and SIRT1 in BALB/c males would indicate a lower insulin sensitivity, which may be detected earlier than at the physiological level. This leads us to propose these genes as possible premature markers of a future metabolic imbalance.

THE TYRO3, AXL, AND MERTK AXIS IN MAINTAINING IMMUNE HOMEOSTASIS IN HUMAN INFLAMMATORY DISEASES

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The tyrosine kinase receptors TYRO3, AXL and MERTK (TAM) and their ligands Protein S (PROS1) and growth arrest-specific 6 are critical players to maintain immune homeostasis by dampening inflammatory response, mediate efferocytosis and to contribute to tissue repair process. The main goal of our research is to understand the relevance of this pathway in human immune-pathological conditions where the immune balance and tissue repair are altered such as in Inflammatory Bowel Disease (IBD), Multiple sclerosis (MS) and in Langerhans cells Histiocytosis (LCH). MS is a chronic inflammatory and autoimmune disorder affecting the central nervous system by infiltration of autoreactive Th1/Th17 cells. Interestingly, it has been shown that patients with MS and helminthes infection exhibited the lower number of relapses and lesion activity compared with uninfected individuals with MS. Our goal is to elucidate if parasite-driven protection mechanisms involve TAM axis to dampen an associated pathological Th1/Th17 response by enhancing negatively regulatory signals, promoting the conversion of Th17 to Tregulatory lymphocyte and inducing tissue repair macrophages. The etiology of LCH is still under scientific discussion, and it is not clear if LCH results from malignant transformation or unbalanced immune response that leads to the proliferation of pathogenic LC-like cells. Considering that TAM axis has been identified as negative regulators of the immune response, but also aberrantly expressed in multiple hematological malignancies we aim to dissect which TAM pathway is activated in one or another condition. Crohn's disease and Ulcerative Colitis are IBD characterized by chronic inflammation and tissue damage. Loss of T cell-derived PROS1, as well as genetic ablation of Axl and Mertk, increases the inflammatory signature with loss of tissue repair macrophage phenotype in a mouse model of IBD. Our goal is to characterize the immune compartments, TAM receptors and PROS1 expression in monocyte/macrophage and lymphocytes of patients with IBD.

SPECIFIC COMPONENTS OF THE HISTAMINERGIC SYSTEM AS POTENTIAL TARGETS FOR LEYDIG CELL TUMOR TREATMENT IN PREPUBERTAL BOYS

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Testicular Leydig cell tumors (LCTs) are rare neoplasms associated with endocrine dysfunctions in boys and adult men. Aromatase (CYP19) overexpression and excessive estrogen (E2) production are known to sustain Leydig cell tumorigenesis. Although mostly benign, LCTs can become malignant and unresponsive to current chemo/radiotherapy, highlighting the need to identify new therapeutic targets. Previously, we reported that L-histidine descarboxylase (HDC), responsible for histamine (HA) synthesis, is overexpressed in human prepubertal LCTs versus control prepubertal testes (CPTs), whereas HA receptor H4 (HRH4) is weakly expressed in these tumors compared to CPTs. Analogously, we observed that HA is an autocrine growth factor stimulating cell proliferation and steroid production through HA receptor H2 (HRH2) in mouse and rat Leydig tumor cells, while selective HRH4 activation decreases proliferation, steroidogenesis and angiogenesis. Recently, we assessed the effect of HDC inhibitors on Leydig tumor cell proliferative, steroidogenic and pro-angiogenic capacities. Experiments were performed in R2C rat Leydig tumor cells (R2C), the best-known in vitro model of Leydigioma. Cell proliferation was assessed using 3H-Thymidine and sulforhodamine B incorporation assays. CYP19 expression was evaluated by qPCR, and CYP19 activity was measured using a tritiated water-release assay. Steroid levels were determined by radioimmunoassay. The angiogenic potential of conditioned media from R2C and human Leydig tumor cells isolated from prepubertal LCTs (n=3) was evaluated in vitro and in vivo, using human umbilical vein endothelial cells and by means of the quail chorioallantoic membrane assay, respectively. Our results show that HDC inhibitors can significantly diminish Leydig tumor cell proliferation (48-h), steroidogenesis (24-h) and angiogenesis (48-h). Also, we provide the first evidence that HA would be one of the pro-angiogenic factors secreted by Leydig tumor cells. Overall, our former and present results suggest that specifically targeting HDC and/or HRH4 may constitute a potential effective neoadjuvant therapy against LCTs, at least in prepubertal patients.

GENETIC DEFECTS IN THE DEVELOPMENT OF THE ANTERIOR PITUITARY GLAND: FROM MOUSE TO HUMAN

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Pituitary hormone deficiency occurs ~1:4,000 live births. Over 30 genes have been implicated in isolated and/or combined pituitary hormone deficiency (IGHD/CPHD). Mutations are estimated to account for ~16% of patient cases, thus the majority of familial and sporadic cases have no known genetic origin. We recently implemented a novel and cost-effective approach based on Molecular inversion probe sequencing (MIPS) to identify novel variants and candidate genes in sporadic trios and familial cases of CPHD and IGHD. We captured 693 coding exons of 30 known genes and 37 candidate genes. We captured genomic DNA from 176 pediatric patients from Argentina with CPHD or IGHD and 133 relatives and conducted next generation sequencing. We obtained a 600X average coverage per sample over targeted regions. We discovered 10 likely pathogenic variants in 6 genes; 8 of them are novel. Mutations in PROP1 are the most common known cause of CPHD, accounting for 11% of total cases worldwide. We determined that PROP1 is essential for stimulating stem cells to undergo an epithelial to mesenchymal transition-like process necessary for cell migration and differentiation. The mechanism whereby PROP1 regulates this process is not completely understood. Our goal is to further our understanding of the factors regulating embryonic pituitary progenitor cells. Gene expression profiling, using GHFT1 cells, revealed that Prop1 upregulates genes that are involved in migration and in degrading extracellular matrix proteins, like matrix metalloproteinases and downregulates tissue inhibitor of metalloproteinases. Using immunoprecipitation coupled to mass spectrometry, we identified 93 proteins that specifically interact with PROP1. These proteins were tested for GO biological process enrichment and two predominant themes were identified: Cell-Cell Adherens Junction and Cell-Cell adhesion. This basic knowledge could implicate candidate genes to explain cases of hypopituitarism with unknown etiology and thereby, yield better diagnosis, together with the identification of potential pathogenic variants.

SAIC – EDUARDO SOTO AWARD

GHRELIN SIGNALING MEDIATES FASTING-INDUCED ACTIVATION OF THE HYPOPHYSIOTROPIC CRF NEURONS VIA RECRUITMENT OF THE NPY/AGRP/GABA NEURONS

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Ghrelin is a stomach-derived hormone that acts on the growth hormone secretagogue receptor (GHSR). Plasma ghrelin increases under fasting, when it promotes appetite and activation of the hypothalamic–pituitary–ad-

renal (HPA) via its action on corticotropin-releasing factor (CRF) neurons of the paraventricular nucleus (PVN). The neuronal circuits by which ghrelin regulates these actions are unclear. Here, we tested in male mice with pharmacological or genetic blockage of GHSR the effect of 48 h fasting on the PVN CRF neurons and on the neuropeptide Y (NPY)/agouti-related protein (AgRP)/GABA neurons of the arcuate nucleus (ARC), which sense plasma factors and regulate the PVN. **Results:** As compared to fed mice, fasted mice had an increase of the number of PVN CRF cells (3.57±0.22 fold increase, p≤0.05, T-test) and of the NPY/AgRP-fiber intensity (NPY-fibers: 0.09±0.02 vs 0.17±0.02 OD; AgRP-fibers:3428±754 vs 10783±1490 intensity; GFP-fibers: 8558±965 vs 16799±1771 intensity in mice expressing GFP in NPY neurons, p≤0.05, T-test). As compared to wild-type (WT) mice, GHSR-deficient mice had lower levels of plasma corticosterone and the marker of neuronal activation, c-Fos, in the PVN (corticosterone: 204±30 vs 113±30 ng/ml; c-Fos: 44±9 vs 13±6 cells/side, p≤0.05, 2-way ANOVA). Similarly, fasted mice with pharmacological blockage of GHSR showed lower c-Fos and of NPY-fiber intensity in the PVN (c-Fos: 119±19 vs 177±13 cells/side; NPY-fiber: 0.21±0.01 vs 0.45±0.04 OD, p≤0.05, T-test). Fasted mice expressing tdTomato fluorescent protein in GABA neurons had more tdTomato fibers in PVN (1.36±0.08 fold increase, p≤0.05 vs fed mice, T-test). As compared to fed mice, PVN explants of fasted mice had a reduction of the basal and KCl-stimulated GABA release (basal: 4.5±0.3 vs 3.1±0.5 and KCl: 6.1±0.3 vs 4.3±0.8 % of total incorporated tracer, p≤ 0.05, 2-way ANOVA). Current results indicate that ghrelin signaling mediates fasting-induced activation of the hypophysiotropic CRF neurons via recruitment of the NPY/AGRP/GABA neurons.

DEGENERATION RATES OF COCHLEAR HAIR CELLS AND NEURONS IN A DFNA2 DEAFNESS MODEL

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DFNA2, an autosomal dominant disease with progressive hearing loss, is caused by mutations in voltage-activated potassium channel KCNQ4. In DFNA2, Inner and Outer Hair Cells (IHC and OHC) remain chronically depolarized leading to impaired cell function, cell death and hearing loss. Transgenic mice with a deletion in Kcnq4 gene (Kcnq4-/-) develop a DFNA2-like hearing loss syndrome.

Our aim is to characterize the degeneration rate of HCs and spiral ganglion neurons (SGN) in Kcnq4-/- mouse as a model of DFNA2.

First, we evaluated mRNA and protein expression of KCNQ4, in different cochlear segments in wild-type (WT) mice. We observed lower expression in apical turn than in basal/middle ones (p=0.0035). Next, we evaluated HCs survival for WT and Kcnq4-/- mice. We constructed cytocochleograms at each age (3-58 postnatal weeks (W)), in which the HCs number in each 5%-segment of cochlear length was plotted relative to their distance from the apex. We observed that, for IHC and OHC, cell death began at basal turn increasing towards the apex with age in Kcnq4-/- mice. Moreover, cell death rate was slower in apical than in basal segments (p=0.0022), but started 30 weeks earlier in OHC than in IHC. Furthermore, using scanning electron microscopy, we observed that in Kcnq4-/- mice, cell degeneration became apparent in basal segments early at 4W, showing hair bundle disorganization an absence of OHCs. Finally, we analysed SGN survival in both genotypes in each segment at different ages. Kcnq4-/- animals showed neuronal loss starting at 40W for basal and apical segments (p<0.0010), which progressed further with age.

Since Kcnq4-/- mouse resembles many DFNA2 features, we concluded it is a suitable model for studying this disease. It would provide a useful platform for testing drugs which could retard hearing loss in DFNA2 and related diseases such as Presbycusis and Noise-related Hearing Loss.

METABOLIC SYNDROME TRIGGERED BY HIGH-FRUCTOSE DIET SHOWS VASCULAR INTEGRITY AND NEURONAL FUNCTIONALITY ALTERATIONS IN MOUSE RETINA

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Diabetes mellitus type 2 is consequence of the metabolic syndrome (MS), being diabetic retinopathy (RD) a serious complication and cause of blindness in the world. We aimed to analyze markers of vascular integrity and neuronal functionality related to early stages of DR in a model of MS.

C57BL/6 (WT) and Apolipoprotein E knockout (ApoE-KO) mice fed with a normal diet (ND) or a 10% w/v fructose diet (FD) in drinking water from 2 months of age were used. Time-dependent kinetic studies were done from 2 to 6 months of diet.

The hypercholesterolemic ApoE-KO showed an increase in LDL-Chol, and being fed with FD, they also showed hypertriglyceridemia and a decrease in HDL-Chol, in addition to hyperglycemia, hyperinsulinemia and altered glucouse tolerance test. Scotopic ERG showed decreased a, b waves and OPs in ApoE-KO DF vs WT DN, which correlated with increased TUNEL positive

cells. High vascular permeability in ApoE-KO FD was evidenced by leakage of Evans blue (e.v.) and extravasation of albumin and α2-Macroglobulin. GFAP expression were observed in astrocytes but not in Müller glial cells (MGCs), so there is no reactive gliosis in retinas of ApoE-KO FD, which correlates with normal expression of the glutamine synthetase, indicating a normal operation of the MGCs. However, GFAP immunoreactivity decreased was observed in retinal flatmounts, which could explain the reduced integrity of the blood-retinal barrier. The expression of HIF and VEGF mRNA was not modified in any group, indicating changes associated with early stages of RD, without characteristics related to stages of neovascularization, at the time evaluated.
The results showed that the ApoE-KO DF mice, which reproduce characteristics of the human SM, presented vascular dysfunction and neurodegeneration, without alterations related to tissue ischemia. Therefore, this model offers the opportunity to study early stages of the DR, whose prevalence increases in the world.

SAI – LEONARDO SATZ AWARD

IN VIVO PHARMACOLOGICAL INHIBITION OF WNT PROTEINS SECRETION DURING THE ACUTE PHASE OF T. CRUZI INFECTION REDUCES THE SEVERITY OF CHRONIC CHAGAS DISEASE CARDIOMYOPATHY IN BALB/C MICE.

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Chagas disease is a major cause of heart disease and cardiovascular-related deaths in endemic areas located in Latin America. Each year there are approximately 12,000 deaths which are attributable to Chagas disease, typically due to severe chronic Chagas disease cardiomyopathy. Wnt signaling, essential for embryonic development, has also recently been involved in the regulation of inflammatory processes. We have previously reported that *T. cruzi* infection induces Wnt pathways activation and that *in vivo* pharmacological inhibition of the Wnt proteins secretion controls the parasite replication and improve the survival of lethally infected B6 mice. To investigate the role on Wnt proteins in determining the outcome of chronic Chagas disease, BALB/c mice were infected with 1,000 trypomastigotes of T. cruzi and treated with the inhibitor of Wnt secretion IWP-L6 (7.5 mg/kg) or vehicle (control) on days 5, 8, 11 and 14 post-infection (pi). During the acute phase of the infection, IWP-L6-treated mice showed lower levels of parasitemia (p<0.05), associated with increased serum levels of IL-12 (p<0.05) and TNF (p<0.001) and decreased function of Treg cells (p<0.05) compared with control mice. At the chronic phase of the infection (180 days pi), the cardiac electrophysiology and global left ventricular function (LV) were studied by electrocardiogram (ECG) and 2D-echocardiogram (ECHO), respectively. The ECGs of IWP-L6-treated mice were improved compared with control mice, because non-treated group presented a significant prolongation in the QT intervals (p<0.001), suggesting intraventricular conduction blockages; meanwhile, this abnormality was not observed in the IWP-L6-treated group. In addition, ECHO revealed systolic disfunction that was only present in control mice (p<0.01). Our results indicate that the inhibition of Wnt proteins secretion during the acute phase of the infection might controls parasite replication, thus preventing the development of chronic cardiomyopathy.

THE ROLE OF BIN1 IN THE EXPRESSION OF PD-L1 IN INFLAMMATORY BOWEL DISEASE AND COLORECTAL CANCER MIGHT EXPLAIN A DIFFERENTIAL REGULATORY T CELL ACTIVITY THROUGH THE PD-1/PD-L1 AXIS.

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Background: We previously described a natural human CD8+HLA-DR+ Treg subset acting through cell-cell inter-

actions, such as CTLA-4 and PD-1/PD-L1. The present study aimed to evaluate the role of the PD-1/PD-L1 axis in the intestinal mucosa from Colorectal Cancer (CRC) and Inflammatory Bowel Disease (IBD) patients, focusing on the regulation of PD-L1 expression within epithelial tissues.
Methods: Colon samples from healthy controls (HC) (n=41), IBD (n=36) and CRC patients (n=31) were obtained from biopsies or surgical specimens. Lamina propria (LP) CD8+HLA-DR+ cells and PD-L1+-enterocytes were assessed by flow cytometry; BIN1 transcripts were analyzed by RT-qPCR; PD-L1 immunofluorescence and immunohistochemistry were performed in paraffin slides.
Results: The frequency of CD8+HLA-DR+ cells in LP of HC was 22.8±1.4% of the CD8+ subset, and they highly expressed PD-1 (CD8+HLA-DR+ 52.4±4.5%vs31.5±3.6% within CD8+HLA-DR-;p<0.0001). Affected intestinal areas from IBD and CRC patients showed a higher frequency of CD8+HLA-DR+PD1+ cells in comparison to non-affected areas (IBD: 73.8±3.3%vs49±5.7%;p<0.001; and CRC: 65.4±4.6%vs47.8±5.8%;p<0.01).

Strikingly, PD-L1 epithelial expression was decreased in the inflamed tissue of IBD patients (25.8±6.7%vs56.3±6.3%;p<0.001), and increased within CRC tumors (74.2±5.7%vs57.5±6.4%;p<0.01) compared with non-affected tissues. Moreover, BIN1 expression, a PD-L1 negative regulator, was increased in IBD inflamed areas (p<0.01) and decreased in the CRC tumor (p<0.05). Finally, we observed BIN1 epithelial expression up-regulated (p<0.05) and PD-L1 decreased (p<0.05) in tumoral tissues of chemotherapy-treated CRC patients.
Conclusions: CD8+HLA-DR+PD1+ Tregs were increased in CRC and IBD affected tissues, whilst the frequency of PD-L1+ colonocytes was up-regulated in CRC and decreased in IBD. Accordingly, BIN1 was inhibited in CRC and increased in IBD patients. These findings could explain the different immune responses observed in CRC and IBD, and should be considered for monitoring the treatment in clinical trials for CRC and IBD. Strikingly, CRC patients receiving chemotherapy showed a reverted pattern with decreased expression of PD-L1 and increased levels of BIN1.

IL-17A REVERTS THE REDUCTION IN CD8+ T CELL RESPONSE GENERATED BY ANTI-CD20 TREATMENT.

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Anti-CD20 therapy, that depletes B cells, is widely used to treat autoimmunity and lymphomas. B cells can regulate T cell responses because they have antibody independent functions, such as antigen presentation and cytokine secretion. To evaluate the effect of anti-CD20 treatment on CD8+T cell response we used the experimental model of *Trypanosoma cruzi* infection, since CD8+T cells are essential for infection control.
For that, anti-CD20 mAb was injected eight days before infection with 5000 trypomastigotes, to deplete B cells and CD8+T cell response was analyzed at different days post infection (dpi). Infected control mice were injected with an isotype antibody.
At 20 dpi, B-cell depleted mice (BcD) exhibited higher parasitism (determined by parasite DNA quantification by RT-PCR) in the spleen, liver and heart than controls (p<0,001). Interestingly, an early contraction of total and T. cruzi-specific CD8+T cell response, measured by FACS using tetramers, was observed in BcD mice. Infected BcD mice had significant lower frequency and number of splenic CD8+T cells (p<0,05), decreased CD8+T cell proliferation (p<0,05), higher levels of apoptosis (p<0,01) and expression of inhibitory receptors (p<0,05) on CD8+T cells, and lower in vivo infected-cell lysis in comparison to controls. CD8+T cells from BcD mice also exhibited reduced cytokine production. When anti-CD20 was injected after 12 dpi, CD8+T cells exhibited the same characteristics than those present in mice injected before infection, suggesting that B cells did not influence CD8+T cell response through antigen presentation. Immunofluorescence studies showed that T cells were in a narrow zone of contact with extrafollicular IL-17A-producing plasmablasts. Moreover, the frequency of splenic IL-17A-producing cells from infected BcD mice was strongly reduced. IL-17A administration to infected anti-CD20 treated mice rescued the overall CD8+T cell response. The results indicate that IL-17A production by B cells or other IL-17A producing cells are key to sustain CD8+T cell response.

RESTORATION OF ANTI-TUMOR IMMUNITY THROUGH ANTI-MICA ANTIBODIES ELICITED WITH A CHIMERIC PROTEIN.

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To overcome tumor immune escape and eradicate established tumors and metastases constitutes a pending challenge in immuno-oncology. Natural killer and cytotoxic CD8+ T cells are major players during tumor immunity. They express NKG2D, an activating receptor that promotes tumor elimination through recognition of the MHC class I chain-related proteins A and B (MICA and MICB) over-expressed on tumor cells. However, tumors shed MICA and mediate tumor immune escape. As anti-MICA antibodies (Ab) may promote the restoration of tumor immunity, we generated a highly immunogenic chimeric protein (BLS-MICA) consisting of MICA fused to the lumazine synthase from Brucella spp. (BLS) and used it to investigate if anti-MICA Ab can reinstate tumor immunity. Active immunization with BLS-MICA and passive administration of anti-MICA Ab triggered by BLS-MICA in C57BL/6 mice significantly delayed the growth of MICA-expressing tumors (n=14, p<0,0001 in both cases). Anti-MICA Ab promoted scavenging of soluble MICA (n=5, p<0,05), induced in vivo Ab-dependent cell-mediated cytotoxicity (n=13, p<0,0001), higher intra-tumoral M1/pro-inflammatory macrophages (n=10, p<0,001) and antigen-experienced CD8+ T cells (n=8, p<0,01), suggesting that these Ab reinstate anti-tumor immunity. Thus, the chimeric protein BLS-MICA constitutes a promising biotechnological approach for cancer patients.

SAFIS – YOUNG RESEARCHERS IN PHYSIOLOGY AWARD

ESOPHAGEAL HIATUS REPAIR: COMPARISON STUDY OF THREE REINFORCEMENT MESHES IN A PRECLINICAL PORCINE MODEL

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Laparoscopic repair of the hiatal hernia is associated with a recurrence rate between 12% and 42% depending on the defect size. The main limitation of hiatal reinforcement has been the risk of adverse events related with the use of synthetic meshes in the vicinity of the esophagus. Aims: To evaluate the biomechanical characteristics and histologic remodeling of a non-absorbable synthetic mesh, an absorbable biosynthetic mesh and an absorbable biologic mesh in a preclinical model of laparoscopic hernia repair.

15 Landrace pigs underwent laparoscopic primary hiatal hernia repair of a simulated defect in the esophageal hiatus. Five pigs were reinforced with a non-absorbable synthetic polypropylene mesh (Prolene®: PP), five with an absorbable biosynthetic scaffold (Gore Bio-A®: PGA) and five with an absorbable biologic extracellular matrix scaffolds, (Matristem®: ECM). Animals were euthanized at 12 weeks. Endpoints included gross morphology with adhesion assessment, mechanical testing and histology. The site of the repaired defect reinforced with ECM scaffolds showed a robust closure of the crura with a smooth peritoneal structure that covers the entire repair, while those repaired with PP or PGA showed thickening and fibrosis at the repair site. Load at failure and stiffness of the PP group were significantly higher than of ECM group (2171.8±607.8 vs. 1196.8±518.1 P<0.05; 676.3±337 vs. 242.9±122.1, P<0.05). In both tests, the PGA group showed intermediate characteristics, without significant differences with PP or ECM group.

In ECM pigs, histology resembled that of native tissue. On the contrary, PP and PGA pigs showed dense fibrotic tissue with large mononuclear infiltrates, foreign body reaction, fibroencapsulation, necrosis, evident remnants of mesh and large areas of disorganized tissue.

Conclusions: In this experimental setting, the ECM meshes showed the most appropriate characteristics for the consolidation of the hiatus, recovering the tissue characteristics that can help reduce the chances of early failure.

THE CAMP EFFLUX, THROUGH MULTIDRUG RESISTANCE PROTEIN 4 (MRP4/ABCC4), IS INVOLVED IN THE ACQUISITION OF FERTILIZING ABILITY IN MAMMALIAN SPERMATOZOA

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In order to fertilize an oocyte, mammalian spermatozoa must undergo a number of physiological modifications in the female reproductive tract, collectively known as capacitation. Activation of PKA is essential for capacitation, and therefore cAMP levels are tightly regulated during this process. Although cAMP levels are mainly determined by its synthesis and degradation, we previously demonstrated that cAMP extrusion through multidrug resistance protein 4 (MRP4) is also involved in this modulation in bovine sperm capacitation. Moreover, supplementation of incubation media with non-permeable-cAMP triggers capacitation, suggesting that cAMP efflux is necessary

to regulate intracellular nucleotide levels and to provide the extracellular space with molecules that promote purinergic signalling.
Our aim was to deepen the role of cAMP/MRP4 efflux system in mammalian sperm capacitation. For this, we propose 1) to elucidate molecular pathways involved in extracellular cAMP-induced in bovine sperm capacitation; 2) to evaluate the possible role of cAMP/MRP4 efflux in mouse sperm capacitation.
Bovine spermatozoa were incubated in non-capacitating media with non-permeable-cAMP (ecAMP; 10nM) and enzymes inhibitors of purinergic signaling pathways, and changes associated with capacitation were assessed. Our results indicated that ecAMP exerts a robust response in bovine sperm capacitation activating PLC, PKC and ERK-1/2. Additionally, ecAMP elicited a fast rise in sperm Ca2+ levels that activated sAC and increased pPKA and pY levels, indicating that cAMP exerts a broad range of responses in this species.
On the other hand, mouse spermatozoa were incubated in capacitating conditions with MK571 (MRP4 inhibitor) and changes associated with capacitation were assessed. MRP4 inhibition increased intracellular cAMP and pPKA levels. However, MK571 inhibited sperm motility, hyperactivation, induced-acrosomal reaction and in vitro fertilization.
Our results suggest that cAMP/MRP4 efflux-system has a critical role in the regulation of cAMP-activated signalling pathways in both species and emerges as an important player in sperm physiology.

ANTIFIBROTIC EFFECT ON LIVER OF A NOVEL TRUNCATED ISOFORM OF THE HUMAN TGF-B TYPE II RECEPTOR FC-TAG PROTEIN

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Liver fibrosis is a hallmark feature of chronic liver diseases, which affects millions of patients worldwide, and leads to liver failure. Current protective options for these patients are limited. We have recently described the presence in human cells of a new splicing variant of TGF-β type II receptor (TGFBR2) lacking the transmembrane and intracellular domains, thus rendering a truncated protein of 80 amino acids known as soluble endogenous TGFBR2 (TGFBR2-SE). It is well established that transforming growth factor beta (TGF-β) promotes liver fibrosis. Thus, the development of agents with a significant potential to achieve a specific and long-lasting interference of TGF-β action in vivo, is of clinical relevance. The aim of this work was to study the effect of lentiviral-mediated overexpression of TGFBR2-SE fused in frame with the human IgG1 (Lv-TGFBR2-SE/Fc), in a carbon tetrachloride (CCl4)-induced liver fibrosis rat model. We compared three experimental groups: vehicle, CCl4 and CCl4 previously administrated with Lv-TGFBR2-SE/Fc intrahepatically. In this way, we observed partial recovery of body weight in rats treated with Lv-TGFBR2-SE/Fc + CCl4 compared to the CCl4 group. In addition, gross appearance of liver in the Lv-TGFBR2-SE/Fc + CCl4 group showed reversion of the irregular shape and shrinkage observed in the CCl4 group. Moreover, administration of Lv-TGFBR2-SE/Fc diminished CCl4-induced liver enzyme increase, indicative of liver injury recovery. Histological analysis of liver sections revealed that Lv-TGFBR2-SE/Fc significantly decreased the deposition of collagen fibers induced by CCl4 as well as practically restored liver architecture. Moreover, immunohistochemical and Western-blot studies showed that the administration of Lv-TGFBR2-SE/Fc significantly diminished α-smooth muscle actin (α-SMA) expression, indicative of a reduced activation of hepatic stellate cells (HSC). These results suggest that lentiviral delivery of TGFBR2 exerts a protective effect against liver fibrosis induced by CCl4 in rats.

SAFIS – CAMILIÓN DE HURTADO AWARD IN CARDIOVASCULAR PHYSIOLOGY

DIFFERENCES IN AQP1 AND AQP4 LOCALIZATION AND MODULATION IN THE HEART OF ADULT PATIENTS UNDERGOING AORTIC VALVE REPLACEMENT SURGERY

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Background: Cardiopulmonary bypass (CPB) is an essential strategy in heart valve replacement surgery; however, it may produce myocardial edema and dysfunction. Aquaporins (AQP) 1 and 4 are selective water channels found in myocardial tissue. In animals, AQP1 increases after CPB and possibly produces myocardial edema. In human subjects information is still scarce. We designed an observational study to evaluate changes in AQP1 and

4 expression after CPB using myocardial biopsies from adult patients undergoing aortic valve replacement surgery. Our previous results suggest that AQP1 expression increases after CPB, while AQP4 expression decreases. The goals of this study are: 1) To identify the cellular localization of AQP1 and 4; 2) To explore possible candidates that may explain changes in AQPs expression during CPB. Methods: 15 patients undergoing aortic valve replacement surgery were enrolled. Biopsies were taken from the anterior wall of the left ventricle before and after CPB using an automatic transmural fine-needle. Immunohistochemistry was performed using anti-AQP1 and anti-AQP4 antibodies. Data from routine blood tests were collected. Data are expressed as mean ± SEM. Results: Immunohistochemistry staining identified AQP1 expression in the endothelium of myocardial capillaries and AQP4 expression in cardiomyocytes, both before and after CPB. Throughout CPB, there was an increase in calculated plasmatic osmolarity (in mOsm: 287.5±2.8 vs 292.0±1.7; p=0.0346) and glycemia (in mg/dl: 118.9±7.5 vs 194.6±10.5; p<0.0001), and a decrease in plasmatic pH (7.43±0.01 vs 7.36±0.01; p=0.0004). Conclusions: We suggest that AQP1 and AQP4 have different cellular localizations and may have specific roles in water handling in the heart. Endothelial AQP1 could contribute to fluid extravasation while AQP4 from cardiomyocytes could participate in cell volume homeostasis. As previously proposed in other cells, AQP1 upregulation could be due to increased plasmatic osmolarity and glycemia, while AQP4 downregulation could be related to reductions in plasmatic pH.

ELECTROGENIC SODIUM BICARBONATE CO-TRANSPORTER ISOFORM 1 (NBCE1) BLOCKADE PROMOTES CARDIOPROTECTION THROUGH THE P38MAPK ACTIVATION-DEPENDENT ATTENUATION OF POSTISCHEMIC MITOCHONDRIAL INJURY.

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Centro de Investigaciones Cardiovasculares Dr. Horacio Cingolani - CONICET- Facultad De Ciencias Médicas- UNLP

The cardioprotective action of the binding of an antibody directed to the extracellular loop 3 (a-L3) of the electrogenic sodium bicarbonate co-transporter isoform 1 (NBCe1) against ischemia-reperfusion injury has been previously demonstrated by us. In order to examine the involved mechanisms, isolated rat hearts were assigned to the following groups: 1) Non-ischemic control (NIC): 110 min of perfusion; 2) Ischemic control (IC): 30 min of global ischemia and 60 min of reperfusion (R); 3) a-L3: a-L3 was administered during the initial 10 min of R; 4) SB + a-L3: SB202190 (p38MAPK inhibitor) plus a-L3. Infarct size (IS), developed pressure (LVDP) and end-diastolic pressure (LVEDP) of the left ventricle were evaluated. Mitochondrial state [membrane potential (ΔΨm), Ca2+ response (CaR) and Ca2+ retention capacity (CRC)] and dynamic (expression of P-Drp1 and OPA1) were measured. The content of P-p38MAPK, P-HSP27, and calcineurin were also determined. a-L3 significantly decreased IS (11 ± 2 % vs 32 ± 2 %) and improved post-ischemic recovery of myocardial function (LVDP = 62 ± 4% vs.18 ± 3 %; LVEDP= 22 ± 3 vs 55 ± 7 mmHg) in comparison to IC. A normalization of ΔΨm (-145 ± 9 vs -95 ± 6 mV), and a greater CaR and CRC 1.3 ± 0.1 vs 0.3 ± 0.1 a.u. and 387 ± 28 vs 11 ± 2 nmol/mg prot) were detected in a-L3 group. The P-p38MAPK, P-HSP27, P-DRP1 and OPA1 levels significantly increased and calcineurin content decreased after a-L3 treatment. SB attenuated all the effects detected by a-L3. These data show that NBCe1 inhibition by a-L3 limits the cell death and improves myocardial post-ischemic contractility by an attenuation of mitochondrial damage through p38MAPK/HSP27-dependent pathway. These results also suggest that the decreased calcineurin expression associated to Ca2+ reduction by a-L3 could be the first stages of the cardioprotective cascade.

INFLUENCE OF AGEING AND MITOCHONDRIAL CALCIUM TRANSPORTERS ON MECHANICAL AND ENERGETIC STUNNING OF FEMALE RAT HEARTS EXPOSED TO ISCHEMIA AND REPERFUSION.

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Myocardial infarction is the leading cause of death in elderly women. Differences in sensitivity to dysfunction in the ischemic heart disease are related to age. Previous results showed that hearts from >20 months aged female rats (AgF) had less postischemic contractile recovery (PICR) and muscle economy (P/Ht) than hearts from young female (YF) rats when exposed to moderate I/R (I/Rm, 20 min I/45 min R), but similar in severe I/R (I/Rs, 30 min I/45min R). In I/Rs the activation of mKATP channels or mNCX blockade improved PICR of AgF (Medicina 77S1-p172R520, 2017). The aim of this work was to evaluate the mechanisms of moderate stunning in AgF. Isolated hearts were perfused inside a calorimeter at 37°C and left ventricular pressure (LVP) and total heat rate (Ht) during I/Rm were measured. The Ht vs P correlation was similar for AgF and YF before I. To evaluate mPTP activation, hearts from AgF were perfused with 0.2 μM cyclosporine-A (Cys-A) during I/Rm. PICR was not increased (45.4±5.2 vs 24.5±10.7% of control at 45´R), as neither were P/Ht (2.5±0.4 vs 1.5±0.6 mmHg. mW-1.g) and LVEDP. Ageing reduced the caffeine-induced Δ[Ca2+] in cardiomyocytes but increased contrac-

ture in caffeine-reperfused ischemic hearts. The role of mNCX was evaluated by selectively blocking AgF hearts with clonazepam (Clzp, 10 µM) before I/Rm. Contrarily to YF, in AgF Clzp decreased PICR (from 45.4±5.2% to 14.5±3.8%, p<0.05, at 45´R), as well as P/Ht (from 2.5±0.4 to 0.9±0.2 mmHg.mW-1.g), while reduced ΔLVEDP at 20´I (from 40.2±6.4 to 9.2±6.3 mmHg). In AgF cardiomyocytes, Clzp reduced cytosolic (Fluo-4 ΔF/Fo) and increased mitochondrial (Rhod-2 ΔF/Fo) [Ca2+]. Results suggest that: a) ageing don´t affect contractile economy; b) the low cardioprotection of elderly under I/Rm doesn´t involve mPTP opening; c) ageing increases the postischemic Ca2+ cycling between mitochondria (through the mNCX) and SR causing leak and stunning. UNLP-X-795.

PRO-SURVIVAL AND PRO-APOPTOTIC MECHANISMS TRIGGERED BY ENDOPLASMIC RETICULUM STRESS IN THE ISCHEMIC-REPERFUSED MYOCARDIUM.

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Myocardial ischemia/reperfusion (I/R) produces a wide spectrum of pathophysiological alterations depending on the duration of ischemia. When ischemia is brief, I/R causes reversible contractile dysfunction and arrhythmias (stunned heart) and when it is prolonged, I/R leads to cell death. Severe I/R challenges the endoplasmic reticulum (ER) protein folding capacity, leading to ER stress, however there is still a gap for its occurrence in the stunned heart. ER stress response (UPR) comprises three pathways: 1) ATF6 induces transcription of XBP1 and GRP78 (main ER chaperone); 2) IRE1α produces spliced XBP1 (sXBP1) increasing GRP78 expression and also leads to apoptosis through JNK and caspase-12 activation; 3) PERK attenuates protein synthesis via eIF2α phosphorylation and promotes CHOP expression, a pro-apoptotic protein. Under mild stress, upregulation of ER chaperones restores ER homeostasis and enhances survival. The severe stress switches UPR to pro-apoptotic signals and cell death. Our aim was to study the presence of UPR in the stunned heart. Isolated perfused rat hearts were subjected to reversible (20/30min) or irreversible (30/60min) I/R. mRNA expression of ER stress markers (qRT-PCR) and proteins of early UPR response (Western blot) were assessed. Myocardial damage was evaluated by lactate dehydrogenase (LDH) release and apoptosis (TUNEL assay). While GRP78, XBP1 and sXBP1 mRNA levels significantly increased in both I/R protocols vs. non-ischemic hearts (Ctrl), CHOP mRNA only increased in irreversible I/R (1.86±0.10 fold change n=13, p<0.05). Caspase-12, JNK and eIF2α activity were similarly increased in both I/R protocols (20/30: 169.2±28.2; 288.8±13.1; 170.7±20.8; 30/60: 167.5±13.6; 219.9±6.7; 163.8±22.6 % Ctrl respectively, n=4-10, p<0.05). As expected, LDH release and apoptosis were only enhanced in irreversible I/R. Results indicate that both pro-survival and pro-apoptotic mechanisms of the UPR are activated in the stunned heart. The lack of cell death suggests that the pro-apoptotic signaling is either ineffective or overwhelmed by the adaptive response.

SAIC SAI SAFIS – CÉSAR MILSTEIN AWARD

THE IL-10/STAT3/SOCS3 AXIS IS INVOLVED IN THE ANTI-INFLAMMATORY EFFECT OF BENZNIDAZOLE

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Chagas disease is the main cause of dilated cardiomyopathy in Latin America. During the acute infection, the inflammatory response is critical for the control of parasite proliferation and its evolution. Benznidazole (Bz), one of the anti-parasitic drugs currently used for its treatment, exerts anti-inflammatory effects. We showed previously that a low dose of Bz reduces the expression of inflammatory mediators through the inhibition of the NF-κB pathway, although the mechanism involved is poorly understood. In order to clarify, and to separate the anti-inflammatory effects from its anti-parasitic properties, mouse neonatal cardiomyocytes were stimulated with 10 µg/ml of LPS. The pre-treatment with 15 µM Bz increased SOCS3 expression (RT-qPCR) (p<0.05). This increase was precluded by 10 µM Stattic, a STAT3 inhibitor, suggesting that Bz increased SOCS3 expression in a STAT3-dependent manner. To assess the participation of SOCS3 in the anti-inflammatory effect of Bz, we accomplished specific knock-down of SOCS-3 with siRNA. The expression of IL-6 and TNFα (RT-qPCR/ELISA) and the release of NO (Griess) could not be restored by Bz in silenced LPS-stimulated cardiomyocytes (p<0.05). Also, in the absence of SOCS3, Bz could not preclude IKK phosphorylation (ICQ) and IκBα degradation (WB), indicating that SOCS-3 is required for the Bz-mediated inhibition of the NF-κB pathway. Previously, we demonstrated that IL-10 increased the expression of SOCS3 in cultured cardiomyocytes. Here we found that Bz increased IL-10 expression (RT-qPCR) (p<0.05). To evaluate if Bz increased SOCS3 in an IL-10-dependent manner, cardiomyocytes from IL-10 knockout mice were pre-treated with Bz and stimulated with LPS. Bz did not inhibit NO release (Griess) nor avoid the phosphorylation of IKK (ICQ) and degradation of IκBα (ICQ), indicating

that IL-10 is required for Bz-mediated inhibition of NF-κB. These results report, for the first time, that the IL-10/STAT3/SOCS3 axis participates in the anti-inflammatory effect of Bz.

EARLY IMMUNE RESPONSE INDUCED BY DIFFERENT TRYPANOSOMA CRUZI INFECTIVE STAGES

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Trypanosoma cruzi is an intracellular protozoan parasite that affects millions of people in Latin America. Infection commonly occurs by vectorial transmission via skin and/or mucous membranes. Immunologic events occurring immediately after the parasite entrance are poorly studied.

Skin constitutes a complex network and includes several population of antigen presenting cells (APCs). The phenotype, localization and functional properties define cellular identity. *Trypanosoma cruzi* infective stages would condition the repertoire of cells recruited into the site of infection.

In the intradermic model, blood and in vitro cultured metacyclic trypomastigotes (bTp and mTp, respectively) not only displayed differences in cell recruitment at the site of infection, but also the populations of APCs and their activation in draining lymph nodes and spleen. Animals inoculated with mTp exhibited 100% of survival with no parasite detection in blood, in contrast with the ones injected with bTp that displayed 80% of mortality and high parasitemia. Infection after mTp inoculation was confirmed by qPCR, not only at the site of infection but also in spleen. Animals infected with mTp and challenged with bTp 15 days later showed APCs with enhanced activation in secondary lymphoid organs compared to controls injected with bTp or not infected mice. These animals also displayed a less number of amastigote nests in cardiac tissue than bTp infected ones. All the results suggest that bTp and mTp differently infect mice. In addition, both stages induce an unequal immune response since the very beginning of the infection.

HIF-1A/PURINERGIC AXIS IS ALTERED IN PATIENTS WITH CHRONIC CHAGAS DISEASE

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Introduction: The molecular mechanisms involved in the development of human chronic Chagas cardiomyopathy (CCC) are still largely unknown. Purinergic system components have taken a robust significance as danger signals and modulators of immunity. Ischemic cells release ATP (inflammasome activator), which is metabolized by CD39/CD73 ectoenzymes to anti-inflammatory adenosine. We have reported that CD73 pharmacological inhibition during acute T. cruzi murine infection reduced progression of CCC. The aim of this study was to explore the hypoxia-inducible factor-1α (HIF-1α)/purinergic system axis in immune cells from infected individuals and in cardiac explants from CCC patients.

Materials and Methods: Seropositive patients (n = 24) were evaluated clinically and by electrocardiogram and chest X-ray. The uninfected control group (n = 24) consisted of age-matched individuals. Cardiac tissue samples from end-stage CCC patients were stained by IHC/IF. The myocarditis degree was determined considering the number of CD68+ plus CD3+ cells, as follow: Severe≥ median number; Moderate 25-50th percentile; or Mild≤ 25th percentile.

Results: In comparison with control donors, infected patients showed higher frequency of HIF-1α+ and IL-1β+ circulating monocytes with increased nitric oxide production ($p<0.05$). Moreover, while lymphocytes exhibited decreased expression of HIF-1α-target molecules, CD39 and CD73 ($p<0.05$), concomitant with higher ATP serum levels ($p<0.05$); the percentage of CD39+ monocytes augmented ($p<0.05$). Heart samples with severe myocarditis showed a prevalence of CD3+ cells over other infiltrating mononuclear cells ($p<0.001$) and the number of T cells positively correlated with HIF-1α expression ($p<0.05$). In addition, severe CCC hearts showed higher HIF-1α expression compared to moderate or mild disease ($p<0.05$). Furthermore, a marked CD73 expression was observed in infiltrating and endothelial cells.

Conclusion: Summing up, infected patients exhibited an inflammatory state potentiated by altered purinergic pathways that may be involved in cardiac dysfunction. These findings suggest that targeting the hypoxic/adenosine axis could be used therapeutically for Chagas disease patients.

STUDY OF THE ROLE OF TISSUE RESIDENT REGULATORY T CELLS DURING TRYPANOSOMA CRUZI INFECTION

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During *T. cruzi* (Tc) infection, a limited CD4+Foxp3+ regulatory T cells (Tregs) response allows the induction of protective CD8+ T cell immunity in peripheral tissues, but it may also facilitate tissue damage and immunopathology. Tissue resident Tregs (tisTregs) are a specialized subset that, besides regulating effector cells, maintains tissue homeostasis. We previously showed that Tc infected mice exhibit a reduced frequency of Tregs, particularly of those expressing the tisTregs markers ST2 and KLRG-1, in Blood, Spleen, Liver and Skeletal Muscle (SM).

Our current aim is to evaluate concentration of tisTregs growth factors during Tc infection and the role of TisTregs in the regulation of effector immune responses and immunopathology. To this end, Foxp3-GFP-C57BL/6 mice infected with 5000 Tc parasites (Tulahuen) were used as infection model. The levels of IL-33 and IL-18, recognized tisTregs growth factors, were determined by ELISA in plasma, and Spleen and SM lysates. IL-33 levels decreased and IL-18 levels increased after 14 and 21 dpi in plasma and spleen, but both cytokines increased after 21dpi in SM ($p<0.05$). Next, we evaluated by flow cytometry the frequency of Tregs, tisTregs and parasite-specific CD8+ T cells in Blood, Spleen, Liver and SM. We found a significant inverse correlation between the frequencies of Tregs, but not tisTregs, with parasite-specific CD8+ T cells in blood, spleen and liver ($p<0.001$). Finally, we determined that several biochemical markers of damage (LDH, GOT, GPT, CK and CK-MB activities) were increased in plasma at 21dpi, corresponding with the lowest frequencies of Tregs and tisTregs. These results suggest that tisTregs and Tregs responses may be restrained during Tc infection, favoring the emergence of an effective antiparasite response but also promoting a greater damage in target organs. Further studies may establish whether boosting tisTregs generation may be useful to limit immunopathology during *T. cruzi* infection.

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RESÚMENES DE LAS COMUNICACIONES / ABSTRACTS MEDICINA (Buenos Aires) 2018; 78 (Supl. III): 70-273

INMUNOLOGÍA / IMMUNOLOGY ORAL SESSION 1

1. (106) **THE INTERLEUKIN-4/SIGNAL TRANSDUCER AND ACTIVATOR OF TRANSCRIPTION 6 AXIS ENHANCES THE LIPOLYTIC ACTIVITY PREVENTING THE FORMATION OF FOAMY MACROPHAGES INDUCED BY TUBERCULOUS PLEURAL EFFUSIONS**

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The ability of *Mycobacterium tuberculosis* (*Mtb*) to persist inside the host relies on its numerous immune evasion strategies such as the dysregulation of the lipid metabolism leading to foamy macrophages (FMs). FMs are cells filled of lipids which fail to control the infection. Recently, we showed that the tuberculous pleural effusions (TB-PE) promote the formation of FMs in uninfected-macrophages leading to an immunosuppressive profile though the activation of the IL-10/STAT-3 axis. Herein, we wondered whether different activation programs in human macrophages differ in their ability to form FMs after the treatment with TB-PE. For that, we polarized macrophages with either IL-4, IL-10 or IFN-γ, treated them or not with TB-PE, and evaluated the intracellular accumulation of lipid bodies (LBs). We observed that M(IL-4) did not accumulate LBs under TB-PE treatment in comparison with unpolarized macrophages (M0), M(IFN-γ) or M(IL-10) ($p<0,05$). Moreover, the addition of recombinant IL-4 inhibited the accumulation of LBs in TB-PE-treated M0 in a dose-dependent manner ($p<0,05$). We hypothesized that the refractoriness of M(IL-4) to become FM could be associated to their higher levels of β-oxidation. So, we compared the metabolism of M(IL-4) vs M0 treated or not with TB-PE and we found that M(IL-4) showed a higher uptake of glucose ($p<0,05$), an increased oxygen consumption ($p<0,05$), and an enhanced lipolytic activity ($p<0,05$). In line with it, when we inhibited the lipases activity, TB-PE-treated M(IL-4) adopted the foamy phenotype ($p<0,05$). Also, the inhibition of the activity of STAT6 led TB-PE-treated M(IL-4) to accumulate LBs ($p<0.05$) and decreased the lipolysis in this profile ($p<0.05$). In conclusion, we showed that the activation of the IL-4/STAT6 axis promotes the lipolysis impairing the accumulation of LBs. Therefore, this knowledge may contribute to the identification of host molecular pathways that could be modulated for patient benefit.

2. (229) **AUTOPHAGY CONDITIONS THE IMMUNE RESPONSE TO PANCREATIC TUMORS**

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Pancreatic ductal adenocarcinoma (PDAC) is characterized by inducing tolerance of the immune system. In this mechanism exosomes participate as intercellular messengers, carrying molecules from the tumor cell to the immune cells. We previously described that autophagy is crucial for PDAC survival and chemoresistance. In this work we investigated the role of autophagy in the composition of tumor exosomes and their impact on the activity of Natural Killer (NK) and Dendritic cells (DC).

We performed the experiments using two PDAC cell lines, MIAPaCa-2 and PANC-1, and two inhibitors of autophagy, 3-Methyladenine (3-MA) and Spautin-1 (SP-1).

First, we demonstrated the presence of exosomes in the supernatant of cells cultured with or without 3-MA or SP-1 by electron microscopy. Besides, the treatment with 3-MA or SP-1 increased CD63 levels observed by western blot.

Then, we evaluated NK cytotoxic activity using K562 cell line as target cells. For that we incubated PBMCs with exosomes obtained from supernatant of MIAPaCa-2 and PANC-1 cells treated or not with SP-1, for 2h. K562 cells were stained with CFSE. Co-cultures of PBMCs:K562 in a ratio 50:1 were performed for 4h. Cytotoxicity of NK cells was evaluated by CFSE/PI stain. Exosomes from supernatant of cells treated with SP-1 increased cytotoxic activity of NK cells ($p<0.05$).

Monocyte-Derived-Dendritic-Cells (MDDC) were treated with the different population of exosomes and after 1h LPS was added. Supernatant were collected 48h after to evaluate cytokine production by ELISA. MDDCs incubated with the exosomes obtained from cell culture without SP-1 secreted TGF-β, meanwhile the exosomes obtained from cell culture with SP-1 induced the secretion of IL-12, and an increment in HLA-DR expression on MDDC membrane (observed by flow Cytometry) ($p<0.01$). No differences were observed in IL-10 profile.

Our results suggest that autophagy could condition exosome-composition, activating NK activity but inducing a tolerogenic profile in DC.

3. (412) **A TLR-4 AGONIST INDUCES THE RECRUITMENT OF TUMOUR INFILTRATING LYMPHOCYTES IN MELANOMA-BEARING MICE AND IMPROVES IMMUNE CHECKPOINT BLOCKADE TREATMENT.**

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Brucella lumazine synthase (BLS) is a homodecameric protein that activates dendritic cells (DCs) via TLR4, inducing the upregulation of costimulatory molecules and the secretion of proinflammatory cytokines and chemokines. Due to BLS structure, proteins can be fused to its 10 N-termini, constituting a proven platform for the development of vaccines. The chimera BLS-OVA (containing ovalbumin peptide 257-264) induces the cross presentation of the peptide and a specific CTL response through TLR4 signalling. We have shown that BLS and BLS-OVA have a therapeutic effect in B16F1-OVA-expressing melanoma-bearing mice only when administrated at early stages of tumour growth. In order to study the triggered mechanisms, we analysed the tumour infiltrating lymphocytes. B16F1-OVA-expressing cells were s.c. inoculated in C57/Bl6 mice and after 2 or 10 days 200μg of BLS was administrated. At day 14 tumours were analysed through flow cytometry. Administration of BLS at day 2 but not at day 10 induces the recruitment of immune cells (10,39%±6,080 and 2,75%±0,6218; $p<0.005$), including DCs, CD8+ and CD4+ T cells. Moreover, we studied PD-L1 expression in bone marrow derived DCs activated with BLS or BLS-OVA and the subsequent effect on PD-1 expression in OT-I CD8+ T cells. Both molecules were upregulated. Therefore, we combined administration of BLS or BLS-OVA at day 2 and anti-PD-1, anti-PD-L1 or both at days 4, 7 and 10 and tumour volume and survival were assessed.

Combined treatment with BLS and anti-PD-1 slows down tumour growth compared to BLS. Interestingly, only anti-PDL1 improves the therapeutic effect of BLS-OVA. Furthermore, serum IFNγ levels 3hs post-administration were only detectable in BLS but not BLS-OVA treated group (318,9±75,47pg/ml and 46,11±35,49pg/ml respectively p<0.005), suggesting that the presence of the tumour associated antigen OVA impacts differently in the anti-tumour response. Hence, we successfully demonstrated two different treatments that overcome the resistance to immune checkpoint blockade.

4. (423) **INTRATUMORAL NK CELLS FROM HUMAN RENAL CARCINOMAS DISPLAY AN UNUSUAL PHENOTYPE AND SUPPRESSED EFFECTOR FUNCTIONS**

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Renal cell carcinoma (RCC) is an aggressive neoplasm, characterized for being silent and asymptomatic during early stages, that becomes evident when metastasis may have already occurred. As RCC displays resistance to radio- and chemotherapy, nephrectomy constitutes the gold-standard treatment. Moreover, RCC patients exhibit a high incidence of recurrence and metastasis. To gain insight into RCC, we previously characterized the expression pattern of the ligands of the NK cell activating receptor NKG2D (NKG2DL) on peripheral blood mononuclear cells (PBMC), tumor infiltrating lymphoid cells (TIL) and tumor cells from RCC patients. Tumor cells showed high expression of MICA, while TIL, not only exhibited high expression of MICA, but also of ULBP-3 and ULBP-4. No expression was observed on PBMC from RCC patients or healthy donors (HD). Additionally, NKG2D was down-regulated on NK and $CD8^+$ T cells in PBMC from RCC patients compared to HD, whereas tumor infiltrating NK cells (TINK) and $CD8^+$ T cells, unexpectedly, displayed increased expression of NKG2D. To further characterize TINK, we assessed their effector functions (degranulation against K562 cells and IFN-γ production upon stimulation with cytokines) and compared them to peripheral blood NK cells (PBNK) from RCC patients using multicolor flow cytometry. PBNK from RCC patients displayed lower degranulation capacity and diminished IFN-γ production than PBNK from HD. Moreover, TINK displayed a more pronounced reduced degranulation (p<0.01) and IFN-γ production (p<0.05) than PBNK from RCC patients. These results suggest that tumor microenvironment drives NK cell suppression and acquisition of an unusual phenotype in terms of NKG2D and NKG2DL expression that might contribute to tumor progression.

5. (582) **TERMINAL GLYCAN RESIDUES INCREASED THE ADJUVANT CAPACITY OF THE S-LAYER PROTEIN FROM LACTOBACILLUS KEFIRI**

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The surface layer (S-layer), a (glyco)-proteinaceous cell envelope constituted by subunits that self-assemble to form a lattice that covers the microbial surface, was ubiquitously found in different bacterial species, including probiotic *Lactobacillus kefiri*. Recently, we have demonstrated that S-layer proteins from *L. kefiri* CIDCA 8348 (SLP-8348) injected subcutaneously in combination with incomplete Freund´s adjuvant induced proliferation of CD4+T cells as well as secretion of IFN-γ in draining lymphoid nodes, being glycans involved in that cellular response. In this study, we aim to investigate the adjuvant capacity of the SLPs from *L. kefiri* strains and to assess the role of the glycosidic moieties in that property. To do that, SLP from two different *L. kefiri* strains (SLP-8348 and SLP-5818) were removed with 5M LiCl and exhaustively dialyzed against PBS. To test the SLPs adjuvant capacity, one dose (10 μg/mouse) of each SLP or oxidized SLP (SLPOx) in combination with Ovoalbumin (OVA at 10 μg/mouse) was subcutaneously injected into the right flank in front of the hind leg on BALB/c mice. After ten days, cells from inguinal lymph nodes were labeled with CFSE and stimulated in vitro with OVA (10 μg/ml) for five days. Proliferation index of CD4+T cells, as well as secretion of IFN-γ, were significantly higher (P<0.05) in the group of mice treated with SLP-8348+OVA respect to OVA-treated mice. Interestingly, oxidation of the glycol groups of SLP-8348 abrogates the adjuvant capacity of this protein, since the combination of SLPOx-8348 with OVA does not modify the cellular response respect to OVA-treated mice. On the other hand, the presence of SLP-5818 or SLPOx-5818 does not enhance the cellular response to OVA. Taken together, these results indicate that differences in the structure of SLPs determine both the immunogenicity and adjuvant capacity of these proteins and demonstrate that glycoconjugates are involved in these properties.

CARDIOVASCULAR Y RESPIRATORIO
CARDIOVASCULAR AND RESPIRATORY 1

6. (342) **HUMAN APOA-I AND ITS NATURAL VARIANTS INDUCE DIFFERENT ENDOTHELIAL CHONDROITIN/ DERMATAN SULFATE PROTEOGLYCAN PROFILE. PROBABLE ROLE IN SETTLEMENT OF AMYLOIDOSIS.**

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Apolipoprotein A-I (apoA-I), the main protein of plasma high-density lipoproteins (HDL), removes excess cell cholesterol and protects against atherosclerosis. Nevertheless, some natural variants (R173P) or their N-terminal fragments (G26R1-93, IOWA) elicit their propensity to suffer misfolding or aggregation.Moreover, amyloidosis due to apoA-I with the native sequence (Wt) has been described deposited in atherosclerotic plaques. We studied the expression of vascular chondroitin/dermatan sulfate proteoglycan (CS/DS-PGs) in the presence of human apoA-I variants to understand whether chemical changes in the glycosylation pattern could elicit extracellular apoA-I aggregation. WT,IOWA and R173P were obtained by molecular biology techniques. Human umbilical vein endothelial cells (HUVEC) were treated with 1.5μg/ml for 24hs. Immunofluorescence of NFκB and zymographic analysis were used to evaluate endothelial activation. The proteoglycan protein cores (PG), and the main enzymes involved in CS/DS synthesis were quantified using RT-PCR. WT, R173P or IOWA treatment did not modify NFκB nuclear translocation and metalloproteinase-2 and -9 activities. Decorin expression was significantly decreased by WT and R173P, 10 and 6 folds respectively, when it was compared with control. Whereas biglycan was increased 4-fold by IOWA variant, versican expression was only detected after R173P treatment. Dermatan-4-O-Sulfotransferase1 (D4ST) expression decreased 2-fold by Wt, and 10 fold by IOWA. While Chrondoitin-4-O-Sulfotransferase1 (C4ST) expression was 5-fold reduced by Wt Dermatan Sulfate Epimerase 1/2 (DS-Epi) decreased 10-fold after R173P treatment and 3-fold after incubation with Wt and IOWA. Our results indicate that apoA-I variants induced substantial modifications in the profile of CS/DS-PGs protein cores without inflammation. These modifications were associated with changes in the expression pattern of the enzymes related to glycosaminoglycans synthesis. In conclusion, glycosaminoglycans polymerization, sulfation and epimerization are influenced by the protein core, modulating the characteristics of the Gagosome components. Changes in PG profile, induced by human apoA-I variants, might be involved directly or indirectly in the apoA-I variants citotoxicity.

7. (521) **INTERDEPENDENT RESPONSE OF CARDIAC WNT AND FOXO3A SIGNALING TO CHRONIC LOSARTAN TREATMENT**

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The Renin-Angiotensin System (RAS) plays an important role in post-ischemic cardiac remodeling. In particular the RAS regulates the decrease in myocardial microvessel density following infarction. Inhibition of Angiotensin 2 signaling through the Angiotensin 2 Type 1 receptor (A2T1R) prevents the rarefaction of the capillary bed after infarction. Wnt signaling and members of the Forkhead Box O mediate some of the effects that Angiotensin 2 has on heart pathophysiology such as is the case with cardiac hypertrophy. This two pathways are regulated by the binding of beta-catenin to Tcf-4 or Forkhead box o transcription factors respectively. Aims: We aimed to characterize the effect that A2T1R inhibition by chronic Losartan treatment has on Wnt signaling and Foxo3a transcription in young healthy mice. Main methods: We measured a significant inhibition in the expression of Foxo3a targets, including enzymes involved in oxygen free radical metabolism, albeit without changes in free oxygen radical concentration as measured by TBARS. Additionally using RT-PCR we found 6 Wnt signaling components whose expression changed due to Losartan treatment. Five of those are described in the literature as involved in angiogenesis. Key findings: Summarizing we have found a coordinated response to a decrease in A2T1R signaling that appears to stimulate an angiogenic response through Wnt signaling while adapting to a smaller oxygen free radical production by Angiotensin 2 signal transduction. Significance: To the best of our knowledge, we have found the first evidence supporting a role for Wnt signaling in mediating cardiac angiogenesis regulation by Angiotensin 2.

8. (770) **CARDIAC FUNCTION IN PERINATAL INDUCED-HYPOTHYROIDISM**

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A close relationship between thyroid status and cardiac function has been well established in adult mammals and humans. Several studies have been carried out to pinpoint those important variables whose manipulation in early postnatal life result in long-lasting effects upon cardiovascular function. Induction of hypothyroidism in rats during the perinatal period leads to hormonal, neuronal, and metabolic disturbances that may influence heart function in adult life. The mechanisms underlying the repercussions of early events during the postnatal period on adult life are not fully known. The aims were to examine: (1) whether postnatal hypothyroidism affects cardiac function during the second month of life and (2) whether postnatal hypothyroidism alters cardiac calcium handling. Male Sprague–Dawley rats (approximately 50 g) were were randomly assigned to one of the experimental groups: (1) euthyroid rats (received SC injections of 0.9 NaCl (0.1 ml/100 g body weight) or (2)hipothyroid rats (received 0.02% methimazole in drinking water during 60 days. Animals were sacrificed by cervical dislocation and hearts were rapidly excised.Cardiacmyocytes were isolated by collagenase-based enzymatic digestion.Ca2+ transient and cardiomyocyte shortening measurements were performed.Perinatal hypothyroidism showed a reduced cardiac contractillity measured by sarcomere shortening and a reduced Ca2+ transient amplitude measured using the Ca2+ fluorophore, Fura-2/AMin isolated cardiomyocytes. Sarcoplasmic-reticulum Ca2+ content was reduced in hypothyroid animals. Hormonal deficit did not change its time to 50% Ca2+ decay.This negative inotropic effect was associated with an increase cardiomyocyte relaxation as revealed by a reduction in the time to 50% relengthening. The number of spontaneous contractions per minute (indicative of a proarrhythmogenic substrate) was significantly increased in the cardiomyocytesfrom hypo rats.We speculate that the proarrhythmogenic substrate observed in Hypo rats could be attributed to the enhanced Ca2+ leak from the SR which could activate the electrogenic NCX which would generate a depolarizing current resulting in DADs and arrhythmias.

9. (121) **CHRONIC ANTIOXIDANT ADMINISTRATION SUPPRESSES INCREASED REACTIVITY OF TRACHEAL STRIPS IN EXPERIMENTAL METABOLIC SYNDROME IN RATS.**

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Consumption of a high-fructose-fat diet (HFFD) in rats promotes the development of pathological characteristics associated with experimental metabolic syndrome (eMS), such as increased systolic blood pressure (SBP), dyslipidemia, insulin resistance (IR), and increased visceral adiposity. In previous studies in high-fructose fed rats we showed an increased tracheal reactivity. This effect was amplified by pre-incubation in a high glucose solution, a situation that results in oxidative stress mainly through superoxide anion (O2-) accumulation.

The present work was designed to evaluate the effects of chronic administration of antioxidant substances (AS) on tracheal reactivity in eMS in male rats.

Male rats were exposed for 5 months to a standard diet –SD- or HFFD with or without antioxidants (each subgroup n=10). Metabolic variables (fasting blood glucose, triglycerides, serum cholesterol levels, insulin and thiobarbituric acid reactive substances –TBARS-) were assayed during and at the end of the experiment. Body weight and SBP were also measured. Lipid peroxides in plasma were estimated by evaluating (TBARS). After euthanasia, abdominal white adipose tissue (AWAT) was extracted and tracheas were cut transversely into segments consisting of 3 ± 5 cartilaginous rings. Each segment was cut longitudinally through the cartilage ring to create a strip. Dose–response curves for Ach-induced contraction (Ach-IC) of tracheal strips were conducted in SD and HFFD groups. Data were analyzed by two-way ANOVA tests adjusted by Bonferroni correction.

Results: HFFD induced an eMS. Significant differences were detected in AWAT, SBP and in metabolic variables (triglycerides, serum cholesterol levels, plasma insulin and TBARS). Ach-IC of tracheal strips increased significantly when compared with those obtained from SD ($p<0.01$). Administration of AS in HFFD treated rats suppressed this effect.

It is concluded that AS counteracts the effect of HFFD probably through the scavenging property of O2- accumulation.

10. (406) **FRACTAL STUDY OF POLISOMNOGRAPHS WITH AND WITHOUT OBSTRUCTIVE SLEEP APNEA HYPOPNEA SYNDROME FOR PROGNOSTIC PURPOSES.**

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The nocturnal polysomnography (PSG) registers the electrical activity of the cerebral cortex (Electroencephalography - EEG) and cardiorespiratory variables, and others. It's the gold standard study to diagnose obstructive sleep apnea hypopnea syndrome (OSAHS). The OSAHS is characterized by the the recurrent collapse of airways; which can induce cardiovascular alterations. The electrical signals translated in PSG are feasible to be analyzed by mathematical algorithms, since they fulfill the fractal properties of complex systems. It is proposed to analyze the PSG behavior through the Higuchi's algorithm (HA) in patients with and without OSAHS, for prognostic purposes. 48 images of patients without OSAHS and 40 with OSAHS were used; adults of both sexes. The HA was used to determine the fractal dimension (FD) and its correlation coefficient (R^2), this constitutes the expression of the adaptability of the system to the environment. The O2 channel of the EEG, the R wave (RW) of the electrocardiogram and nasal flow (NF) were studied. Outcomes:

They were expressed as mean (M) and standard deviation (±) of the coefficient (R^2) of the FD: in patients without OSAHS: O2-(R^2): M=0,7±0,19; RW-(R^2): M=0,75±0,09; NF-(R^2): M=0,8±0,25; in patients with OSAHS: O2-(R^2): M=0,25±0,01; RW-(R^2): M=0,44±0,3; NF-(R^2): M=0,34±0,2. The Pearson correlation (r) between R^2 of the O2 channel with R^2 of RW and R^2 of NF resulted: in patients without OSAHS: O2-(R^2) vs RW-(R^2): r=0,46(p <0,29), O2-(R^2) vs NF-(R^2): r=0,6(p <0,077), in patients with OSAHS: O2-(R^2) vs RW-(R^2): r=0,93(p <0,022), O2-(R^2) vs NF-(R^2): r=0,89(p <0,029). It is concluded that HA could demonstrate maladaptative behavior. In patients with OSAHS, it's suggested loss of fractal properties of the studied variables and a significant association between them. These changes could be brought forward in advance through fractal algorithms and, this way, we can anticipate irreversible modifications.

11. (656) **ROLE OF SEX CHROMOSOME COMPLEMENT AND THE ORGANIZATIONAL GONADAL HORMONAL EFFECTS IN THE VASOPRESSINERGIC PRESSOR AND ANTIDIURETIC SEXUAL DIMORPHIC RESPONSES**

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This study aimed to explore the role of the sex chromosome complement (SCC:XX/XY) and/or the organizational hormonal effects of gonadal steroids in the sexually dimorphic response to the antidiuretic desmopressin administration (vasopressin V2 receptor agonist) on urinary osmolarity and in the pressor response induced by systemic vasopressin infusion. To carry out the aforementioned experiments we used gonadectomized male (XX and XY) and female (XX and XY) mice of the "four core genotypes" model, in which the effect of gonadal sex and sex chromosome complement (SCC) is dissociated, allowing comparisons of sexually dimorphic traits among XX and XY females, as well as in XX and XY males.
Mice aged 60-65 days old were gonadectomized and forty two days later were subcutaneously injected with vehicle solution or desmopressin (1 mg/kg). During the following four hour period mice had no access to water or food and immediately after urine samples were obtained for subsequent determination of osmolarity. Desmopressin infusion showed a significant effect of treatment and the interaction with sex factors {F (1,35) = 5.0650, p = 0.03080}. Desmopresine-male mice showed irrespectively of the SCC (XX-male and XY-male mice) a significant increase in urinary osmolarity when compared to desmopressine treated-females (both XX-females and XY-females). Furthermore, the analysis of blood pressure changes in response to a 30-minute vasopressin infusion (0,2 UI/ml, infusion volume 100μl) revealed a SCC modulatory effect; regardless of sex (male or female); XX-SCC mice showed a greater increase in blood pressure when compared to XY-SCC mice (XY-male and XY-female).
This evidence may indicate that in the absence of the activating hormonal effect, the organizational hormonal factor would define the sexually dimorphic urinary osmotic phenotype, while sex differences in the pressor response to vasopressin infusion may be driven by the sex chromosomal complement factor.
Support: ANPCyT, CONICET and Roemmers Foundation.

12. (769) **A SWIMMING ROUTINE IMPROVES CARDIAC MITOCHONDRIAL PHENOTYPE IN SPONTANEOUSLY HYPERTENSIVE RATS.**

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The hypertension-induced pathological cardiac hypertrophy is characterized by mitochondrial dysfunction, oxidative stress, decreased mitochondrial membrane potential (Ψm) and increased sensitivity to permeability transition pore (PTP) opening. Exercise training induces myocardial adaptations that include physiological cardiac hypertrophy mediated by humoral factors such as insulin like growth factor (IGF-1) and apelin. However, the molecular mechanisms underlying training-induced mitochondrial adaptations remain elusive. Aim: To determine whether regular exercise (swimming routine, 6 wk) or exogenous apelin (50 nmol/L) improves the mitochondrial phenotype affecting Ψm in the hypertrophied myocardium of the spontaneously hypertensive rats (SHR). Methods: The effect of chronic training or exogenous apelin were explored in SHR isolated cardiac mitochondria or cardiomyocytes, respectively. Results: The hypertrophic parameter (left ventricular weight/tibia length) was not significantly different between sedentary and swim-trained rats (Sed:0.341±0.00827 n=6 vs Swim: 0.332±0.0267, n=4), however, the Ψm measured by rodhamine 123 was improved by the swimming routine (in mV, Sed: -132.9±2.74, n=13 vs Swim: -142.56±3.49, n=17 of 5 or 6 rats, respectively) and cardiac mitochondrial swelling seems to be reduced in trained rats compared to the Sed group (%, Sed: 100±13.47, n=8 vs Swim: 49±7.3, n=3, p=0.0547). Pretreatment with apelin or bongkrekic acid of isolated cardiomyocytes loaded with TMRE 1 nmol/L prevented H_2O_2 –induced mitochondrial depolarization (F/F_0, control: 0.842±0.044, n=12, apelin 0.985±0.0247 n=13, bongkrekic acid: 1.16±0.056, n=5). Conclusion: Exercise training seems to be a good strategy to improved cardiac mitochondrial phenotype in SHR hearts, being apelin a putative mediator of these beneficial effects.

13. (786) **PHYSIOLOGICAL CARDIAC HYPERTROPHY INDUCED BY VOLUNTARY WHEEL TRAINING: EFFECTS ON CELLULAR ALKALINIZING MECHANISMS**

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The Na+/HCO3- cotransport (NBC) and the cardiac Na+/H+ exchanger (NHE1) are the main alkalinizing mechanisms in cardiomyocytes. NHE1 hyperactivity is known to induce intracellular sodium overload critical for pathological cardiac cardiac hypertrophy (CH). Regarding NBC, cardiomyocytes express two isoforms: the electroneutral (NBCn1) and the electrogenic (NBCe1) that introduces one Na+ with two HCO3-.
We explored NBC function in physiological CH induced by 5 weeks of voluntary wheel training in C57 mice (Results are expressed as mean ± ES, and compared by t-test). The exercise mice (Ex) runned 3.98±0.23 hs/day (4.45±0.37 Km/day) and developed CH: biventricular weight/tibial length (mg/mm): 8.61±0.23, n=16 vs. 6.65 ±0.26, n=9 and cardiomyocyte area (μm2): 282±24, n=480 vs. 201±8.92, n=270, Ex and sedentary (Sed), respectively; p<0.05. NBC activity (ΔpHi/Δt at pHi 6.8) determined during pHi recovery after amonium pulse under inhibition of NHE1 with cariporide was greater in Ex (0.05±0.004, n=11 vs 0.03±0.004, n=6). To specifically investigate NBCe1 activity, action potential duration at 90% (APD90 in mseg) with/without bicarbonate in the media was determined. APD90 was significantly reduced exclusively in Ex (75.5±8.4, n=7 vs. 128.1±19.5 n=8, with/without bicarbonate respectively, p<0.05), in agreement with NBCe1 protein up-regulation in these mice (Ex: 202.8±73.4 vs. Sed 100±13.8, n=6).
These results support that NBCe1 is up-regulated and hyperactive in CH induced by voluntary training, differentiating from pathological CH. NBCe1 up-regulation, because of its stoichiometry, would represent a protection from intracellular Na+ overload during pHi recovery from acidosis.

14. (18) **DIFFERENT MECHANISMS OF CARDIOPROTECTION IN REMOTE ISCHEMIC PRE AND POSTCONDITIONING**

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Background: It is known that brief episode(s) of ischemia/reperfusion applied prior or after myocardial ischemia to peripheral tissue

located at a distance from the heart significantly reduce myocardial infarct size. This phenomenon is called remote ischemic conditioning (RIc).
Objective: The aim was compared the efficacy of RIc in protecting the heart when the RIc stimulus is applied prior to myocardial ischemia (remote ischemic preconditioning; rIPC), or at the onset of myocardial reperfusion (remote ischemic postconditioning; rIPost).
Methods: In order to induce myocardial infarction, we used mice (FVB, 24 g) which were subjected to 30 min of left coronary artery occlusion followed by 120 min of reperfusion. rIPC and rIPost were induced by 3 cycles of 5 min of ischemia followed by 5 min of reperfusion of the left femoral artery. The infarct size was measured using Evans blue (Risk Area) and Triphenyl-tetrazolium chloride (Infarct size).
Results: The ischemia/reperfusion protocol (30 min of ischemia and 120 min of reperfusion) induced an infarct size of 69.13±3.22 %. The rIPC and rIPost significantly reduced infarct size to 51.17±4.36 ($p<0.05$) and 33.33±4.10 % ($p< 0.05$), respectively. Bilateral vagotomy completely abolished cardioprotection induced by rIPC but not by rIPost. Both the femoral vein occlusion (FVO) and the administration of DPCPX (A1 adenosine receptor blocker) abolished the beneficial effect of rIPost.
Conclusions: These results indicate that RIc confers potent cardioprotection when is applied before myocardial ischemia or at the onset of myocardial reperfusion. Cardioprotection by rIPC is critically dependent on parasympathetic efferent innervation (vagal nerve), while rIPost appears to rely on a different signaling pathway(s) that involved the A1 adenosine receptor activation.

15. (552) **ROLE OF ADENOSINE A1 RECEPTOR IN CARDIAC MITOCHONDRIAL FUNCTION AT EARLY REPERFUSION PHASE, IN A REMOTE ISCHEMIC PRECONDITIONING MODEL**

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Adenosine is involved in classic preconditioning in most species and acts especially through adenosine A1 and A3 receptors. The aim of our study was to evaluate whether remote ischemic preconditioning (rIPC) activates A1 adenosine receptors and improves mitochondrial function, thereby reducing myocardial infarct size. Isolated rat hearts were subjected to 30 min of global ischemia and 60 min of reperfusion (I/R). In a second group, before isolation of the heart, a rIPC protocol (3 cycles of hindlimb ischemia/reperfusion) was performed. The infarct size was measured with tetrazolium staining, and eNOS expression/phosphorylation and mitochondrial function were evaluated after ischemia at 10 and 60 min of reperfusion. As expected, rIPC significantly decreased the infarct size. This beneficial effect was only abolished when DPCPX (A1 receptor blocker) and L-NAME (NO synthesis inhibitor) were administered during the reperfusion phase. At the early reperfusion phase, rIPC induced significant eNOS phosphorylation, which was abolished by the perfusion with an A1 receptor blocker. I/R lead to impaired mitochondrial function, which was attenuated by rIPC and mediated by A1 adenosine receptors. In conclusion, we demonstrated that rIPC limits myocardial infarct by activation of A1 adenosine receptor at early reperfusion in the isolated rat heart. Interestingly, rIPC appears to reduce myocardial infarct size by improving mitochondrial function during myocardial reperfusion.

16. (373) **M1/M2 POLARIZATION IN EPICARDIAL ADIPOSE TISSUE AND ITS ASSOCIATION WITH MMP-1 IN CORONARY ARTERY DISEASE.**

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Epicardial adipose tissue (EAT) is a visceral AT, surrounding and infiltrating myocardium and coronary arteries. An increase in EAT volume is directly related to coronary artery disease (CAD). EAT expansion is accompanied by inflammatory cells infiltration, mainly macrophages. These macrophages could polarize to non-inflammatory (M2) or to pro-inflammatory phenotypes (M1). M1 could lead to metalloproteinases (MMPs) expression, involved in extracellular matrix degradation. Little is known about macrophages phenotypes and MMP-1 expression in EAT. Objective: to evaluate infiltration and polarization of macrophages and its association with MMP-1 expression in EAT of CAD and No CAD patients. Methods: in EAT from patients undergoing coronary artery bypass graft (CAD, n=19) or valve replacement (No CAD, n=10) infiltration of inflammatory cells was evaluated by immunohistochemistry. Markers of M1 (MCP-1, IL-6 and IL-12) and M2 (IL-10 and TGF-β) were measured by qRT-PCR. MMP-1 localization and levels were evaluated by immunohistochemistry and qRT-PCR respectively. Serum lipid profile, glucose and insulin were assessed. The study was approved by the Ethic Committee of the Hospital de Clínicas, UBA. Results: In comparison to No CAD, EAT from CAD patients presented macrophages infiltration and higher MCP-1 levels ($p=0.05$). MMP-1 was mainly localized in perivascular connective stroma and in the basement membrane surrounding adipocytes and its levels were higher in CAD ($p=0.033$). MMP-1 was directly associated with MCP-1 ($r= 0.556$, $p=0.02$) and IL-6 ($r= 0.723$, $p=0.001$). Conclusion: In EAT from CAD the increase of MCP-1 and MMP-1 levels could suggest that M1 macrophages are the predominant phenotype. This could be partially responsible for the expansion and inflammation of EAT in CAD. Therefore, it could be suggested that the most important factor for the inflammatory state of EAT could be not only the number of infiltrating macrophages but also the macrophage polarity.

Transducción de Señales y Mecanismos Moleculares de Enfermedad / Signal Transduction 1

17. (73) **ALTERATIONS IN EXTRACELLULAR MATRIX AND CONNEXIN 43 IN THE OFFSPRING HEART FROM DIABETIC RATS**

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Maternal diabetes leads to the programming of metabolic and cardiovascular alterations in the adult offspring. The pathogenesis of diabetes-induced heart alterations involves remodeling of extracellular matrix (ECM) components and deposition of collagen. In this context, the role of matrix metalloproteinase 2 (MMP2) is relevant. MMP2 overactivation is involved in pathological processes. Besides ECM components, MMP2 is able to cleave intracellular molecules as Connexin 43, main component of the gap junctions and hemichannels in myocytes, affecting the physiological function of the channels. Our objective was to evaluate whether there are alterations in the collagen deposition, MMP2 activity and expression of connexin 43 in the heart of the offspring from diabetic rats. Methodology: Pregestational diabetic rats were obtained by neonatal streptozotocin administration (90 mg/kg) and, at 3 month of age, were mated with healthy males. Heart of male adult offspring of 5 month of age from control and diabetic rats were explanted and left ventricles were store for histological determinations, qRT-PCR and Western blot.
Results: mRNA levels of Collagen I and III were increased (2.0 and 3.0 fold respectively, $p<0.05$) in concordance with an increased interstitial collagen deposition observed in Masson's Trichrome staining in the left ventricles of the offspring from diabetic rats compared to controls. Activity of both proMMP2 and MMP2 was increased in the left ventricles of the offspring´s heart from diabetic rats (148% and 140% times respectively, $p<0.05$). The expression of connexin 43 was found reduced in the offspring´s left ventricle from diabetic rats compared to controls (25%, $p<0.05$). Conclusion: Maternal diabetes induced cardiac alterations in the adult offspring affecting collagen deposition and increasing the activity of MMP2 that may be involved with a reduced expression of connexin 43 in the heart of

offspring from diabetic rats.

18. (94) **EGFR LIGANDS EXPRESSION ARE MODULATED BY THE CFTR CHANNEL**

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Cystic fibrosis (CF) is an autosomic recessive disease characterized by mutations in the CFTR (cystic fibrosis transmembrane conductance regulator) gene. Interestingly, CFTR functions, not only as a regulated chloride channel, but also as a signaling molecule modulating different genes. Previously, we found different genes which are regulated by CFTR expression or activity ($p<0.05$), such as SRC (a tyrosine-kinase which in turn regulated MUC1), MTND4 (a mitochondrial gene encoding a subunit of the mitochondrial Complex I) and CISD1. The aim of the present work was to study if a failure in the CFTR activity (or expression) alters the expression of the EGF receptor and its ligands and identify the possible signalling pathways involved. We use a cellular model consisting of Caco-2 cells, expressing high levels of wt-CFTR, that were previously selected and cloned after transfections with short hairpin RNA interference (shRNA) directed against different regions of CFTR (CaCo-2/pRS26) or with its control plasmid (CaCo-2/pRSctrl). The results obtained, by real time PCR, suggested that CFTR modulates significantly ($p<0.05$) the expression of TGF-alpha, amphiregulin and epiregulin but not the EGFR expression (or other ligands). As we observed an important regulation of epiregulin (EREG) ligand by CFTR, we continued to study the possible signalling pathways involved. We observed that IL-1β increased significantly ($p<0.05$) EREG expression. Moreover, the EREG upregulation presented in CF-cells was significantly ($p<0.05$) reduced by JNK inhibitor (SP600125). EREG overexpression is also modulated by the EGFR activation, as it was observed by using AG1478 and PD168393 inhibitors ($p<0.05$). In conclusion, CFTR channel activity failure or CFTR inhibition regulate the expression of different EGFR ligands, particularly EREG, possible involved in phenotype changes present in CF-like cells. This work was supported by ANPCYT (PICT 2012-1278), CONICET (PIP 2015-2017 GI 11220150100227CO and PUE 22920160100129CO), and research fellowships from CONICET.

19. (127) **CFTR ACTIVITY MODULATES MITOCHONDRIAL FRAGMENTATION AND AFFECTS MITOCHONDRIAL COMPLEX I ACTIVITY IN CYSTIC FIBROSIS AIRWAY EPHITELIAL CELLS**

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Mitochondria are dynamic organelles that continuously join (fusion) and divide (fission), regulating cellular homeostasis. CFTR is the gene responsible for Cystic Fibrosis (CF) disease and encodes a cAMP-activated chloride (Cl-) channel, causing a variety of alterations such as differential gene expression and mitochondrial dysfunction. Impairment of the CFTR channel activity causes a decreased mitochondrial complex-I activity (mCx-I) and an increased ROS production. CFTR activity could regulate mitochondrial function through the modulation of mitochondrial dynamics, explaining mitochondrial defects observed in CF. The mitochondrial network morphology was studied in the bronchial epithelial IB3-1 cells (CF) and compared with IB3-1 CFTR corrected cells (S9). Cells incubated for 24 h in serum-free medium were stained with the fluorescent mitochondrial probe TMRM and analyzed by confocal microscopy. Small mitochondria population was significantly increased ($p<0.05$) in IB3-1 cells compared to S9 cells, and treatment with a mitochondrial fission inhibitor (Mdivi-1) rescued the decreased mCx-I activity observed in IB3-1 cells. To test if CFTR activity modulates mitochondrial morphology, C38 cells (IB3-1 cells expressing CFTR) transfected with pMito-YFP were treated with two pharmacological CFTR inhibitors (GlyH101 and CFTRinh-172) and analyzed by confocal microscopy in live cells. Both CFTR inhibitors increased ($p<0.05$) the percentage of cells with a fragmented mitochondrial network (2 h of treatment). In these experimental conditions, the analysis by Western blot showed an increased phosphorylation of the DRP1 protein (involved in mitochondrial fission), while the expression of MFN-1 protein (involved in mitochondrial fusion) was decreased. These data suggest the modulation of the mitochondrial dynamics by the activity of CFTR. Also, results observed with the inhibitor Mdivi-1 on mCx-I activity highlight mitochondrial dynamics regulation as a possible therapeutic target in CF. Supported by PIP 2015-2017, PUE 22920160100129CO, and PICT-2015-1031 to AGV.

20. (146) **REGULATION OF THE SPHINGOLIPID METABOLISM BY MALONDIALDEHYDE-ACETALDEHYDE PROTEIN ADDUCTS AND COMPLEMENT REGULATORS IN HUMAN RETINAL CELL LINES**

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We previously reported increased levels of serum ceramides in patients with age-related macular degeneration (AMD), a common sight-threatening condition with complex etiology. We observed that genetic variant rs1061170 (p.Y402H) in the complement factor H (CFH)/factor H-like protein 1 (FHL-1) gene correlates with Cer d18:1/16:0 levels in AMD. The aim of this study was to evaluate the cellular sphingolipid metabolism under treatment with malondialdehyde-acetaldehyde (MAA) adducts, a product of oxidative stress shown to be highly elevated in AMD retinas, and the modulation by FHL-1, an alternative splicing isoform of CFH. Gene expression, survival and sphingolipid levels were evaluated in WERI-Rb1 and ARPE-19 cells exposed to MAA-BSA or BSA (control). We evaluated the influence of FHL-1 and CFH on gene expression by quantitative RT-PCR. Lipids were quantified by electrospray ionization tandem mass spectrometry (ESI-MS/MS), and cell survival was estimated by the MTT assay. Data statistics were done by t-tests or ANOVAs. The expression of *SMPD2* and *SMPD3* which are involved in the synthesis of sphingomyelin (SM) from ceramide decreased with addition of MAA-BSA in ARPE-19 cells ($p<0.05$). No statistical differences were observed in cell survival and ceramide levels, and only two minor SM species decreased upon MAA-BSA addition ($p<0.05$). WERI-Rb1 cells revealed a decreased survival and upregulation of *SPTLC1*, *DEGS1*, *CERS2*, *CERS6* and *UGCG* ($p<0.01$) under MAA-BSA, pointing to an upregulation of ceramide and glucosylceramide synthesis. Five ceramide and two SM species increased after MAA-BSA treatment in this cell type ($p<0.05$). Notably, WERI-Rb1 cells exposed to the non-risk isoform FHL-1:Y402, but not the AMD risk associated isoform FHL-1:H402 or CFH, revealed a downregulation of *CERS2* and *UGCG* in the presence or absence of MAA-BSA ($p<0.05$). Together, our findings suggest that ceramide levels are influenced by AMD associated risk variants and oxidative stress by-products.

21. (147) **DESMOGLEIN-4 DEFICIENCY ENHANCES SKIN LESIONS IN A RAT PSORIASIS MODEL**

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Desmogleins are proteins involved in cell adhesion and structural integrity of different tissues. In skin, desmogleins modulate keratinocyte (KC) activation and may control key molecules that participate in signaling pathways. It is known that psoriasis is a chronic inflammatory skin disease, characterized by KC hyperproliferation, vasculature growth, and leukocyte infiltration into the dermis and epidermis. However, the role of desmoglein-4 in psoriasis development is unknown. Our aim was to assess the impact of desmoglein-4 deficiency in the histological structure of the skin. To this end, we used a rat psoriasis model using Imiquimod (IMQ), a TLR7 ligand that induces skin lesions closely resembling human psoriasis. IMQ was admin-

istered to the shaved skin for four days to generate psoriasis-like lesions in OFA hr/hr (desmoglein-4 deficient) and Sprague-Dawley (SD) rats. Skin biopsies from treated and untreated OFA and SD rats were processed to obtain histological sections that were dyed with hematoxylin/eosin. Histopathological analysis showed that IMQ treated OFA hr/hr rats displayed more severe changes in epidermis, dermis and sub-cutis with increased KC apoptosis compared with IMQ treated SD rats. Furthermore, lymphocytic perifollicular and perivascular infiltration and vasodilatation were more intense in IMQ-treated OFA rats than in SD rats. In contrast, adhesion molecules such as cadherin-VE, cadherin-N and cadherin-E qPCR analysis did not show changes between all experimental groups. These results suggest that desmoglein-4 deficiency contributes to experimental psoriasis development, promoting increased vasculature growth and leukocyte infiltration into the dermis and epidermis. Although further research is needed, these results could have a potential impact on prognostic and diagnostic parameters in psoriasis.

22. (155) **STRESS PROMOTES AUTOIMMUNITY IN EXPERIMENTAL TYPE 1 DIABETES**

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Autoimmune diseases are multifactorial disorders in which environment, hormones and genetics are involved. Immune cells can be modulated by several endocrine mediators, Prolactin (PRL) and glucocorticoids among them. It is known that stress influences the immune system; however the role of psychological stress exposure on autoimmunity is still controversial. Our aim was to investigate whether chronic unpredictable stress (CUS) enhances development of Type 1 Diabetes autoimmunity in NOD mice. For this, NOD and BALB/c female mice were exposed to CUS consisting in restraint, isolation, forced swim, 24 hours light and tilting the home cage at 45° inclination for 24 hours, during 3 months. We found an increase in CD45+ cell infiltration of the pancreas mainly conformed by CD4+ T cells in CUS compared to untreated (UT) NOD mice (UT 0,78±0,12 vs CUS 2,17±0,50; t test; p< 0,05). Furthermore, the CUS group displayed a 3,2 fold increase in CD4+ T cell infiltration of the pancreas compared to the UT group (UT 0,207±0,039 vs CUS 0,75±0,19; p< 0,05). Histological studies showed that the CUS treated NOD group had an increase in immune cells infiltration located mainly in interlobulillar areas. Additionally, we found that CUS increased the long form of PRL receptor mRNA in the spleen (p<0.05) while the short form S3 was unaffected. These results suggest that chronic psychological stress enhances the development of autoimmune Type 1 diabetes which may be driven by CD4+ T cells. The up-regulation of PRL receptor long form in the spleen of NOD mice in response to CUS may shed light to the underlying mechanism of stress-induced autoimmunity. These data suggest that prolonged stress may alter hormonal sensitization of immune cells, increasing risk of autoimmune pathology in susceptible hosts.

23. (164) **HP24 REGULATES PRO-INFLAMMATORY AND PRO-ANGIOGENIC MEDIATORS BY PPARΓ -DEPENDENT AND -INDEPENDENT MECHANISMS IN T. CRUZI INFECTED MACROPHAGES**

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Chagas disease is caused by *T. cruzi* infection and represents a major public health problem in Latin America. Macrophages (MΦ) are one of the main infiltrating leukocytes arriving early to the myocardium in response to the infection. They persist as an important immune cell population in the heart of patients with chronic Chagas disease. HP_{24}, a 3-hydroxy-4-piridinecarboxylic acid derivative, is a new PPARγ ligand. It exerts significant anti-inflammatory and pro-angiogenic effects. In this work, we analyzed whether HP_{24} exerts its effects through PPARγ-dependent or -independent mechanisms in *T. cruzi* infected macrophages. For this purpose, we used specific knock-down of this nuclear receptor, by small interfering RNA (PPARγ siRNA). In the absence of PPARγ, HP_{24} was unable to exert its anti-inflammatory effects on NOS2 expression (Wb and ICQ) and NO release (Griess method) (P<0.05, HP_{24}-treated MΦ vs. PPARγ siRNA-HP_{24}-treated MΦ). Moreover, HP_{24} was unable to increase VEGF-A expression in a PPARγ-silenced cells (Wb) (P<0.05, HP_{24}-treated MΦ vs. PPARγ siRNA-HP_{24}-treated MΦ). Then, we analyzed whether the AKT pathway was involved in the increase of pro-angiogenic mediators exerted by HP_{24}. The effect of HP_{24} on the expression of VEGF-A and NOS3 in *T. cruzi*-infected macrophages was reverted upon disruption of the AKT signaling pathway using the specific PI3K inhibitor LY940002 (P<0.05, HP_{24}-treated MΦ vs LY940002-HP_{24}-treated MΦ). These results show that HP_{24} not only exerts its anti-inflammatory and pro-angiogenic effects through PPARγ-dependent mechanisms, but also through the AKT pathway. Since inflammation and microvascular abnormalities are crucial aspects of Chagas heart disease, the study of the mechanisms of action of new PPARγ ligands, opens up new perspectives towards an integrative approach, using these agonists as possible coadjuvants of the classic antiparasitic treatment.

24. (169) **INTRACELLULAR CHLORIDE MODULATES NLRP3 INFLAMMASOME AND IL-1B SECRETION**

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The impairment of the CFTR activity induces intracellular chloride (Cl^-) accumulation. The anion Cl^-, acting as a second messenger, in turn stimulates the secretion of IL-1B, which starts an autocrine positive feedback loop. As the NLRP3 inflammasome is involved in IL-1B maturation and secretion, the aim of this study was to investigate if the NLRP3 inflammasome is modulated by Cl^-. In that way we studied the three proteins that form this complex: NLR family pyrin domain containing 3 (NRLP3), caspase-1 (CASP1), and apoptosis-associated speck-like protein containing a CARD (ASC/PYCARD). Here we show that NLRP3 and CASP1 are modulated by the intracellular Cl^- concentration, showing a biphasic response with maximal expression and activity at 75 mM Cl^- (p<0.05, n=3). The expression of the third NLRP3 inflammasome component ASC/PYCARD remained constant from 0 to 125 mM Cl^-. The CASP1 inhibitor VX-765 and the NLRP3 inflammasome inhibitor MCC950 completely blocked the Cl^--stimulated IL-1B mRNA expression and most of the IL-1B secretion. DCF fluorescence (cellular reactive oxygen species, cROS) and MitoSOX fluorescence (mitochondrial ROS, mtROS) also showed maximal ROS levels at 75 mM Cl^- (p<0.05, n=3), a response strongly inhibited by the ROS scavenger N-acetylcysteine (NAC) or the NOX inhibitor GKT137831. These inhibitors also affected CASP1 and NLRP3 mRNA and protein expression. In addition, the SGK1 inhibitor GSK650394 completely abrogated the IL-1B mRNA response to Cl^-, in agreement with its recently identified responsiveness to Cl^-. In conclusion, these results emphasize the relevance of Cl^- and SGK1 in modulation of the NLRP3 inflammasome.

25. (180) **ROLE OF THE ANGIOTENSIN TYPE 2 RECEPTOR ON INSULIN SENSITIVITY AND ACTION.**

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Introduction. The renin-angiotensin system is a hormonal cascade that controls important physiological functions through the actions of angiotensin II (Ang II) which acts through two types of receptors, the Ang II type 1 receptor (AT1R) and the Ang II type 2 receptor (AT2R).

The AT1R mediates the main physiological effects of Ang II. Chronic elevation of Ang II interferes negatively with the metabolic actions of insulin. Treatment with AT1R antagonists improves this condition. In contrast, little is know about the role of the AT2R on the modulation of insulin actions. The evidence obtained by the stimulation or antagonism of the AT2R, indicates that the AT2R is involved in the glucose homeostasis, but the information obtained so far using AT2RKO mice is not consistent.
Objective. To analyze the impact of the global elimination of the AT2R on glucose homeostasis. It is postulated that the elimination of the AT2R will generate a reduction of insulin sensitivity.
Methods. Male and female AT2KO mice (4 months), were provided by Dr. Pedro Miguel Geraldes, University of Sheerbroke, Montreal, Canada. We evaluated the effect of the absence of the AT2R on insulin sensitivity (insulin tolerance test), glucose tolerance (glucose tolerance test) and circulating metabolic parameters. The in vivo status of main components of the insulin pathway was analyzed by western blotting.
Results. Female AT2KO mice showed a reduction in insulin sensitivity while males did not present differences when compared to their respective controls. Female AT2KO mice displayed a reduction in the insulin-stimulated phosphorylation of both the insulin receptor and Akt at activating residues in white adipose tissue compared with respective controls.
Conclusions. Our findings show that female AT2RKO mice display decreased insulin sensitivity, associated with a reduction in the response to insulin at the phosphorylation of the insulin receptor and Akt in adipose tissue.

26. (199) **CROSS-TALK BETWEEN BMP AND WNT SIGNALING PATHWAYS IN HAIR FOLLICLE STEM CELLS DIFFERENTIATION. IMPLICATION IN ANDROGENETIC ALOPECIA.**

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Hair follicle (HF) cyclical growth is governed by interactions between dermal papilla cells (DPC) and epidermal HF stem cells (HFSC). During androgenetic alopecia (AGA) androgens deregulate these interactions and impair HFSC differentiation through inhibition of the Wnt/ B catenin signaling pathway. BMPs and WNTs act on DPC to maintain hair-inducing activity. We studied the role of BMPs on DPC spheres (DPC Sph)- induced HFSC differentiation. The activity of alkaline phosphatase, marker of hair inductivity, decreased in DPC Sph treated with DHT and the addition of BMP2 restored it. Conditioned media from DPC Sph induced HFSC hair-linage differentiation. When these media were conditioned in presence of DHT, HFSC-differentiation was impaired, however, when DPC Sph media were conditioned in presence of DHT and BMP-2, HFSC-differentiation was recovered, suggesting that BMPs can overcome the inhibitory effect of DHT on HFSC differentiation. To deepen in the mechanism by which BMPs could be exerting this pro- differentiating activity, we analyzed the beta-catenin nuclear translocation in HFSC. When BMP2 was added to the DPC Sph conditioned media, beta-catenin translocation was favored in differentiating HFSC compared with conditioned media alone or with DHT, implicating a cross-talk between BMPs and WNT signaling pathway in HFSC. We then analyzed, two WNT pathway inhibitors. CXXC5 mRNA was downregulated in differentiated HFSC independently of the presence of DHT or BMP2 in conditioned media. GSK-3 phosphorylation was not modified by BMP2, as it was in presence of LiCl, a known inhibitor of GSK-3. Even if further studies are necessary to elucidate at which level the cross-interactions of BMP and Wnt signaling may occur, BMPs contribute to DPC inductivity and HFSC differentiation. We conclude that BMPs are critical factors of the complex epithelial-mesenchymal interaction and their downregulation would favour AGA development.

27. (228) **MOLECULAR EFFECTORS AND MECHANISMS INVOLVED IN THE SUSTAINED CAMP RESPONSE MEDIATED BY CRHR1**

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Corticotropin-releasing hormone (CRH) plays a central role in the stress response and dysregulation of its action is linked to psychiatric disorders as anxiety and depression. CRH is a high affinity ligand for corticotropin-releasing hormone receptor type 1 (CRHR1) whose activation is associated to a sustained increase in cAMP levels and ERK1/2 activation in cellular models that recapitulate contexts of CRHR1 action. In a hippocampal neuronal model, CRHR1-mediated ERK1/2 activation triggered by CRH is biphasic, with an early phase B-Raf and PKA dependent, and a late phase of sustained ERK1/2 phosphorylation dependent on β-Arrestin2 and CRHR1 internalization. The aim of this work is to characterize the mechanisms implicated in the sustained cAMP response to CRH mediated by CRHR1. As a model, mouse hippocampal neuronal cell line HT22 stably expressing CRHR1 (HT22-CRHR1) was used together with the FRET-based biosensor Epac-S^{H187} to monitor cAMP levels in real time in living cells. By means of pharmacological and genetic tools, we revisited the role of PKA and β-Arrestin and explored PI3K/Akt signaling pathway in our system. In HT22-CRHR1 cells, expression of a β-arrestin dominant negative mutant altered Akt and CREB responses to CRH. Furthermore, Akt activation proved to be dependent on cAMP in HT22-CRHR1 cells as forskolin or 8-CPT-cAMP treatment promoted its phosphorylation. Pharmacological inhibition of PI3K, Akt and PKA triggered a further increase in cAMP levels in CRH-stimulated cells. Besides, PI3K/Akt inhibition led to a decrease in CRH-induced ERK1/2 and CREB activation, similar to what had already been reported when PKA activity was repressed. Moreover, our results showed that PKA inhibition is associated to increased Akt activation in response to CRH. These results suggest that PKA and PI3K/Akt may play a role in the mechanisms involved in CRH-elicited/CRHR1-mediated sustained cAMP response in a hippocampal context.
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28. (343) **THE CROSS-REGULATION BETWEEN H1 AND H2 HISTAMINE RECEPTORS MODULATES THE BEHAVIOR OF H2 RECEPTOR BLOCKERS**

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Histamine, modulates several biological processes, including allergy and gastric acid secretion, through H1 and H2 receptors. H2 blockers (H2B) are mainly used to treat gastrointestinal disorders, and much interest is focused on their repositioning for other pathologies. For it, deep understanding of their mechanisms of action is needed. We have previously described that H1 and H2 agonists (H1A and H2A) induce the receptor's co-internalization and cross-desensitization. We have also reported that H2B lead to desensitization and internalization of H2 receptor. Now, we hypothesize that H2B may also induce H1 receptor's cross-desensitization and co-internalization and that the cross-regulation induced by H1A will affect the behavior of H2B. In HEK293 cells transfected with both receptors (HEK-H1-H2), pretreatment with the H2B (cimetidine, ranitidine and famotidine) significantly reduced the activation of H1 receptor, evaluated through IL-6 promoter´s activity. Similar results were obtained for COX-2 and IL-8 gene expression, by qPCR in U937 cells. Also, we analyzed the impact of H2B in the antiproliferative/apoptotic response induced by H1A. Proliferation, cell cycle and annexin V assays showed that H2B reduced the antiproliferative/apoptotic response induced by H1A. Regarding the effect of H1 receptor activation on H2B response, H1A prevented the reduction of cAMP

levels induced by H2B, in U937 and HEK-H1-H2 cells. Additionally, saturation-binding assays showed that H2B lead to a decrease in H1 receptor binding sites. Finally, we analyzed the cross-regulation induced by H2B in presence of the receptor internalization inhibitor, dynasore. In this condition, none of the H2B used were able to modify the IL-6 promoter´s activity induced by H1A. This indicates that the co-internalization process is responsible of the cross-regulation induced by H2B. Our study provides new insights in the mechanisms of action of H1 and H2 receptors that explain the effect of antihistamines and opens up new venues for novel therapeutic applications.

ONCOLOGÍA / ONCOLOGY 1

29. (93) **BORON NEUTRON CAPTURE THERAPY (BNCT) COMBINED WITH BCG IMMUNOTHERAPY IN AN ECTOPIC COLON CANCER MODEL, IMMUNOLOGICAL RESPONSE AND CYTOTOXIC EFFECT**

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BNCT combines selective tumor uptake of ^{10}B compounds and neutron irradiation. The abscopal effect refers to the inhibitory action of radiotherapy on tumor growth at a site distant from the area of irradiation. Bacillus Calmette-Guerin (BCG) is known to induce a potent cytotoxic immune response. Having demonstrated the capacity of BNCT alone to induce abscopal effect, the aim of the present study was to evaluate the local therapeutic efficacy of BNCT combined with BCG immunotherapy in the BDIX rat ectopic colon cancer model and assess the abscopal effect and the tumor specific cytotoxicity. BDIX rats were inoculated with syngeneic colon cancer cells in the right leg. Four weeks post-inoculation, tumor was locally irradiated at RA-3 employing borono-phenyl-alanine (BPA): BNCT-group and BNCT+BCG-group (three intratumoral applications of BCG). BCG-group: BCG only; Beam only-group (BO-group): irradiated without BPA; BO+BCG-group; Sham-group: untreated tumor-bearing animals (tumor in both legs); Naive: untreated tumor-bearing animals (tumor in left leg). To evaluate abscopal effect, two weeks post-BNCT, colon cancer cell were inoculated in left leg. Once weekly for 7 weeks post BNCT tumor volume was measured in both legs. A significant local therapeutic efficacy was observed in the BNCT-group and BNCT+BCG-group vs. Sham-group $p < 0.05$. An abscopal effect, defined as tumor volume ≤ 50 mm^3, was observed in the BNCT-group (22%), BCG-group (44%) and BNCT+BCG-group (38%) compared to Sham-group (3%). A specific cytotoxicity assay against colon tumor cells using splenocytes from different experimental groups was performed. The cytotoxicity median (range) were: Normal rats 67% (60-70); Naive 20% (8-36). The 20% of BNCT and 43% of BCG+/-BNCT rats restored the normal cytotoxicity. The present study demonstrates that the therapeutic efficacy of BNCT could be improved if it is combined with BCG immunotherapy. This combination increases immune tumor cytotoxicity inducing an abscopal response in a higher number of animals.

30. (102) **BORON NEUTRON CAPTURE THERAPY MEDIATED BY BORIC ACID COMBINED WITH ELECTROPORATION IN THE HAMSTER CHEEK POUCH ORAL CANCER MODEL**

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BNCT is based on the selective incorporation of ^{10}B carriers in tumor followed by neutron irradiation. The biodistribution of boron carriers in tumor in terms of absolute and relative ^{10}B concentration, retention in tumor, targeting homogeneity and microdistribution conditions the therapeutic efficacy of BNCT. Given that electroporation (EP) can act as a non-specific system to administer anti-tumoral agents, the aim of this study was to evaluate if EP optimizes the delivery of the boron compound Boric Acid (BA), improving the therapeutic efficacy of BNCT in the hamster cheek pouch oral cancer model. Exophytic tumors (Squamous Cell Carcinoma) were induced in the pouch of Syrian hamsters by topical application of the carcinogen dimethyl-benzanthracene (DMBA) twice a week for 3 months. For boron biodistribution and BNCT studies we administered BA (50 mg ^{10}B/kg iv) followed by EP (1000 v/cm, 8 pulses of 100μs) 10 min. post-administration. Three hours after the administration of BA we sacrificed the animals for boron biodistribution studies or performed BNCT/BA studies. We observed a statistically significant increase ($p < 0.0001$) in boron uptake in tumors corresponding to the protocol BA+EP (47±10 ppm) versus control BA without EP (36±7 ppm) whilst no changes were observed in normal pouch and precancerous tissues. The ongoing studies of BNCT/BA+EP showed a high therapeutic efficacy (94%), similar to that observed with BNCT/BA (84%). In addition, these results are statistically significant ($p<0.0001$) compared to those of BNCT/GB-10 (48%), a boron compound with characteristics similar to BA, although we observed an increase in radiotoxicity. Biodistribution studies showed that EP induced an increase in mean gross boron concentration in tumor and would contribute to BNCT/BA induced tumor response.

31. (98) **OLIGO-FUCOIDAN ENHANCES THE THERAPEUTIC EFFICACY OF BORON NEUTRON CAPTURE THERAPY (BNCT) IN THE ORAL CANCER AND ECTOPIC COLON CANCER EXPERIMENTAL MODELS**

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Introduction BNCT combines selective tumor uptake of 10-B compounds and neutron irradiation. We demonstrated the therapeutic effect of BNCT in the hamster cheek pouch oral cancer and BDIX rat ectopic colon cancer models. We also studied the radiotoxic effects of BNCT, mucositis and dermatitis, respectively. Oligo-Fucoidan is extracted from seaweeds and has exhibited anticancer and anti-inflammatory properties. The aim of the present study is to evaluate the radioprotective and therapeutic effect of Oligo-Fucoidan+BNCT in both cancer models. Materials and Methods BDIX rats were injected subcutaneously in the right hind flank with DHD/K12/TRb syngeneic colon cancer cells. The tumor-bearing legs were treated locally with BNCT mediated by BPA (boronophenylalanine) at RA-3 Nuclear Reactor, with or without Oligo-Fucoidan (200mg/ml, oral administration during 16 days). Cancerized hamster cheek pouches (DMBA in mineral oil, applied twice a week for 8 weeks) were exposed to BPA-BNCT with or without Oligo-Fucoidan (200mg/kg, 16 days). Results Oligo-Fucoidan was nontoxic. It did not reduce the percentage of animals with severe dermatitis or mucositis, but it did enhance BNCT therapeutic effect on tumors. BDIX rats treated with BNCT+Oligo-Fucoidan exhibited a mean ratio tumor volume Post-BNCT/Pre-BNCT significantly lower than the BNCT group (0.35±0.31 vs 1.09±0.76 respectively, $p= 0.0002$). In the oral cancer model, BNCT+Oligo-Fucoidan group exhibited a higher Overall tumor response than the BNCT group and also enhanced tumor complete response (94% vs 67%; 71% vs 41% respectively). Conclusion Oligo-Fucoidan enhances the therapeutic efficacy of BNCT in the hamster oral cancer model and BDIX rat ectopic colon cancer model. Acknowledgments We gratefully acknowledge the provision of Oligo-Fucoidan by Hi-Q Marine Biotech International Ltd (Taiwan), and the efforts of Ming-Chen Hsiao to promote these studies.

32. (220) **ANTITUMOR EFFECTS OF THE VITAMIN D ANALOGUES UVB1 AND EM1 ON BREAST CANCER PATIENT DERIVED XENOGRAFTS**

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Despite advances in the treatment of HER2 positive and Triple Negative (TN) Breast Cancer (BC), mortality remains high due to intrinsic or acquired resistance to therapy. Therefore, new candidates to treat these subtypes of tumors are needed. Patient-Derived Xenografts (PDX) generated from recent tumor samples recapitulate the diversity of breast cancer being a powerful preclinical tool for testing new drugs. Previously, the Vitamin D analogues UVB1 and EM1 have demonstrated antitumoral effects in preclinical studies employing cell lines and animal models. Hence, the aim of the present study was to evaluate the antineoplastic effects of UVB1 and EM1 on cells derived from HER2-positive and TNBC- PDXs. The results showed that the analogue UVB1 reduced cell viability of the skin metastatic HER2-positive BC PDX118 and its Trastuzumab-emtansine PDX118 resistant cells (crystal violet assays, $p<0.001$). Also, both analogues decreased the viability of the brain metastatic HER2-positive BC PDX554 (UVB1: $p<0.001$, EM1: $p<0.01$). The cell cycle analysis of PDX118 cells treated with UVB1 and stained with propidium iodide showed an induction of cell cycle arrest in GO/G1 phase ($p<0.001$). In accordance with this result, UVB1 decreased Cyclin D1 expression in these cells by western blot. Regarding TNBC-PDXs, UVB1 and EM1 reduced the viability of cells derived from the primary tumors PDX410, PDX575 and PDX549. And, UVB1 also decreased the viability of TNBC-PDXs: PDX570 ($p<0.05$), PDX347 ($p<0.001$) and PDX454 ($p<0.01$). Finally, the combination of UVB1 and an Antibody Drug Conjugate (ADC) displayed a better effect than ADC treatment alone in PDX410, PDX570 and PDX575 ($p<0.001$). Altogether, these results suggest the potential use of these vitamin D analogues as antitumor agents to treat HER2 positive and TNBC.

33. (221) **PRECLINICAL STUDIES OF THE COMBINATION OF EM1 CALCITRIOL ANALOGUE WITH PACLITAXEL IN TNBC**

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Triple negative breast cancer (TNBC) does not respond to current targeted therapies as it lacks the expression of hormone receptors and epidermal growth factor receptor HER2. Therefore, current treatment options include cytotoxic drugs such as taxanes, which are limited in terms of extending patient survival or improving patient's quality of life. The vitamin D receptor is a nuclear transcription factor whose natural ligand, calcitriol, has antitumor activity. Unfortunately, calcitriol anticancer utility is limited by its hypercalcemic effects and thus, vitamin D analogues are being developed to try to solve this problem. We hypothesize that the analogue of calcitriol, EM1, has synergistic effect with Paclitaxel in TNBC without exerting additional toxicity. This would allow to use lower doses of cytotoxic chemotherapy, with the consequent reduction of adverse effects. Also, this combination could minimize or slow the development of resistance to chemotherapeutic agents. We first studied the activity of EM1 on cell count and observed that EM1 strongly decreases the viability of the 4T1 TNBC murine cell line ($p<0.001$), while not affecting significantly that of MDA-MB-231 cells. In addition, EM1 was able to retard 4T1 cell migration in the wound healing assay ($p<0.001$) and to inhibit the invasion through Matrigel ($p<0.001$). The subsequent combination assays showed that EM1 and Paclitaxel did not display antagonistic effects in the cell count of the MDA-MB-231 cells. In addition, a pilot experiment of syngeneic transplantation of 4T1 cells in Balb/C mice showed that mice did not develop hypercalcemia or other signs of toxicity, following 30 days of EM1 treatment. Importantly, although no reduction in the growth of the primary tumor was observed, a significant decrease in the number of lung metastases ($p<0.0024$) was obtained. Altogether, these results suggest that EM1 could be an effective agent in combination with Paclitaxel in TNBC, by targeting the metastatic process.

34. (266) **ANTICANCER MECHANISMS OF CALCITRIOL AND MENADIONE ON BREAST CANCER CELLS**

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Calcitriol (D), the active form derived from vitamin D3, presents antineoplastic properties in several types of cancer. However, its use as an antitumoral agent may promote secondary effects. We have demonstrated that menadione (MEN) potentiates the antiproliferative effect of D on breast cancer MCF-7 cells, at least in part, by cell cycle alteration and increasing intracellular calcium and ROS and NOS production, although the mechanisms are still under study. Our working hypothesis was that the combined treatment of D and MEN could increase the antitumoral effect of D, mainly via activation of autophagy and reduction of the migration of the MCF-7 cells. The aim of the present study was to investigate further the mechanisms involved in the antiproliferative action of the combined treatment. MCF-7 cells were treated with 100 nM D, 10 μM MEN, both or vehicle (96 h). Gene and protein expression of PMCA1b were measured by qRT-PCR and western blot, respectively. Induction of autophagy was evaluated through detection of acidic vesicular organelles (AVOs) by fluorescence microscopy and the protein expression of LC3II by western blot. Cell migration was estimated by the wound healing assay. Statistical analyses were performed by ANOVA/Bonferroni. Differences were considered significant at $p<0.05$. Both D and combined treatments diminished the gene expression of PMCA1b. However, its protein expression was increased with MEN+D. Drug combination enhanced the formation of AVOs and modified LC3II protein expression. In addition, MEN plus D treatment decreased wound closing. In conclusion, MEN increases the anticancer effect of D on MCF-7 cells partly due to changes in the expression of PMCA1b, a molecule closely related to calcium regulation and thus modifying its intracellular concentration, as we previously reported. Increased AVOs formation and LC3II expression changes suggest the activation of autophagy. The combined treatment also decreased the capacity of the cells to migrate.

35. (105) **MUSCARINIC RECEPTORS AS THERAPEUTIC TARGETS IN TRIPLE NEGATIVE BREAST CANCER TREATMENT. PACLITAXEL PLUS CARBACHOL ADMINISTERED IN A METRONOMIC SCHEDULE.**

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We have demonstrated that the long term activation of muscarinic receptors (MR) decreases tumor cell viability pointing to MR as possible therapeutic targets in breast cancer. Metronomic therapy consists in the administration of low doses of cytotoxic drugs, alone or combined with repositioning drugs with short intervals inter-doses.

Here, we investigated the effect that a metronomic combination of paclitaxel (PX) plus the muscarinic agonist carbachol (CARB) exerts on tumor growth and on "ATP binding cassette" (ABC)G2 transporter and epidermal growth factor receptor (EGFR) as mediators of chemotherapy resistance in breast cancer treatment. MDA-MB231 cells derived from a triple negative (TN) breast tumor express functional MR, and the combination of subthreshold concentrations of PX (10-8M) plus CARB (10-12M) in 2 cycles of 48 h reduced cell viability by 24% in each cycle ($p<0.05$ vs. control; determined by MTT assay), similarly to PX at a pharmacological concentration (10-6M, $p<0.05$). This effect could be mediated partially by a decrement in the expression of ABCG2 and EGFR (56±2% and 35±3% respectively vs. control; $p<0.01$) measured by Western blot. We also analyzed the effect of the systemic administration (i.p.) of PX plus CARB to MDA-MB231 NUDE mice tumor bearers. A significant reduction in tumor growth was observed after 2 cycles of treatment (90±19%; $p<0.001$ vs. untreated tumor bearers). The effect was prevented by treating animals with atropine. This combined treatment has the advantage to not affect non tumorigenic mammary MCF-10A cells. These results suggest the possibility to consider MR as therapeutic targets in a metronomic therapy schedule in TN breast cancer.

36. (168) **EFFECT LAPATINIB AND ALL TRANS RETINOIC ACID (ATRA) COMBINED TREATMENT ON MAMMARY CANCER STEM CELLS DERIVED FROM HER2 NEGATIVE CELL LINES**

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Cancer stem cells (CSC) are resistant to both chemo and radiotherapies and are also considered as the metastasis seed. In order to validate CSC as new therapeutic targets in breast cancer, we have analyzed whether the CSC component of three HER2 negative cell lines (4T1, MCF-7 and T47D) express this receptor and which is the effect of Lapatinib (Lp, HER2 inhibitor) and ATRA (Vitamine A active metabolite) or the combined treatments, on the growth and development of primary mammospheres (CSC enriched cultures).

HER2 expression was analyzed by Western blot and immunofluorescence using monolayers and primary mammospheres from 4T1, MCF7 and T47D cell lines. We have determined that HER2 is overexpressed only in the CSC subpopulation of all the analyzed cell cultures. Moreover, primary mammospheres were treated for 96 h with Lp1μM for 4T1 cells; 5μM for MCF-7, 2μM for T47D cells combined or not with ATRA 1μM. Although we have previously shown that ATRA did not induced 4T1 growth inhibition, the treatment with ATRA alone or combined with Lp significantly reduced the diameter of 4T1 mammospheres ($p<0.05$ Anova test). Important signs of cell death were also observed in the combined treatment condition. Similar results were obtained in MCF-7 ($p<0.05$ Anova test) and T47D cell lines (NS).

In the present work we have demonstrated that the CSC component of HER2 negative cell lines overexpress such receptor. Moreover, the Lp and ATRA combined treatment can successfully reduce mammospheres growth. Our work provides for the first-time in vitro evidences for the potential use of the Lapatinib and ATRA combined therapy in HER2 negative tumors, having as main target the stem component.

37. (207) **EVALUATION OF BETA-EMITTING DEVICES AS A COMPLEMENTARY TOOL OF BNCT FOR THE TREATMENT OF SUPERFICIAL CANCER**

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Introduction: BNCT based on the nuclear reaction 10B (n,4He)7Li restarted in 2015 after modifications in the nuclear reactor RA6. Some materials such as rhodium, silver and indium have a high effective neutron capture section, rapid decay activation products and high energy beta particles emission. As beta radiation has a short range of tissue penetration these devices beta enhancers (BE) can be used to compensate or even significantly increase the surface dose gradient BNCT. Objective: Evaluation of radiotoxicity and effectiveness of three BE devices (rhodium, silver and indium) as a complementary tool of BNCT. Materials and Methods: Sixty NIH nude mice were implanted subcutaneously with cells from the HT-29 colon cancer human cell line, developing tumours between 150 and 200 mm3 of size at day 15. The animals were divided into 4 groups: 1) Control; 2) BNCT + BE rhodium; 3) BNCT + BE silver; 4) BNCT + BE indium. The mice were irradiated for 42.5 minutes with a neutron flux of 4.96 x 108 n/cm2sec. Results: The animal did not show any signs of radiotoxicity. Complete inhibition of tumor growth was observed in the three BNCT-BE groups during the first three weeks. The histological studies showed lower percentage of the viable area in the three treated groups, being even lower for the group 4. The analysis of mitosis showed that the number was lower in the three treatments. The CD133 and CD166 positive cells indicated the presence of cancer stem cells (CSCs) in the tumour. Its persistence, although diminished, would seem to be associated with tumour proliferation and loss of tumor growth control after one month of treatment. Conclusions: These studies demonstrated that the three devices would be non-toxic and effective as complementary tools to BNCT for the treatment of superficial tumours.

38. (290) **"METRONOMICS" WITH CYCLOPHOSPHAMIDE (CY) AND LOSARTAN (LOS) FOR THE TREATMENT OF M-406 TRIPLE NEGATIVE MURINE MAMMARY ADENOCARCINOMA**

Mónica Carolina Grillo, Cintia Kaufman, Antonela Del Giúdice, María Virginia Baglioni, Viviana R. Rozados, O. Graciela Scharovsky, María José Rico, Leandro Mainetti

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Metronomic chemotherapy (MCT) refers to the chronic, equally spaced, delivery of low doses of chemotherapeutic drugs, without extended interruptions. Drug repositioning (DR) in oncology make mention of the use of drugs originally formulated for other indications that showed antitumor potential. "Metronomics" is defined as the combination of MCT and DR. Cy is an alkylating drug with toxic action on proliferating cells. Los is an antagonist of angiotensin II receptor, used to treat hypertension. Our aim was to study the effect of metronomic Cy+Los treatment, on M-406 bearing animals. CBi female mice were challenged s.c. with M-406 (day 0) and distributed on day 6, into 4 groups (n=6-7/group). GI: Control, non-treated; GII: Treated with Cy 25mg/kg/day in the drinking water; GIII: Treated with Los 150mg/kg/day in the drinking water; GIV: Treated as GII+GIII. Mice were weighted and tumor volume measured, 3 times/week. When tumors were exponentially growing, mice were euthanized, and blood samples were taken to evaluate immune cells populations. On day 21, tumor volume in GIV (mm3, mean ± SEM: 284.4± 91.4) was lower than in GI (3552±745.7), GII (1101±344.3) and GIII (1033±306.6) ($P<0.001$). All the groups differed in tumor volume doubling time ($P<0.0001$), and in survival ($P<0.001$), showing GIV the highest values of both variables. No general toxicity was evinced during treatment. Flow cytometry analysis indicated that there was no changes in CD4+ and CD8+ circulating lymphocytes in any group, while the number of Th17+ cells increased ($P<0.005$) and a non-significant decrease of Foxp3+ lymphocytes was observed in treated groups. Conclusions: 1) MCT with Cy+Los inhibited M-406 growth and increased mice survival without general toxic effects; 2) The combined treatment was more effective than the individual ones. 3) The antitumor effect obtained would be due, at least in part, by the modifications induced in Th17+ and Foxp3+ cell populations.

39. (378) **EFFECT OF NORCANTHARIDINE TREATMENT ON TRIPLE NEGATIVE MAMMARY TUMORS PROGRESSION**

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Triple negative breast tumors (TNBC) are a subgroup of breast cancer that do not express estrogen or progesterone receptors and neither overexpress the HER2 receptor. These tumors present a very aggressive clinical course with high rate of recurrence and metastasis incidence.
It was previously described that Norcantaridine (NCTD) can inhibit the progression of several cancers types including lung and liver. However, the effect on breast cancer has not been studied yet. Here we analyzed the effect of NCTD in in vitro and in vivo processes associated with malignant progression and metastatic capacity, using human (HS578T) and murine (4T1) TNBC cell lines.
NCTD exhibited an important antiproliferative effect, with an Inhibitory Concentration 50 (IC50) of 35uM for 4T1 and 56uM for HS578T cell lines, obtained by MTS assay. By flow cytometry we have demonstrated an increase in the Sub-G0 cell cycle fraction, compatible with the presence of apoptotic cells. Next, we studied the effect of NCTD on adhesion, migration (wound healing), and metalloproteinases (MMPs) secretion (zymography). Moreover, we performed an in vivo lung colonization assay using the 4T1 murine model, syngeneic in BALB/c mice.
In both cell lines, NCTD significantly reduced adhesive and migratory capacities ($p<0.05$ Anova test) also displaying a significant reduction in MMP-9 secreted activity. Although all these parameters could have a direct implication in the malignant progression impairment, in vivo assays showed the pretreatment of 4T1 cells with NCTD induces an increase in the number of lung metastatic nodes ($p<0.05$ Kruskal-Wallis test).
Even though some results obtained are encouraging, we must seek the appropriated therapeutic strategy that allow the safe and effective use of NCTD for the treatment of triple negative breast cancer either as a single drug or in combination with existing therapies.

40. (609) **INVOLVEMENT OF ER STRESS AND MAPK PATHWAYS IN THE APOPTOTIC EFFECT INDUCED BY A TRIAZOLYL PEPTIDYL PENICILLIN IN MURINE MELANOMA CELLS**

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The triazolyl peptidyl penicillins (TAPs) are novel hybrids compounds having in their structure a penicillanic core linked to a peptide portion via a triazole group. In a previous study, we showed that the derivative containing the dipeptide Leu-Phe (TAP7f) behaves as a selective and potent antitumor agent that induces an apoptotic response. In order to further investigate the mechanism of action of this compound, we first examined the possible contribution of the ER stress to the cell death process. The significant increase in the expression levels of the ER chaperones Calnexin, Bip, ATF4 and CHOP (~2 fold) observed after 3-6 h of incubation with TAP7f suggested the activation of an unfolded protein response. We then evaluated the effect of TAP7f on the modulation of signaling pathways involved in cell proliferation. By Western blot analysis, we found a significant increase in the phosphorylation levels of p38MAPK, JNK and AKT after 15-30 min of incubation with 20 μM of TAP7f. We also demonstrated a decrease of ERK 1/2 phosphorylation after 30-45 min. To study the participation of these pathways in the antitumor effect of TAP7f, cell viability was determined after pre-incubating cells with specific pharmacological inhibitors (Inh). Results showed that cell viability increased from 36±3% to 51±4% or 55±7% after pre-treatment with SP600125 (JNK Inh) and SB203580 (p38 Inh), respectively. When cells were pre-incubated with PD98059 (Erk 1/2 Inh) or wortmannin (PI3K Inh), cell proliferation diminished from 36±3% to 21±7% or 9±2%, respectively.
Taken together, our results suggested the contribution of ER stress, JNK and p38 MAPK pathways to the apoptotic cell death induced by TAP7f, whereas Erk 1/2 and PI3K cascades would be related to survival responses. The knowledge of the role played by the intracellular signals regulating cell death could certainly contribute to find new intracellular targets for melanoma treatment.

ONCOLOGÍA / ONCOLOGY 2

41. (167) **CIRCULATING MIRNAS AS BIOMARKERS ASSOCIATED TO BREAST CANCER AND METABOLIC SYNDROME**

Paula Lucia Farre, Guillermo Nicolás Dalton, Flavia Piccioni, Cintia Massillo, Karen Daniela Graña, Georgina Daniela Scalise, Paola De Luca, Adriana De Siervi
IBYME-CONICET

Breast cancer (BrCa) is the main cause of death by cancer in women worldwide. Metabolic syndrome (MeS) is an important risk factor in the development of BrCa, since it correlates with high grade tumors, increased recurrence and metastasis progression. miRNAs are non-coding RNAs that silence the expression of mRNAs and can be released to bloodstream. The aim of this work was to identify new biomarkers based on circulating miRNAs for diagnosis of BrCa associated to MeS. Balb/c mice (n=30) were chronically fed with high fat (HFD) or control (CD) diets. After 15 weeks, 10 animals of each group were inoculated *s.c.* with 4T1 cells on the mammary fat pad. When tumors were palpable, 5 HFD and 5 CD mice were sacrificed (Early Stage=ES). The remaining animals were sacrificed when tumors reached a volume around 600 mm^3 (Advanced Stage=AS) together with the non-tumor group (Control=C). Blood and tumors samples were collected for RNA isolation. The expression of several miRNAs associated to BrCa or MeS were analyzed by *stem-loop* RT-qPCR. We identified two groups of BrCa and MeS associated miRNAs in allografts: one group of miRNAs (miR-125b-5p, miR-21-5p, miR-106b-5p, miR-221-3p, miR-34a-5p) was increased in mice with AS BrCa compared to ES tumors. The other group of miRNAs (miR-10b-5p, miR-138-5p, miR-143-3p, miR-127-3p, miR-205-5p, miR-146a-5p, miR-195-5p) was diminished in mice with AS BrCa compared to ES tumors. Remarkably, MeS increased miR-195-5p and diminished miR-146a-5p expression at ES BrCa. We also identified circulating miRNAs in plasma from mice: i) miR-10b-5p was downregulated in AS vs C plasma; ii) miR-195-5p and miR-106b-5p were upregulated in HFD vs CD fed mice in ES; iii) miR-195-5p and miR-106b-5p were downregulated in HFD vs CD in AS; and iv) miR-21-5p, miR-125b-5p, miR-138-5p, miR-205-5p, miR-146a-5p and miR-221-3p were upregulated in HFD vs CD in ES.

42. (329) **BIOINFORMATICS ANALYSIS SHOWS ASSOCIATION BETWEEN A GENE EXPRESSION SIGNATURE AND BIOCHEMICAL RELAPSE AFTER RADICAL PROSTATECTOMY IN PATIENTS WITH PROSTATE CANCER**

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Prostate cancer (PCa) is the second most incident type of cancer in men and the fifth leading cause of cancer-related deaths worldwide. Surgical resection of the entire gland by radical prostatectomy is one therapeutic option with curative purposes for men with organ confined PCa. However, 30–40% of patients will have a biochemical relapse (BCR) after surgery, which may indicate clinical recurrence of an aggressive disease. Current clinical indicators of PCa recurrence after radical prostatectomy have limited sensitivity and specificity. Thus, the ability to establish the risk of progression after surgery is of high importance for patient management and to avoid overtreatment. We used transcriptome data from the dataset GSE54460 publicly available at GEO-NCBI to study differential gene expression

between patients with and without BCR post radical prostatectomy. We obtained a list of 1021 genes differentially expressed (adjusted p<0.05). In order to increase the confidence of the results, we ran the stability selection algorithm on the 1021 genes selected on the previous step. This algorithm is a resampling procedure to assess the stability of variables for high-dimensional data. We obtained a set of 4 genes that resulted as differentially expressed in >40% of the subsamples. The selected genes were: CRISP3, APOE, CEL and DDTL; and they all were significantly overexpressed in the tumors of patients who relapsed. The analysis of BCR-free survival showed significant poorer survival for patients with high-tumoral expression of these genes. Moreover, the risk of BCR is >2-fold for patients with high-tumoral expression compared to patients with low expression. The analysis of the combined expression showed a HR=15 (95%CI=3.5-63.9) for patients with high-tumoral expression for two or more genes. In conclusion, the expression signature of these genes might have the potential to detect aggressive tumors and predict BCR at the time of radical prostatectomy.

43. (364) **THE MIRNAOME ASSOCIATED TO CTBP1 AND METABOLIC SYNDROME IMPACTS ON THE OUTCOME OF BREAST CANCER PATIENTS.**
Rocío Belén Duca, Paula Lucia Farre, Karen Daniela Graña, Guillermo Nicolás Dalton, Georgina Daniela Scalise, Cintia Massillo, Adriana De Siervi, Paola De Luca
Laboratorio de Oncología Molecular y Nuevos Blancos Terapéuticos - IByME – CONICET

Breast cancer (BrCa) is the leading cause of cancer death in women and metabolic syndrome (MeS) constitutes a risk factor for this disease. C-terminal binding protein 1 (CTBP1) is a co-repressor of tumor suppressors activated by low NAD^+/NADH ratio. Previously, we generated a MeS model by chronically feeding mice with high fat diet. We found that CTBP1 and MeS induced tumor growth and progression regulating the expression of let-7e-3p, miR-494-3p, miR-146a-5p, miR-194-1-5p, miR-381-5p and miR-378a-3p in BrCa xenografts. The aim of this work was to investigate the role of the miRNAs modulated by CTBP1 and MeS in BrCa. We analyzed the effect of the CTBP1/MeS-associated miRNAs in survival of BrCa patients through the bioinformatics tool PROGmiR. We found that in almost all cases, miRNAs effects depend on ER and PR status. Thus, low expression of let-7e-3p correlated with diminished survival in patients with BrCa ER^+ and PR^+, while high expression levels are associated with a lower metastasis-free survival in patients with BrCa ER^-. Low expression of miR-494-3p is associated with decreased metastasis-free survival in patients with BrCa PR^+. Low expression of miR-146a-5p correlated with low metastasis-free in ER^+ BrCa patients. In BrCa PR^- patients, miR-146a-5p and miR-194-1-5p expression is related to increased relapse-free survival, while miR-381-5p expression is associated with reduced metastasis-free survival. Interestingly, miR-378a-3p expression is associated with low metastasis-free survival in BrCa global population. Based on this, we selected miR-378a-3p to evaluate its effects in proliferation, adhesion and migration of BrCa cells through *in vitro* assays. Thus, miR-78a-3p was cloned into the expression vector pSM30. We generated MDA-MB-231 stable-transfected cells with overexpression of miR-378a-3p or control cells and selected positive MDA-MB-231-derived clones by RT-qPCR. To identify microRNAome associated with tumor growth and progression constitutes the first step for the development of targeted therapies for BrCa associated to MeS.

44. (414) **THEORETICAL AND BIOLOGICAL STUDY OF NEW POTENTIAL BRAFV600E INHIBITORS**
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Around 50% of melanoma patients express the mutated protein kinase BRAFV600E which in turn induces cell survival and proliferation through ERK pathway activation. Lately, two small BRAF inhibitors (BRAFi) have been approved for the treatment of metastatic melanoma: Vemurafenib and Dabrafenib. Considering that tumors become resistant after a few months of treatment and in some cases tumors are intrinsically resistant to BRAFi, new therapeutic options should be analyzed. Thus, by a combination of theoretical and experimental studies our aim was to find new potential BRAF inhibitors. Based on virtual screening, docking and molecular dynamics approaches we selected a panel of 20 different compounds. To test its potential BRAFi activity, biological assays were conducted in melanoma cell line Lu1205 which express the mutant kinase BRAFV600E, and Vemurafenib was employed as positive control of all the experiments performed. In particular, ERK phosphorylation, an indirect measure of BRAFV600E activity, was determined by western blot. In addition, MTT assay was conducted to study the effect of the compounds on cell viability. Our results show that 6-OH-2-carboxianilide derivatives 10C and 10F reduce significantly ERK phosphorylation at 1 μM (p<0.05). In addition, compound 10C also reduce cell viability (p<0.001). Taking together, these results allowed us to identify the compound 10C as a new potential BRAFi that reduce ERK phosphorylation and cell viability. Moreover, this compound can be modified in order to design new chemical structures with improved activity.

45. (322) **TUMOR ORGANIZATION: A MULTIDIMENSIONAL APPROACH**
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Tridimensional (3D) culture of cancer cells has become a useful technique to replicate as close as possible the conditions found in living tissue. Along with it came new methodological challenges to visualize cell organization and progression. We present an open source Python toolbox applied to transmission and fluorescence 2D/3D images, obtained with a variety of techniques such as Confocal and Single Plane Illumination Microscopy (SPIM). Using glioblastoma multicellular spheroids, we were able to quantify their morphology through time with automatic segmentation based on object recognition. We expect, by making this tool available to the scientific community, to improve 3D cell culture characterization to assess the effect of drug screening assays.
Supported by ANPCyT, CONICET and FOCEM (COF 03/11)

46. (295) **STUDY OF AHCYL1 GENE IN A TUMOR CELL PLASTICITY MODEL**
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Development and tumorigenesis involve cell plasticity events, where cells may undergo differentiation/dedifferentiation processes. TGF-beta/SMAD proteins and the core transcriptional factors (Oct4, Sox2 and Nanog) modulate the behaviour of stem cells as well as the tumor cells. Our aim is to study oncogenic reprogramming as a cell plasticity model and we chose A549 human lung adenocarcinoma cell line. This cell line is capable of growing as three-dimensional (3D) culture (a property of stem-like cells) and is induced to undergo TGFbeta-driven Epithelial-Mesenchymal Transition (EMT). We have developed INSECT, a bioinformatic tool to identify genes potentially co-regulated by the core factors and SMAD proteins in stem-like cells. Using this tool we identified several candidate genes and we selected and started to characterize the high-scored gene Ahcyl1. Results showed that after 48-hour-TGFbeta1 treatment Ahcyl1 transcriptional variant A is downregulated while Snail expression as a known target is enhanced. Moreover, we found that in 3D-culture vs. monolayer assays Ahcyl1 expression is downregulated in A549 spheres, suggesting its expression could be associated with a more differentiated phenotype. These results are consistent with INSECT in silico predictions about Ahcyl1 expression being modulated by TGFbeta1 and associated with cell plasticity events.
Supported by ANPCyT, CONICET and FOCEM (COF 03/11)

47. (428) **CHARACTERIZATION OF INFILTRATING MACROPHAGES IN ORGANOTYPIC 3D CULTURE MODEL OF GLIOBLASTOMA**

Ivana Gisele Estecho, Maria Jose Gattas, Onias Castellanos Collazo, María Amparo Lago Huvelle, Tomás Laporte, María Inés Díaz Bessone, Andrea E. Errasti, Eugenio Antonio Carrera Silva, Marina Simian
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Glioblastoma multiforme (GBM) is one of the most devastating cancer types with very limited therapeutic options and tumor-associated macrophages have been described to play a critical role in its growth and progression.
The aim of this study was to set up a 3-dimensional (3D) organotypic human model of GBM to characterize macrophages infiltration and to identify potential therapeutic targets. We have used U-87 MG human glioblastoma cells together with human primary monocytes, or the THP-1 monocyte cell line. We established that hanging-drop cultures originating from ~3000 GBM cells resulted in reproducible spheroids. Once they were formed, the spheroids were transferred into the wells and co-cultured with 40.000 primary human monocytes or THP-1 cells. Monocytes invaded the spheroids as established by tracking with a live-cell fluorescent dye. Moreover, flow cytometry analysis of dissociated spheroids co-cultured with monocytes during 7 days, clearly shows CD11b+ macrophages skewed to M2 phenotype with high levels of CD14, CD206, MERTK and CD163 compared to the mean intensity fluorescence of monocyte-derived macrophages alone ($p<0.01$). M2 polarization was also observed when primary monocytes were exposed to U-87 MG conditioned media in 2D culture. The development of the co-culture-based 3D model of GBM will allow us to test new therapeutic strategies that specifically target M2 macrophages under physiologically relevant settings.

48. (72) **MUTATIONS IN IDH1 AND TERT GENES IN GLIOMAS AND THE PROGNOSIS VALUE**

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Gliomas are the largest group of aggressive primary CNS tumors, they occur predominantly in adults. Purpose: correlation of mutations in isocytratedehydrogenase (IDH1) and telomerase reverse transcriptase (TERT) genes with tumor progression. DNA was isolated from tumor and blood for PCR amplification of IDH1 exon 6 and TERT promoter. PCR products were sequenced to identify the most frequent mutation in codon 132 of IDH1 and the C to T transitions at -124 and -146bp upstream the TERT ATG codon. Clinical data included age, gender, symptoms, tumor localization, histopathology, tumor growth and survival. Nineteen cases with an average age of 49 years were studied, 7 patients presented with low grade glioma and 12 patients presented with high grade. These 12 patients did not show mutations in IDH1 and showed tumor growth, whereas six patients with low grade glioma had a mutated IDH1 and showed no tumor growth. Mutations in TERT gene were identified in 7 out of 17 analyzed patients, they were C to T transition at -146bp, 4 of these patients had grade 4 glioma, 2 showed tumor growth and one of them died 3 months after surgery. Six patients with grade 4 glioma did not carry mutations in TERT gene. Twelve tumors showed a polymorphism C to T transition at -245bp which was also present in blood. Neither IDH1 nor TERT mutations were found in constitutional DNA. Comparison between IDH1 and TERT mutations indicated a correlation between the wild type state of IDH1 and the presence of mutation in TERT in four of the studied cases and these data were related to the high tumor grade. Our results point out that the presence of mutations in IDH1 can be used as a good prognosis. Analysis of IDH1 and TERT mutations is valuable in prediction of patient´s evolution and chemotherapy response.

49. (109) **COOPERATION BETWEEN THE MITOGEN-ACTIVATED PROTEIN KINASES (MAPK) PATHWAY AND THE TUMORAL PROTEIN MAGEC2**

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MageC2, a MAGE family member, is frequently expressed in a wide range of solid tumors and is associated with a non-favourable clinical course. We recently detected Ras as a potential oncogene that collaborates with MageC2, increasing its stabilty and activity (as repressor of p53 function). Here, we explored two of the main cellular pathways in which the RAS protein operates: the mitogen-activated protein kinases (MAPK) and phosphoinositide-3 kinase (PI3K) pathways and we observed that the main pathway by which Ras stabilizes MageC2 is the MAPK since the ability of activated Ras to enhance transfected Myc-MageC2 protein stability was severely impaired by the MEK inhibitor PD098059, but it was not affected by the Akt inhibitor LY294002. A similar behavior was seen by inhibiting both pathways in human melanoma A375 cells expressing endogenous MageC2. Moreover, transfected Myc-MageC2 stability was strongly increased by activation of endogenous Raf-1 (by Phorbol 12-myristate 13-acetate-PMA treatment, a PKC activator) and transfection with a constitutive active ERK1 also increased Myc-MageC2 stability. To understand if ERK activities lead to MageC2 stabilization, we mutated two highly conserved Ser to Ala on MageC2 (S85 and S86), that are within a consensus phosphorylation sequence for the ERK kinase, and we observed that S85A/S86A MageC2 mutant was still up-regulated after expression of RasV12. Furthermore, we did not observe MageC2/ERK interaction in an immunoprecipitation assay. In order to explore ERK downstream signaling, we inhibited an ERK-activated kinase RSK, but Myc-MageC2 was still stabilized with Ras activation. The mechanism by which the MAPK pathway stabilizes MageC2 is still under study. Our findings have an important biological relevance, since it proposes for the first time that MageC2 tumor protein could act as a link between the activation of the Ras oncogene and the negative regulation of p53, thus enhancing the oncogenic effect of Ras.

50. (790) **DENDRITIC CELL VACCINES GENERATED FROM NAIVE OR BREAST CANCER-BEARING MICE ARE PHENOTYPICALLY SIMILAR**

Alejandro Nicola, Araceli Abt, Antonela Asad, Camila Zuccato, Mercedes Insem, Sofia Sagripanti, Elisa Bal de Kier Joffé, Flavia Zanetti, Adriana Seilicovich, Marianela Candolfi
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The use of dendritic cells (DCs) for antitumor immunotherapy has been widely studied in preclinical and clinical settings showing good safety profiles. Although these strategies exert robust antitumor effects in preclinical cancer models, clinical efficacy has been lower than anticipated. This discrepancy could rely in part in the fact that DC precursors are extracted from cancer patients to generate autologous vaccines, while in preclinical studies antitumor vaccines are commonly generated from DC precursors from healthy animals. Considering that there is discrepancy on the functionality of CDs collected from cancer patients, we aimed to characterize DCs cultured from the bone marrow of naïve and breast tumor-bearing mice. In order to optimize the culture conditions, we first evaluated the yield and purity of bone marrow cultures from naïve Balb/c mice grown for 7 days in medium supplemented with recombinant GM-CSF (rGM-CSF, 10 ng/ml) or 30% conditioned medium (CM) from GM-CSF-producing mouse B myeloma J558 cell cultures. The number of DCs obtained from these cultures was significantly higher when precursors were grown in J558 CM (2.1-3.0x106/mouse with 95% CD11c+ cells) than in rGM-CSF (0.6-1.8x106/mouse with 55% CD11c+ cells). We next characterized bone marrow cultures obtained from immunocompetent Balb/c naïve mice or mice bearing s.c. LM3 murine breast carcinomas. Cells were grown for 7 days in J558 CM, exhibiting similar yield between naïve (1.3-7.6 x106/mouse) vs tumor-bearing mouse bone marrow (0.6-5.0 x106/mouse). DCs from naïve or tumor bearing mice were then incubated with TLR9 agonist CpG1826 (10 μg/ml) and their activation status was assessed 48h later. Both cohorts of DCs exhibited comparable levels of IL-12 and IL-10 secretion, as assessed by ELISA, as well

as CD86 expression, as determined by flow cytometry. Our results suggest that DC precursors obtained from tumor-bearing mice retain the features of their normal counterparts.

51. (157) **SIRNA TARGETED KNOCK-DOWN OF NOX4 DECREASES MIGRATION IN AN ANAPLASTIC THYROID CARCINOMA CELL LINE.**

Romina Oglio, Luciano Rossich, Leonardo Salvarredi, Mario Alberto Pisarev, Guillermo Juvenal, Lisa Thomasz
National Atomic Energy Commission

BACKGROUND:
Overexpression of NADPH oxidase isoform 4 (NOX4) has been implicated in promoting cell survival, migration and invasion in many cancers. Anaplastic thyroid cancer (ATC) is one of the most lethal human malignancies. In the present study, we studied the effect of suppressing NOX4 by RNA silencing on the survival and migration on the ATC cell line, 8505C.
METHODS:
Small interfering RNA (siRNA) constructs targeting NOX4 were validated and used to develop clonal derivatives of the ATC cell line, 8505C. Cell viability was measured by MTT (thiazolyl blue tetrazolium bromide) assay. The wound healing assay was used to determine the migration of cells in culture. The expression of mesenchymal markers such as vimentin and E-cadherin was detected by Western blot. Transforming growth factor β1 (TGF-β1), FOXO1 and FOXO3 expression was determined by quantitative RT-PCR
RESULTS:
Targeting NOX4 expression in 8505C cells caused a 30 % decrease in migration and Western Blot analysis shows an increase of E-cadherin expression. However the cell viability has not changed. NOX4 siRNAs decreased mRNA levels of TGF-β1 (30%) and increased FOXO1 (20%) and FOXO3 (90%) mRNA expression.
CONCLUSION:
Targeting NOX4 in combination with other tumor-targeted drugs could be enhances the anticancer therapies in ATC.

52. (177) **4-METHYLUMBELLIFERONE EXERTS ANTI-TUMORAL EFFECTS IN A DEPENDENT AND INDEPENDENT MANNER TO HYALURONAN SYNTHESIS INHIBITION IN HUMAN GLIOBLASTOMA CELLS.**

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4-methylumbelliferone (4MU) is a non toxic coumarins derivative that shows anti-tumoral effects on several neoplasms. Despite being widely used as hyaluronan (HA) synthesis inhibitor, HA-independent effects have been recently reported. HA is the main glycosaminoglycan of extracellular matrix and it is strongly involved in tumor progression, favoring cell proliferation and migration, processes that are directly related with glioblastoma, the most common primary tumor of central nervous system. Given that current therapy for this type of cancer is ineffective and highly toxic, new drugs are required for glioblastoma treatment. Our hypothesis is that 4MU exerts anti-tumoral effects on glioblastoma as a consequence of dependent as well as independent mechanisms of hyaluronan synthesis. Therefore, the aim of this work was to evaluate the effect of 4MU, alone and in combination with hyaluronan, on cell migration and metabolic as well as metalloprotease (MMP) activity in LN229 and U251 human glioblastoma cell lines. Metabolic activity was evaluated by XTT assay, migration by wound healing assay, MMPs activity by zymography and cell death by FDA/PI using flow cytometry. 4MU reduced metabolic activity in a dose dependent manner in both cell lines ($p<0.05$), however this drug slightly modified the percentage of PI+ LN229 and U251 cells. Besides, 4MU decreased gap closure and MMP-2 activity in both cell lines ($p<0.05$). Furthermore, the addition of HA partially prevented the 4MU effect on migration and MMP-2 activity ($p<0.05$) without modifying metabolic activity respect to 4MU alone in LN229 and U251 cells. In conclusion, we demonstrate that 4MU anti-tumoral effects on glioblastoma cell migration and MMP-2 activity seem to be dependent of hyaluronan synthesis inhibition, while 4MU effect on metabolic activity showed signs of being independent of hyaluronan metabolism. Altogether, these results highlight the potential use of 4MU for glioblastoma therapy.

METABOLISMO Y NUTRICIÓN / METABOLISM AND NUTRITION 1

53. (227) **FATTY ACID COMPOSITION OF HUMAN EPICARDIAL ADIPOSE TISSUE: FROM LIPOPROTEINS TO ADIPOCYTES.**

Magali Barchuk, Veronica Miksztowicz, Jessica Luquez, Rosario Mecozzi, Graciela Lopez, Marta Aveldaño, Miguel Rubio, Laura Schreier, Maria del Carmen Fernandez Tome, Gabriela Berg
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Epicardial adipose tissue (EAT) is a visceral AT, surrounding and infiltrating myocardium and coronary arteries. An increase in EAT volume is directly related to coronary artery disease (CAD); this increase would be in part a consequence of greater fatty acids (FA) influx to adipocytes from lipoproteins, which hydrolysis is mediated by Lipoprotein Lipase (LPL). It is known that FA species in AT would determine its properties. To date, little is known about LPL behavior in EAT, and whether FA released from lipoproteins would determine its composition. Our aim was to evaluate LPL activity and FA composition in EAT, and their association to circulating lipoprotein characteristics and FA profile.
Methods: in EAT and subcutaneous AT (SAT) from patients undergoing coronary artery bypass graft (CAD, n=40) or valve replacement (No CAD, n=24) LPL activity was evaluated by a radiometric assay. Serum lipid profile and glucose were assessed. Circulating VLDL and HDL were isolated by ultracentrifugation and characterized in their lipid and protein composition. FA composition from EAT and lipoproteins were assessed by Gas Chromatography. The study was approved by the Ethic Committee of the Hospital de Clínicas, UBA.
Results: LPL activity was higher in EAT than in SAT in both groups ($p<0.001$). EAT LPL activity was higher in CAD compared to No-CAD ($p=0.01$). No differences were observed in VLDL and HDL characteristics between groups. EAT, but not SAT, LPL activity was inversely associated to VLDLs TG content ($R=-0.529$, $p=0.05$), mass ($R=-0.541$, $p=0.04$) and TG/protein index ($R=-0.691$, $p=0.05$). No differences in FA species was observed in EAT nor lipoproteins between groups, although FA pattern was conserved between EAT and lipoproteins.
Conclusion: The increase in EAT LPL activity in CAD would be responsible of VLDL catabolism, supplying FA to the tissue. Deeper knowledge in FA from EAT would help understand its behavior in CAD.

54. (232) **IS ENDOTHELIAL LIPASE A SUPPORTING ACTOR OF LIPOPROTEIN LIPASE? STUDY FROM THE GENE TO THE ACTIVITY IN AN OBESITY MODEL.**

Magali Barchuk, Veronica Miksztowicz, Ágata Carolina Cevey, Valeria Zago, Graciela Lopez, Rosario Mecozzi, Silvia Maria Friedman, Nora Goren, Celina Morales, Laura Schreier, Gabriela Berg
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Lipoprotein lipase (LPL) and endothelial lipase (EL) are involved in lipoproteins metabolism. In insulin-resistance (IR), with visceral adipose tissue (AT) expansion and cardiac steatosis, this enzymes behavior is altered. Peroxisome Proliferator Activated Receptors

(PPARs) and apoproteins (apo)CII and CIII could be partly responsible for these alterations. Aim: evaluate LPL and EL mRNA, protein levels and activity in AT and heart, association with lipoprotein profile and the role of PPARs and apoCs in an obesity model.
Methods: Male Wistar rats were fed with standard diet (Control,n=14) or High Fat Diet (HFD,n=14) during 14 weeks. Glucose, lipoprotein profile and IR markers were measured. Histological studies were performed in heart and epididymal AT. LPL and EL mRNA levels were assessed by RT-qPCR, proteins levels by Western Blot and enzymes activities by radiometric assays. Cardiac and AT PPARs expression were measured by Western Blot and hepatic apoCs mRNA by RT-qPCR. The study was approved by CICUAL-FFYB(UBA).
Results: In HFD, fat depots were observed in hearts, whereas AT presented higher adipocyte size. In heart and AT, no differences were found in EL mRNA levels between groups, while AT LPL mRNA and protein levels were decreased in HFD (p=0.04 and p=0.01,respectively), without differences in heart. In both tissues, EL protein levels and activity were increased (p=0.05 and p=0.001,respectively) and inversely associated with decreased LPL activity(heart: R=-0.64,p<0.001,AT: R=-0.53,p=0.004), being partially responsible for the atherogenic lipoprotein profile in HFD. PPARγ expression in AT was decreased in HFD, without differences in cardiac PPARδ expression and hepatic apoCs mRNA.
Conclusion: This is the first time that three levels of regulation of EL and LPL are reported. The increase in EL activity could be an alternative pathway for fatty acids release from lipoproteins and uptake in tissues with decreased LPL activity. In adipose tissue, PPARγ could be involved in enzymes regulation.

55. (249) **SATURATED FATTY ACIDS DIETS AND THE EFFECT OF SUPPLEMENTATION WITH DIFFERENT SOURCES OF OMEGA 3 FATTY ACIDS. STUDY IN EXPERIMENTAL MODEL.**

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A balanced and varied diet is important to maintain optimal health status and prevent diseases. Diet's fatty acids profile has an essential function as immune regulator. Thymus is a biological marker of nutritional disorders.
The objective was to analyze the effect of diet containing butter, as fat source, with and without the supplementation with omega 3, on serum and thymus' fatty acid profiles of growing rats. Weanling Wistar rats received during 10 days normocaloric diet; fat was provided by butter (B). The others groups received the same diet supplemented with 24mg/day of fish oil (BF) or chía oil (BCh). Control group (C) received diet AIN´93. Serum and thymus fatty acids profiles were determined by gas chromatography. Statistical analysis used ANOVA and Dunnett test. Results were (%Area):
SERUM: OLEIC B:17.21±1.68b BF:18.85±2.66b BCh:20.07±2.94b C:10.36±1.85a. LINOLEIC(LA) B:7.82±1.83b BF: 8.23±0.88b BCh:8.82±0.59b C:19.59±2.92a. Alfa-linolenic(ALA) B: 0.29±0.12c BF:0.30±0.10b BCh:0.73±0.23a C:0.94±0.28a. EPA B:0.95±0.21a BF: 2.23±1.06b BCh: 2.74±0.45b C: 0.93±0.33a. DHA: B:1.90±0.30a BF:3.67±1.11b BCh:3.44±0.73 b C:1.33±0.19a .
THYMUS: OLEIC B:22.32±5.21 BF:25.58±2.66 BCh:22.38±4.79 C:18.42±2.82. LA B:3.95±0.58b BF:3.60±0.87b BCh:4.83±0.45b C:10.86±2.23a. ALA B:0.33±0.05b BF:0.26±0.06b BCh:0.54±0.06a C:0.57±0.13a. EPA B:0.49±0.17a BF:0.51±0.15a BCh:0.89±0.29b C:0.50±0.10a. DHA: B:0.62±0.14a BF:0.66±0.17a BCh:0.85±0.17b C:0.58±0.17a. Media that didn't present a letter(a,b) in common, were different(p<0.01).
In sera, B, BF and BCh groups showed lower LA levels and higher oleic acid levels, compared to C. Only B and BF present low values of ALA. BF and BCh groups presented high levels of EPA and DHA. In thymus, B, BF and BCh groups showed lower levels of LA than C. ALA present the same behavior than sera. Only BCh group increased EPA and DHA.
The results suggest that dietary lipids provoked changes in serum and thymus fatty acids profiles and the supplementation with omega 3 fatty acid provided by chía oil is better than that provided by fish oil.

56. (273) **TRANS FATTY ACIDS INTAKE, INCORPORATION INTO CIRCULATING LIPIDS AND LIPID PROFILE MODIFICATION IN UNIVERSITY STUDENTS**

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The effects of high intake of industrial trans fatty acids (t-FAs) can have a high long-term impact on human health. These t-FAs produce among others, alterations in lipid profile: increase of triglycerides (TG), total cholesterol (TC), LDL-cholesterol and Lip-a in plasma, and decrease levels of HDL-cholesterol. These isomers can be measured by gas chromatography in serum or plasma, erythrocytes and adipose tissue (AT) as biomarkers of t-FAs intake. However, the availability of AT limits their use in epidemiological studies. Therefore blood samples, that are most available, are widely used. This study describes the correlation between recent t-FAs intake calculated by a 24 hours recall (R- 24 hours) and their incorporation into circulating lipids, as well as the association between t-FAs intake and lipid profile modification in 116 university students of Santa Fe, Argentina. It was observed that 72.2% of students exceeded 1% total daily caloric value (TDCV) recommended by World Health Organization (WHO) for t-FAs intake and there is a significant correlation ($p < 0.05$) between "total t-FAs intake" vs "total t-FAs in serum". In relation with lipid profile, the effect which showed the strongest association with t-FAs intake was the decreased levels of HDL-cholesterol, with an OR of 2.26 ($p = 0.23$), followed by increased TG (OR 2.01, $p = 0.53$), increased LDL-cholesterol (OR 1.80, $p = 0.27$) and finally, the increased CT (OR 1.34, $p = 0.67$). The incorporation of t-FAs into circulating lipids was confirmed. Regarding the deleterious effect of them on the lipid profile, a certain association could be observed although it was not significant. Therefore, chronic t-FAs intake should be evaluated. Although the results were not conclusive, it is very important to avoid the consumption of t-FAs from the first stages of life.

57. (282) **CDK4 INHIBITION IMPAIRS BROWNING OF INGUINAL ADIPOSE TISSUE**

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Browning of adipose tissue (AT) is the appearance of thermogenically competent adipocytes in white AT depots upon cold exposure. Here, we aimed to assess if browning is regulated by cyclin-dependent kinase 4 (CDK4) activity. To this end, CDK4 was inhibited with simultaneous browning stimulation. C57BL/6J male mice were gavaged CDK4 inhibitor (Palbociclib (PAL), 50mg/Kg) or vehicle for 10 days. At day 3, groups were subdivided: half remained at 22°C (PAL and CTR) and the others were housed at 4°C (PAL-C and CTR-C). Body weight and caloric intake were daily recorded. At day 10, mice were euthanized and inguinal AT (IAT) was collected. Besides, stroma vascular fraction of IAT from CTR and PAL groups was differentiated to adipocytes. At day 8 post-differentiation, a subgroup of CTR and PAL differentiated adipocytes was treated with Forskolin for thermogenesis stimulation (CTR-F and PAL-F). qPCR was used in all experiments for analysis of thermogenic markers (Ucp1, Pgc1a, Prdm16). Results showed no differences in body weight between groups, whereas cold induced a rise in caloric intake (CTR-C vs CTR and PAL-C vs PAL). In IAT, PAL reduced expression of Ucp1, Pgc1a and Prdm16 in both basal and browning stimulated conditions ($p<0,05$, PAL vs CTR and PAL-C vs CTR-C). Similarly, Ucp1 and Pgc1a were downregulated in differentiated adipocytes of PAL group in basal conditions ($p<0,05$ PAL vs CTR). Thermogenic stimulation of Pgc1a was also reduced by PAL ($p<0,05$ PAL-F vs CTR-F). To assess the direct effect of CDK4 inhibition in AT, matures and differentiated adipocytes from IAT of CTR animals were treated in vitro with PAL or DMSO for 48 hs. qPCR analysis showed a decreased expression of Ucp1 in PAL matures and differentiated adipocytes ($p<0,05$ PAL vs CTR). Overall, these results show that CDK4 inhi-

bition impairs browning of IAT in vivo and in vitro. PICT2015-2352.

58. (294) **HYDROXYTYROSOL SUPPLEMENTATION IMPROVES ALTERATIONS IN ADIPOSE TISSUE FROM MICE FED A HIGH-FAT DIET MODULATING THE TRANSCRIPTION FACTORS NRF2, NF-KB AND SREBP-1C**

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Adipose tissue (AT) have relevant metabolic and inflammatory functions. Overfeeding condition lead to significant increment in AT mass, accompanied by oxidative stress, pro-inflammatory status and depletion of n-3 long chain polyunsaturated fatty acid (LCPUFA). Hydroxytyrosol (HT) is a polyphenol with proven cytoprotective effects, able to confer antioxidant protection, promote the activation of transcription factors and expression of genes involved in redox homeostasis and inflammation. Therefore, the purpose of this study was evaluate the anti-oxidant, anti-inflammatory and anti-lipogenic effects of HT supplementation and the molecular adaptations involved, in AT from high-fat diet (HFD)-fed mice. Male C57BL/6J mice received: control diet (CD) (10% fat); CD+HT (daily doses: 5 mg/kg body weight), HFD (60% fat); or HFD+HT for 12 weeks, constituting 4 experimental groups. In AT was evaluated: (i) localized and total adipose mass; (ii) fatty acid composition; (iii) oxidative stress markers; (iv) glutathione levels, glutamate-cysteine ligase (GCL) and glutathione-S-transferase (GST) mRNA levels and activity of antioxidant enzymes; (v) tumor necrosis factor alpha (TNF-α) and interleukin 6 (IL-6) mRNA levels; (vi) binding activity and gene expression of transcription factors: nuclear factor erythroid 2–related factor 2 (Nrf2), nuclear-κB (NF-κB) and sterol regulatory element-binding protein 1c (SREBP-1c). HT supplementation mitigated the impairment observed in AT of HFD-fed mice: (i) decreased localized and total AT weight; (ii) preserved the n-3 LCPUFA levels; (iii) reduced the TBARS, F-2 isoprostanes and protein carbonyl levels; (iv) restored GSH and GSSG content, increased GCL and GST gene expression and augmented enzymatic activity of superoxide dismutase, catalase, glutathione peroxidase and reductase; (v) increased TNF-α and IL-6 mRNA levels; (vi) up-regulated the Nrf2 and down-regulated the NF-κB and SREBP 1c. HT supplementation improve the AT alterations induced by HDF in mice through the modulation of transcription factors as well as their target genes, involved in inflammation, antioxidant defenses and lipogenesis.

59. (393) **HORMONAL MODULATION OF CANALICULAR ABC TRANSPORTERS EXPRESSION IN A MURINE MODEL OF OBESITY INDUCED BY HIGH FAT DIET (HFD)**

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Obesity is a metabolic disease characterized by low grade inflammation as well as alterations in serum levels of different hormones. Prolactin (PRL) is a pleiotropic peptide hormone released from the lactotrophs of the anterior pituitary gland that participates at different levels to regulate the metabolism. Previous studies showed that PRL was able to induce biotransformation systems and some hepatic transporters. There is no consensus regarding the plasma levels existing in the different experimental models of obesity and insulin resistance, so in this work we aimed to evaluate the plasmatic levels of PRL and the expression of canalicular ABC transporters in a murine model of obesity induced by HFD. Five-week-old C57BL/6 wild type mice were fed with regular chow diet (CHOW) (n=4) or a 40% high fat diet (HFD) (n=4) for 16 weeks. PRL plasma levels were measured using an ELISA kit, and the results showed to be increased in HFD (CHOW: 50.5±11.4 pg/mL; HFD: 180.4±37.7 pg/mL; $p<0.01$). Canalicular expression of Breast Cancer Resistance Protein (Bcrp) and P-glycoprotein (P-gp) were higher in HFD (+103%; $p<0.001$ and +331%; $p<0.0001$, respectively), analyzed by western blot in enriched fractions of canalicular membranes. In line with this, evaluation by RT-qPCR exhibited an increase in mRNA levels of Bcrp (abcg2) in HFD (+34%; $p<0.001$), but there were no changes for the genes that encode the different P-gp isoforms mdr1a and mdr1b. Our results showed that HFD was able to increase plasmatic levels of PRL and canalicular expression of Bcrp and P-gp. In this regard, we suggest that the restoration of plasma levels of hormones such as PRL could contribute to normalize the expression and activity of metabolic and transport systems, with favorable consequences on the efficacy of drugs used by obese patients.

60. (404) **METABOLIC PROFILE ASSOCIATED WITH ALTERED GUT MICROBIOTA IN A HIGH FAT DIET-INDUCED ANIMAL MODEL.**

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Currently, the gut microbiota is recognized as an emerging factor of cardiometabolic risk. Changes in diet have a significant effect on the composition and function of the gut microbiota, which can contribute to the development of an atherogenic metabolic profile. However, metabolic alterations associated to intestinal dysbiosis induced by high fat diet (HFD), with special focus on intestinal chylomicrons (QM), have not been deeply studied. Methods and results: Twelve male Wistar rats (180-200 g) were fed with standard diet (Control, n=6) or standard diet plus 40% fat (HFD, n=6) throughout 14 weeks. The study was approved by the Comité Institucional para el Cuidado y Uso de Animales de Laboratorio (CICUAL)-FFYB-UBA. Glucose, free fatty acids (FFA), lipoprotein profile and lipopolysaccharide (LPS), as altered gut microbiota marker, were measured in serum. Isolated QM by ultracentrifugation ($d<0.95$g/ml) were characterized by chemical composition and Cholesterol/Triglycerides (chol/TG) index was calculated. Epididymal adipose tissue, visceral adipose tissue (VAT) estimator, was removed and weighted. Compared to Control, HFD showed higher LPS levels ($p=0.011$), TG ($p=0.030$), and non HDL-chol levels ($p=0.028$); as well as surrogate markers of insulin-resistance: TG/HDL-chol ($p=0.045$), FFA ($p<0.05$) and VAT ($p < 0.01$). No differences in glucose were observed. QM chemical composition of HFD showed a higher proportion of TG and chol/TG ratio ($p=0.001$ and $p=0.013$, respectively). LPS levels were directly associated to QM-TG ($r=0.74$;$p<0.05$), and non-HDL-chol ($r=0.72$;$p=0.04$) and inversely correlated with QM-chol/TG ratio ($r=-0.77$;$p=0.04$). Conclusion: intestinal dysbiosis induced by high fat diet promotes an increase in triglyceride over-enriched QM particles, which would constitute a better lipoprotein lipase substrate, generating smaller remnant particles of higher atherogenicity.

61. (255) **EFFECT OF A FUNCTIONAL MILK FAT ON BIOMARKERS OF HEPATIC OXIDATIVE STRESS IN RATS FED HIGH FAT LEVELS.**

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Risk of cardiovascular diseases has been associated with a consumption of high fat levels, including milk fat (MF) attributed mainly to the presence of high amounts of saturated fatty acids (SFA). Milk presents bioactive compounds with beneficial effects on health and, in addition, MF can be modified, reducing SFA and increasing bioactive FA such as conjugated linoleic acid and vaccenic acid leading to a functional milk fat (FMF). This modification can be achieved through modifications of the feeding of dairy cows with oils rich in polyunsaturated FA. The aim was to investigate some biomarkers involved in hepatic oxidative stress, in rats fed diets containing MF and FMF at high fat levels. Male Wistar rats were fed: S7 (soybean oil,7%), S30 (soybean oil, 30%), MF30 (soybean oil, 3%+MF27%) or FMF30 (soybean oil3%+FMF27%). After 60 days the following eval-

uations in liver were performed: levels of reactive oxygen species (ROS) by fluorometry; ratio reduced glutathione / oxidized glutathione (GSH/GSSG) by capillary electrophoresis; enzymatic activity of catalase (CAT), glutathione peroxidase (GSH-Px) and glutathione reductase (GR); lipoperoxidation (LPO) by kinetic and colorimetric methods. To estimate antioxidant capacity (AC), the concentration of Uric Acid (UA) in serum was measured. Statistical differences ($p<0.05$) were tested by ANOVA (1X3). ROS, LPO, GSH-Px and CAT increased (30%) in S30 and MF30 compared to S7. FMF30 showed similar values to S7. AC decreased in both groups (35%) with respect to S7, while for FMF30 group a AC value similar to S7 was found. GSH/GSSG ratio, decreased in S30, MF30 and FMF30 compared to S7. FMF30 did not improve this parameters . FMF30 was able to attenuate hepatic oxidative stress originated by consumption high levels of fat through the modification of biomarkers of hepatic oxidative state. In addition, FMF30 would improve AC, associated to attenuating the decrease of UA in plasma.

62. (192) **GHRELIN CONCENTRATION, GASTRIC PATHOLOGY AND NUTRITIONAL STATUS AFTER HELICOBACTER PYLORI ERADICATION**

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Ghrelin is an appetite modulating peptide mainly produced in the stomach. We have reported that gastric *Helicobacter pylori* infection was associated to lower ghrelin levels. We aimed to evaluate the effect of *H.pylori* eradication on gastric histopathology, serum ghrelin concentrations, appetite sensation and nutritional status. Dyspeptic adults referred for an upper-digestive-endoscopy were included. Gastric biopsies were obtained for histopathology and *H. pylori* diagnosis. We evaluated appetite sensation using a validated questionnaire and nutrient intake with 24h-dietary-recalls. We assessed weight and height. Ghrelin concentration was determined by ELISA. Eradication therapy was administered to *H. pylori* positive patients, who returned 12 weeks later. The protocol was approved by the Ethics Committee of the Hospital Udaondo. Statistical analysis was performed using the Proportion, Wilcoxon-Signed-Rank and Kruskal-Wallis Tests. We included 117 adults (43.3±12.6y). *H. pylori* infection prevalence was 68.4%(CI95%;59.5-76.1%). 47 patients returned for control. Eradication rate was 59.6%. Appetite sensation, nutrient intake and anthropometric status did not differ significantly after eradication. Ghrelin concentrations significantly decreased in eradicated patients [345.0pg/mL(IQR;373.0-517.8) before vs 298.5pg/mL(IQR;251.0-383.5) after-eradication, P=0.0007], but did not differ in those patients who remained infected (P=0.11). Severity of gastric pathology was lower after treatment in eradicated patients in antrum (P=0.00001) and corpus (P=0.00001), but remained unchanged in the uneradicated group (P=0.10 and P=0.53). Percent change in ghrelin concentration from baseline to follow-up did not differ significantly according to the anthropometric nutritional status (P=0.63) or the gastric pathology of the antrum (P=0.84) or corpus (P=0.50) at baseline; however, variation was significantly higher in eradicated patients with the *H. pylori cagA*+ genotype (P=0.011). Although anthropometric nutritional status and appetite sensation were not altered after *H. pylori* eradication, serum ghrelin concentration and severity of gastric pathology decreased significantly after treatment in *H. pylori* eradicated patients, with a higher ghrelin variation in patients carrying the more virulent strains.

63. (296) **EFFECT A YOGURT CONTAINING GALACTOOLIGOSACCHARIDES (GOS), SYNTHESIZED FROM MILK LACTOSE BY ENZYMATIC ACTION ON CALCIUM ABSORPTION (CAABS) AND RETENTION DURING GROWTH**

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GOS are natural prebiotics of human milk. In addition, GOS could be synthesized in dairy products, such as yogurt, by β-galactosidase action on lactose, giving a reduced-lactose product. GOS fermentation by the acid resistant gut microbiota produces short-chain fatty acid (SCFAs) that could favor CaAbs and Ca bone retention. The effect on CaAbs and retention of the yogurt containing GOS was evaluated during normal growth. Male weaning Wistar rats (n=10 per group) received during 30 days AIN´93-G control diet (CD) or the yogurt containing GOS diet (ED). Food consumption was evaluated three times per week; body weight (BW) and faecal Lactobacilli (LB) colonies weekly; CaAbs during the last 3 days of the experience and caecal pH, SCFAs and total skeleton bone mineral content (BMC) and bone mineral density (BMD) at the end of the study. Result (mean±SD). Food consumption and BW were similar between diets (16.6±2.7 vs. 16.4±0.6 g and 229±13 vs. 214±5 g, for ED and CD, respectively). ED compared to CD group showed an increased in faecal LB colonies ($p<0.05$) from the first week of experience; a significant lower caecal pH (5.2±0.1 vs. 6.8±0.1; $p<0.01$) and higher SCFAs production (propionic acid: 14.59±3.62 vs. 4.33±1.01 mg; butyric acid: 14.45±3.02 vs. 2.76±0.75 mg; $p<0.001$). CaAbs percentage was higher (84.8±1.4 vs. 78.3±9.4%; $p<0.05$) while BMC (1.258±0.052 vs. 1.195±0.069 g) and BMD (0.317±0.002 vs 0.309±0.004 g/cm2) showed no significant differences. Conclusion: The results evidence the functional characteristics of the assayed yogurt. Moreover, the higher CaAbs and the similar bone mass suggest a beneficial effect on bone health, especially in subject with lactose intolerance.

64. (175) **ANALYSIS OF CONCENTRATION OF PLASMA CHOLESTEROL AND EVALUATION OF HEMORHEOLOGICAL PARAMETERS IN HYPERLIPEMIC WISTAR RATS TREATED FOR 7 AND 10 DAYS WITH LIGARIA CUNEIFOLIA (LC)-PROANTOCIANIDIN ENRICHED FRACTION**

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We were demonstrated that Ligaria cuneifolia (Lc) crude extract increased blood viscosity and decreased plasma cholesterol in rats. In the present study, we analysed the effect of Lc- proanthocyanidin enriched fraction (PLc) on cholesterol (Cho) and blood fluidity in adult male Wistar rats (aged 70 days, n=24) fed with standard diet added with 40% bovine meat juice during 28 days (HFD). The rats were administered i.p. each 24hr during 7 and 10 days, with either physiological solution (controls C, C7 and C10, n=6 each one) or PLc 3mg /100g body weight (treated T; T7 and T10, n=6 each one), in day 8 and 11 they were anaesthetized i.p. with Ketamine/ Xylazine (100mg/kg/3mg/kg) to obtain blood samples by cardiac puncture. Plasma assays: Cho (enzymatic method with cholesterol oxidase esterase (ChoOE), TG, HDL-Cho, and LDL-Cho (enzymatics methods). Blood assays: rigidity index (RI) by filtration method, lipid dynamics and order the erythrocyte membrane were studied by electron paramagnetic resonance and the measured hyperfine parameter Amax was used to compare membrane fluidity between samples. Erythrocyte membrane cholesterol (ChoM), after hypotonic lysis and lipid extraction, was determined by ChoOE. Results: (mean ± SE). (C7 and C10 show no significant differences) Plasma: Cho(mg%): C:203.33±21.54; T7:119.50±28.53*; T10:109.83±11.14*; ChoHDL: C:22.40±1.66; T7: 21.25±1.70; T10:19.62±0.84; ChoLDL: C:28.30±1.68; T7 : 19.25±0.95*; T10:16.27±0.71*;T:133.00±9.68*; Blood: RI:C:6.37±0.47; T7: 6.52±0.23(n.s.) T10: 7.25±0.41(n.s.); Amax:C: 56.51± 0.16;T7: 56.67± 0.11(n.s.); T10: 56.77±0.13(n.s.);

ChoM: C: 115.66± 13.37; T7: 127±10.27(n.s.); T10: 132.17±7.84 (n.s.). (*p<0.05 vs. C; Student's t Test for unpaired data). The treatment with PLc shows a lipid-lowering effect, without any significant changes in the hemorheological parameters in rats fed with HFD. There are no significant differences between the two times of administration. We have obtained a fraction of the crude extract of Lc, which lowers the total Co and LDL-Co in plasm without altering the fluidity of the blood.

MEDICINA REGENERATIVA / REGENERATIVE MEDICINE 1

65. (606) **INDUCED PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTES PROLIFERATE BY EXPOSURE TO CONDITIONED MEDIUM FROM MESENCHYMAL STEM CELLS**

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In human heart, shortly after birth, cardiomyocytes (CM) undergo one last round of division followed by escape from cell cycle. Production of induced pluripotent stem cell-derived CM (iPSC-CM) is a potentially promising strategy for regenerative therapies. Cardiomyocytes in adult mammalian heart are characterized by lack of proliferation, thus shaping a growing interest in identifying factors relevant in the regulation of iPSC-CMs cell cycle. In recent years, accumulating evidences highlighted the regenerative properties of mesenchymal stem cells (MSC), a well-known type of multipotent cell. Particularly, their secretome has considerable pro-mitotic potential. Hence, the aim of this work was to study iPSC-CM ability to re-enter cell cycle after exposure to conditioned medium from MSC. iPSC-CMs were obtained by the implementation of a previously reported protocol and cell proliferation was monitored by stable introduction of a fluorescent ubiquitination cell cycle indicator (FUCCI) in iPSC (Fucci-iPSC). FUCCI construction consists of mCherry and mVenus fluorescent proteins fused to different regulators of cell cycle: cdt1 and geminin, respectively. Shift in color from red to green allow us to follow and quantify cell division events.
Fucci-iPSC-CMs were treated or not with 48h-conditioned medium for 24, 48 and 72h. Images were captured in fluorescent microscope. Number of green fluorescent cells increased from 15%±2.36 to 29%±12.76 in the first 24h and from 5%±2.94 to 23%±4.23 at 48h. Differential proliferation rates were only significant at 72h of treatment with conditioned medium, showing a change from 5%±2.8 to 23%±2.07 ($p<0.01$, $n=3$). Interestingly, conditioned medium maintained the proliferative state while replication activity decreases over time with control medium. Proliferative capacity was confirmed by incubation with EdU and subsequent analysis by immunofluorescence. Finally, analysis of images showed that cells were able to re-enter cell cycle with cytokinesis. Taken together, our data indicate that exposure to contidioned medium from MSC induces proliferation of iPSC-CMs.

66. (739) **CHARACTERIZATION OF LNCRNAS EXPRESSION PROFILE IN CARDIOMYOCYTES DERIVED FROM PLURIPOTENT STEM CELLS**

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Differentiation of human induced pluripotent stem cells (iPSC) into cardiomyocytes (CMs) is a resourceful approach for clinical and biological studies. Gaining insight into gene regulatory networks (GRN) that govern cardiac differentiation will allow a better understanding of cardiac development and commitment. Over the past ten years, long non-coding RNAs (lncRNAs), a heterogeneous group of non-coding transcripts, have gained importance as key modulators of these networks by fine tuning epigenetic, transcriptional and post-transcriptional expression, although their precise molecular mechanisms are not fully clarified.
This work focused on characterizing the expression profiles of lncRNAs along cardiac differentiation by high throughput RNA next generation sequencing.
CMs were generated from an iPSC line (FN2.1) developed in our laboratory by implementation and optimization of a previously reported protocol. Three cellular populations were studied: day 0 (pluripotent state); day 3.5 (early mesoderm progenitor, isolated by cell sorting); day 21 (immature cardiomyocyte, isolated by enrichment with selection medium - >80% CMs). Population identity was confirmed by qRT-PCR of specific gene markers and long non-coding markers previously reported in the literature. High-quality, total RNA was purified from three independent experiments on the three populations mentioned before and subjected to deep sequencing (~50 million 100bp-long pair-end reads) on Illumina platform. Initial assessment of data revealed homogeneous FPKM distribution and high interreplicate correlation indices (d0: r=0.90; d3.5: r=0.9; d21: r=0.85; p<2.2e-16). Next, we aligned good-quality reads to a reference genome with STAR followed by identification of lncRNA expression profiles (~5000 lncRNA genes). Conclusively, we identified distinctive clusters of lncRNAs with specific behavioural patterns across cellular populations. Our next step will be the study of epigenetic marks that give insight on the functions of differentially expressed lncRNAs in cardiac differentiation, to discern those with most compelling roles, such as enhancers and super-enhancers.

67. (756) **MIR-520A INDUCE RE-ENTER IN S PHASE OF PLURIPOTENT STEM CELL DERIVED CARDIOMYOCYTES**

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Production of induced pluripotent stem cells derived cardiomyocytes (iPSC-CM) and controlling their proliferation is a potentially promising strategy for regenerative therapies. MicroRNAs (miRNAs) are key regulators of gene expression at the post-transcriptional level and play essential roles in diverse biological processes. A role for miRNA in cardiomyocyte proliferation and regeneration was revealed in several studies. Forced overexpression of certain synthetic miRNAs can promote CM proliferation. The aim of this study was to analyze the ability of iPSC-CMs to re-enter cell cycle after exposure to miR520a-mimic. We generated iPSC with cell cycle indicator Fucci. Fucci-iPSCs were constructed by a transposable recombination approach, using a system that employs both a red (mCherry) and a green (mVenus) fluorescent protein fused to different regulators of the cell cycle: cdt1 (G1) and geminin (S/G2/M), respectively. iPSC-CMs were obtained by the implementation of a previously reported protocol (Lian *et al.* 2012). We performed an Ago-IP in iPSC-CMs transfected with 10nM miR-520a mimic or 10nM negative control-mimic. That experiment showed 152 genes differentially expressed. Gene-Ontology analysis revealed mRNAs involved in the regulation of microtubule polymerization or depolymerization and mitotic cell cycle (GO:0000278). For example, SUGT1 and SKA2 are genes that encode proteins involved in kinetochore function and required for the G1/S and G2/M transitions. Then, iPSC-CMs were transfected with miR-520a-mimic or a control-mimic and cells proliferation was observed at 48hs. Our results show that only a small proportion of iPSC-CMs were cycling in control condition (~3%), but this percentage increased when exposed to miR-520a-mimic (>10%). iPSC-CMs were able to duplicate their DNA and re-enter S phase, but generated multinucleated cells. With control-mimic we observed 6% of multinucleated cells, while with miR-520a-mimic they increase to 12%. Taken together, our data indicate that miR-520a-mimic let IPSC-CMs re-enter cell cycle without cytokinesis.

68. (408) **SUITABILITY OF A BACULOVIRAL VECTOR FOR**

GENE DELIVERY IN A RABBIT MODEL OF PERIPHERAL ARTERY DISEASE. PRELIMINARY RESULTS

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Introduction. Peripheral artery disease (PAD) is a condition with no effective treatment. Gene therapy emerged as a possible strategy to restore blood flow to the ischemic zone. We hypothesize that baculovirus (Bv), a virus of insects not pathogenic to humans, is a suitable vector for angiogenic gene delivery in PAD. Objectives. To study the transduction efficiency (TE) in skeletal myoblasts (Msk) of rabbit hindlimb and assess the virus presence and expression profile in ischemic muscle of rabbits with PAD at different times. Methods. Msk were isolated from biopsies and cultured on a feeder layer of autologous macrophages. Then, Msk were transduced at different multiplicities of infection (MOI) with Bv encoding the green fluorescent protein (GFP) reporter gene (Bv.pCMV.GFP). TE was measured by flow cytometry. Besides, 6 rabbits with PAD were injected with 109 viral particles of Bv.pCMV.GFP and sacrificed at 3, 7 and 14 days (n=2 per time). Three additional animals injected with PBS (n=1 per time) were used as control. Injected limb biopsies were harvested and the presence of viral DNA or GFP mRNA was analyzed by RT-qPCR. GFP protein was observed by fluorescence microscopy. Results. TE was 0.3%, 9.7%, 24.2%, 43.3%, 59.4% and 74.4% for MOI 0, 25, 50, 100, 200 and 500, respectively. Viral DNA was detectable at 3 and 7 days but not at 14 days after injection. Similar profile was observed for GFP mRNA expression. GFP protein was detectable at all 3 time points post-injection. Conclusion. These preliminary results suggest that, on account of its TE and low virus persistence in the tissue, Bv is a suitable vector for angiogenic genes delivery in this model of PAD. Further safety studies to detect undesired expression in remote organs remain to be performed.

69. (625) **SCREENING OF OVINE CARDIOMYOCYTE CELL CYCLE REGULATORS AS POSSIBLE TARGETS FOR CARDIAC REGENERATION**

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Ischemic heart disease, including acute myocardial infarction, is the main cause of death worldwide. Considering the poor results of clinical trials on cardiac regeneration using stem cells, there is a growing interest in developing gene therapies aimed at inducing the adult cardiomyocyte (CM) mitosis, an approach that would warrant efficient electromechanical coupling of new cells to the myocardial sincitium. Hence, disclosing novel CM cell cycle mediators that could be targeted with these therapies is essential. Thus, our objective was to analyze the ovine CM transcriptome and compare the expression levels of cell cycle regulator's mRNAs in fetal sheep at two time points of gestation [90 days, when the CM is still mitotic (F90), and 130 days, when the CM has exited the cell cycle (F130)] and adults (A).
Fetal (F90, n=3 and F130, n=3) and A (n=3) sheep were euthanized and samples from the left ventricle were collected. Total RNA isolation was performed and mRNA was purified. Samples were delivered to Macrogen Inc. for cDNA library construction and Next Generation Sequencing. Raw RNAseq reads of the three experimental groups were quantified and classified using bioinformatic tools according to expression levels and proposed function. Several genes related to cell cycle regulation showing significant expression differences were selected and their expression levels re-evaluated by Real Time PCR. For statistical analysis we used ANOVA-Bonferroni (significance: $p < 0.05$).
We detected 30024 mRNAs (1641 expressed only in F90, 1506 only in F130 and 655 in A). The cell-cycle genes diferentially expressed in all groups (meis2, meis3, p16, p21, nkx 2.5, gata4, cdk2, cdk4, cdk5, cyclinE1, cyclinD, cyclinA, hgf, sav1, vegfA and yap1) showed expression levels in Real Time in accordance with RNAseq.
The group of evaluated genes may be possible targets for studies aimed at modulating the cardiomyocyte cell cycle with a regenerative approach.

70. (742) **MYOCARDIAL HOMING, DIFFERENTIATION AND ANGIOGENIC EFFECT OF HUMAN MUSE CELLS XENOTRANSPLANTED IN NON-IMMUNOSUPPRESSED SHEEP WITH ACUTE MYOCARDIAL INFARCTION.**

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Introduction. Multilineage differentiating stress-enduring (Muse) cells are non-tumorigenic endogenous pluripotent-like stem cells. Recently it was shown that human Muse cells (hMuse) were mobilized into the peripheral blood in patients with acute myocardial infarction (AMI). In addition, the therapeutic efficiency of xenotransplanting hMuse in non-immunosuppressed rabbits with AMI was recently demonstrated. However, this novel approach has not been tested in a large mammalian model closer to the clinical setting. Thus we examined the retention of hMuse 7 days after xenotransplantation, their ability to induce angiogenesis and their capacity of differentiation into myocardiocytes, in sheep with AMI. Methods. Under ethical approval and written informed consent, hMuse were isolated from abdominal lipoaspirates using severe cellular stress procedure. hMuse were stained with PKH26 Red Fluorescent Dye for in vivo tracking. Ten million pre-stained hMuse were delivered in 10 intramyocardial injections in the peri-infarct zone (hMuse group, n=4). Additional sheep received PBS (Placebo, n=4). One week later, animals were sacrificed, hMuse were tracked, and capillary and arteriolar densities were measured in representative myocardial samples (immunohistochemistry). Results: Confocal microscopy revealed the presence of fluorescent cells in hMuse group, located mainly in the infarct border. Arteriolar density was higher in hMuse group than placebo (21.3±2.1 vs. 10.4±1 arterioles/mm2, $p<0.01$, X±SD, t-Test). Capillary density was higher in hMuse group than placebo (852.7±89.6 vs. 443.9±47.87 cap/mm2, $p<0.01$, X±SD, t-Test). Some of the fluorescent cells were lightly positive for cardiac markers such as sarcomeric α-actinin, desmin but negative for connexin 43, suggesting ongoing differentiation after homing. Conclusions: Human Muse cells xenotransplanted in immunocompetent sheep with AMI are retained in the target tissue, induce angio-arteriogenesis and exhibit early signs of differentiation into cardiomyocytes. Further studies assessing potential effects on infarct size limitation and left ventricular function improvement are needed. Keywords: AMI; human Muse cells; angiogenesis

71. (762) **EXPRESSION PROFILE OF TBX20 AND OTHER CELL CYCLE REGULATOR GENES AT DIFFERENT PROLIFERATE STAGES OF NEONATAL RAT CARDIOMYOCYTE CULTURES AND POST-NATAL RAT MYOCARDIUM**

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Background and objectives: To induce cardiac regeneration with preservation of the physiologic electro-mechanical connection between contractile cells, the best approach would be to induce the adult cardiomyocyte to re-enter the cell cycle and divide into daughter cells. This requires disclosing positive and negative cell cycle regulator molecules that could be targeted to induce cardiomyocyte mitosis and eventually cytokinesis. In this regard, it has been recently

reported that transgenic mice overexpressing Tbx20 displayed cardiomyocyte proliferation. We thus aimed at analyzing the expression of Tbx20 and other cell cycle regulatory genes at different proliferative stages of neonatal cardiomyocyte cultures and rat myocardium. Methods: Using the MTS assay, we first assessed cell proliferation in 2, 5 and 9 days old cultures of neonatal rat cardiomyocytes. Gene expression of the cell cycle stimulator genes Tbx20, Cyclin D1 and repressor genes p21 and p27 was evaluated (RT-qPCR) in 2, 5 and 9 days post-natal cardiomyocytes and in the myocardium of days 2, 5 and 9 post-natal rats and adults. Results: cardiomyocyte proliferation was maximal at day 5 of culture (3.87±0.14 area under the curve) vs day 2 (1.56±0.02AUC) and day 9 (1.59±0.1, $p<0.001$, one-way ANOVA-Bonferroni). Consistently, expression of Tbx20 and Cyclin D1 (stimulator genes) was maximal at day 5 and decreased at day 9, and expression of p21 and p27 (repressor genes) displayed the opposite behavior. As concerns gene expression in myocardium, Tbx20 and Cyclin D1 was highest at postnatal day 2 and declined overtime being minimal in the adults. Conclusion: In proliferative stages Tbx20 and Cyclin D1 expression peaks, while p21 and p27 decrease. These results confirm that Tbx20 is a positive cell cycle regulator and its overexpression may be potentially useful to encourage adult cardiomyocytes to reenter the cell cycle.

72. (138) **IN VIVO EVALUATION OF A CHISTOSAN-BASED SCAFFOLD FOR BONE TISSUE ENGINEERING**

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As an effort to find new biomaterials with improved biocompatibility properties, to be used on bone regeneration for large defects our group have previously developed a scaffold derived from a fumaric - polymer combined with chitosan (PVF-CHI-B). The in vitro studies showed that PVF-CHI-B was a noticeable candidate to extend the studies to an in vivo model, since it presented very low cytotoxicity and excellent osteoinduction [Lastra et al. 2017, Macromolecular Bioscience 17 (5)]. The objective of the present work was to study their biocompatibility, osteoinduction and cytotoxicity using an in vivo model of bone regeneration. Methods: PVF-CHI-B scaffolds were obtained by lyophilization and cutted into 2 mm round pieces; a circular craniotomy (2 mm diameter) was made in each parietal bone of WKAH / Hok rats. The sterilized biomaterial was placed into the right defect, while the left lesion without scaffold was an internal control of regeneration. After 30 day post surgery, animals were sacrificed, parietal bone were dissected and processed for bone histomorfometric analysis (bone regeneration, wound stabilization, limphocyte infiltracion), after hematoxilin/ eosin stain. Results: Although there were no differences in the percentage of bone regeneration between the wound with and without the scaffold, the morphological analysis showed that the stabilization of the lesion (assessed as the presence of fibrous connective tissue) was better in the presence of PVF-CHI-B than in control bone lesion. We also found no limphocyte infiltration. On the other hand, the scaffold was partially filled with fibroblastic-like cells indication integration with surrounding tissues. Conclusion: PVF-CHI-B was a suitable biomaterial to be used in bone tissue regeneration.

73. (208) **CRISPR-ON SYSTEM FOR THE ACTIVATION OF ENDOGENOUS HUMAN ATOH1, POU4F3, SOX2 AND GFI1 INNER EAR GENES: BASES FOR A POSSIBLE CELL REPLACEMENT THERAPY IN HEARING LOSS.**

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Hearing loss is a major issue in human health. Inner ear hair cells are the main sensory receptors responsible for hearing. Defects in hair cells are one of the major causes of deafness. The new CRISPR-on technology may provide an attractive cell-based strategy to regenerate hair cells and treat deafness in humans. The CRISPR-on system is a RNA-guided system that can turn on specific endogenous gene expression. This consists of the inactive DNA nuclease Cas9 (dCas9) fused to activation domains and co-expressed single guide RNAs (sgRNAs) that are designed to hybridize a target sequence and activates a specific gene. Here, we aimed to determine whether the CRISPR-on system fused with transcriptional activators (dCas9-VP160) could activate gene expression of inner ear hair cells genes (ATOH1, POU4F3, SOX2 AND GFI1) on HEK293T cell line and human mesenchymal stem cells (hMSC). Therefore, four sgRNAs were designed to target proximal promoter for activation of each gene using CRISPR Design Tool (Feng Zhang Lab, MIT). The sgRNAs target sequences were cloned on the sgRNA expression plasmid (Addgene #47108). The dCas9-VP160 plasmids (Addgene #48226) were transfected at a mass ratio of 1:1 to a mixture of equal amounts of the different sgRNAs expression plasmids. Control group cells were co-transfected with the dCas9-VP160 plasmid and an empty guide-plasmid. By RT-qPCR at day 4 post-transfection we observed that the CRISPR-VP160 system significantly activated SOX2, ATOH1 and POU4F3 in HEK293 ($p<0.05$). We did not detect a significant activation of GFI1, maybe due to the preexisting expression of this gene in that cell line. The hMSC were characterized by immunocytochemistry and flow cytometry. We are currently focused on improving different lipo transfection methodologies on these cells. In the future, the CRISPR-VP160 system could be applied to improve reprogramming strategies of cells, to develop new cellular therapies in hearing loss patients.

74. (302) **ENDOTHELIAL REGENERATION OF SPLENIC SCAFFOLD WITH HUVEC CELLS.**

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Tissue engineering has had a great boom in the last decade. The possibility of generating bioartificial organs has been demonstrated with the ultimate goal of solving lack of organs for transplantation. Here we present a bioartificial spleen project that will allow the evaluation of drugs, both, in their biological efficacy and in their immunogenic potential. Rat spleens (n=10) were decellularized with 1% SDS (2 ml/min) solution admistrated by the splenic artery for 18 h, then 0.1 % Triton X-100 for 18 h (2ml/min) was perfused to remove DNA, finally spleen were perfused with saline solution. Our decellulation protocol did not show any cellular components (H&E and DAPI) but preserved the extracellular matrix architecture. We also evaluate by fluorescence the presence of laminin and fibronectin in the matrix and did not found any difference with native spleen. Developing the vascular system before developing the rest of the tissue is critical in any bioartificial organ for these reason 2.107 HUVEC cells were administered by the splenic artery and could be maintained under culture conditions for 6 days with medium with growth factors for endothelial cells (1 ml/min). It was possible to observe both by H&E and by CD31/DAPI staining that viable cells are present at 6 days in the scaffold. Although the degree of coverage was low, we believe that by increasing the number of cells seeded we could achieve a better result. Our next steps in this line of work, considering that the spleen is an organ of the immune system itself, will be to evaluate recellularization with hematopoietic stem cells, peripheral blood mononuclear cells and isolated populations of lymphocytes and monocytes. The present study demonstrates that spleen scaffold can be prepared successfully and can be use for endothelial cells culture.

Farmacología / Pharmacology 1

75. (356) **A SUNFLOWER MANNOSE-BINDING LECTIN MODULATES THE RELEASE OF CYTOKINES FROM HUMAN PERIPHERAL BLOOD MONONUCLEAR CELLS.**

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The immunomodulatory therapies are based in the stimulation of specific host immune responses against microbes rather than target microbe viability or virulence. Thus, induction of the immune system can be useful in the treatment of infectious processes. Changes in the production of cytokines promoted by treatment with synthetic drugs or natural compounds are essential for the activation and recruitment of the immune response. Among the natural products, plant lectins are known as potent immunomodulatory agents, able to act in both the innate and adaptive immune system. They modulate the production of cytokines and other mediators of immune response (such as reactive oxygen and nitrogen species), improving the defenses against microbes. We have previously isolated a sunflower mannose-binding lectin of the jacalin family, which was called Helja (Helianthus annuus jacalin). The purpose of this study was to assess the effect of Helja on the activation of human peripheral blood mononuclear cells (PBMCs) with focus on the production of inflammatory mediators. Human PBMCs were incubated with Helja for 24 h and the cytokines TNFα, IL-1β, IL-6 and IL-10 were quantified by ELISA technique. Helja (1 and 10 *μg*/ml) showed the ability to induce the release of pro-inflammatory cytokines TNF-α, IL-1β and IL-6; and the anti-inflammatory cytokine IL-10. Thus, Helja displayed a modulatory profile compatible with a polyclonal activation, since both the levels of proinflammatory cytokines and suppressors of inflammation were increased. These preliminary results point out the immunomodulatory activity of Helja and its potential application as pharmacological tool.

76. (369) **THE BINDING OF A SUNFLOWER LECTIN TO CANDIDA ALBICANS CELL WALL: THE KEY TO ITS ANTIFUNGAL ACTIVITY?**

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Candida albicans (Ca) is a major fungal pathogen causing systemic infections in immunosuppressed patients. High mortality rates induced by candidiasis are usually associated with the development of biofilms. The cell wall of Ca is composed of an outer layer containing mannoproteins, which are essential for the adhesion to host cells and biofilm formation. In addition, non-specific unions attributed to hydrophobic forces, have been implicated in the adhesion to biotic and abiotic surfaces. We have previously isolated a sunflower mannose-binding lectin called Helja that exerts antifungal effect, antibiofilm activity and reduction of the adherence of Ca to primary host cells from buccal epithelium. The aim of this work was to investigate the interaction of the lectin with yeast cell surface. Therefore, to assess whether Helja (0.1 μg/μl) induces changes in the cell surface hydrophobicity (CSH), the method of microbial adhesion to hydrocarbons was used. Interestingly, the CSH of the yeast cells was drastically reduced from 40 % in the controls to 4 % in Helja treated cells. To explore the direct interaction of the lectin with C. albicans cells, Helja was conjugated to fluorescein isothiocyanate (FITC) and monitored by confocal laser scanning microscopy. The cells were completely labeled with the green fluorescence of Helja-FITC, revealing the binding of the protein to the fungal cell surface. Simultaneous staining with the cell wall marker Calcofluor White showed the Helja-FITC fluorescence as a thin and outer layer relative to the CFW signal, which was abolished after mannose addition. These results point out that the mode of action of Helja would be based on the binding to the mannoproteins of the Ca cell wall, which could limit the exposure of both, the mannoproteins and the hydrophobic residues necessary for cell-cell adhesion and consequent establishment of biofilm.

77. (402) **PARAMETERS OF OXIDATIVE STRESS IN SALIVA FROM DOWN SYNDROME PATIENTS WITH PERIODONTITIS ARE RELATED WITH PERIODONTAL STATUS.**

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Down syndrome (DS) is a genetic disorder associated with increased risk for a number of systemic conditions. Regarding dental care, Down syndrome patients (DSP) characteristically have increased gingivitis and periodontal disease (PD). PD is a pathologic inflammatory condition due to bacterial colonization that triggers a host pathogen defense mechanism leading to the destruction of the soft and hard tissues. There is greater oxidative damage in the advanced phases of PD and an increase in the number of reactive-oxygen species (ROS). The deterioration caused by free radicals is regulated by an antioxidant defense system (oxidative stress index: oxidant/antioxidant).
The aim of this study was to investigate the contribution of oxidative stress in the pathology of periodontitis and its relation with the clinical periodontal status in the saliva of DSP.
Methods: Study group: 35 adult patients with DS, Control group with periodontitis (CG): 35 healthy individuals; age range: 19-51 years for both groups. Presence and severity of PD (attachment level, pocket depth, and bleeding index) and panoramic radiography were assessed. Samples of total saliva were taken at rest. TRAP, which represents the capacity of all antioxidants to neutralize free radicals, and the production of ROS were measured as described by Lissi et al. and by a chemiluminescence assay using luminol, respectively.
Results: Three groups were found according PD status: mild, moderate and severe periodontitis. DSP presented 20%, 23.3% and 56.6% of mild, moderate and severe PD compared to 53.3%, 26.6% and 20% of the CG patients, respectively ($p<0.0001$). ROS values (oxidant species) were significantly higher in DSP saliva sample compared with control subjects ($p<0,001$); whereas TRAP (antioxidant capacity) measurements showed a significant decrease in the saliva of DSP ($p<0,001$).
Conclusion: DSP are more vulnerable to oxidative stress as indicated by the relationship oxidative/antioxidative measurements in saliva and the critical periodontal status.

78. (481) **PHARMACOLOGICAL POTENTIAL OF ANTIOXIDANT POLYPHENOLS FROM POTATO PEEL WASTE**

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Oxidative stress is associated with many pathologies going from cancer to neurodegenerative diseases, and antioxidants such as polyphenols have an impact on them. Potato peel waste (PPW) is the major leftover from the potato processing. We have previously demonstrated that the total level of phenolic compounds was up two to four times more in peel compared to flesh, which is correlated with a major antioxidant activity. The aim of this study was to characterize the phenolic composition and biological activities of antioxidant polyphenols from PPW in vitro. The HPLC-DAD analysis of PPW extracts indicated the presence of three isoforms of chlorogenic acid (CGA), 5-CGA, 4-CGA (cryptochlorogenic) and 3-CGA (neoclorogenic), being 5-CGA the major one. Caffeic acid was also detected, although as a minor component. The content of total phenols of PPW extract was 2650.00 ± 1092.26 μg eq. CGA.g^{-1} dry weight determined by the Folin-Ciocalteu method, and the antioxidant capacity was 2113.94 ± 1089.89 μg eq. Trolox.g^{-1} dry weight. In order to test the biological activities, we assayed different doses of PPW extract on a human neuroblastoma cell line or neuronal differentiated cells injured by hydrogen peroxide (H_2O_2) or amyloid peptide ($A\beta_{25\text{-}35}$). First, we observed a dose-dependent cytotoxic effect on the human neuroblastoma cell line, where doses greater than 20 μg eq. CGA.mL^{-1} induced a significant decrease in the cell viability of the tumor cells ($p=0,0063$). Then, pretreatment with low doses of PPW extracts was observed to protect injured-H_2O_2 or $A\beta_{25\text{-}35}$ neuronal differentiated cells. These findings demonstrated a dual effect of polyphenols from PPW, where the phenolic compounds quantity and the cellular physiology are important to induce different effects. Our results suggest that potato peel waste would be a good source of neuroprotective antioxidants polyphenols to development a dietary

supplement with some impact in human health.

79. (534) **ALPHA-1 ANTITRYPSIN DECREASES CX43, NFKB AND AS160 EXPRESSION THROUGH PI3K/AKT PATHWAY IN ARPE-19 CELLS EXPOSED TO HIGH GLYCEMIA**

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Diabetic retinopathy (DR) is associated with persistent inflammation and with damage to the vascular bed. The ophthalmic therapy for this retinal disease is focused on severe stages of the illness. Previous results obtained by our group show that Alpha-1 Antitrypsin (A1AT) acts like an anti-inflammatory agent that could play a role on DR treatment. However, it is important to know the effect of A1AT on proteins that are relevant to retinal function and the molecular mechanisms involved. The retinal pigment epithelium (RPE) forms the outer component of the blood-retinal barrier. Connexin43 is a major gap junction protein expressed in RPE cells. Cx43 upregulation have been implicated in edema and loss of vascular integrity, leading to neuronal death. AS160 is involved in insulin signaling affecting GLUT proteins.
We evaluated Cx43, NFkB and AS160 expression and proteins implicated in different signaling pathways in an in vitro diabetic retinopathy cell model.
ARPE-19 cells (ATCC® CRL-2302TM, USA) were maintained in DMEM/F12 (Invitrogen, USA) containing 2µM L-glutamine, 100U/ml penicillin, 100µg/ml streptomycin, and 10% fetal bovine serum. ARPE-19 cells (passages 9-12) were incubated 16h with DMEM 5,5mM glucose (Control), DMEM 5,5mM glucose + 4.5mg/ml A1AT (Control + A1AT), DMEM 30mM glucose (Diabetic), DMEM 30mM glucose + 4.5mg/ml A1AT (Diabetic + A1AT). Cells were harvested with RIPA for Western blot or fixed for immunohistochemistry.
A1AT diminished levels of Cx43 and AS160, A1AT also reduces AKT and pAKT1/2/3 expression levels, indicating PI3K/AKT pathway participation, and a possible crosstalk with Wnt and Insulin signaling. Besides, we could also observe a lower expression of NFkB p65 and iNOS, both proteins involved in the inflammatory response.
Results support the hypothesis that A1AT regulates Cx43 and AS160 expression through PI3K/AKT, Wnt and Insulin signaling pathway. Taking together, these results indicate that A1AT is a promising molecule to treat DR.

80. (751) **ADESMIA BORONIOIDES AND SOLIDAGO CHILENSIS, TWO NOVELS HERBAL INFUSIONS WITH TOXIC EFFECTS AGAINST COLON CANCER DERIVED CELLS**

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The development and progression of colon cancer is strongly influenced by diet substances that enter in the digestive tract. Herbal infusions from medicinal plants usually contains phytochemicals that can restrain the development and progression of colon cancer in various ways. Flavonoids, an important group of these phytochemicals, report a recognized anti-inflammatory, antioxidant and signal-regulating properties. Adesmia boronioides and Solidago chilensis are two native medicinal plants that contain flavonoids and have reported promising antiproliferative activity against T-84 cells.
Our aim was to study the toxic effects of herbal infusions obtained from A. boronioides and S. chilensis on Caco-2 and HT-29 cells as models of colon cancer.
We observed by MTT assay (after 72 h) that the percentage of viable cells decreased with the increase in the concentration of freeze-dried infusions of both plant species (0 to 50 mg/ml) ($p<0.05$, n= 3). S. chilensis had a higher antiproliferative effect (EC50 (mg/ml): 0.57±0.06 and 0.18±0.02) in comparison with A. boronioides (EC50 (mg/ml): 1.27±0.08 and 2.87±0.21), for Caco-2 and HT-29 cells, respectively. Colchicine was used as positive control. Similar results were obtained by Trypan blue exclusion technique ($p<0.05$, n= 3). After staining the cells with AO and Et/Br, apoptosis cells (orange cells) were observed under the fluorescence microscope. The basal apoptotic percentage (24h) was increased in A. boronioides (35.0±4.1 and 46.2±8.2%) and in S. chilensis (47.2±6.7 and 35.8±4.0%) with respect to control (3.8±4.9 and 2.7±3.1%) in Caco-2 and HT-29, respectively ($p<0.05$, n=3). The Procaspase-3 expression was also checked.
We conclude that the infusion of the both species exert strong antiproliferative activity on cells derived from colon cancer, partly due to the modulation of basal apoptosis. Its effects show to be much greater in comparison with other species studied. These results provide a direction for further researches about the antitumoral potential of these native plants.

81. (570) **DEVELOPMENT OF THERAPEUTIC IMMUNORADIOPHARMACEUTICALS BASED ON CAMELID NANOANTIBODIES (VHH) AGAINST EGFR**

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Radioimmunotherapy (RIT) is a type of cancer cell targeting therapy which uses monoclonal antibodies against tumor-associated antigens labeled with radionuclide. The epidermal growth factor receptor (EGFR) is often overexpressed in various types of human cancers, for which it is a good antigen to be used in RIT. Lutetium-177 (Lu-177) has a half-life of 6,7 days and a maximum negative beta emission of 497 KeV. Hence, this radionuclide is ideal for therapeutic radiopharmaceuticals developments. The aim of this work is to develop novels Lu-177 radiopharmaceuticals based in camelid nanobodies or VHH. We propose to label a VHH against EGFR (VHH-EGFR) with Lu-177 to treatment EGFR(+) tumors. At this stage, we have obtained VHH-EGFR and labeled it with Lu-177, which was generated in the Centro Atómico Ezeiza. Previously, VHH-EGFR was conjugated to the bifunctional chelating agent pSCN-Bn-DTPA in order to label it by Lu-177. The specific activity of 177Lu-VHH-EGFR radiopharmaceutical was of 2,11 mCi/mg and the radiochemical purity was of 99,2% at the time of purification and 80,6% seven days later. We used different EGFR (+) or EGFR (-) human tumor cell lines in order to prove the selectively of our radiopharmaceutical. In addition, cells were blocked for 2 h with cold Cetuximab (monoclonal antibody against EGFR) as negative control. After that, cells were exposed to radiopharmaceutical for 4 and 24h at 37 °C. Fractions of non-internalized (supernatant and washed with PBS) and cell-associated (washed with trypsin) were collected. The fractions were manually measured in a well-glass radiometric detector. As conclusion we obtain 177Lu-VHH-EGFR with high specific activity and high radiochemical purity that was able to bind selectively to EGFR(+) cells lines. In future experiments, we will advance improving the affinity of the VHH-EGFR, obtaining a bivalent VHH-EGFR, which will also be labeled with Lu-177 and compared with the monovalent VHH-EGFR.

INMUNOLOGÍA / IMMUNOLOGY 1

82. (27) **IMMUNE-MEDIATED INFLAMMATION PROMOTES SUBCLINICAL ATHEROSCLEROSIS IN RECENT-ONSET PSORIATIC ARTHRITIS PATIENTS WITHOUT CONVENTIONAL CARDIOVASCULAR RISK FACTORS**

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Objectives. To evaluate markers of cardiovascular risk in cutaneous psoriasis (CPs) and recent-onset PsA patients.
Methods. Men and women 25-75 years old attending the Hospital JM Ramos Mejía (2012-2016) were recruited. Following a selection process, 32 patients entered the study: C (9 control healthy patients), CPs (9 low-intermediate CPs patients to account for psoriasis inflammation), and PsA (14 patients with recent PsA onset following low-intermediate CPs). Blood biochemistry (glucose, cholesterol, uric acid, lipid profile and apolipoprotein B) was analysed using standard kits. Pro-atherogenic inflammation markers, C-reactive protein (CRP) and interleukin-6 (IL-6), and endothelial activators monocyte chemoattractant protein-1 (MCP-1) and soluble intercellular adhesion molecule-1 (sICAM-1), were determined by ELISA. Ultrasound images evidenced carotid intima-media thickness (cIMT).
Results. Only PsA patients (0.590 ± 0.045), unlike CPs patients (0.621 ± 0.100), showed higher cIMT values compared with C (0.436 ± 0.051). The PsA group showed a dramatic increase in hs-CRP level compared with C ($p<0.01$) and CPs ($p<0.05$). Also, sICAM1 increased by 54 % ($p<0.05$) in PsA compared with C. Other inflammatory biomarkers as IL-6, TNF-α (2.2 ± 0.9 in C, 1.4 ± 0.7 in CPs, 3.6 ± 2.1 in PsA) or MCP-1 (142.2 ±7.6 in C, 160.5 ± 14.4 in CPs, 142.3 ± 11.5 in PsA) showed no differences. The erythrocyte sedimentation rate (ESR, mm/h) increased in CPs (85.8 ± 9.1, $p<0.01$) and PsA (53.0 ±17.7, $p<0.01$) compared with C (12.7 ± 5.1).
Conclusion. Our study first shows an increase in cIMT, and in serum levels of sICAM-1 and CRP in recent-onset PsA patients not presenting conventional CVRFs over the non-medicated time-period, from disease diagnosis to the beginning of pharmacological treatment, compared with C, highlighting the importance of monitoring serum level of sICAM1, CRP, and cIMT in PsA patients with no history of cardiovascular events.

83. (28) **AN INNOVATIVE MUCOSAL VACCINE BASED ON BTAF ADHESIN FROM BRUCELLA SUIS.**
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Currently, there are no human or porcine vaccines against Brucella infection, which is mainly transmitted through mucosae. We have shown that the BtaF adhesin is necessary for full virulence of B. suis after intragastric and respiratory murine infection. Here we characterized the cellular immune response elicited after intranasal immunization and its potential protectivity against brucellosis.
Six-week-old Balb/c mice were weekly immunized for 3 weeks with BtaF (10ug) plus c-di-AMP (10ug), c-di-AMP alone or saline.One week after last immunization lungs and cervical lymph nodes were harvested, and the cells obtained were cultured in the presence of BtaF (10ug) or RPMI. After 24h lymph node cells were stained with anti-CD3, anti-CD4, anti-CD8, anti-CD44 and anti-CD62L antibodies for flow cytometry analysis. In vitro production of IFN-γ by lung cells was determined after 72h of stimulation. Other mice from the immunized and saline groups were injected intradermally in opposite footpads with BtaF or saline to evaluate DTH (48 and 72h). Two weeks after last immunization Balb/c mice were challenged with B. suis through the intragastric or intratracheal route. CFU counts were determined in lungs, liver and spleens 20 days after challenge.
Pulmonary cells from immunized mice secreted high levels of IFN-γ after stimulation with BtaF (mean: 8651pg/ml, $p<0.0001$ vs RPMI). Immunized mice showed higher percentages of central and effector memory CD4+T lymphocytes in lymph nodes ($p<0.01$ vs saline), in concordance with a specific DTH response ($p<0.01$; $p<0.5$ vs saline). The immune response elicited after intranasal immunization did not protect against respiratory challenge with B. suis. However, it conferred sterilizing protection in 7/9 intragastrically challenged mice, and reduced significantly ($p<0.001$) the spleen burden in other two.
The immune response elicited by intranasal vaccination with BtaF/c-di-AMP, characterized by central and effector memory CD4+T cells, protected against intragastric infection with B. suis, but not against respiratory infection

84. (46) **GAUCHER HMSCS PRESENT AN ALTERED OSTEOBLAST AND ADIPOSE DIFFERENTIATION**
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Gaucher disease (GD) is caused by mutations on the gene encoding the lysosomal enzyme glucocerebrosidase. Type I GD (GD1) patients present diverse symptoms as chronic inflammation, anemia, hepatosplenomegaly and bone alterations. The most widespread treatment for GD, enzyme replacement therapy (ERT), cannot completely reverse bone problems. Despite mechanisms leading to bone damage are not fully described, reports suggest that alterations in osteoblasts and osteoclast, as well as a chronic pro-inflammatory state, could be involved. It is known that mesenchymal stem cells (MSCs) differentiate to osteoblasts and adipocytes, so the aim of this work was to evaluate, in an in vitro model, the potential of MSCs from control and GD patients to differentiate towards the osteoblast (GDOb) and adipocyte (GDAd) lineage. Furthermore, we sought to analyze released cytokines from differentiated osteoblasts and the capacity of these cells to generate osteoclasts. The expression of differentiation markers were lower in GDOb at 14 days compared to control Ob: BMP-2 ($p=0,001$), Runx2 ($p=0,01$), ALP ($p<0.0001$) and ColA1 ($p<0.0001$). Reduced mineralization, collagen deposition and alkaline phosphatase activity were revealed (all $p<0.0001$). We also observed that GD MSCs supernatants promoted osteoclastogenesis ($p=0,04$) and presented higher levels of IL-1β ($p=0,004$). However, we did not observe any difference in TNF-α concentration. GD MSCs produced lower levels of lipid droplets than control MSCs at 7 ($p=0,001$) and 14 ($p<0.0001$) days of adipose differentiation. Our results show an alteration in GD MSC differentiation towards osteoblast and adipose lineage as well as an increased osteoclast differentiation capacity and an altered cytokine secretion. Therefore, we suggest that impairment in GD MSCs and its subsequent cytokine profile could contribute to bone damage in GD.

85. (48) **TRISOMY 21 DISRUPTS T CELL HOMEOSTASIS WITH IMPAIRED TREG SUPPRESSION AND ENHANCED CYTOTOXICITY.**
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Individuals with Trisomy 21 (T21) have a unique disease spectrum and a hyperactivated Type-I interferon signaling, which could be a result of increased gene dosage of the four IFN receptor subunits encoded on chr21. T21 causes widespread alterations in gene expression across the genome, including a consistent activation of the interferon transcriptional response and increased levels of potent inflammatory cytokines and chemokines known to act downstream of INF signaling, which leads an imbalance between immune response and regulation. Based on this, we dissected the T cells functionality in individuals with T21. We characterized the circulating T cells subsets using multiparametric flow cytometry. Individuals with T21 present alterations in their T cell distribution, while they have a reduced frequency of naïve T cells ($p<0,05$), they have an increased on TEMRA CD8+ T cells ($p<0,05$) and memory Tconv cells ($p<0,01$), however they have similar distribution on the Treg subpopulations compared with their controls. In addition, these individuals present more CD8+ T cells that produce effector proteins (IFN-γ $p<0,05$ and GzmB $p<0,05$) and are more readily proliferative compared with the controls (Ki-67 $p<0,01$). Also, individuals with T21 have an increased frequency of inhibitory receptors (TIGIT and KLRG1 $p<0,01$, PD-1 $p<0,001$) and senescence markers ($p<0,01$). Interestingly, individuals with T21 have a higher frequency of IL-17+ Tconv cells ($p<0,05$)

and the Treg cells shows higher expression of Foxp3 (p<0,0001), without showing any other significant changes in their phenotype. When an in vitro autologous suppression assay was performed, an impaired Treg suppression was clearly observed in individuals with T21 compared with their controls (p<0,001), these differences disappear when we perform an allogenic suppression assay.
Thus, individuals with T21 have hyperactivated T cells which are resistant to be suppressed by their Tregs. We hypothesize that this could explain why individuals with T21 have an altered disease spectrum.

86. (55) **CHARACTERIZATION OF THE AGONIST INNATE RESPONSE OF PHARMACEUTICAL PRODUCTS OBTAINED FROM BACTERIAL LYSATES**

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Immunostimulation with bacterial lysate-based products has been worldwide used for the treatment of several recurrent or chronic infectious diseases for more than 50 years. However, the way these products may exert their effects is not fully characterized. The Instituto Biológico Argentino S.A.I.C. (BIoI) has developed formulations based in glucidolipids extracted from *Escherichia coli* and *Salmonella typhi*: GL01 and GL02 respectively, that are successfully used in Argentina and Latin-American countries since the 60s, indicated to prevent recurrent respiratory infections.
Here we analyzed the composition of GL01 and GL02 by Fourier Transformed Infrared Spectroscopy (FTIR) and the agonist activity of innate immunity using reporter cell lines.
Our FTIR results show that both contain mainly LPS and proteins, however GL02 displays higher molecular complexity than GL01. Both GL01 and GL02 mediate activation of hTLR4 evaluated in HEK-BlueTM hTLR4 cells, $1.9x10^{-7}$ μg of KDO of GL02 shows the same activity than $1.2X10^{-5}$ μg of KDO of GL01. Stimulation of THP1-XBlue TM-def MyD cells indicates that both products respond to receptors whose signaling is dependent on MyD88. Transfection of HEK cells with hTLR2 alone or in combination with hTLR1 or hTLR6 showed that only *S. tyhpi* products are able to mediate hTLR2 (and combinations) activation; while hTLR9 or hTLR5 were not activated by either GL01 or GL02. Activation of ASC-dependent inflammasomes was analysed in THP1 ASC-GFP cells by confocal microscopy; 2.37 $x10^{-2}$ μg of KDO of GL01 activate 1.6 ± 0.5 cells/100 total cells and 3.9 $x10^{-1}$ μg of KDO of GL02 activate 8.6 ± 0.6 cells/100 total cells.
These results show that GL02 derived from *S. typhi* exhibits better stimulation capacity of innate response compared with *E. coli* derived product under our experimental conditions. These results are relevant to understand the mechanism of action of immunostimulatory commercial products, up to now largely unexplained.

87. (69) **LOCAL REGULATION OF ADRENAL STEROIDOGENESIS: IS IL-1B INVOLVED?**

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Adrenal steroidogenesis and immune responses (IR) are mutually regulated through the production of specific molecules helping to orchestrate an optimal response. Under pathological conditions, the IR attempts to eliminate the noxious stimulus and repair the damage whereas the endocrine response pursues to avoid an excessive inflammation. As an infectious disease, Tuberculosis is characterized by a substantial secretion of inflammatory cytokines like IL-1β, which is mainly produced by macrophages. While IL-1β modulates the adrenal steroidogenesis at the central level, it is not clear whether it exerts a local effect on the adrenal gland. Several studies suggest that Nuclear Receptors NR4As are potential key factors in inflammation and metabolism, for instance in steroidogenesis by modulating steroidogenic enzyme expression. Our former studies in the human cell line NCI-H295R (derived from a carcinoma of adrenal cortex), revealed that treatment with IL-1β 1.25 pg/ml + Fk (Forskolin), 24 h, increased cortisol and DHEA production respect cultures stimulated with Fk or left untreated. Cells exposed or not to Fk or IL-1β expressed type 1 and type 2 IL-1β receptors. Fk treatment increased IL-1R2 mRNA respect to IL-1R1 ($p<0.05$). Analysis on the expression of steroidogenic enzymes and NR4As mRNA together with the microRNAs (miRNAs) profile, according to different treatments showed that Cyp17A1 mRNA was augmented in Fk+IL-1β ($p<0.05$). NR4A1 and 2 mRNA were also increased in Fk and IL-1β+Fk cultures ($p<0.001$ vs. basal, both cases; and $p<0.01$ respect IL-1β-stimulated cultures). IL-1β+Fk-stimulated cultures, displayed higher NR4A2 mRNA levels than Fk counterparts ($p<0.05$). Two out of 10 analyzed miRNAs had a differential expression, being MiRNAS 26a-5p and 484 augmented in IL-1β+Fk cultures. Four independent cultures with biological triplicates were analyzed. IL-1β induced changes in hormonal secretion, receptor expression, and modifications in the miRNA profile on NCI-H295R cells are likely to be involved in the regulatory mechanisms underlying adrenal steroidogenesis.

88. (76) **ECHINOCOCCUS GRANULOSUS MODULATES GLOBAL TRANSLATION AND THE IMMUNE RESPONSE IN DENDRITIC CELLS BY ACTIVATION OF THE MTOR PATHWAY**

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Background: The target of rapamycin (mTOR), represents a key biological switch in the modulation of cell metabolism and environmental signals. It has recently been recognized as a regulator in the immune system. In the present work, we show the modulation exerted by Echinococcus on signaling of the mTOR pathway and its relation to the functionality of dendritic cells. Methods: BMDCs were cultured in complete RPMI supplemented with 100 ng/ml Flt3-L. Hydatid cysts were collected from the liver of infected cattle slaughtered. Hydatid fluid (HF) was punctured from the cysts. The germinal layer was removed from purified laminar layers (pLL) by washing with 2M NaCl. Flow cytometry: FITC or PE-conjugated mAbs directed to CD11c, CD40, CD80, CD86, MHC I and MHC II were from eBioscience. Immunobloting and confocal microscopy: mouse anti-puromycin and rabbit anti-Phospho-mTOR antibodies were used. Results: Protein synthesis was monitored using puromycin labeling followed by confocal microscopy. Cells not treated with puromycin, cycloheximide or rapamycin were used as controls. Both stimulus, pLL (MFI:_979) and HF (MFI:_835), showed an increase in translation levels compared to its counterpart without stimulation (MFI:_686, n=3). LPS was used as a positive control. To confirm if the mTOR pathway was involved in the recognition of parasitic antigens, we monitored the phosphorylation of MTORC1 by WB and confocal microscopy. We have observed that DCs cultured without SFB or FLT3L, and stimulated with pLL or HF induce an increase in mTOR phosphorylation levels compared to untreated CDs (n=3, p=0,035 Anova test). Finally, we evaluated the phenotype of DCs. The pLL of Echinococcus induce an up-regulation of MHCII and CD86 (n=3, $p<0.05$ vs Ctrl). There were no significant changes in CD40, CD80, and MHCI. Conclusions: These data suggest that Echinococcus antigens are recognized by DCs and induce an activation of mTOR pathway favoring global translation and their maturation.

89. (78) **BRUCELLA ABORTUS RNA CONTRIBUTES, ALONG WITH ITS LIPOPROTEINS, TO THE DOWN-REGULATION OF THE IFN-Γ-INDUCED MAJOR HISTOCOMPATIBILITY COMPLEX (MHC) CLASS II MOLECULES EXPRESSION ON HUMAN MONOCYTES, EVADING THE HOST T CELL SURVEILLANCE.**

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In order to persist inside the host, *B. abortus* must trigger different strategies to evade the adaptive T cell response. Previously, we demonstrated that *B. abortus* inhibits the IFN-γ-induced MHC-II surface expression on human monocytes. Consequently, the infected macrophages show a diminished antigen presentation to $TCD4^+$ lymphocytes. *B. abortus* outer membrane lipoproteins –bacterial structural components- are involved in MHC-II down-modulation through IL-6 secretion. Nevertheless, this phenomenon was less marked than that observed in the infection. This led us to think that there should have been another component associated to live bacteria implicated in MHC-II down-modulation. Actually, we have recently showed that *B. abortus* RNA, a PAMP associated to bacterial viability (*vita*-PAMP), is responsible for MHC-I down-modulation. Thus, the aim of this study was to analyse whether *B. abortus* RNA could also contribute to MHC-II down-modulation. For this, human monocytic THP-1 cells were incubated with *B. abortus* RNA in the presence of IFN-γ for 48 h. MHC-II expression (HLA-DR) was evaluated by flow cytometry. *B. abortus*RNA significantly ($p<0.001$) down-regulated the IFN-γ-induced MHC-II surface expression in a dose-dependent fashion. Furthermore, this was also reproduced in peripheral blood human monocytes ($p<0.05$). Accordingly, MHC-II down-modulation in cells treated with *B. abortus* lipoprotein (L-Omp19) and RNA was even higher than in those stimulated with merely RNA or L-Omp19 ($p<0.01$). Eukaryotic RNA was incapable of inhibiting IFN-γ-induced MHC-II expression. The expression of co-stimulatory molecules up-regulated by IFN-γ (CD80, CD86 and CD40) were significantly stimulated on *B. abortus* RNA-treated THP-1 cells ($p<0.05$; $p<0.001$ and $p<0.001$, respectively), showing that MHC inhibition is not a global effect on IFN-γ-induced molecules. By confocal microscopy, we demonstrated that not only does *B. abortus* RNA inhibit MHC-II surface expression but also inside the cells. Therefore, *B. abortus* RNA and lipoproteins down-regulate MHC-II expression contributing to the establishment of a chronic infection

90. (82) **TOXOPLASMA GONDII CHRONIC INFECTION MODULATES THE FIRST STAGES OF ATOPIC DERMATITIS SENSITIZATION**

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We previously showed that chronic *T. gondii* infection diminishes the susceptibility to develop atopic dermatitis by using a murine experimental model with OVA as allergen. Skin histopathology of mice infected before allergic sensitization was similar to normal mice. Diminished OVA specific IgE and IgG1 levels and reduced systemic and local Th1/Th2 cytokines were detected. These results suggested that regulatory cells induced by the parasite may account for the immunomodulatory effect. However, no significant differences were observed in regulatory cytokine production or $CD4^+Foxp3^+$ T cells between groups. Based on these results, we hypothesized that the parasite could modulate the allergic sensitization by regulating the innate immune response. Therefore, the aim of the present work was to study the first stages of the sensitization process. Adult BALB/c mice chronically infected with *T. gondii* cysts were epicutaneously sensitized with OVA (TDA) and euthanized at 0, 2, 6 or 24hs later. Non-infected mice sensitized with OVA (DA) where used as control. Systemic cytokine levels were analyzed in splenocytes ex vivo stimulated with PMA/ionomycin. *Toxoplasma gondii* infected mice showed decreased IL-4 (TDA=89.3±31.6//DA=523±41.7), IL-5 (TDA=42.2±23.4//DA=329.3±43.8) and augmented IFN-γ levels (TDA=2961±474.7//DA=1660±134.6) compared to control mice at 24hs ($p<0.05$). Though not significant, this profile was detected in the other tested time points. The same trend was observed in draining lymph nodes and skin homogenates of the treated sections ($p<0.05$). We next analyzed by flow cytometry the expression of CD80 and CD86 on spleen $MHCII^+CD11c^+$ dendritic cells (DCs). In contrast to infected mice, control mice showed increased mature DCs percentage. This difference resulted statistically significant at 6hs (TDA=16.4±5.4//DA=38.1±0.9) ($p<0.05$). Altogether, these results suggest that reduced local and systemic type 2 early innate responses and diminished mature DCs count observed in chronically infected mice may account for the lower susceptibility to develop atopic dermatitis.

91. (116) **DELETERIOUS EFFECTS OF ACTIVATED NEUTROPHILS AND NETS ON TROPHOBLAST CELL FUNCTION IN A MODEL OF MATERNAL FETAL INTERACTION**

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Trophoblast cells (Tb) interact with different maternal immune cell populations at early pregnancy promoting an anti-inflammatory and tolerogenic response. Uterine vasculature remodeling occurs to accommodate the increasing demand of the growing fetus. Circulating neutrophils are found activated during pregnancy and even more during pathological pregnancies. Vasoactive Intestinal Peptide (VIP) is a pleiotropic peptide with immunomodulatory effects through its action on VPAC1 and VPAC2 receptors, both found in neutrophils.
We have already shown that VIP and conditioned media (CM) from human first trimester Tb (Swan-71 cell line) inhibit PMA-induced neutrophil extracellular trap(NET) formation, promote neutrophil apoptosis and revert the anti-apoptotic effect of LPS.
Our aim was to evaluate the effect of VIP and trophoblast derived factors on neutrophils and how these conditioned cells could impact likewise on Tb function and profile.
Neutrophils were isolated from healthy donors blood. We found that both neutrophils and monosodium urate crystals (MSU)-induced NETs increased the expression of trophoblast IL-8 and decreased TGF-β explored by RT-qPCR ($P<0.05$). Pre-treatment of neutrophils with 10nM VIP reversed TGF-β diminished expression (arbitrary units±SE: Tb: 0.78±0.13; Tb+neu: 0.53±0.07; Tb+neu+VIP: 0.81±0.24; $P<0.05$). Consistently, neutrophils impaired trophoblast migration, evaluated using the wound healing assay (%±SE: Tb alone: 52.0±1.8; Tb+neu: 30.7±4.1; $P<0.05$) and the conditioning neutrophils with VIP partially reversed this effect (neu+VIP: 42.8±4.6; $P<0.05$). NETs produced by MSU-stimulated neutrophils also impaired trophoblast migration (%±SE: Tb alone: 60.4±1.0; Tb+NETs: 52.5±1.8; $P<0.05$). In addition to this, we studied the impact of 10nM VIP and trophoblast derived factors on the pro-angiogenic capacity of neutrophils, assessing specific markers by RT-qPCR and its effect in tubulogenesis, using the hybrid endothelial cell line EA.hy926. Neutrophils cultured with VIP or CM increased the expression of VEGF, Arginase-1, TGF-β and CCL2 ($P<0.05$).
We conclude that activated neutrophils adversely affect trophoblast cell function and might alter vascular transformation processes required during placentation.

92. (117) **DECIDUAL MACROPHAGES AND TROPHOBLAST CELL PHENOTYPE AND FUNCTION ARE REGULATED BY VIP IN HUMAN FIRST TRIMESTER PLACENTA**

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Deep placentation disorders are associated with impaired invasiveness of extravillous trophoblast cells (EVT) and vascular remodeling in a pro-inflammatory microenvironment. The vasoactive intestinal peptide (VIP) has anti-inflammatory, pro-secretory and vasodilating effects. However its effect on decidual macrophages (dMA) and pregnancy outcome is still unclear. Here we studied the role of VIP in human first trimester placenta as a regulator of dMA and EVT

function.
Positive selection with CD14-immunomagnetic beads was used to isolate dMA from human placental explants (5-9 weeks). Explants or dMA were cultured ±VIP (10-100 nM). Gene expression and protein secretion was studied by qPCR and BioPlex assay, respectively. Tubulogenesis assay was performed in uterine endothelial cells (EC) in the presence of supernatant from dMA. The first trimester trophoblast derived cell line, BeWo (Tb), was knocked-down for VIP (VIP-KD) with a specific siRNA and spiral artery (spA) remodelling was studied by immunostaining assays.
VIP increased IL-10 (116±37 vs. 81±32 pg/ml; P<0.05) and reduced IL-2 and IL-12 in dMA and it also increased metalloprotease (MMP)-2 expression. The supernatant of dMA treated with VIP or VIP alone, reduced the tubulogenesis in EC. In this context, VIP increased the outgrowth of EVT and the effect was prevented by α-VIP (P<0.05). Additionally, VIP increased ZEB2 expression (fold change±SEM: 6.6±1.0; P<0.05) in Tb and Vimentin which are important markers for trophoblast transition to an invasive phenotype. In contrast, VIP-KD Tb showed a reduction in ZEB2 and MMPs expression.
Moreover, CD45 and HLA-G/VIP positive cells were found in both the walls and lumens of spA being remodeled.
The results suggest that VIP regulates dMA phenotype increasing IL-10 and MMP-2 production and reducing pro-inflammatory cytokines and tubulogenesis in the early maternal-placental interface. Results also confirm that both endogenous and exogenous VIP modulates trophoblast cell function.

93. (119) **TEAR FILM HYPEROSMOLARITY DISRUPTS OCULAR MUCOSAL TOLERANCE IN A NF-KB-DEPENDENT FASHION IN DRY EYE DISEASE**

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Introduction: Tear film hyperosmolarity resulting from increased evaporation is commonly observed in dry eye and is known to have proinflammatory effects on the ocular surface. Dry eye progression involves disruption of ocular mucosal tolerance, but the events that lead to it are unclear.
Objective: To evaluate the effect of hyperosmolar stress on ocular mucosal tolerance.
Methods: 8- to 12-week-old female Balb/c mice were instilled isoosmolar (0.3 Osm) or hyperosmolar (3 Osm) saline on both eyes 3 times daily for 5 days. Ovalbumin (OVA) was instilled on both eyes at different time points. After subcutaneous immunization with OVA in adjuvant (day 8), induced T cell responses were measured by the delayed-type hypersensitivity (DTH) response in the footpad (day 15). In some experiments, T cells in draining lymph nodes (day 5) were evaluated by flow cytometry. In other experiments, mice were exposed to hyperosmolar stress for 72h and then epithelial NF-kB activation levels were analyzed by confocal microscopy in conjunctival whole-mounts (ImageJ software).
Results: Compared to non-instilled immunized mice, OVA-instilled mice developed reduced DTH responses, as did their OVA+isoosmolar saline-instilled cage mates (n:6; p<0.05). By contrast, OVA+hyperosmolar saline-instilled mice exhibited full DTH responses and their T cells in draining lymph nodes showed increased expression of activation (CD69, CD25) and memory (CD44) markers (p<0.01). We observed increased nuclear translocation of the NF-κB p65 subunit, which is indicative of NF-κB activity, in the conjunctival epithelium exposed to hyperosmolar stress (p<0.01).
Conclusion: Tear hyperosmolarity per se is sufficient to disrupt ocular mucosal tolerance towards a harmless antigen in Balb/c mice. Hyperosmolar stress is a likely environmental trigger for dry eye's underlying immune response.

94. (124) **PEPTIDES FROM AMARANTH CONTROLLED THE NF-KB PATHWAY ACTIVATION ON EPITHELIAL CELLS AND SUPPRESSED INTESTINAL INFLAMMATION**

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Biological, nutritional and health benefits of amaranth have been highlighted in the last years. Proteins of amaranth exert anti-hypertensive, anti-oxidant, anti-thrombotic and anti-proliferative effects. The aim of this study was to analyze the anti-inflammatory effect of peptides from amaranth on NF-kB-intracellular pathway activation in intestinal epithelial cells, and in experimental intestinal inflammation, such as colitis and food allergy.
Colon cell lines (Caco-2 and Caco-luc) were cultured with flagellin and amaranth peptides. CCL20-expression was evaluated by qPCR and NF-κB modulation was evaluated by light emission and qPCR, along with p65-nuclear traslocation. In vivo studies included the oral administration of a formulation containing the peptide during the allergic sensitization or the colitis induction phase in Balb/c mice. Treatment efficacy was in vivo and in vitro evaluated.
We found several peptides with anti-inflammatory capacity and we selected that with the highest ability to suppress cell activation (decrease in CCL20 and light emission p<0.05). In vivo studies showed, an amelioration of the clinical score (p<0.01) in the food allergy mouse model, with inhibition of specific-IgE secretion (p<0.05) and negativitization of the cutaneous test (mean increase in footpad thickness control: 0.6mm vs peptide-treated: 0.3mm; p<0.05); intestinal nf-kb gene expression was reduced (fold change=3; p<0.01) along with up-regulation of tfg-b and foxp3. In the colitis mouse model, we found a decrease of the histologic score with a decrease expression and production of pro-inflammatory cytokines (IL-1b, TNF and IFNg, p<0.05) and a decrease in the myeloperoxidase activity in the peptide-treated group compared to control (p<0.05). NF-κB pathway was also abrogated in the gut.
In conclusion, our findings indicated that peptides from amaranth endowed mucosal anti-inflammatory properties that suppressed the intestinal activation of NF-kB in Th1- and Th2-mediated inflammation. These findings led us to propose that this peptide might be included in the composition of a functional food.

95. (140) **OUTCOME OF HEMATOPOIETIC STEM CELL TRANSPLANTATION (HSCT) IN PATIENTS WITH CHRONIC GRANULOMATOUS DISEASE: SINGLE CENTRE REPORT**

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Chronic granulomatous disease (CGD) is a primary immunodeficiency caused by mutations in the genes encoding subunits of the phagocytic NADPH oxidase system. Patients present severe, recurrent bacteria or fungi infections and inflammatory manifestations. Hematopoyetic stem cells transplantation (HSCT) is the only cure for CGD. Aim: To describe clinical and immunological outcome in CGD patients(P) under HSCT treatment. Materials and Methods: Clinical and laboratory data was collected retrospectively from 6 CGD patients: 5 X-linked (P1-P5) and 1 Autosomic recessive CGD (P6). Results: Median age at diagnosis 1,1yo (range: 0.08-3yo). Clinical features: 4/4 P had BCG vaccine complications, 5/6 had lung infections; 4/5 due to Aspergillus and 3/5 required pulmonar lobectomy. 4/6 presented inflammatory manifestations. Median age at HSCT: 7,1yo (range: 3.7-13.1yo); 1 HLA-identical related donor (bone marrow (BM)), 5 HLA-identical unrelated donor (2 BM, 1 peripheral blood, 2 umbilical cord), 5/6 were conditioned with myeloablative regimen, P6 received reduced intensity regimen. All received graft-versus-host disease (GvHD) prophylaxis. P6 had graft failure, developed medular aplasia, died 2yo 6m after HSCT. Of 5 alive patients, median follow-up 1346 (184-4027) days. 3 P had acute GvHD. 3/5 CMV infection. 2P underwent a second HSCT: P3 because of a medular aplasia and lost of donor chimerism and P4 due to an early graft failure. Both engrafted after the second HSCT, P4 lost it due to infection, he is in plan of a third HSCT. P2 had EBV associated post-transplant lymphoproliferative disease. P5 was recently trasplanted without complications.4/5 alive P had normal

DHR test. P1 and P2 had T and B cells reconstitution 12 months post HSCT and discontinued IVIG. Conclusion: Most of our P are cured from CGD, but are still having complications related to HSCT. It is important to highlight that half of our P had graft failure.

96. (158) **EXACERBATION OF EXPERIMENTAL PSORIASIS SYMPTOMS IN DESMOGLEIN-4 DEFICIENT RATS IS MEDIATED BY INCREASED INFLAMMATION**

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IMBECU-CONICET

Psoriasis is a chronic inflammatory skin disease, characterized by keratinocyte hyperproliferation, vasculature growth and leukocyte infiltration into the dermis and epidermis. Although it is known that desmogleins are proteins involved in cell adhesion mechanisms, their role in psoriasis has not been addressed. The aim of our work was to assess the impact of desmoglein-4 deficiency in the immunological response of the skin. To this end, OFA hr/hr rats, which are mutant for the desmoglein-4 gene and Sprague-Dawley (SD) wild type rats were used. Imiquimod (IMQ), which is an immune response modifier that acts via toll-like receptor 7 pathway, was administered to both rat strains in shaved skin for four days to generate psoriasis-like lesions. Skin biopsies from treated and untreated OFA and SD rats were weighed, minced, stained with PE-Cy5-anti CD45 and FITC-anti CD3 monoclonal antibodies and analyzed by flow cytometry. We observed that in both strains, CD45+, CD3+ cells in increased in IMQ-treated groups, but the rise was higher in OFA rats ($p<0.05$). Similarly, qPCR analysis of skin mRNA showed that pro-inflammatory genes such as IL-1β, IL-8, and CCR1 were increased in both IMQ-treated groups, SD and OFA, compared to untreated groups but the increase was also higher in OFA rats ($p<0.05$). Furthermore, TNF-α, CCR2, CCR3, CCR5 and CXCR5 mRNA expression rose only in the OFA IMQ treated group ($p<0.05$). When anti-inflammatory genes were evaluated, we found that both IMQ treated groups increased TGF-β expression similarly but OFA IMQ showed higher levels of IL-10 than SD IMQ and untreated groups ($p<0.05$). These results suggest that desmoglein-4 deficiency contributes to experimental psoriasis progress, promoting expansion of leukocyte population and increasing different pro-inflammatory genes mRNA expression in skin. Although further research is needed, these results could have a potential impact of desmoglein-4 on the diagnosis and prognosis of psoriasis.

97. (181) **VACCINE CANDIDATE BASED ON OUTER MEMBRANE VESICLES FROM BORDETELLA PERTUSSIS TRIGGERS THE CANONICAL INFLAMMASOME ACTIVATION**

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The resurgence of the respiratory disease named pertussis has urged the need to develop a new vaccine. Under this context we have already identified and characterized with very good results, a vaccine candidate based on outer membrane vesicles (OMVs). Recent advances in the field have shed light on some of the multifaceted roles of OMVs in host-pathogen interactions. In this study, we investigated the ability of OMVs derived from B.pertussis, to activate the inflammasome pathway. To this end we evaluated the secretion of IL-1beta and the ASC-speck formation after the activation of THP-1 cells with different quantities of OMVs (5ng/mL to 5ug/mL). The cytokine levels were measured in culture supernatant by ELISA and the specks formation from THP1-ASC-GFP cells were observed and quantified by fluorescence microscopy.
By these assays we detected that the release of IL-1b from THP1 cells stimulated with at least 100ng OMVs was significantly higher than non treated cells. Release of IL-1b was independent of previous priming of cells with TLR agonists. In particular when 200ng OMVs was used, the IL-1b level was 72,3±2,1 pg/mL vs. 4,10±0,02 pg/mL ($p\leq0,001$) detected in the non treated cells. The percentage of ASC+/total THP1-ASC-GFP cells was also significantly higher for cells stimulated with at least 200ng OMVs in comparison with non-stimulated cells (6,8±0,6 vs. non detected $p\leq0,001$).
These results show for the first time that our vaccine candidate based on the OMVs derived from B. pertussis activate the iflammasome in a human macrophage cell line. Furthermore, this data would strengthen the concept of inflammasome activation as one of the innate immune pathways with the ability to profile the protective adaptive response of our vaccine.

98. (189) **BRUCELLA ABORTUS-INFECTED PLATELETS ACTIVATE THE ENDOTHELIUM AND PROMOTE THE MIGRATION OF MONOCYTES TOWARDS THE SITE OF INFECTION**

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Brucellosis is an infectious disease elicited by bacteria of the genus *Brucella*. Platelets have recently got involved in the modulation of innate and adaptive immune responses. We have previously reported that platelets act as carriers of bacteria, promoting the invasion of monocytes. Thus, the aim of this study was to further investigate the role of platelets in the immune response against *Brucella*. First, we wondered whether the presence of platelets modulates the time course of *B. abortus* infection. For this, THP-1 cells (human monocytic cell line) were infected with *B. abortus* in presence or absence of platelets. Then, extracellular bacteria were killed and cells were incubated for different times. Our results demonstrate that the presence of platelets significantly increased the percentage of *B. abortus*-infected THP-1 cells at early time-points ($p<0.001$). Nevertheless, the presence of platelets subsequently improved the contention of the infection. Taking into consideration that *B. abortus* localization within different tissues requires its extravasation across the endothelium, our next aim was to study the role of platelets in the modulation of monocytes extravasation in the context of *B. abortus*-mediated infection. We first studied the ability of platelets to recruit monocytes. Our results showed that supernatants collected from infected platelets promote the transmigration of monocytes ($p<0.01$). Moreover, the pre-treatment of monocytes with this supernatant enhance the responsiveness of monocytes towards other chemoattractant stimuli ($p<0.01$). Finally, we studied the ability of platelets to activate the endothelium. For this, HMEC cells (human endothelial cell line) were stimulated with supernatants collected from *B. abortus*-infected platelets. This supernatant stimulated the expression of ICAM-1 (CD54) ($p<0.01$). At the same time, it enhanced the secretion of both IL-8 and MCP-1 ($p<0.01$). These results showed that infected platelets are able to activate the endothelium and promote the migration of monocytes towards the site of the infection

99. (191) **SLPI IS TAKEN UP BY MONOCYTES AND IMPAIRS THEIR DIFFERENTIATION TO DENDRITIC CELLS**

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Secretory leukocyte proteinase inhibitor (SLPI) is a serine protease inhibitor produced mainly by epithelial cells. It has anti-inflammatory and antimicrobial activity, and enhances wound healing. We have previously described that SLPI inhibits lymphocyte proliferation which depends on the presence of monocytes. The aim of the present study was to assess whether SLPI is captured by monocytes and its effect on monocytes differentiation to dendritic cells.
To study SLPI uptake, human myelomonocytic U937 cells and human peripheral blood mononuclear cells (PBMC) were treated or

not with 10 μg/ml of rhSLPI for 1 h. Then, the cells were fixed and permeabilized. Afterwards, the cells were incubated with an anti SLPI-FITC antibody and the fluorescence was analyzed by flow cytometry. To evaluate the effect of SLPI on monocytes differentiation to dendritic cells, human monocytes were isolated from PBMC and incubated with GM-CSF + IL-4 in the presence or absence of rhSLPI for 5 days. The cells phenotype was characterized by flow cytometry using anti-MHC I and II, CD86 and CD14 monoclonal antibodies.
We observed that 45-50% of U937 cells cultured with SLPI but not without SLPI, were stained with SLPI-FITC but not with isotype control monoclonal antibody. The same was observed for just only 15-20 % of CD14+ human mononuclear cells. These results suggest that monocytes can incorporate SLPI from the medium. Moreover, as expected, monocytes treated with IL-4 + GM-CSF (5 days) showed high expression of CD86 and MHC II, intermediate expression of MHC I and very low expression of CD14. In contrast, when monocytes were incubated with IL-4 + GM-CSF in the presence of SLPI, cells showed a high expression of CD14, low expression of CD86 and MHC II.
Overall, these results demonstrate that monocytes can incorporate SLPI from the microenvironment and suggest that SLPI alters their differentiation to dendritic cells.

100. (240) **DECIDUALIZED CELLS RESPOND DIFFERENTIALLY CONTRIBUTING TO A TOLEROGENIC MICROENVIROMENT ACCORDING TO BLASTOCYST QUALITY**

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During decidualization process endometrial stromal cells modulate their secretome and display the ability to secrete many immune-regulatory and implantatory factors. Based on this ability, decidualized cells sense embryo quality allowing the implantation of normal development (ND) blastocysts while limit those with impaired development (ID). We propose that decidualized cells promote a tolerogenic microenviroment in response to ND blastocyst-derived factors; in contrast, decidualized cells response to ID-blastocyst derived factors sustaining a hostile microenvironmet that could trigger menstruation. Here we used an in vitro decidualization model based on the Human endometrial stromal cell line (HESC) treated with medroxiprogesterone+dbcAMP in absence/presence of Blastocyst Conditioned Media (BCM) for 24h. BCM were recovered from 5 days individually cultured blastocyst obtained from women with IVF or ICSI indication according to male evaluation, and classified as ND or ID blastocyst according to clinical standards. We observed that ND-BCM increased the frequency of decidualized cells producing the inmunomodulator peptide VIP evaluated by FACS, while ID BCM decreased expression of its receptor VPAC1 relative to decidualized cells not treated with BCM ($p<0.05$, ANOVA). Both BCM reduced the production of proinflamatory cytokine IL 1β ($p<0.05$, ANOVA). Additionally, CXCL12 expression was increased by ND BCM ($p<0.05$, Student T test), which was accompanied by a selective recruitment of regulatory T cells evaluated by transwell migration system. In opposite, ID BCM reduce Tregs recruitment ($p<0.05$, ANOVA), and the secretion of proimplantatory cytokine IL 6 ($p<0.05$, ANOVA), while MMP9 activity quantified by zymography was increased, highlithing a different microenvironment for blastocyst implantation.
The present results suggest that soluble factors released by blastocyst are differentially sensed by decidualized cells according to embryo quality. While decidualized cells promote a tolerogenic microenviroment in the presence of ND BCM, ID BCM decreased proimplantatory factors and might be involved in the disruptium of the decidualized tissue.

101. (285) **IDENTIFICATION OF CORTADERIA SELLOANA ALLERGENS AND CROSS REACTIVITY WITH LOLIUM PERENNE (POACEAE)**

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The pollen of grasses is the fourth in quantitative importance in Bahía Blanca and is a significant cause of pollinosis. *Cortaderia selloana* is a widely distributed plant often used as an ornamental. Unlike *Lolium perenne*, little is known about its allergenicity. The aim of this work is to identify the main allergens of *C. selloana* and establish relationship with *L. perenne*. The serum of 16 patients with allergic symptomatology, positive skin tests for grasses and specific IgE for *C. selloana* and/or *L. perenne* (ELISA) were analyzed. The allergen profiles were revealed for each patient by western blot. The mean inhibitory concentration (IC50) of each extract was established by ELISA preadsorbing the sera with pollen proteins. *C. selloana* presented a profile of 15 allergens (13-100 kDa). The major one, with 33 kDa, was detected in 91% of patients. Those of 42, 48, 51, 62, 68 and 71 kDa were observed in 82% of patients. *L. perenne* showed 15 allergens (20-100 kDa). The 57 kDa allergen was present in all patients and those of 34, 48, 71 and 76 kDa in 87% of them. For *C. selloana* pooled positive sera, the IC50 was 0.28 µg/ml using *C. selloana* extract and 1.02 µg/mL using *L. perenne* extract. For *L. perenne* pooled positive sera, the IC50 was 0.45 µg/mL using *L. perenne* extract. At a maximum concentration of 200 µg/mL of *C. selloana* extract only IC14 was reached. *C. selloana* allergens mentionated would be included in groups 1 and 4 of grass pollen allergens. The cross reactivity observed is broad. Qualitative and quantitative differences detected may be due to the existence of multiple isoforms. This work comprises a first attempt in the molecular characterization of *C. selloana* allergens and its implication in the design of vaccines and the effectiveness of the grass immunotherapy.

102. (455) **SERUM AUTOANTIBODIES IN PATIENTS WITH RELAPSING REMITTING MULTIPLE SCLEROSIS (RRMS)**

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Multiple sclerosis (MS) is a chronic inflammatory disease of the Central Nervous System (CNS) that usually presents an acute focal inflammatory demyelination and axonal loss with limited remyelination. The autoimmune response against myelin is initiated by unknown mechanisms, and the auto-reactive activated T and B lymphocytes cross the blood-brain barrier to respond locally to their target antigen triggering the inflammatory cascade, demyelination and neurodegeneration. We here designed an study to evaluate the presence of autoantibodies against CNS myelin in the peripheral blood of relapsing remitting MS (RRMS) patients. The detection of the antibodies was carried out using three strategies: Indirect immunofluorescence on slices containing in vitro cultured astrocytes and oligodendrocytes; indirect immunofluorescence from prepared with rat cerebellum slices and immunoblot assays from rat cerebellum extracts. A total of 99 samples were analyzed, 64 from RRMS patients (Group 1) and 35 healthy control subjects (Group 2). Both groups were tested by indirect immunofluorescence on the in vitro cell culture of astrocytes and oligodendrocytes and resulted non-reactive. However, in rat cerebellum slices, group 1 showed reactivity in 84.4% of the samples. Typical positive images were detected against the cerebellar white matter showing different fluorescence patterns that we classified in homogeneous (26,6%), thin granular (21,9%), and thick granular (35,9%). Group 2 showed no reactivity. All samples were simultaneously tested in triple tissue slices (rodent liver-stomach-kidney) to exclude false positive results due to other autoantibodies that may be present in the samples. The immunoblot assays showed several positive bands in the group 1 and the molecular weight level were similar to the myelin proteins present in

cerebellum extracts. Our results show that RRMS patients have circulating autoantibodies that can be detected in serum samples and probably the different fluorescence patterns reflect different stages of the disease and/or response to the treatment. Grant: PDTS-CIN-CONICET.

103. (479) **VIRUS LIKE PARTICLES OF THE JUNIN VIRUS Z PROTEIN (JUNV Z-VLPS) AS A BIOLOGICAL VEHICLE OF ANTIGENS AND VACCINE ADJUVANTS.**

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Virus-like particles (VLPs) are non-genomic nanostructures assembled from viral structural proteins. VLPs are incapable of infection or self-replicate due to the lack of genome yet their protein retain the antigenicity capable of induce cellular and human immune responses which makes VLPs potential viral-based vaccines. Currently, several licensed VLP vaccines are already being used against human and zoonotic pathogens.
Arenavirus matrix Z protein plays an important role in virus budding and is able to generate enveloped virus-like-particles (Z-VLPs) in absence of any other viral proteins. We previously demonstrated that Z-VLP induce maturation of dendritic cells (DCs) derived from bone marrow balb/c mice testified by a boost of surface markers MHC class II and CD86 levels.
The antigen presentations cells (APCs), DCs and macrophages, are found throughout the body as sentinels. They main aim of these cells is to continuously search for pathogens and present different antigens to activate the induction of an adaptive immune response.
We hypothesized that Z-VLPs could be efficient immunogens and excellent tools for vaccine purposes due to the fact that VLPs may promote adaptive immune response through the activation of APCs. In this work we study the expression of different surface markers in different subset of DCs treated with Z-VLP for 24 h. We demonstrated an increased level of CD40 ($p<0.05$) and CD80 ($p<0.05$) on bone marrow derived dendritic cells (bmDCs) of Balb/c mice by flow cytometry. On the other hand, we started to study the effect of Z-VLP on cytokines secretion in macrophages after 24 h Z-VLP treatment by ELISA. These nanoparticles induced the secretion of IL-12 ($p<0.01$) and TNF-α ($p<0.05$).
We conclude that Z-VLPs could be used as delivery systems that combine good safety profiles with strong immunogenicity.

104. (509) **RESPIRATORY EPITHELIAL CELLS AS AN ALTERNATIVE NICHE FOR BORDETELLA PARAPERTUSSIS**

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B. parapertussis (Bpp) is one of the two causative agents of whopping cough. Humans are the only natural reservoir for this respiratory non-invasive pathogen. However, its location within the host during asymptomatic periods is still unclear. We previously showed that Bpp survives intracellularly in neutrophils and macrophages. These cells have been proposed as a niche of persistence. Here we investigated the potential of the epithelial airway cells, also relevant at the infection sites, as another niche of persistence for Bpp. We used the 16HBE40- bronchial cell line that partially retained the characteristics and functions of the airway epithelium. Culture conditions were set up to promote cell polarity and the formation of tight junctions. Cells were infected with Bpp and the outcome of this interaction was evaluated. We used fluorescent probes to investigate bacterial internalization and intracellular trafficking by confocal microscopy. Intracellular survival was evaluated by polymyxin B protection assays. Statistical differences were analyzed by ANOVA ($p<0.05$).. Microscopy analysis showed a great proportion of cell-associated bacteria (52,5 ± 9,7 %) located intracellularly at 5 h post-infection. About 17,9 ± 1,2 % of these bacteria avoided lysosomal pathway and remained in compartments with access to extracellular nutrients. Accordingly, 14,5 ± 2,1 % of phagocytosed bacteria were found viable at this time point. 24 h post-infection the number of viable intracellular bacteria remained unchanged. These results suggest that Bpp survives intracellularly by avoiding lysosomal pathway. In addition, we found that after complete elimination of the extracellular bacteria with polymyxin B, intracellular Bpp repopulate the extracellular medium. These results suggest that Bpp is able to enter and survive within respiratory epithelial cells potentially contributing to host immune evasion and persistence.

105. (728) **HUMORAL IMMUNE RESPONSE INDUCED BY A PRIME-BOOST VACCINE STRATEGY AGAINST RECOMBINANT PMPD FROM CHLAMYDIA TRACHOMATIS USING LIPOSOMAL FORMULATIONS WITH AMINOACIDIC AMPHIPHILES OR CPG-ODN AS IMMUNOSTIMULANTS**

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Chlamydia trachomatis (Ct) is a frequently sexually transmitted bacterial worldwide. Vaccine development is strongly needed. The selection of antigen and novel adjuvant play a crucial role in vaccine effectiveness. Our aim was to evaluate the ability of a prime boost strategy to induce antibodies against Polymorphic membrane protein D (PmpD). A selected fragment of PmpD containing B- and T-cell epitopes was expressed in *Escherichia coli*. The coding sequence was subcloned in a eukaryotic expression vector (pVAX1). Recombinant protein was formulated with cationic liposomes (Lip) with CpG oligodeoxynucleotide (CpG-ODN) or an aminoacidic amphiphile (AA) as immunostimulants. Female Balb/c mice were immunized with a first intradermal dose of nude plasmid. Two protein boosters were administered, every three weeks, by intranasal and subcutaneous route, simultaneously. Mice were divided in groups receiving: Lip+CpG-ODN+rPmpD, Lip+AA+rPmpD, rPmpD alone, Lip+CpG-ODN or Lip+AA. Mice were bleed before starting the protocol and ten days after the last dose. Sera anti-rPmpD IgG, IgG1 and IgG2a antibodies were assessed by indirect ELISA (OD_{450nm}, mean±SEM). Liposome suspensions were stable with a mean particle size from 209.0 to 248.7nm. Anti-rPmpD IgG were higher in Lip+CpG-ODN+rPmpD(0,52±0,16) and Lip+AA+rPmpD(0.41±0.14) immunized mice compared to Lip+CpG-ODN(0,19±0,03) and Lip+AA(0.11±0.01) ($p<0.05$, $p<0.001$, Mann Whitney test; respectively). Moreover, rPmpD was able to induce higher levels of IgG(0.41±0.14) than Lip+CpG-ODN($p<0.05$) and Lip+AA($p<0.001$). IgG1 and IgG2a were higher in Lip+AA+rPmpD group compared to Lip+AA (0.49±0.22 vs. 0.08±0.02; 0.49±0.130 vs. 0.14±0.02; $p<0.05$,$p<0.001$; respectively). rPmpD alone was able to produce high levels of IgG2a(0.69±0.28) and IgG1(0.33±0.08) compared to Lip+AA ($p<0.001$,$p<0.05$; respectively). All the formulations with PmpD induce antigen specific humoral response. Although, liposomal formulations led to higher levels of antibodies, these are not significant compared to rPmpD alone. Therefore, PmpD is an attractive candidate for the design of a vaccine and liposomal formulations could be added to induce an effective immune profile against *Ct*.

106. (753) **THE NON-NEURONAL CHOLINERGIC SYSTEM IN THE GLIOBLASTOMA CELLS**

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Glioblastoma multiforme (GBM) is the deadliest and most common type of human primary brain tumor. This tumor is defined by the hallmark features of uncontrolled cellular proliferation, diffuse infiltration, robust angiogenesis and resistance to apoptosis. Acetylcholine is a neurotransmitter which can also modulate cell survival, proliferation and differentiation in neuronal and non-neuronal cells such as immune cells and tumors cells, among others, which has been referred to as a "non-neuronal cholinergic system". The aim of this work was to elucidate the relevance of the non-neuronal cholinergic system in GBM cells and the relevance of the dendritic cells (DC). First, we observed the expression of acetylcholine receptors in human GBM cell lines by fluorescence microscopy and found that both U251 and U373 human GBM cells express acetylcholine muscarinic receptors M1 and M3. The expression of the M3 was also observed by immunohistochemestry in experimental GL26 tumors growing in the brain of immunocompetent mice.
We analyzed whether the cholinergic system modulates the apoptotic response of human GBM U373 cells. Apoptosis was assessed by flow cytometry by annexin propidium iodidestaining. We observed a clear increased of the apoptosic rate when U373 cells were incubated with carbachol (10-9M) ($p<0.05$).
Finally, we evalueted the relevance of the co-culture of DC with U373 (DC+U373) in the activity of metalloprotease 9 (MMP-9) with or without carbachol. Thereby that the mononuclear cells were isolated from buffy coats of volunteer and CD14+ cells were then isolated and then were cultured with GM-CSF and IL-4. The supernatant of the co-cultured was collected and the MMP-9 activity was evaluated. We did not find differences in the activity of MMP-9 when co-cultured were cultured with or without carbachol.
Conclusions: our findings suggest that the non-neuronal cholinergic system is present in GBM cells and could modulate his function.

107. (761) **POTENTIAL USE OF RV2626C ANTIGEN FOR DEVELOPING VACCINES AGAINST LATENT TUBERCULOSIS**

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Tuberculosis is one of the most prevalent infectious diseases. Nearly 1/3 of the world population is latently infected (LTBI) with Mycobacterium tuberculosis (Mtb) and at risk of disease reactivation. The aim of this work was to study the potential of Rv2626c, an antigen secreted by Mtb during LTBI, to develop therapeutic vaccines. Previously, we demonstrated that rRv2626c induced IFN-γ responses in peripheral blood mononuclear cells (PBMCs) from LTBI. In this work, we investigated the generation of polyfunctional responses (IFN-γ/IL-2/TNF-α) against Rv2626c. The results showed that 31% of responder $CD4^+$LT produced more than one cytokine in LTBI after treatment with rRv2626c. However, only 19% of $CD4^+$LT polyfunctional cells were identified in Tuberculosis Patients (TB) and Healthy Donors (HD). Due to recent works have proposed that humoral immunity against Mtb might have a role in the defense against the pathogen, we decided to analyze the levels of IgG against Rv2626c in plasma from LTBI, TB and HD. We found that the levels of IgG secreted by LTBI (42.29±12.39ng/ml) and TB (84.36±28.86 ng/ml) were higher as compared to HD (13.71±2.96ng/ml)($p<0.05$). Moreover, in the LTBI group, we could identify two population of subjects according the production of IgG against Rv2626c: healthy workers (76,73±22,64ng/ml) and close contacts (CC) (13,58±4,77ng/ml) ($p<0.01$). We hypothesized then that CC are individuals recently infected with Mtb who have not been exposed to Rv2626c yet. Considering our results in humans, we initiated experiments in BALB/c mice using rRv2626c and rAg85A (another deeply studied protein from Mtb) as immunogens and IL-12 as an adjuvant. After immunization, splenocytes were obtained and stimulated ex vivo with these proteins. We 66observed an increased production of IFN-γ in cells cultured either with rRv2626c or rAg85A. Overall, our findings suggest that Rv2626c could be a candidate for the development of new therapeutic vaccines for the treatment of LTBI.

CARDIOVASCULAR Y MEDICINA REGENERATIVA / CARDIOVASCULAR AND REGENERATIVE MEDICINE ORAL SESSION

108. (43) **VASCULAR ACTIONS OF THE BISPHOSPHONATE ALENDRONATE ON CELLULAR EVENTS INVOLVED IN VESSEL REMODELING**

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Bisphosphonates are drugs used for postmenopausal osteoporosis treatment. Cardiovascular disease is a pathophysiological condition prevalent in menopausal women. Within artery wall, atherosclerosis lesion and subsequent plaque calcification mainly compromise vascular architecture. Neovascularization is a survival option for damaged tissue. In this work, we investigate the effect of the bisphosphonate alendronate (ALN) on cellular events that affect vessel remodeling such as cell proliferation and migration, and angiogenesis under pro-calcifying conditions. Primary cultures of endothelial cells (EC) and vascular smooth muscle cells (VSMC) isolated from murine aorta were employed. In order to induce vascular calcification, VSMC were cultured for 21 days in osteogenic medium. Tube formation assay was used to evaluate angiogenesis. Total tube length of vessel segments was quantified using optical microscopy and ImageJ software. The bisphosphonate (5 μM) significantly enhanced tube formation after 4 days of treatment (41% above control, $p<0.05$). The presence of osteogenic medium did not affect the stimulatory action of ALN on angiogenesis. Under an inflammatory environment (LPS treatment) the stimulation of tubular structures formation elicited by ALN was sustained. Although nitric oxide (NO) modulates vascular angiogenesis, we found that ALN action was independent of NO, since the presence of L-NAME, a nitric oxide synthase inhibitor did not modify the ALN action. VSMC proliferation and migration towards endothelial layer represents the initial steps that conduct to atherosclerotic vascular calcification. Using co-culture of EC and VSMC, we did not find significant changes after 5 µM ALN treatment. We found that LPS induce VSMC migration, meanwhile in the presence of ALN no VSMC mobilization was observed. Using MTT colorimetric assay, we show that ALN significantly inhibited VSMC proliferation induced by osteogenic medium at all concentrations tested (5-35% of inhibition, 0.01-10 µM respectively, $p<0.05$). In conclusion, ALN exhibits a potential beneficial effect on cellular processes that compromise the vascular architecture.

109. (680) **GENERATION OF PLAKOGLOBIN EDITED PLURIPOTENT STEM CELLS FOR ARRYTHMOGENIC CARDIOMYOPATHY DISEASE MODELLING**

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Fundacion para la Lucha contra Enfermedades Neurológicas de la Infancia. (FLENI-CONICET)

The arrhythmogenic cardiomyopathy (ACM) is a heart muscle disease that evoques ventricle arrhythmias due to progressive replacement of contractile myocardium by fibro-fatty adipose tissue. The ACM has a genetic origin with mutations in desmosomal genes, including non-sense mutations in the plakoglobin gene (*Pkg*). Our aim is to generate a *Pkg* knockout (KO) iPSCs line by CRISPR/Cas9 and

to model the disease *in-vitro*. First, we analyzed the *Pkg* expression during the differentiation of iPSCs to cardiomyocytes. No differences of *Pkg* gene expression were seen by RT-qPCR among day 0, day 3.5, day 7 and day 21 of differentiation. However, wester blot (WB) analysis showed an increase of 15 to 50 times of PKG protein expression between day 0 and day 21 ($p<0.05$). To generate an early stop codon in the *Pkg* gene, we designed 2 RNA guides (gRNA1 and gRNA2) directed to the exon 1 of the gene and a single strand oligo DNA (ssODN) of 70 bp complementary to the sequence containing the desired mutation. 1ug of the CRISPR system and the ssODN were co-transfected to $2x10^5$ iPSCs. After puromycin selection, the cells were clonally expanded and evaluated by PCR and Sanger sequencing. We obtained different efficiencies of ssODN incorporation depending on the gRNA used: 15.4% (n=2/13) and 71.4% (n=10/14) for the gRNA1 and gRNA2, respectively. Among these clones, 2 of them were homocygotes for the desired mutation, which was confirmed by WB and immunofluorescence, not observing the expression of PKG. In summary, the increase of PKG protein expression during cardiomyocyte differentiation shows the importance of this protein in the cardio-system. In addition, the generation of 2 KO iPSCs lines for this protein will allow us to model the ACM *in-vitro* after differentiating these KO lines to cardiomyocytes.

110. (593) **HIGH DOSE INTRAMYOCARDIAL HMGB1 PROTEIN INDUCES CARDIOMYOGENESIS AND PRESERVES VENTRICULAR FUNCTION IN SHEEP WITH ACUTE MYOCARDIAL INFARCTION**

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Aims. In rodents with acute myocardial infarction (AMI), high mobility group box-1 (HMGB1) injection has been demonstrated to be cardioprotective. Contrarily, some reports show that cardioprotection is achieved with HMGB1 inhibition. Given the lack of data in large mammals, we searched the dose that would promote angiogenesis and expression of cardiac specific regenerative genes in sheep with AMI (protocol 1) and subsequently, use this dose to study long-term effects on infarct size, microvessels density, cardiomyogenesis and echocardiographic left ventricular (LV) function (protocol 2). Methods and Results. Protocol 1: Sheep with AMI received 250 µg (high-dose, n=7), 25 µg (low-dose, n=7) HMGB1, or PBS (placebo, n=7) in 10 intramyocardial injections (0.2 ml each) in the peri-infarct area. Seven days later, as compared with placebo, only the high-HMGB1 dose group exhibited higher microvascular densities (2828±511 vs. 1711±194 capillaries/mm2, P<0.01; and 39±14 vs. 23.2±4 arterioles/mm2; P<0.05), ki67-positive cardiomyocytes (4.6±1.1 /mm2 vs. 0.3±0.6, P<0.05, one-way ANOVA-Bonferroni) and overexpression of VEGF, Tbx20, Ckit, Nkx2.5 and Gata4. Protocol 2: Sheep with AMI received HMGB1 250 µg (n=6) or PBS (n=6). At 60 days HMGB1-treated sheep showed smaller infarcts (8.5±2.11 vs. 12.2±1.97 %LV area, P<0.05, t-test) and higher microvascular density (capillaries: 1798±252 vs. 1266±250/mm2; arterioles: 18.3±3.9 vs. 11.7±2.2/mm2, both P<0.01, t-test). Global LV function as assessed by % ejection fraction improved in HMGB1 group from day 3 to day 60 (39.8±8.6% vs. 54.4±6.9%, P<0.05). Similarly, as regards LV regional function, % anterior wall thickening improved in HMGB1 group between days 3 and 60 (11.5±3.2 vs. 35.3±8.3, P<0.05) and % septal wall thickening from day 3 and 30 (10.5±8.9 vs. 30.9±16.7, P<0.05, all 2-way ANOVA-Bonferroni). Conclusion. In this large mammalian model of AMI, high-dose HMGB1 induces angio-arteriogenesis and cardiomyogenesis due to expression of specific regenerative genes, reduces infarct size and preserves LV function at 2 months post-treatment.

111. (741) **CHROMOSOME 19 MICRORNA CLUSTER (C19MC) REGULATES EARLY HUMAN PLURIPOTENT STEM CELL CARDIAC DIFFERENTIATION**

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Fundacion para la Lucha contra Enfermedades Neurológicas de la Infancia. (FLENI-CONICET)

Human pluripotent stem cells (PSC) have the capacity to self-renew indefinitely in-vitro and to differentiate into any cell type of the three germ layers. The primate-specific Chromosome 19 microRNAs Cluster (C19MC) is composed of 56 microRNAs located within a 100 kbp-long locus. We previously found that C19MC microRNA expression rapidly falls upon PSC differentiation. However, little is known about C19MC function. We then investigated the role of these microRNAs in the pluripotency state. Analysis of different small RNA-seq data confirmed that C19MC is downregulated in PSC upon differentiation. We performed Ago-IP-seq for mir-520a-3p in order to study gene targets and identified more than a hundred genes. Gene Ontology (GO) analysis revealed cell proliferation and embryonic differentiation pathways, in particularly cardiac differentiation (CD), as main targets. Two strategies for microRNA downregulation failed: LNAs for a few microRNAs and CRISPR-OFF techniques proved not to be effective. We then generated a clone by CRISRP/Cas9 to knockout the whole C19MC and confirmed undetectable microRNA expression. Fifty clones were picked and one of them turned out to be positive for the C19MC deletion. C19MC(-)-PSC grew as usual and major features of undifferentiation were mostly unchanged. Minor increased in G1 was detected and slight changes in the expression of pluripotency factors, D1 and D2 Cyclines, FZD3 and MAPK genes were observed. All of them showed up to four-fold change upregulation compared to wild type cells. Cardiac differentiation was dramatically compromised in C19MC(-) cells (74% vs 1%). MESP1, a master gene for CD, was not upregulated in C19MC(-) cells. A lower increased in Zeb1 was also seen. Of note, neural differentiation was not compromised. These results suggest that C19MC is a master regulator of early cardiac differentiation.

112. (529) **DEEP LEARNING/ARTIFICIAL INTELLIGENCE HIGHLY PREDICTS EARLY CHANGES IN PLURIPOTENT STEM CELLS**

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There is an ongoing revolution with the use of powerful algorithms collectively known as machine learning. One of its branches, deep learning (DL), allows to predict unique features based on the relation of numbers through the application of vast neural networks. Digital images, as a plain collection of numbers, are susceptible to be processed by DL. Hence, DL can recognize image forms and classify them. We then applied DL to phase contrast images of pluripotent stem cells (PSC) under two different models previously developed in our labs. In a first model of cell differentiation, naïve mouse PSC were differentiated to Epiblast-like cells by removing stemness factors. In a second model of cell death, human PSC were incubated with camptothencin, a topoisomerase inhibitor, which rapid and massively induces apoptosis. Both models were validated through standard assays, including real time PCR and fluorescent staining. We took hundreds of microscopic phase contrast images in an EVOS microscope with a 10x objective. Image processing was done in AWS using Keras as frontend and TensorFlow as backend. We trained deep residual networks under different settings. We found that DL training can correctly classify images in both models with an accuracy close to 1. Independent test on non-trained images and validation in new replicates confirmed the high accuracy of both DL in both cell models. Importantly, such high accuracy was reached after approximately 30 minutes of cell differentiation and 3 hours after cell death induction. In summary, we found that applying deep learning algorithm to plain, non-stained microscopic images can readily detect morphological changes in almost all cases and correctly clas-

sify them. Our findings predicts potent applications of deep learning/ artificial intelligence in cell image detection and classification.

NEUROCIENCIAS / NEUROSCIENCE ORAL SESSION

113. (40) **EARLY EXPOSURE TO A HIGH FAT DIET PROMOTES COGNITIVE IMPAIRMENT, NEUROINFLAMMATION AND DECREASED HIPPOCAMPAL PLASTICITY. POSSIBLE IMPLICATION OF MICROGLIA- DERIVED EXOSOME-LIKE EXTRACELLULAR VESICLES.**

Angeles Vinuesa[1, 2], Melisa Bentivegna[1, 2], Gastón Calfa, Fabia Filipello, Carlos Javier Pomilio[1, 2], María Marta Bonaventura[1], Victoria Lux-Lantos[1], Amal Patricio Gregosa Merlino[2, 1], Jessica Lorena Presa[1, 2], Michela Matteoli, Juan Beauquis[1, 2], Flavia Saravia[1, 2]

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Western dietary habits including high fat-industrialized foods are increasingly represented in juvenile populations and constitute one of the factors that affect brain health, potentially leading to long lasting effects. Adolescence, characterized by brain maturation and behavioral changes, is particularly vulnerable to the environment. Hence, our aim was to study the impact of an early exposure to a high fat diet (HFD) on mouse hippocampal plasticity and cognition. To that end, C57BL/6J male mice were exposed to HFD for 6 weeks since weaning. Glycemia and seric proinflammatory IL1β were higher in HFD mice ($p<0.05$) without differences on body weight. In HFD hippocampus, neuroinflammation was evidenced by Iba1+ cells reactivity together with a higher expression of TNFα and IL1β and the neurogenic capability was found strongly reduced: both Ki67+ proliferative cells and immature newborn DCX+ neurons in the subgranular zone of the dentate gyrus were decreased ($p<0.05$). We also found a predominance of immature DiI-labeled dendritic spines from CA1 neurons, along with diminished levels of the scaffold protein Shank2, suggesting a defective connectivity. Moreover, the HFD group exhibited spatial memory alterations in the novel object location recognition test ($p<0.05$). To elucidate whether microglia could be mediating HFD-associated neuronal changes, the lipotoxic context was emulated by incubating primary microglia with palmitate, a saturated fatty acid present in HFD. Palmitate induced a proinflammatory profile as shown by secreted cytokine levels. Exosome-like extracellular vesicles were isolated by ultracentrifugating the conditioned media from palmitate stimulated microglia and induced an immature dendritic spine phenotype in primary GFP+ hippocampal neurons, in line with the *in vivo* findings. These results provide novel data concerning microglia-neuron communication and highlight that fat excess during a short and early period of life could negatively impact on cognition and synaptic plasticity in a neuroinflammatory context, where microglia-derived exosomes seem to be directly implicated.

114. (242) **AMYLOID-B PEPTIDES DISRUPT BLOOD-BRAIN BARRIER PROPERTIES BY ACTING DIRECTLY ON BRAIN ENDOTHELIAL CELLS AND INDIRECTLY THROUGH INTERACTION WITH GLIAL CELLS.**

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Alzheimer's disease (AD) is the leading cause of dementia. It is characterized by the presence of intraneuronal deposits of Tau and the extracellular accumulation of Amyloid- β (Aβ) fibrils, composed mainly of Aβ1-40 and Aβ1-42 peptides. Moreover, glial activation, neuroinflammation and alterations on brain vasculature were evidenced both in patients and animal models of AD. In this study, we hypothesized that Aβ1-40 (mostly present in perivascular deposits) can directly affect endothelial cells, while Aβ1-42 (the principal component of parenchymal deposits) act mainly through modulating glial activation. To test this, we employed human brain microvascular endothelial cells (HBMEC cell line) exposed to Aβ1-40 and Aβ1-42. Acute exposition to both fibrilar and soluble Aβ-40 (but not Aβ1-42) induced activation of HBMEC as it was shown by nuclear translocation of NFkB. This activation was mediated by interaction with the Receptor for Advanced Glication End-Products (RAGE), as it was prevented by the use of a competitive inhibitor. The nuclear translocation of NfkB was associated with a reduced location of the tight junction protein Occludin at the plasmatic membrane. Moreover, the conditioned medium from astrocytes (C6 cell line) exposed to fibrilar Aβ1-40 and also Aβ1-42, activated endothelial cells in a RAGE-dependent way. However, conditioned medium from microglial cells (BV-2 cell line) activated endothelial cells when they were exposed either to fibrilar Aβ1-40 or Aβ1-42, in a RAGE-independent way. Employing PDAPP mice, an animal model of AD, we detected an early reduction in the proportion of astrocytes in direct contact with hippocampal vasculature, suggesting that astroglial-endothelial interaction can be altered in pre-symptomatic stages of AD. We conclude that disruption of blood-brain barrier properties can be caused directly by the action of Aβ peptides but also indirectly by the activation of glial cells. In both cases, the damage-associated receptor RAGE plays a critical role on endothelial activation.

115. (467) **INTRA-SPINAL ADMINISTRATION OF NETRIN-1 PROMOTES RECOVERY OF VOLUNTARY LOCOMOTION AFTER COMPLETE SPINAL CORD INJURY IN A RAT MODEL.**

Gabriela Barrientos, Ana Uceda, Alejandra Sgariglia, Graciela Giardina, Jorge Toblli, Ramiro Quinta

Hospital Alemán - UBA - CONICET

For decades, axon regeneration has been considered the Holy Grail for spinal cord injury (SCI) repair. The "key" has been thought to be the regeneration of the long motor and sensory tracts that are interrupted by SCI. Worldwide scientists focus their research in promoting re-growth of these axonal tracts to achieve recovery of paralysis, restoration of autonomic function and re-gain of sensation.

According to this background, we focus the treatment of this traumatic pathology by using an axonal chemoattractant protein, which is involved in axonal growth during the embryonic development. This protein, Netrin-1, was described 20 years ago, as an axonal repulsive molecule expressed in embryonic tissues in the form of gradients. However, it has been described that it also participates in axonal guidance by promoting axonal growth as opposed to what was believed.

Using a transection SCI rat model, it was observed a significant recovery of locomotion in rats treated with Netrin-1 intra-spinally. Locomotion scores 21 days after injury showed a significant improvement of locomotor function in rats treated acutely with Netrin-1 as compared to control animals treated with vehicle, which showed a complete absence of locomotor recovery. This result correlated with an improvement in the control of voluntary locomotion assessed by using the ladder rung test. Rats treated with Netrin-1 showed a decreased number of missed steps and slipped steps in the ladder as compared to controls.

At the histology, it was observed a significant regeneration of axonal tracts at the injury site in Netrin-1 treated animals. Moreover, treated animals showed decreased axonal "die-back" upstream of the injury site, and maintained synaptic vesicles. These effects were found exclusively in treated rats.

In summary, Netrin-1 administration after SCI promotes a sharp recovery of locomotor activity that was correlated with reduced axonal die back and increased axon outgrowth.

116. (422) **EFFECTS OF TAURINE MIXED WITH ALCOHOL IN A MURINE MODEL OF ALCOHOL HANGOVER. BEHAVIORAL AND MORPHOLOGICAL APPROACH**

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The amino acid Taurine (TAU) is one of the main components of energy drinks. Its use has become popular, mixed with alcohol (OH). The objective of this work was to study the possible behavioral and neurodegenerative effects of TAU in an experimental model of al-

cohol hangover (AH) in mice. Neuromuscular coordination, muscle tension and presence of early signs of neurodegeneration were evaluated in male mice (n=10-12/group) treated with OH (3.8 mg/kg, i.p.) and/or TAU (190 mg/kg, i.p.). Controls were injected with saline (SAL). The treatment was carried out at 8 am (ZT1) and the behavioral studies were performed at ZT 0 (7 am, basal) and ZT 7 (2 pm, beginning of the AH). The Tightrope and Hanging wire tests were used to study neuromuscular coordination and muscle tension, respectively. Another groups of mice with the same treatment (n=8) were intracardially perfused and their brains were subjected to immunofluorescence by using an anti-GFAP antibody (glial fibrillary acidic protein) to identify reactive astrocytes and anti-NeuN (Neuronal nuclei marker) antibody to determine early signs of neurodegeneration. The confocal images were analyzed with the Image-J and GraphPad Prism software. At the beginning of AH, it was detected a greater loss of neuromuscular coordination (> score in sec) (SAL+SAL: 2.2±0.5; TAU+OH: 14.1±3.0, $p<0.01$). In addition, mice showed a significant decrease in latency to fall (SAL+SAL: 58.3±0.8; TAU+ OH: 10.1±2.1, in sec, $p<0.01$). A significant astrocytic reaction was observed in the internal capsule of TAU+ OH treated mice (SAL+SAL 15.70±0.49 vs TAU+OH: 25.60±1.00 , in IOD, $p<0.05$). Likewise, the NeuN neuronal marker was displaced to the cytoplasm in a significant number of neurons in TAU+ OH (SAL+SAL: 3.38±0.85 vs. TAU+ OH: 35.50±4.70, in %, $p<0.05$). The results suggest that TAU combined with OH decreases neuromuscular coordination and muscle tension and also produces early signs of neurodegeneration.

117. (485) **ALTERED SYNAPTOGENESIS AND SYNAPSE REMODELING UNDERLIE HIPPOCAMPAL HYPOCONNECTIVITY IN AN EXPERIMENTAL MODEL OF AUTISM**

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Autism spectrum disorders (ASD) are characterized by impairments in social interaction and repetitive-stereotyped behaviours. Although image studies have described local hyperconnectivity in the prefrontal cortex (PFC) of ASD patients, reports in the hippocampus are still not conclusive. This structure is implicated in exploration, learning and memory but also in emotion and social behaviour. Applying the well-validated ASD animal model by prenatal exposure to valproic acid (VPA 450mg/kg IP), we previously reported in the hippocampus of juvenile VPA rats: a decrease in the synaptic marker synaptophysin (SYN) along with an increased expression of the neural cell adhesion molecule (NCAM) and a decrease in its polysialylated form (PSA-NCAM). The aim of this study was to evaluate synapse formation and remodeling of primary hippocampal neurons either from VPA or control male pups (postnatal days 1-2), in the absence of glia. Cytoskeletal and synaptic markers were evaluated (DIV7-14) by immunocytochemistry and Western Blot. At DIV 14, neurons from VPA animals displayed a reduced dendritic tree (reduced MAP2 area), a reduced number of glutamatergic synapses (decreased vGLUT and PSD-95 puncta number and area) and NMDA receptor clusters (decreased NR1 puncta number and individual puncta area). These neurons also exhibited reduced number of functional synapses (FM4-64 labelling) which contained smaller vesicular pools; total NCAM expression increased while PSA-NCAM decreased. While in neurons from control animal glutamate (5μM-3min) induced an NMDA-dependent dendritic retraction and SYN puncta number reduction, neurons from VPA animals were only capable of dendritic retraction without any change in synapse number. Our results indicate that neurons from VPA animals form a lower number of glutamatergic synapses that exhibit a more adhesive and resistant profile to synaptic remodeling. Unlike to the hyperconnectivity proposed for the PFC, our findings suggest that neuronal alterations would contribute to hippocampal hypoconnectivity and reduced synaptic plasticity.

118. (576) **ENVIRONMENTAL ENRICHMENT INFLUENCES OLIGODENDROCYTE LINEAGE PROLIFERATION AND BENEFITS BEHAVIOURAL ALTERATIONS IN THE VPA MODEL OF AUTISM SPECTRUM DISORDERS**

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Autism Spectrum Disorders (ASD) are a group of neurodevelopmental disabilities characterized by alterations in brain connectivity. The valproic acid (VPA) model of ASD resembles behavioural characteristics of these disorders and evidences of altered brain connectivity. Oligodendrocytes are specialized glial cells responsible of axonal tracts myelination, a fundamental process for connectivity of neural circuits. It is known that myelination is influenced by the environment. Since corpus callosum (CC) is the main structure that connects both brain hemispheres, the aim of this work was to evaluate the glial profile in the CC of VPA rats, and the therapeutic effects of an environmental enrichment (EE) strategy on the behaviour and oligodendrocyte lineage of these animals. On PND 21, VPA and control rats were weaned and housed in standard (C-S/V-S) or enriched (C-EE/V-EE) environment. Behaviour was evaluated in two stages: on early postnatal days (PND) 7-16 and on juvenile stage (PND 30-36). On PND 36, expression levels of GFAP and tomato lectin, and number of PDGF+ and CC1+ cells were evaluated. Myelin sheet ultrastructure was studied by electron microscopy. VPA animals presented behavioural deficits in juvenile stages that were prevented by EE. Number of mature oligodendrocytes (CC1+) was diminished in the CC of VPA animals, accompanied with an increment in the number of immature oligodendrocytes (PDGF+). This was in the absence of astrogliosis (GFAP) or microgliosis (tomato lectin). CC ultrastructure of VPA rats exhibited a decreased percentage of myelinated axons and an aberrant myelin sheet. When VPA animals were raised in EE, myelin ultrastructural alterations were fully prevented, number of immature oligodendrocytes reached control levels with no changes in number of mature oligodendrocytes. Our results demonstrate that EE is an effective strategy for modulating behaviour and myelination in VPA rats. Also, this work suggests that EE affects the proliferation of oligodendrocytes in VPA animals.

INMUNOLOGÍA / IMMUNOLOGY ORAL SESSION 2

119. (89) **PROTEIN DEFICIENCY MODIFIES THE GUT HUMORAL RESPONSE TO TRICHINELLA SPIRALIS AND THE FECUNDITY INDEX OF ADULT WORMS**

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We have previously demonstrated that protein deficient rats infected with *Trichinella spiralis* suffer a severe infection with a delayed expulsion of adult worms (AW) from the gut and a higher parasite burden in muscles.

Intestinal *Trichinella*-specific IgE and IgA are known to reduce the fecundity of the AW.

The aim of this work was to analyze the effect of a protein deficient diet on the production of intestinal anti-*Trichinella* antibodies and on AW fecundity index.

Weaning Wistar rats received protein deficient (PD, 6.5% casein) or control (C, 20% casein) diets. After ten days, both groups were orally infected with *T. spiralis* muscle larvae (PD_I and C_I groups, respectively). Intestine tissue extracts were obtained (PERFEXT method) and specific antibodies against AW excretory/secretory products (AW-ESP) were detected by ELISA. To determine the fecundity index AW were obtained from the intestine of PD_I and C_I groups and were incubated during 3 h in RPMI medium. Female AW and newborn larvae (NBL) shed by female AW were counted. The fecundity index was calculated as the number of NBL/female AW. Results were analyzed using the two-way ANOVA test.

All antibody isotypes against AW-ESP were increased in intesti-

nal extracts of the C_I group but only IgG2a had a significant rise in PD_I. The PD_I group had a higher fecundity index than C_I starting at day 9 p.i. (30.58±3.39 *vs.* 14.32±2.95, NBL/female AW $P<0.05$). At day 20 p.i., only the AW obtained from the PD_I group continued to release NBL (14.05±2.30 *vs.* 0.00±0.00 NBL/female AW $P<0.05$). Overall, these results suggest that the higher parasite burden previously found in the PD_I group may be associated with the longer persistence of AW in the intestine and the higher fecundity index of female AW due to the lower specific immune response in these animals.

120. (178) **HUMAN B CELLS: A NEWLY DESCRIBED TARGET OF INFECTION BY OLD AND NEW WORLD HANTAVIRUSES**

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Hantavirus are enveloped RNA viruses that belong to the Hantaviridae family. Old World hantaviruses cause Haemorrhagic Fever with Renal Syndrome (HFRS), whereas New World hantaviruses are responsible for Hantavirus Pulmonary Syndrome (HPS). The main target of infection are microvascular endothelial cells. In previous studies, we observed a massive polyclonal activation of circulating B cells in HPS patients from Argentina and an increased risk for B cell lymphoma after HFRS in Sweden, which prompted the question whether these cells may constitute an alternative target for hantavirus infection.
To test this hypothesis, a B cell line (BJAB) and purified B cells from healthy blood donors (n=4) were exposed to HTNV -Old World- and ANDV -New World- species (MOI 1) or UV-inactivated virus, as negative controls. Supernatants and cells were collected at subsequent time points (6-96h). To asses for infection, the presence of nucleocapsid protein (NP) in fixed cells (confocal microscopy) or cell lysates (western blot) was determined. Genomic RNA S-segment was quantified by qRT-PCR to measure viral replication. For production of progeny virus, supernatants were assessed by a focus forming unit assay.
NP from both species was detected by WB in both BJAB and purified B cells, as well as by microscopy in BJAB, confirming that human B cells are indeed susceptible to infection by both hantaviruses. Furthermore, both species replicated in the two cell types, confirmed by increasing copy number ($\Delta\Delta$Ct) over time ($p<0.01$). Moreover, both hantaviruses caused productive infection in the BJAB, shown by increased progeny titers over time ($p<0.05$), whereas no productive infection was readily detected for purified B cells.
Altogether, these results indicate that human B cells constitute an alternative target for hantaviruses. This novel finding sets the stage for further studies to assess a possible role of infected B cells in disease pathogenesis.

121. (337) **UV PHOTOTHERAPY AS AN ADJUVANT TREATMENT FOR METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS CUTANEOUS INFECTION**

Eliana Cela, Camila Ledo, Valeria Campo, Juliana Leoni, Marisa Gómez, Daniel González Maglio
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Staphylococcus aureus, a colonizer of 30% of the population, is the most common cause of skin and soft tissue infections. The skin is the main target of UV radiation (UVr), which modulates cutaneous immune responses. The increasing incidence of resistant S. aureus (methicillin resistant S. aureus-MRSA), has major implications for current as well as future treatment options for this pathogen. This study was aimed at evaluating the use of UVr as an adjuvant treatment for MRSA skin infection.
To select the UVr dose, Balb/c mice were irradiated on their back with 0, 25, 50, 100, 200 and 400 mJ/cm2, and sacrificed at 24, 48, 72 or 96 hours post-exposure. Histological evaluation and quantification of pro-inflammatory cytokines and chemokines (epidermis and dermis) was performed. The dose of UVr selected was 100 mJ/cm2, since it moderately increased pro-inflammatory cytokine and chemokine production (TNF-α, IL-6 and KC-1: 30, 80 and 1500 pg/mg protein, respectively, $p<0,05$ vs 0 mJ/cm2), promoted an inflammatory infiltrate (neutrophils) in the irradiated area (maximum 72 hs post-UVr) and produced just slight alterations of the skin architecture. To assess the potential of UVr as adjuvant therapy, mice were subcutaneously inoculated with 50 μl of MRSA (USA300LAC, 1x109 UFC/ml). Forty-eight hours post-inoculation, one group of mice was exposed to UVr, whereas the other remained as control. The infection was monitored by measuring the abscess size and mice weight daily for 14 days. UVr significantly reduced the area of the abscesses (89% of reduction at time 14 compared with time 0) whereas the non-irradiated group showed a reduction of 60% in the abscess size ($p<0,05$). UVr phototherapy may be a new strategy for MRSA treatment, since it promotes a local inflammatory response that favours neutrophil recruitment, necessary for bacterial clearance from the abscess and does not generate new antimicrobial resistance.

122. (585) **SIGNALING LYMPHOCYTIC ACTIVATION MOLECULE (SLAM): A MOLECULE THAT COUNTERACTS M. TUBERCULOSIS MACROPHAGES' IMMUNE EVASION?**

Angela Maria Barbero[3], Rodrigo Emanuel Hernández Del Pino[3], Josefina Celano[3], Martin Estermann[3], Aldana Trotta[1], Melanie Genoula[1], Luciana Balboa[1], Paula Barrionuevo[1], Virginia Pasquinelli[3]
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Far from being an eradicated disease, Tuberculosis is nowadays the leading cause of death from a pathogen worldwide. *Mycobacterium tuberculosis* (*Mtb*) has smartly manipulated the immune system to survive within macrophages over ages.
The costimulatory molecule SLAM is a self-ligand receptor that can internalize Gram-negative bacteria and regulate macrophages' phagosomal functions. In tuberculosis SLAM promotes Th1 protective responses.
Here we studied SLAM modulation during *Mtb* infection and its role on macrophages' functions.
Human monocyte-derived macrophages were obtained from healthy donors by CD14 positive selection. After 2h of adherence, cells were cultured in complete media overnight before stimulation with sonicated *Mtb*. THP-1 cells differentiated with PMA and stimulated with *Mtb* were also used. In some experiments macrophages were additionally stimulated with rhIFN-γ, rhIl-4, rhIl-10 or agonistic anti-SLAM antibody.
Our results showed that *Mtb*-induced SLAM expression, determined by flow cytometry, was increased by IFN-Y and IL-10 treatment. No changes where observed with IL-4. Moreover, IFN-γ increased TNF-α secretion in *Mtb*-stimulated THP-1 cells as measured by ELISA.
Rhodamine-stained *Mtb* (*Mtb-R*) was used to study SLAM role on bacterial uptake by flow cytometry. Anti-SLAM treatment further induced *Mtb-R* phagocytosis ($p<0.05$). Moreover, these cells showed high expression of activation markers (HLA-DR and CD86) and very low levels of CD163 (M2 marker). More than 50% of the phagocytic cells expressed SLAM and this percentage increased with IFN-γ stimulation.
To elucidate SLAM role as a microbial sensor, SLAM colocalization

with bacteria and endosome markers (EEA1 and CD107b) was evaluated by confocal microscopy. Interestingly, we observed colocalization between SLAM/*Mtb-R*, SLAM/EEA1 and CD107b/*Mtb-R*, suggesting that SLAM actively participates in macrophages' key microbicidal processes such as phagocytosis and phagolysosome maturation.
Taken together, these results provide evidence of SLAM as a potential microbiological sensor and regulator of macrophages' functions against *Mycobacterium tuberculosis.*

REPRODUCCIÓN / REPRODUCTION 1

123. (26) **COCAINE ALTERS MOUSE GERM CELLS EPIGENOME WITH DIRECT IMPACT ON H4AC EXPRESSION AND DNA METHYLATION IN THE SPERM**
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Cocaine intake is associated with testicular toxicity and significant reproductive function impairment. There is accumulating evidence that cocaine administration can trigger nongenetic inheritance through the male germ line affecting development and behavior of the offspring. The influence of environmental factors on the epigenome of male germ cells appears to be most impactful if it happens during a developmental phase when these cells are epigenetically reprogrammed. In the present study, we measured epigenetic marks in isolated germ cells of adult mice treated with cocaine (10 mg/kg) or vehicle, in an intermittent binge protocol (3 i.p. injections, 1 h apart, one day on/off for 13 days). We found that chronic cocaine intake disrupts male germ cell epigenetic homeostasis, increasing global methylated citocine (5-mC) levels in DNA from germ cells and cauda epididymal sperm (germ cells: vehicle 13.15 ± 0.99 vs cocaine 20.113 ± 2.28; sperm: vehicle 15.81 ± 1.08 vs cocaine 21.35 ± 1.52). Cocaine also increased acetylated histone 4 (H4ac) protein levels and decreased class I deacetylases HDAC1/2 mRNA and protein expression ($p<0.05$). The mRNA expression levels of class IIa and IIb HDACs were also altered ($p<0.05$). Immunolocalization studies showed that HDAC1/2 were mainly expressed in primary spermatocytes and H4ac was immunolocalized in late meiotic stages in vehicle mice while it was detected in primary spermatocytes and in successive stages until round spermatid in cocaine-treated mice. We observed altered mRNA expression of DNA methylation markers in isolated germ cells showing decreased levels of Dnmt3b and Tet1 gene expression after cocaine treatment ($p<0.05$). TET1 was mainly immunolocalized in primary spermatocytes in vehicle and cocaine-treated mice. The results presented here broaden the basic knowledge of the impact of addictive stimulants on testicular pathophysiology, fertility and male reproductive health and imply that altered epigenetic homeostasis by cocaine may have potential consequences on future generations.

124. (592) **ANANDAMIDE IMPAIRS THE SYNCYTIALIZATION OF HUMAN CYTOTROPHOBLAST CELLS**
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The syncytiotrophoblast (STB) is the main structural and functional layer of the human placenta. The underlying cytotrophoblast cells (CTB) fuse to form this multinucleated syncytium in a process called syncytialization. Disturbance in this event is associated with pregnancy pathologies such as preeclampsia and intrauterine growth restriction.
Endocannabinoids (eCBs) are a group of bioactive lipid mediators which, together with their receptors and the enzymes involved in their metabolism, constitute the endocannabinoid system (ECS). Anandamide (N-arachidonylethanoalmine, AEA) and 2-Arachidonoylglycerol (2-AG) are the major eCBs, having both important roles in human placentation. Recently it has been reported that 2-AG impairs CTB fusion. However, the role of AEA in this process remains unknown.
The aim of this work was to study the impact of AEA on syncytialization and to elucidate the mechanisms involved in this process.
BeWo cell line was cultured with 25μM forskolin (FSK) to induce syncytialization. CTB (BeWo) and STB (BeWo+FSK) were treated with or without R-(+)-Methanandamide (0.01μM-10μM Met-AEA), a stable analogous of AEA. Cell viability was evaluated by MTT assay and LDH activity. We studied different trophoblast syncytialization markers: Glial cells missing-1 (GCM-1) mRNA levels by RT-qPCR; human chorionic gonadotropin (hCG) concentration by chemiluminescence immunoassay; syncytin-1 protein expression, cell size and DNA content by flow cytometry; and E-cadherin distribution by immunocytochemistry. Incubation with 1μM Met-AEA diminished the mRNA levels of GCM-1($p<0.05$ n=4) and the secretion of hCG ($p<0.05$ n=3), syncytialization markers increased by the forskolin-induced cell fusion. This was partially reverted by incubation with AM630, an antagonist of cannabinoid receptor 2. Additionally, Met-AEA produced a decrease in cell fusion evidenced by E-cadherin distribution and impaired the increase in the number of syncytin-1-positive cells stimulated by FSK.
These results suggest that increased AEA levels may disturb human trophoblast syncytialization.

125. (704) **THE ENDOCANNABINOID SYSTEM IS PRESENT IN THE CERVIX OF PREGNANT MICE AND IS INVOLVED IN PRETERM LABOR INDUCED BY LIPOPOLYSACCHARIDE ADMINISTRATION.**
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The endocannabinoid system (ECS) is one of the numerous signaling pathways that have been involved in the pathophysiology of pregnancy. In our laboratory, we have previously demonstrated the presence of the ECS in implantation sites and infiltrating immune cells in a murine model of lipopolysaccharide (LPS)-induced embryonic resorption. On the other hand, in an in-vivo LPS-induced preterm labor model, we have observed that the immune challenge produced an augmentation of matrix metalloproteinase (MMP) activity in the cervix. Based on these premises, the objective of the present study was to investigate the presence and possible modulation of the ECS in cervical tissue in LPS-induced preterm labor model. Furthermore, we proposed to study the metalloproteinase activity changes induced by LPS and its possible modulation for the ECS in vitro. In first place we evaluated protein levels of CB1, CB2, NAPE-PLD and FAAH in cervical tissue from pregnant balb/c mice on 15 day of pregnancy, treated or not with LPS. We demonstrated the presence of all the components of de ECS in the cervix and we found that the immune challenge downregulates the expression of FAAH protein. On the other hand, we utilized an in vitro model to evaluate MMP9 and MMP2 activity. Briefly, cervix from 15 day pregnant mice were obtained and cultured in presence or not of LPS for 6 hours. MMPs gelatinase activity was evaluated by zymography. We found that LPS increased significantly MMP9 and MMP2 gelatinase activity in cervix ($P<0.05$) and preliminary data showed that an specific antagonist of CB1 could be modulating MMPs activity, reducing it to control levels. Collectively, we showed that the ECS is present in the cervical tissue and probably it is involved in cervical remodeling.

126. (677) **ENDOCANNABINOID SYSTEM CARACTERIZATION IN DECIDUAL TISSUES FROM 15-DAY PREGNANT MICE**
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Endocannabinoids, like anandamide (AEA), are the endogenous

ligands for cannabinoids receptors CB1 and CB2 that are part of the endocannabinoid system (ECS). Similarly to prostaglandins, endocannabinoids are implicated in different aspects of reproduction, such as maintenance of pregnancy and parturition. In our laboratory, we have previously seen that lipopolysaccharide (LPS) systemic administration increased the level of prostaglandin F2α production and the protein level of cyclooxygenase (COX)-2 in deciduas from 7-day pregnant mice.
The aim of the present study was to investigate the presence and possible modulation of the ECS in decidual tissue in an LPS-induced preterm labor model.
We demonstrated the presence of ECS in deciduas from pregnant Balb/c mice in 15-day of gestation, comprising the enzyme that synthesizes AEA, (N-acylphosphatidylethanolamine-specific phospholipase D, NAPE-PLD,), the enzyme that hydrolase AEA (fatty acid amide hydrolase, FAAH), and the receptors CB1 and CB2. When we administred LPS intraperitonealy on 15 day of pregnancy we observed an increase in COX-2 decidual protein levels vs deciduas from control 15-day pregnant mice ($p<0.05$). This could be associated with an increase of prostaglandin levels. Furthermore, we utilized an in vitro model to evaluate COX-2 expression. Briefly, decidua from 15-day pregnant mice were obtained and cultured in presence or not of LPS for 6 hours. Consistently with our previously results with observed an increase in COX-2 protein levels in 15-day of pregnant mice decidual explants ($p<0.05$).
In summary, we showed that the ECS is present in decidual tissue and we proposed an in vitro approach to study the changes induced by LPS and its possible modulation for the ECS.

127. (280) **SHIGA TOXIN TYPE 2 (STX2) INHIBITS MIGRATION, INVASION AND TUBES FORMATION IN TROPHOBLAST CELLS OF THE FIRST TRIMESTER (SWAN 71 CELL LINE). POSSIBLE ROLE OF INDUCIBLE NITRIC OXIDE SYNTHASE (INOS) IN THE DAMAGE PRODUCED BY STX2.**

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Successful placentation involves migration, invasion and remodeling of uterine arteries by trophoblast cells. Failures in these processes are related to serious obstetric complications, as placental dysfunction or miscarriage. We propose was that Stx2, the main virulence factor of Shiga toxin producing Escherichia coli, can cause feto-placental damages through a misbalance of nitric oxide production by inducible nitric oxide synthase (iNOS). Our aim was to evaluate the effects of Stx2 (0.1 μg/ml) at 24 h on migration, invasion and tubulogenesis of first trimester trophoblast cells and to analyze if aminoguanidine (AG), a selective inhibitor of iNOS can modulate Stx2 effects. Swan 71 cell line was used as a first trimester trophoblast model. Cell migration was determined as percentage of wound closure in wound-healing assays. Invasiveness was evaluated as the number of cells invading *tanswells* coated with Matrigel. Finally, tubulogenesis were analyzed by counting the number of extremities and branch formation. Stx2 significantly decreased cell migration (64.8 ± 3.1 *vs* 27.5 ± 6.9 %), cell invasion (nº of invading cells: 107.3 ± 19.5 *vs* 41.5 ± 6.9) and tubulogenesis (nº of extremities: 538 ± 58 *vs* 304 ± 28, branch formation: 335 ± 41 *vs* 139 ± 4). AG partially reverted the Stx2 effects compared to control (n.s.). These results support the hypothesis that Stx2 could impair placenta formation by a process involving iNOS

128. (357) **IMPACT OF ZIKA VIRAL INFECTION ON FIRST-TRIMESTER TROPHOBLAST CELL FUNCTION AND ITS RELEVANCE IN THE REGULATION OF THE IMMUNE MICROENVIRONMENT**

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The placenta may become exposed to micro-organisms throughout pregnancy such as viruses which pose a substantial threat to the embryo/fetus development. Zika virus (ZIKV) infection during pregnancy leads to an increased risk of fetal growth restriction and fetal central nervous system malformations. Recently it has been reported that ZIKV infects first-trimester human trophoblast (Tb) cells enabling transplacental transmission. Since trophoblast cell function and metabolism are crucial to allow fetal growth and development, our aim is to determine the effect of ZIKV infection on first-trimester human Tb cells function and its implication in immune homeostasis maintenance. We infected the first-trimester human Tb-derived cell line Swan-71 with the local isolated ZIKV strain INEVH116141. High intracellular levels of viral RNA, with an extracellular viral production of 2.7 105 UFP/mL was detected. ZIKV induced an important antiviral response in Tb cells increasing the expression of IFN-beta/BST2/RIG/TRAF6 and viperin mRNAs. Also, an increased mRNA expression of pro-inflammatory cytokines (TNF-alpha/IL-6/IL-1beta) was found in Tb ZIKV infected cells. Alterations in the AHR/IDO1 pathway, crucial for the immune homeostasis maintenance and a dysregulation in the expression of specific glucose/amino acid carrier proteins GLUT1/3 and SNAT1/2, respectively were also determined. On the other hand, assays with synthetic analog of viral RNA, Poly I:C, induced a decrease of cellular migration after 24h of culture in wound healing assays ($n=5$; $p<0.05$) and a decrease of glucose uptake measured by flow cytometry using the fluorescent analogue 2-NBDG ($n=4$; $p<0.05$) without changes in cell viability measured as the percentage of apoptotic/necrotic cells by annexin V/propidium iodide staining.
Taken together these preliminary results suggest that RNA viral infection might impact early pregnancy by affecting trophoblast migration capability and metabolism.

129. (360) **LPS FROM PORPHYROMONAS GINGIVALIS AFFECTS TROPHOBLAST CELL FUNCTION AND IMMUNE-TROPHOBLAST INTERACTION**

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Placentation is associated with trophoblast cell differentiation into invasive phenotypes, activation of matrix metalloproteinases (MMP) as well as trophoblast-immune cell interaction to maintain immune homeostasis. Porphyromonas gingivalis is considered to be an important pathogen of periodontal disease that has been also implicated in adverse pregnancy outcome (APO). However the pathophysiology remains unknown.
The aim of this study was to examined the effect of a virulence factor such as lipopolysaccharide from Porphyromonas gingivalis (PgLPS) on trophoblast cell function and trophoblast-immune interaction.
Cell migration was studied in the human trophoblastic cell line from 1st trimester Swan-71 by wound healing assays and invasiveness in Matrigel-covered transwells. MMPs, TIMPs and cytokine expression was measured by RTqPCR. Whole blood was obtained from healthy donors and neutrophil purified on Ficoll-Triyosom gradient and Dextran sedimentation. The effect of Swan-71 treated with PgLPS conditioned media (CM) on neutrophil migration through transwells was quantified by Flow cytometry.
PgLPS treatment (10ng/ml) significantly reduced cell invasion with more than five times increased expression of tissue inhibitor of matrix metalloproteinase -1 and -2 ($P<0.05$). This effect was associated with 11.5 ±3.0 fold increased expression of TGFb1 with PGLPS vs. basal; $P<0.05$). The migration of neutrophils to PGLPS treated - trophoblast CM was decreased vs trophoblast CM ($P<0.05$). In line with these results PGLPS treatment strongly inhibited the expression of IL-8, a potent chemoattractant of neutrophils.
These results suggest a pathogenic effect of low concentrations of PgLPS on trophoblastic cell function affecting the invasion capability and the trophoblast-immune interaction.

130. (562) **KRÜPPLE-LIKE TRANSCRIPTION FACTOR 6 (KLF6) TRIGGERS PLACENTAL TROPHOBLAST CELL FUSION AND MODULATES CELL MIGRATION**

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Proper placenta development is critical for foetal well-being and pregnancy outcome. Trophoblasts differentiate into the multinucleated syncytiotrophoblast and the migratory/invasive cytotrophoblasts, through the villous and extravillous pathways, respectively. KLF6 is a ubiquitous transcription factor highly expressed in placenta. Klf6-/- mice die at day E12.5 showing impaired placenta development. We have demonstrated that KLF6 is required for cell–cell fusion in human primary villous cytotrophoblast as well as in the BeWo trophoblast-derived cell line. Additionally, KLF6 immunoreactivity is higher in the placental bed of preeclamptic than in those of uncomplicated pregnancies. We hypothesize that KLF6 is a key transcription factor involved in both differentiation pathways. Cell-cell fusion was analysed by immunofluorescence in BeWo cells overexpressing or not KLF6 and treated or not with 30 μM forskolin, an inducer of BeWo fusion. Migration was evaluated through wound-healing and transwell assays inHTR8/SVneo extravillous cells transfected with a specific KLF6 siRNA or control scramble siRNA. Cell proliferation and viability was evaluated by BrdU labelling, MTT assay and cell count. Transcript and/or protein level of differentiation markers were evaluated by RT-PCR and western blot, respectively. The syncytialization index, as well as βhCG, syncytin-1 and p21 expression were statistically significantly higher in cells overexpressing KLF6, even in the absence of forskolin treatment. On the other hand, increased migration and expression of β-catenin and connexin-43 was observed in HTR8/SVneo KLF6-silenced cells, whereas proliferation was reduced. Present results reveal that KLF6 can initiate and induce BeWo cell fusion and syncytiotrophoblast genes expression, suggesting that it is a master regulatory gene of cell differentiation into syncytium. While the enhanced migratory capacity of KLF6-silenced HTR8/SVneo cells and the increased KLF6 immunoreactivity detected in the placental bed of preeclamptic pregnancies characterized by impaired cytotrophoblast invasion into the decidua, suggest that KLF6 modulates trophoblast differentiation into the extravillous invasive pathway.

131. (737) **EFFECTS OF METFORMIN ON OOCYTE MATURATION AND CUMULUS OOPHORUS APOPTOSIS DURING IVM OF PORCINE COC**

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The insulin-sensitizing drug metformin has antioxidant and antiapoptotic properties in a variety of models. We have previously shown that metformin added to in vitro maturation (IVM) medium containing insulin- transferrin- selenium (ITS) has antioxidant effects on porcine COC. It has been shown that metformin plus insulin during in vitro culture of porcine embryos increases the blastocyst rate. The aim of this work was to determine if metformin added to IVM medium has antiapoptotic effects and if it has any effect on oocyte maturation rate. Porcine COC were obtained from slaughterhouse ovaries and matured in vitro during 44-46 h in TCM medium supplemented with ITS (group I), metformin (group M), ITS + metformin (group I+M) or nothing (control). COC were treated with hyaluronidase for obtaining cumulus cells. Early and late apoptotic, necrotic and viable cumulus cells were detected by flow cytometry using annexin-V and PI. Oocyte maturation rate was determined using Hoechst under UV microscope. Data were analyzed by Chi square and Fisher exact test for each pair of columns: The group M had lower and the group I had higher number of apoptotic cells than the other groups ($p<0.0001$). I+M showed higher maturation rate than the other groups ($p<0.01$). Given that metformin added to IVM medium had antiapoptotic effects on cumulus cells and that it increased oocyte maturation rate, we conclude that metformin is a good supplement for IVM media. It remains to evaluate the potentially beneficial effect of metformin on IVF and in vitro embryo development.

132. (162) **THE EXPRESSION OF SOX2, SOX17 AND STELLA IN OVARIES OF ADULT VIZCACHAS SUGGEST THE DE NOVO RENEWAL OF THE GERMINAL MASS**

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At each estral cycle, vizcachas exhibit a massive follicular recruitment and ovulate up to 800 oocytes per cycle although the maximum size of an eventual litter is 2. Unlike most mammals, vizcachas show no signs of apoptosis of their follicular structures, and so the ovary continuously remodels its architecture to maintain the availability of quiescent primordial follicles throughout their reproductive life. Our laboratory has shown that the pool of primordial follicle remains relatively constant along the different reproductive stages despite the continuous massive recruitment and ovulations that characterizes this species. Therefore, we hypothesize a permanent renewal of the ovarian germinal mass of the adult vizcachas (oogenesis de novo) to maintain the follicular reserve. Herein, we examined the immunoexpression of the pluripotency markers Sox2 and Sox17 as wells as the germinality marker Stella in ovaries of early-, mid-, term- and non-pregnant vizcachas. Oocytes of primordial follicles and nuclei of rounded stromal cells of early- and mid-pregnacy were positive for Sox2. Sox17 was observed in stromal cells located near the ovarian epithelium in the same groups of animals. Interestingly, although Stella was not detected in follicular structures, it exhibited a marked expression in certain conspicuous cellular packages within the stroma at all the evaluated reproductive stages. The expression of pluripotency and germinality markers in ovaries of adult vizcachas indeed supports the hypothesis of the de novo oogenesis. However, the scarce reactivity of Sox2 and Sox17 is not sufficient to explain the constant size of the pool of primordial follicles throughout their reproductive life, suggesting that there should be other(s) factor(s) involved in inducing the renewal of the germinal mass in adult female vizcachas.

133. (720) **EFFECTS MEDIATED BY ANDROGEN RECEPTOR ON THE UTERINE STROMA IN A RAT PCOS MODEL**

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Polycystic ovarian syndrome (PCOS) is associated with uterine abnormalities. Here, we investigated the effects mediated by androgen receptor (AR) on the uterine stroma in a rat PCOS model. Wistar rats were injected sc with sesame oil (control group), dehydroepiandrosterone (DHEA) 6mg/100g bw (PCOS group) or DHEA 6mg/100g + flutamide (AR antagonist) 2mg/100g bw (PCOS+FLU group) from 21 to 41 days of age. In PCOS rats we showed an increase of the subepithelial stroma and myometrial thickness associated with a decrease in cell density compared to controls rats in both uterine compartments. Then we studied the extracellular matrix and demonstrated that this was modified in PCOS rats: the organization of the collagen fibers increased in the subepithelial stroma and the myometrium and the uterine water content was increased in association with changes in aquaporin expression (AQP). In PCOS rats, the expression of AQP3, 7 and 8 decreased in uterine epithelium whereas AQP8 was induced in myometrium. When flutamide was administered together with DHEA (PCOS+FLU group) the myometrial thickness was decreased without changes in cell density compared to PCOS rats (PCOS:360±33 vs PCOS+FLU:231±16

μm; p<0.05). In PCOS+FLU rats, the organization of the collagen fibers did not show differences with both PCOS and control animals. Also, the water content in PCOS+FLU rats did not show differences with PCOS rats. However, the expression of AQP8 in PCOS+FLU rats decreased in the myometrium showing similar values to control rats. Our results show that the inhibition of AR inhibited the increase of the myometrial thickness observed in PCOS rats, and suggest that this effect could be, at least in part, due to changes in collagen organization. In addition, we showed that AQP8 expression in the myometrium is mediated by AR and that this protein is not regulating the water imbibition in the uterus of PCOS animals.

134. (623) **AQUAPORIN-3 EXPRESSION IN PLACENTAL EXOSOMES ISOLATED FROM PLASMA OF FIRST TRIMESTER PREGNANT WOMEN**

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Aquaporin-3 (AQP3) is expressed from early stages of gestation to term placenta. Evidences have described the participation of this protein in physiological processes and in diverse clinical dysfunctions. Regarding the human placenta, we recently found that AQP3 participates in the migration of the extravillous trophoblast cells and in the apoptosis of the villous trophoblast. In addition, we also described a decreased expression of AQP3 in preeclamptic placentas. Among the strategies that have arisen for the study of placental pathologies, exosomes derived from placenta have been proposed as the candidates that could best represent the changes that occur in the trophoblast throughout pregnancy. These extracellular vesicles derived from the syncytiotrophoblast are present in maternal circulation from week 6 to the end of gestation.
Our hypothesis is that the AQP3 normal expression is crucial for an appropriate placental development.
Our objective is to study the presence of AQP3 in placental exosomes isolated from the plasma of pregnant women to evaluate its potential use as an indicator of placental function.
Plasma samples (n=5) from pregnant women before 20 weeks of gestation were obtained after the approval of the bioethics committee and the signing of the informed consent.
Exosomes were isolated by differential centrifugation from the plasma of pregnant women during the first trimester of pregnancy. Samples were positively selected by binding to anti-CD63 (exosome marker). Then, the isolated exosomes were analyzed for AQP3 and PLAP (syncytiotrophoblast marker) by quantitative RT-PCR and western blot.
The results showed that the expression of mRNA and protein of AQP3 is detectable in exosomes obtained from the plasma of pregnant women in the first trimester.
Therefore, the level of AQP3 may be useful as an indicator of placental function throughout pregnancy and potentially correlate with the development of placental pathologies.

ENDOCRINOLOGÍA / ENDOCRINOLOGY 1

135. (67) **NARINGIN, NATURAL FLAVONOID, PREVENTS BONE ALTERATIONS INDUCED BY A FRUCTOSE RICH DIET**

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There is a considerable evidence that fructose rich diet (FRD) causes adverse metabolic perturbations. Recently, we have demonstrated that FRD inhibits the intestinal Ca2+ absorption, which was avoid naringin (NAR). The aim of this study was to know the effect of NAR on bone alterations in FRD rats. Male Wistar rats were used: 1) controls, 2) treated with FRD, 3) FRD treated with 40 mg NAR/kg b.w. for 30 days. Histomorphometric parameters were measured in distal femur and proximal tibiae. Parameters of oxidative stress were measured in bone marrow from femur. Adipocytes and osteocytes were counted in tibiae histological sections. Osteocalcin(OCN) was determined in bone and serum. The data showed that serum OCN levels were reduced by FRD, and NAR treatment returned them to the control values. FRD rats presented reduced bone volume, thickness and intertrabecular spaces in proximal tibiae. All these changes were normalized with NAR. There are no differences in the histomorphometric parameters from distal femur. An increase in the number of adipocytes in tibiae from FRD rats was blocked by NAR. In the proximal tibiae from FRD rats, the number of OCN(+) cells and osteocytes decreased as compared to that of control rats. NAR treatment significantly increased the number of OCN(+) cells and osteocytes. In FRD rats, the GSH content was similar to the control, but NAR treatment increased total GSH in comparison with that from the control and FRD rats. .O2- levels were highly augmented by the FRD and NAR could not normalize them. CAT activity decreased in FRD and NAR administration avoided this response. In summary, NAR protects the bone alterations triggered by FRD. The OCN normalization, the reduction in the number of adipocytes and the increase in the number of osteocytes suggest that NAR is acting as a possible bone protector in FRD rats.

136. (281) **DOPAMINE AND ESTRADIOL REGULATE PITUITARY ACTIVIN AND TGFB1 SYSTEMS IN 11 DAYS-OLD RATS**

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TGFβ1 and activins are known inhibitors of lactotroph function. We previously studied the pituitary expression of several components of these inhibitory systems during postnatal development in rats. We found that 11 days-old females present stronger pituitary TGFβ1 and activin systems compared to males and older females. Only in females pituitary expression of those systems inversely correlates with serum prolactin levels during postnatal development. Since dopamine (DA) and estradiol (E2) are the main regulators of lactotroph function, the aim of the present work was to study the estrogenic and dopaminergic regulation of pituitary TGFβ1 and activin systems at early postnatal age. To this end, 11 days-old Sprague Dawley rats were injected with E2 valerate (0.2mg/kg, sc), cabergoline (DA agonist, 2mg/Kg, ip), sulpiride (DA antagonist, 5mg/kg, ip) or vehicle (castor oil or saline). After three hours, animals were euthanized and pituitary expression of TGFβ1 and activin systems components was evaluated by RTqPCR. Statistical analysis: two-way ANOVA, followed by *post hoc* Tukey test. We found that E2 increased pituitary mRNA expression of most of TGFβ1 and activin systems components evaluated (TGFβ1, TβRII, βA and βB subunits, ActRIIB, ALK4 and FST) in both females and males. On the other hand, sulpiride treatment significantly decreased pituitary TGFβ1, TβRII, βA-subunit and FST expression in both genders; while cabergoline treatment had no effect on TGFβ1 and TβRII pituitary expression but increased expression of βA-subunit and FST. Taken together, the present results indicate a strong positive regulation by E2 and DA on both inhibitory systems of lactotroph function at early postnatal days, and suggest that the hormonal environment at 11 days-old could be determining the gender differences found in the pituitary expression of TGFβ1 and activin systems.

137. (70) **CARDIOMETABOLIC CHANGES IN HYPOGONADIC ADULT FEMALE RATS CAUSE BY MILD HYPERURICEMIA AND EXPOSURE TO A HIGH-FRUCTOSE DIET**

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In postmenopausal women metabolic syndrome increase cardiovascular mortality. The objective was to evaluate the effect of hyperuricemia and high-fructose diet on cardiovascular morphological and functional issues in hypogonadic female rats. Ovariectomized adult female Wistar rats were divided (n=7/group) into four groups receiving during 5 weeks: a) Control (C): standard commercial diet, b) Fructose (F): control diet plus 10% fructose in the drinking water, c) Oxonic acid (OA): control diet and water plus the uricase inhibitor OA (750 mg/kg/d), d) Fructose and Oxonic Acid (FOA): control diet with 10% (w/v) fructose in the drinking water plus oxonic acid. Plasmatic creatinine, uric acid (UA), glucose, lipid profile and systolic blood pressure (SBP) was measured. Cardiovascular morphometric analysis was done by measure cardiomyocyte volume (H&E and PAS) and fibrosis (Masson's trichrome), intima media aorta, total arteriolar medial area and media/lumen ratio. $p<0.05$ was considered significant. Results: UA levels were significantly higher in OA and FOA vs C ($p<0.001$). There was no difference in plasmatic creatinine between groups. Glycemia increases in F and FOA vs C ($p<0.05$). FOA group showed higher total cholesterol (TC), triglycerides (TG), no-HDLc levels and TG/HDL index vs C ($p<0.05$). F group showed a significant increase in the index TG/HDL ($p<0.05$). SBP increase in C during the experiment ($p<0.01$), but at the end it was higher in all treatments vs C group ($p<0.001$). Animals with OA, FOA and F showed greater myocyte volume ($p<0.001$) and fibrosis percentages ($p<0.001$) vs C. Intima media was thicker in FOA, OA and F vs C ($p<0.001$). Arteriole Media/Lumen (M/L) Ratio show no differences between treatments. In conclusion, in hypogonadic adult female rats, hyperuricemia and high-fructose diet conditions increase SBP and cause cardiovascular morphological changes such as cardiac hypertrophy, fibrosis and increased thickness of the intima media, in addition to related metabolic changes.

138. (71) **THE KEY GLUCONEOGENIC ENZYME CYTOSOLIC PHOSPHOENOLPYRUVATE CARBOXYKINASE IS EXPRESSED IN PANCREATIC ISLETS**

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Phosphoenolpyruvate carboxykinase (PEPCK) is a cataplerotic enzyme codified by 2 separate nuclear genes, which give rise to mitochondrial (M-PEPCK) and cytosolic (C-PEPCK) isoforms. C-PEPCK is mainly expressed in liver and kidney where it participates in gluconeogenesis. C-PEPCK transcription is induced by glucagon and repressed by insulin, being a critical modulator of glucose homeostasis during fasting. However, increased C-PEPCK expression during diabetes allows hyperactivation of endogenous glucose production, exacerbating hyperglycemia. To date, it is generally accepted that C-PEPCK is not expressed in pancreas, but this assumption is the result of inconclusive experiments. We hypothesized that C-PEPCK is expressed in pancreas and used several techniques to prove it, assessing liver as control. 10 Sprague Dawley male rats were fasted for 16 hs and then 5 of them were fed for 6 hs. Pieces of pancreas and liver were collected for formalin fixation, RNA extraction and protein preparation. Real time PCR and Western blot analysis of C-PEPCK showed the expected increased expression in liver from fasted compared to fed rats. Notably, C-PEPCK mRNA was not only detected but followed a similar pattern of expression in pancreas. Although at lower levels, C-PEPCK protein was also detected in pancreas. Immunoperoxidase analysis revealed the well-known periportal zonation of C-PEPCK distribution in liver but also allowed us to detect it in pancreas, specifically in Langerhans' islets. Multiple immunofluorescence analysis of C-PEPCK and hormones specifically secreted by each cell population in the Langerhans' islets revealed the exclusive expression of C-PEPCK in glucagon-producing α-cells. Most significantly, we observed the same results in biopsies from cancer patients. This is the first study where the expression of C-PEPCK is demonstrated in pancreas, specifically in α-cells. We postulate that C-PEPCK modulates glucagon production and/or secretion, contributing to hyperglucagonemia in diabetic subjects. This hypothesis is currently under investigation.

139. (319) **A NATURAL ANTIOXIDANT ATTENUATES THE VASCULAR CALCIFICATION IN A RAT MODEL OF TYPE 1 DIABETES MELLITUS**

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Vascular calcification (VC) is one important complication of type 1 Diabetes mellitus (D.M.). Several studies suggest that the antioxidant naringin (NAR) supplementation is beneficial for treatment of D.M, but its effect on the VC has not been investigated. The aim of this work was to know whether NAR could attenuate the VC in Wistar male rats with D.M. Three groups of animals were used: 1) controls, 2) diabetic rats (treated with 60 mg streptozotocin /kg b.w.: STZ), 3) diabetic rats treated with NAR (40 mg/kg b.w.). After 30 days of treatment, plasma was withdrawn and rats were sacrificed to obtain the aortas. Endothelial cells (EC) from aortas were cultured and NO•, indicator of vascular health, was measured by the Griess´s method. NO• production was significantly reduced in STZ rats, which was highly blocked by NAR (213,40 ± 33.3; 143,69±19.88*; 184,66±11.99; C; STZ; STZ + NAR 40; *$p<0.01$). In control aortas, estrone (E1) and genistein (Gen) stimulate NO• synthesis via estrogen receptor, but in aortas from STZ rats there is lack of NO• stimulation by those hormones. However, NAR restores the capability to stimulate NO• production under E1 and Gen. Isolated aortas from the different groups of animals were exposed to a pro-calcific medium (DMEM + glicerophosphate) for 7 days; the aortas were decalcified and the released calcium was measured by a commercial kit. Calcium content from aortas of STZ rats was 74% higher ($p< 0.01$) than that from the control rats. NAR treatment reduced calcium incorporation to values closed to the control ones. These data were confirmed by AgNO3 staining. Aortas from STZ rats showed multiple sites of calcification, effect that was abolished by NAR treatment. All data suggest that NAR could prevent damage of the vascular architecture and functionality in diabetic rats.

140. (589) **METABOLIC SYNDROME AND DENTAL PULP PROGENITOR CELL PLASTICITY.**

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Metabolic syndrome (MS) is a global health problem which has been associated with skeletal tissue alterations such as osteopenia and osteoporosis. In dental pulp there are mesenchymal progenitor cells (DPPC), with the capacity to differentiate to several phenotypes. The objective of this study was to evaluate the effect of the MS on DPPC plasticity. A murine model of MS with a high fructose diet was used. The DPPC were extracted from dental pulp of lower incisors and kept in DMEM-20%FBS at 37°C and 5%CO2. Proliferation at 48h (Crystal violet) of the DPPC-MS was lower than in the DPPC-C ($p <0.001$). Additionally DPPC-MS showed a reorganization of the actin filaments compared to the actin network of CPPD-C. Then DPPC-MS was differentiated to the osteo/odontoblastic lineage and it was found that collagen type I (Sirius Red), alkaline phosphatase and mineral nodules (Alizarin Red) were lower than those obtained for DPPC-C ($p <0.01$). In agreement, the semiquantitative analysis of RT-PCR also showed a decrease in the expression of both osteo (alkaline phosphatase, type I collagen, osteocalcin, Runx2) and odontogenic markers (dentinsialoprotein). DPPC-MS culture in an adipogenic medium, exhibited a significant increase (37%) of Oil red O with respect to DPPC-C, and an increase in the expression of adipogenic markers (PPARγ, Adiponectin and Foxo1). After incubation in a chondrogenic medium, it was found that extracellular matrix production (Alcian Blue) in DPPC-MS was lower than the basal condition. The PCR analysis showed that the expression of SOX9, aggrecan and type 2 collagen decreased by 50% with respect to DPPC-C. We conclude that the metabolic syndrome directed the fate of DPPC to the adipogenic phenotype while inhibited the differ-

entiation to the osteoblastic and chondrocytic lineages.

141. (286) **GENDER DIFFERENCES IN PITUITARY KALLIKREIN-KININ SYSTEM IN NORMAL AND TUMORAL PITUITARIES**

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TGFβ1 is a known inhibitor of lactotroph function and its biological activity is reduced in prolactinomas. We previously demonstrated that a pharmacological recovery of the local cytokine activity counteracts tumor development and decreases the hyperprolactinemia, representing an interesting tool for new treatments. Tissue Kallikrein (KLK1) was described as an important activator of latent TGFβ1. The Kallikrein-Kinin System (KKS) is complex; kininogens are cleaved by KLK1, releasing kinins: bradikinin and kallidin. Kinins exert their effect throw its receptors: B1 (B1R) and B2 (B2R), and are rapidly degraded by kininases I and II. Whereas B2R is constitutively expressed, B1R is inducible in pathological conditions and has a higher affinity for kinin metabolites. The aim of this work was to characterize pituitary KKS in normal pituitaries and prolactinomas. To this end, 8 month-old female and male mice lacking the dopamine receptor type 2 (Drd2KO) and WT counterparts were used. Only KO female develops pituitary hyperplasia and hyperprolactinemia. Pituitary expression of KKS was evaluated by RTqPCR. Statistical analysis: two-way ANOVA, followed by Tukey test. We found interesting gender differences: pituitary mRNA expression of most components of KKS was found significantly increased in males compared to females (KLK1, B2R, B1R, KininaseII). Regarding genotype differences, only KO females presented decreased pituitary expression of Klk1, with increased levels of Klkbp (KLK1 local inhibitor) and B1R compared to WT. In addition, the expression of KininaseII and B2R was found decreased in both male and female KO mice. Taken together, our results show a higher expression of pituitary KKS in males that correlates with higher levels of TGFβ1 and absence of prolactinoma development. We propose that the weakened KKS found in KO female pituitaries could be involved in prolactinoma development. Nevertheless, more in-depth studies are necessary to fully elucidate the role of KKS in the pituitary gland.

142. (444) **TESTOSTERONE MODULATES HYPOXIA-INDUCED CELL PROLIFERATION IN BENIGN PROSTATIC HYPERPLASIA**

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Benign Prostatic Hyperplasia (BPH) is characterized by a hyperproliferative state, mainly in the stromal compartment of the prostate, with androgens being classically ascribed as the main trigger of prostatic growth. However, BPH occurs in older men while testosterone levels are usually declining. Evidence has shown that BPH is associated with hypoxia microenvironment which might be responsible for cell proliferation in this condition. Our objective was to analyze in vitro the effect of hypoxia on stromal cell proliferation and the regulatory role of testosterone on hypoxic actions. Prostatic stromal cells surgically harvested from patients with BPH (n=10) were isolated, cultured in MCDB medium, and stimulated with $CoCl_2$ 200 µM, a stabilizer of hypoxia-inducible factor-1 (HIF-1) that mimics hypoxia, alone or in combination with testosterone 10^{-5}- 10^{-9}M for 24hs. Cell proliferation was assessed by immunocytochemistry of Ki67 and BrDu incorporation whereas cell phenotype was confirmed by transmission electron microscopy (TEM) and western blot of cytoskeletal markers for stromal cells. The hypoxic stimulus upregulated the expression of HIF-1α and increased cell proliferation up to 300% compared to the vehicle (ANOVA, p>0.001). TEM analysis revealed cellular edema, dilation of the endoplasmic reticulum, and disorganization of the contractile apparatus after $CoCl_2$, indicating a strong stimulation compatible with a myofibroblastic profile. The presence of testosterone at low (10^{-9}M) and physiological doses (10^{-7}M) dampened the proliferation induced by $CoCl_2$ while higher doses were associated to enhanced cellular proliferation. Interestingly, the treatment with testosterone 10^{-7}M in hypoxic conditions was related to a decrease in HIF-1α.
Together, our results suggest that a hypoxic microenvironment promotes cell proliferation in BPH while testosterone plays a dual role in this condition, which depends on the androgen levels. Moreover, HIF-1α inhibition would likely be a molecular mechanism involved in the homeostatic effects of testosterone on cell proliferation.

143. (448) **EFFECTS OF ESTRADIOL ON GNRH RECEPTOR SIGNALING PATHWAY AND LH RELEASE IN THE SOUTH AMERICAN PLAINS VIZCACHA (LAGOSTOMUS MAXIMUS).**

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Pituitary expression of gonadotropin-releasing hormone (GnRH) receptor (GnRHR) is regulated by estradiol mainly through its receptor ERalpha. The activation of GnRHR pathway involves the induction of transcription factors such as Egr-1 and Sf-1, resulting in luteinizing hormone (LH) expression. Vizcachas show reproductive axis activity during gestation with increased LH delivery at mid-pregnancy. The aim of this work was to determine the effect of estradiol on the GnRH signaling pathway and its final target LH. Adult vizcachas were used under two experimental conditions: 1) Non-pregnant females (NPf); 2) Ovariectomized NPf (OVX) treated with low (OVX-5) or high (OVX-15) doses of estradiol (5ug/kg or 15ug/kg, respectively). SHAM females were used as control (n=4/group). The success of estradiol treatments was corroborated by serum estradiol measure by ELISA. Pituitary LH pulsatility was assayed with or without GnRH supplementation and LH released measured by RIA, whereas pituitary GnRHR, ERalpha, Egr-1 and Sf-1 were studied by immunohistochemistry and PCR. A significant induction of LH release was determined in pituitaries of NPf supplemented with GnRH ($p<0.001$). In addition, the pituitaries incubated with GnRH showed a significant increase in GnRHR and Egr-1 ($p<0.05$) protein confirming the activation of GnRHR intracellular signaling. The second approach depicted a significant increase of LH release in OVX and OVX-15 animals compared to SHAM and OVX-5 ($p<0.01$). These changes were matched by decreases of GnRHR and Egr-1 in OVX but an increase in OVX-15 ($p<0.05$). Finally, a concordant coexpression was determined between ERalpha and GnRHR in OVX. These results suggest that estradiol affects GnRHR signaling pathway and LH delivery in a dose-dependent manner. (PIP110/14, PICT1281/2014, Fundación Científica Felipe Fiorellino).

144. (683) **VITAMIN D FAVOURS THE THERAPEUTIC ACTION OF HISTAMINE RECEPTOR H4 AGONISTS IN HYPERPLASTIC AND NEOPLASTIC LEYDIG CELLS**

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Testicular Leydig cell (LC) tumors (LCT) are steroid-secreting tumors of unknown etiology. Several clinical features are shared with those of the androgen insensitivity syndrome (AIS), in which 46XY patients show virilization defects and develop LC hyperplasia (LCH). Vitamin D (VD) is believed to play a role in the prevention and treatment of many extra-skeletal diseases, such as cancer. Over one billion

people worldwide are VD deficient. VD receptor (VDR) knock-out mice develop 50% more LCH. The histamine receptor H4 (HRH4) is considered a promising target for cancer therapy. Previously, we reported that VUF8430 (HRH4 agonist) inhibits proliferation and steroidogenesis in MA-10 and R2C tumor LC. Likewise, calcitriol (VD's active form) inhibits R2C proliferation and steroidogenesis, while it promotes HRH4 expression. Accordingly, we detected VDR and HRH4 in LCT.Objectives:To analyze the concomitant expression of VDR and HRH4 in LCH and xenograft murine models.To evaluate a possible synergism between calcitriol and VUF8430 in vitro, in tumor LC.Methods:R2C were treated with: calcitriol(10-9M), VUF8430(10-5M), or calcitriol(10-9M)+VUF8430(10-5M). Cell proliferation was assessed using sulforhodamine B assay. Aromatase activity was measured using a tritiated water-release assay. Tumor xenograft models were developed in athymic mice (R2C) and C57 mice (MA-10). Plasma steroids were measured by chemiluminescence. Formalin-fixed, paraffin-embedded (human and murine) sample sections were evaluated for VDR and HRH4 expression using immunohistochemistry.Results:Calcitriol-, VUF8430- or calcitriol+VUF8430-treated R2C showed diminished proliferation ($p<0.05$). Aromatase activity was only lowered by calcitriol+VUF8430 treatment ($p<0.05$).Athymic mice inoculated with R2C had elevated sex steroids levels ($p<0.001$) and normal VD level. Both murine tumors expressed VDR and HRH4. AIS samples exhibited different staining intensity, but in all cases, when VDR expression was weaker, HRH4 also was.Conclusion: These results suggest that targeting HRH4, in combination with VD treatment, may represent a novel therapeutic approach against LCH and LCT.

145. (437) **PITIUTARY RELEASE OF LH IS MODULATED BY PRL IN A TIME EXPOSURE-DEPENDENT MANNER IN LAGOSTOMUS MAXIMUS**

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The progressive decline of circulating progesterone (P4) during the first half of gestation elicits surges og GnRH and LH, followed by an ovulatory event that leads to the development of numerous accessory corpora lutea (CL). The naturally calcified and steroidogenically poor placenta strongly points to CL as the main source of P4 during pregnancy. For other rodents, an essential role of prolactin (PRL) in CL maintenance and P4 production has been clearly established. To investigate PRL relevance over the hypothalamus-hypophysis-ovarian axis performance, we analyzed PRL effect over LH release in vizcachas under two experimental conditions: 1) LH release of pituitary after one week in vivo treatment with Sulpiride 20mg/kg i.m. twice a day (PRL Chronic treatment, CRtx); 2) 6 h-culture of pituitary explants with serum of animals before/after CRtrxt animals (PRL Acute treatment, ACtx). Success of Sulpiride treatment in inducing a hyperprolactinemic condition was corroborated by the increase of both PRL transcription and immunoexpression compared to that of control pituitaries ($p<0.05$, n=5). LH released significant decreased in CRtx vs control pituitaries, while ACtx explants released significantly more LH than that of controls ($p<0.05$, n=5). CRtx and control ovaries showed similar number of primordial, primaries and CLs, but slightly higher of secondaries and pre-antral follicles. Circulating estradiol (E2) was higher in CRtx animals ($p<0.05$, n=5), yet no differences were detected in the P4. Immunoexpressions of both PRL-receptor and LH-receptor showed similar levels in CRtx and controls ovaries. We conclude that LH release is indeed modulated by PRL in a time exposure manner: initially PRL induces LH release however a sustained exposition of the pituitary to PRL diminishes LH release. Our results suggest that at ovarian level, PRL has a more predominant role over the follicular maturation rather than at luteal activity. Grants: PIP 110/14, PICT 1281/2014, Fundación Científica Felipe Fiorellino.

146. (321) **ACTIVATION OF MEMBRANE PROGESTERONE RECEPTORS (MPRS) REPRESENTS A NOVEL TOOL FOR PROLACTIN INHIBITION IN ANIMAL MODELS OF PROLACTINOMAS**

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Membrane progesterone receptors (mPRs) are known to mediate rapid non-genomic progesterone (P4) effects in different cell types. We recently demonstrated that mPRα is highly expressed in the rat pituitary, being primarily localized in lactotrophs and mPRα/β activation leads to a decrease in prolactin (PRL) secretion. The role of P4 in prolactinoma development remains unclear. In the present work, pituitary expression of mPRs was studied in a well-known model of prolactinoma, transgenic D2 dopamine receptor-deficient mice (Drd2 KO). Expression of mPRs and the classical P4 receptor (nPR) was found significantly decreased in female Drd2 KO pituitaries compared to their WT counterparts. However, the relative proportion of mPRα and mPRβ was increased (about 60% of total pituitary PRs) in tumoral pituitaries. This elevated proportion of mPR to total PR was also observed in other two animal models of prolactinoma. We also found gender differences: male pituitaries express higher levels of mPRs than females, without genotype differences. Males do not develop prolactinoma, even in the absence of dopaminergic inhibition. Finally, as P4 also regulates PRL secretion indirectly by acting on dopaminergic neurons, we studied mPR expression in hypothalamus. We found that the hypothalamus has high expression of mPRs, representing about 80% of total PRs, without genotype or gender differences. Interestingly, the mPR agonist increased dopamine release in hypothalamic explants. Taken together these findings suggest mPRα/β activation could represent a potential tool for hyperprolactinemic patients, especially those that present resistance to dopaminergic drugs.

ONCOLOGÍA / ONCOLOGY 3

147. (450) **ANGIOTENSIN II: KEY ROL IN MAMMARY GLAND INVOLUTION AND MAMMARY TUMOURS.**

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Angiotensin (Ang) II, the main effector peptide of the renin-angiotensin system, has been implicated in multiple aspects of cancer progression such as proliferation, migration, invasion, angiogenesis and metastasis. Our previous studies have shown that AngII through AT1 receptor induces STAT3 activation, epithelial apoptosis and MMP-9 activation during mammary gland involution. The lack of AT1 receptor caused a delay in mammary involution. In this study, we show that AngII through AT1 receptor has a key role as tumour promoter on breast cancer cell lines. We found that AngII induced cell invasion (2 fold change $p<0.01$), MMP-9 activity and VEGF expression (2,5 fold change $p<0.001$) on MDA-MB231 breast cancer cells. In addition, migration induced by AngII was inhibited with the treatment of cells with an anti-angiogenic VEGF antibody (bevacizumab) (2 fold change $p<0.05$). On the other hand, AngII induced activation of Rac1 (a small Rho-GTPase involved in migration, proliferation, tumorigenesis and metastasis) on T47D breast cancer cells. We have performed an analysis on public databases TCGA (The Cancer Genome Atlas) that contains information of numerous samples of patients with different human mammary tumor subtypes. We found that AT1 receptor expression is increased in women with estrogen receptor positive (ER+) breast cancer tumors. Together, these results suggest that AngII could be involved in breast tumorigenesis with a preferential rol on ER+ tumors.

148. (456) **INVOLVEMENT OF PHOSPHO-SRC AND TGF-B TYPE I RECEPTOR IN THE ENHANCEMENT OF MESENCHYMAL FEATURES INDUCED IN BREAST CANCER CELLS BY CONDITIONED MEDIA FROM NORMAL MAM-**

MARY CELLS

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Conventional radiotherapy for breast cancer is delivered in fraction doses of 2 Gy post-mastectomy or post conservation surgery. We have previously demonstrated that 2 Gy irradiation of MDA-MB-231 and MCF-7 tumor cells produces epithelial-mesenchymal transition (EMT) related changes in surviving cells, through a signaling path that involves Src phosphorylation/activation. We also found that conditioned media from normal mammary MCF-10A cells irradiated or not (2GyCM or CM) increase mesenchymal characteristics in both irradiated (I) and non-irradiated (NI) tumor cell lines.
In this work, we investigated whether Src could be involved in the increase of mesenchymal characteristics induced by CM and 2GyCM in MDA-MB-231 and MCF-7 cells (I or NI). Our results showed that CM and 2GyCM increased phospho-Src levels determined by Western blot in both I and NI tumor cell lines ($p<0.05$). PP2, an inhibitor of Src phosphorylation, blocked the increase produced by CM and 2GyCM in 2 EMT markers: cell migration evaluated by transwell units ($p<0.01$) and nuclear localization of transcription factor Slug evaluated by indirect immunofluorescence ($p<0.01$). Since TGF-β1 is the main promoter of EMT, we also investigated whether TGF-β1 in CM and 2GyCM enhance the mesenchymal phenotype. We detected TGF-β1 in CM, and its level was increased in 2GyCM ($p<0.01$). Besides, an inhibitor of the kinase activity of the type I receptor of TGF-β (A8301) hindered the increase in cell migration and in nuclear localization of Slug prompted by CM ($p <0.01$, both) and 2GyCM ($p< 0.01$, both) in tumor cell lines (I and NI). In summary, factors secreted by MCF-10A cells (irradiated or not) favor the acquisition of mesenchymal characteristics in tumor cells (I and NI) at least in part by the activation of the type I receptor of TGF-β and Src phosphorylation.
Our results expose the relevance of the tumor-host interaction in the response to radiotherapy

149. (457) **METABOLIC SYNDROME ALTERS THE EXPRESSION OF CRITICAL MIRNAS FOR BREAST CANCER DEVELOPMENT IN THE MAMMARY GLAND FROM METABOLIC SYNDROME MICE**

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Breast cancer (BrCa) is the first cancer in incidence and mortality in women in the world, excluding skin cancers. Metabolic Syndrome (MeS) is a risk factor for BrCa and increases its aggressiveness and metastasis. Previously, we generated a MeS experimental model by chronically feeding mice with a high fat diet (HFD). This diet induced alterations in the mammary glands such as prominent duct patterns with high expression of CTBP1, a tumor suppressor gene. Moreover, we found that CTBP1 and MeS increased tumor growth and progression from MDA-MB-231 xenografts generated in athimic *nu*/*nu* mice modulating the expression of 42 miRNAs. Inflammation induced by MeS is a crucial feature which impacts on cancer. The aim of this work was to assess the miRNA expression profile induced by MeS in mammary glands from immunosuppressed and immunocompetent mice.
We investigated MeS effect in mammary gland from *nu*/*nu* mice and immunocompetent BALB/c mice fed with HFD or control diet. Thus, we determined expression levels by RT-qPCR of miR-378a-3p, miR-146a-5p, miR-223-3p, miR-381-5p, miR-433-3p, miR-194-1-5p and miR-494-3p in mammary gland from mice fed with HFD or control diet. MeS significantly repressed the expression of miR-194-1-5p while induced miR-433-3p in mammary tissue of *nu*/*nu* mice. On the other hand, MeS repressed the expression of miR-378a-3p in breast tissue of BALB/c mice. Using the bioinformatics tool ChemiRs, we found that these miRNAs are involved in several molecular pathways including cancer, developmental biology, adherent junction and apoptosis. Finally we evaluated the effect of these miRNAs on BrCa patients survival using the bioinformatics tool PROGmiRV2. We found that a low expression of miR-378a-5p increased survival free of metastasis and relapse, while low levels of miR-194-1-5p increased survival free of relapse. Our results suggest that MeS alters miRNA expression profile which could be critical in breast carcinogenesis.

150. (458) **BONE MARROW-DERIVED MESENCHYMAL STROMAL CELLS INDUCE THE RELEASE OF FREE CIRCULATING MICRORNAS BY TRIPLE NEGATIVE BREAST CANCER CELLS.**

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The main cause of death in patients with breast cancer (BC) is due to the metastasis of the primary tumor. Particularly, 60-70% of patients with advanced BC develop bone metastases. It has been reported that mesenchymal stromal cells (MSCs), that include mesenchymal stem cells, precursor and progenitor cells, stimulate breast cancer cells to induce proliferation, invasion and migration to other niches, through several factors. MicroRNAs (miRNAs) are key regulators of tumorigenesis and tumor progression, and it is now known that they can circulate in the peripheral blood, which makes them promising biomarkers. Previous results from our group established a list of miRNAs obtained from breast allografts generated by 4T1 triple-negative cells in mice, which are differentially expressed in advanced stage vs. early stage. In addition, they were also found in the plasma of these mice. In this work, our aim was to identify the miRNAs released by MSCs and 4T1 tumor cells interaction. We generated a conditioned medium (MCo) from a primary culture of bone marrow MSCs (MCo-MSCs) from mice. Then, 4T1 cells were exposed or not to MCo-MSCs. After 24 h, 4T1 cells were washed and incubated with culture medium for 48 h (MCo-MSCs 4T1). Total RNA was isolated from these MCo. MiRNAs released to the medium were detected using stem-loop RT-qPCR. Results showed that MCo-MSCs exposure increased the release of miR-125b-5p, miR-221-3p and miR-21-5p by 4T1 cells. Furthermore, miR-221-3p and miR-21-5p were released by MSCs. We conclude that MSCs induce the release of miRNAs by 4T1 cells, mainly miR-21-5p and miR-221-3p, which are known to be involved in the processes of mesenchymal-epithelial transition, proliferation and migration of breast tumor cells, as well as in the activity of tumor-associated fibroblasts.

151. (477) **RET RECEPTOR TYROSINE KINASE CONTROLS MOUSE MAMMARY GLAND REMODELLING DURING THE POST-LACTATIONAL TRANSITION AND ITS DEREGULATION INCREASES CANCER POTENTIAL**

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Loss of normal development is a hallmark of cancer. Thus, understanding the mechanisms of tissue-specific, normal development regulation and the changes that occur during tumorigenesis may provide insights of both diagnostic and therapeutic importance. In breast cancer, several members of the receptor tyrosine kinases (RTK) family that are well known to promote aggressive breast cancers also have roles in normal breast. We found that endogenous Ret, a RTK member, is highly expressed in the mouse glands during transition to involution, a well know stage that drives cancer progression. Involution is the period with high inflammation which returns the lactating mammary gland to a quiescent state after weaning. Recently, using a doxycycline-inducible transgenic mouse model (Ret/MTB) we determined that chronic expression of Ret is oncogenic in the mammary epithelium. Ret is overexpressed in about 40% of human breast tumors. However, the stage of development at which Ret expression results in increase mammary tumor incidence has not been identified. To address this, we used the Ret/MTB system,

to conditionally overexpress Ret during discrete stages of mammary gland development. We found that Ret is required for efficient transition to involution. We determined that the induction of Ret in Ret/MTB females promotes the expression of factors that drives involution, including specific inflammatory molecules identified by cytokine arrays and Stat3 activation. RNA-seq data in Ret-overexpressing glands is supporting these findings, which were confirmed by several techniques. In addition, sustained expression of Ret during post-lactation enhances cancer potential showing an increase in pre-neoplastic lesions, defective milk recycling and disrupting Stat3 signaling. These results demonstrate that Ret is essential for mammary gland post-lactational transition and its deregulation increases cancer potential.

152. (523) **AN N4-ARYL SUBSTITUTED THIOSEMICARBAZONE MODULATES MIRNAS AND DECREASES INVASIVENESS AND DISSEMINATION IN 4T1 TRIPLE NEGATIVE MURINE MAMMARY CANCER**

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Triple negative (TN) breast cancer represents an urgent unmet clinical need for treatment options due to its aggressive nature and lack of suitable therapeutic targets. Thiosemicarbazones are synthetic compounds that exhibit several pharmacological activities. Previously, we found that T2, an *N*⁴-aryl substituted thiosemicarbazone, inhibited some of metastasis-related properties in 4T1 TN mouse mammary tumor cells. Now, we have investigated the action of T2 on invasion and metastasis in vivo and explored T2 effects on miRNA expression in order to shed some light on T2 anti-tumoral mechanism/s. We found that T2 treated cells were significantly less invasive, as determined by a transwell invasion assay, than untreated control cells (T2 2.5μM= 44.7±18% and T2 10μM= 32±3% of control). Then, BALB/c female mice bearing a subcutaneous 4T1 tumor were treated with vehicle or T2. The necropsy revealed that 4T1 tumor invaded the peritoneal cavity in 63.3±3% of control untreated mice. This percentage decreased markedly when mice were treated with T2 (T2 5mg/kg= 40%; T2, 25mg/kg =22.5±2%, p≤0.05). We also observed that treatment with T2 reduced number and size of 4T1 lung spontaneous metastasic nodules ($p<0.05$). Finally, we investigated the expression of two miRNAs (related to invasion and metastasis in other tumors). Our results from stem loop PCR showed that T2 significantly increased miR-200c expression (T2, 2.5μM= 2.4±1 and T2, 10μM= 2.9±3-fold respect to control) while it decreased miR-182 expression (T2, 2.5μM= 35.5±11% and T2, 10μM= 21.7±3% respect to control) in 4T1 cells.
In conclusion, our results show that, in adittion to its cytotoxic activity, T2 inhibits invasion and dissemination of 4T1 cells. Interestingly, this inhibition was associated to an opposite modulation of miR-182 y miR-200c. In agreement with previous reports in which these molecules act as modulators of invasiveness and metastases, these findings place miR-182 and miR-200c as possible molecular targets of T2.

153. (524) **NON-NEURONAL ALPHA 7 NICOTINIC RECEPTOR REGULATES CELL SIGNALING IN HUMAN MAMMARY EPITHELIAL CELL LINES**

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Nicotine acetylcholine receptors (nAChRs) constitute a heterologous family of ion channels that mediate fast synaptic transmission in neurons. They are also expressed in numerous cell types, where they have other functions well beyond neurotransmission such as cell proliferation or migration. Nicotine has been implicated in cancer progression by activation of nAChRs, mainly through pentameric α7 and α9 receptors. Here we examine the possible downstream signaling pathway of the α7 nAChR in human breast cell lines. For this purpose, MCF-10A, MCF-7, MDA-MB-231 and T47D cells were used. By using RT-PCR and Western Blot, we determined the expression of the α7 nAChR subunit in all cell lines. No expression of the α9 nAChR subunit was detected. Relative quantitation (qPCR) demonstrated up-regulation of the α7 subunit expression in MCF-7 cells after 48 hr incubation with nicotine (10 μM). Kinetics of α7 activation by a specific agonist PNU-282987 demonstrated an increase of ERK1/2 and Akt phosphorylation, in all cell lines, measured by Western blot. Moreover, PNU-282987 incubation stimulated significantly MCF-7 cell proliferation, and this effect was reversed by the α7 specific antagonist bungarotoxin. These results highlight the pathophysiological role of α7 receptors in promoting cell growth and intracellular signaling and provide a framework for the development of new drugs that specifically target these receptors.

154. (691) **RELEVANCE OF HYPOXIC TUMOR MICROENVIRONMENT ON ACQUIRED RESISTANCE IN HER2+ BREAST CANCER IMMUNOTHERAPY**

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Although the success of trastuzumab immunotherapy along with alternative strategies such as trastuzumab emtansine (T-DM1) in the treatment of HER2+ breast cancer patients, de novo or acquired resistance is the major obstacle in clinical practice. Since hypoxic tumor microenvironment plays a central role in cancer treatment resistance, our hypothesis is that hypoxic conditions are involved in the acquisition of resistance to trastuzumab and T-DM1 in HER2+ breast tumors. First, we established an hypoxic tumor model with human mammary carcinoma BT-474 (HER2+) and MCF-7 (control) cell lines. Since both hypoxic chamber and cobalt chloride (CoCl2, 100 μM), a chemical hypoxia mimetic, produced similar effects upon cell viability, we chose CoCl2 method due to its operative advantages. The hypoxic status of the cells was confirmed by a Western blot analysis showing a peak of HIF-1α expression 6 hours after CoCl2 treatment that correlated with VEGF induction, as measured by RT-qPCR. Furthermore, we observed increased levels of nitric oxide production in hypoxic conditions versus normoxia by Griess assay ($p<0.0001$). Then, we studied the hypoxia-mediated effect on trastuzumab and T-DM1 cell treatment. Comparing the IC50 values calculated from concentration-response curves, we observed that hypoxia reduced trastuzumab (0.10 [0.06-0.17] μg/mL vs. 0.64 [0.20-2.17] μg/mL) and T-DM1 (0.21 [0.12-0.38] μg/mL vs. 12.27 [2.70-54.41] μg/mL) effects on BT-474 cell viability ($p<0.01$) compared to normoxia, while drug-effects on MCF-7 cells were not modulated. A similar effect was observed in a clonogenic assay done with BT-474 cells treated with trastuzumab ($p<0.0001$). By flow cytometry, we found that hypoxic conditions reduced drug-mediated apoptosis ($p<0.05$), while effects on the cell cycle did not change. In turn, a Western blot analysis showed that hypoxia could maintain activation of AKT pathway in the presence of trastuzumab. In summary, our results suggest that hypoxic microenvironment promotes trastuzumab and T-DM1-resistance in HER2+ breast cancer cells.

155. (584) **BREAST CANCER CIRCULATING EXOSOMES EXPRESS CD175**

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Tumor cells secrete exosomes, small vesicles involved in tumor dissemination and immune evasion. It has been found that glycoproteins may be O-glycan carriers at the exosomal membrane. One of them is CD175 (Tn antigen), a well-known tumor antigen constituted by the attachment of GalNAc to Ser/Thr. Also, Tn is recognized by the major galactose binding lectin (MGL). With the aim to study Tn antigen expression in circulating exosomes, we isolated exosomes from 27 plasma samples from breast untreated cancer patients. We employed size exclusion chromatography in a 20 mL

CL4B agarose column from which we collected the void volume in 0,5 mL fractions. Protein content was analyzed employing a Qubit assay and 330 and 260/280 absorbance ratio was calculated for fraction selection; high 330 and 260/280 fractions were evaluated by electron microscopy. Presence of exosomal markers CD9 and CD6, as well as Tn expression was assessed by dotblot. Tn positive samples were selected for Western blot. From 27 samples 11 (41%) were positive for CD63, 13/27 (48%) for CD9 and 7/27 (26%) were Tn+. All Tn+ samples were also CD63+ and CD9+ and showed exosome like microvesicles by electron miscroscopy. CD63+ samples had more protein content than CD63- (5,7 vs 3,5 ug/mL). Western blot analysis showed high to low molecular weight bands (110 to 60 kD), suggesting that Tn may be attached to different glycoproteins in breast cancer exosomes.
In conclusion, we could detect Pan-cancer Tn antigen in breast cancer plasma circulating exosome containing fractions.

156. (792) **USE OF QUANTITATIVE MULTIPLEX IMMUNOHISTOCHEMISTRY TO CORRELATE IRF8 EXPRESSION WITH THE IMMUNE INFILTRATE IN BREAST CANCER**
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The interferon regulatory factor-8 (IRF-8) is crucial for regulating the antitumor immune response and acts as a tumor suppressor gene. Previous results using a statistical mining tool of published annotated genomic data showed a positive correlation between genes related with the antitumor immune response and high expression of IRF8 inside the tumor. The main goal of this work was to use quantitative multiplex immunohistochemistry technology and computational image processing workflow to analyze the immune infiltrate and correlate its composition with IRF8 expression in breast cancer specimens. We performed sequential immunohistochemistry approach in tissue microarrays containing 30 breast cancer surgical specimens in formalin-fixed paraffin-embedded (FFPE). We used three panels of 12-antibody biomarkers each to audit quantitatively and simultaneously lymphoid and myeloid lineages and the functional status of T cells in three FFPE tissue microarrays. Our preliminary results show that high frequencies of CD45+CD3+CD8-PDL1+ cells, CD8+PDL1+ cells and CD45+CD3+CD8-FOXP3+PD1+ regulatory T cells were associated with weak expression of IRF8 in breast cancer specimens ($p<0.05$). The use of this platform will allow us to dissect the complexity of the immune infiltrate that correlates with IRF8 expression, supporting our hypothesis that IRF8 could be a prognostic biomarker in breast cancer.

157. (447) **EVALUATION OF ANTITUMORAL ACTIVITY OF YERBA MATE EXTRACT ON MURINE AND HUMAN BREAST CANCER CELLS.**
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Yerba Mate (Ilex paraguariensis A.St.-Hil., Aquifoliaceae) is a native tree growing in the subtropics of South America. This plant contains several active phytochemicals, which are responsible for its health benefits. Cancer is a global health problem with high mortality and disability rates. Breast cancer is considered to be one of the most prevalent and deadliest cancers in women. This type of cancer has many differences between patients which are associated with clinical characteristics such as, patient age, tumor size and molecular subtype. In this context, novel approaches for effective cancer treatment are necessary. The aim of this study was to evaluate the effects of a Yerba Mate extract on specific steps of tumor progression using preclinical in vitro and in vivo models. The tumor cells panel evaluated with the Yerba Mate extract was extending to MDA-MB 231 (triple-negative human breast cancer cells), MCF7 (ER/PR+ human breast cancer cells), F3II (sarcomatoid murine mammary carcinoma cell line) and 4T1 (murine mammary carcinoma cell line). We found that Yerba Mate extract reduces tumor cell viability, shows negative modulatory effect on cell adhesion and migration of tumor cells and reduces invasiveness capacity. In addition, using in vivo protocols (orthotopic and heterothopic models), we tested the ability of the extract to interfere in processes such as tumor growth and latency. Model of subcutaneously transplanted tumor was established by inoculating 5.104 F3II cells into the right flank (heterotopic model) and mammary fat pad (MFP) transplantation was established by inoculating 1.105 F3II cells into the abdominal mammary gland. The extract was administrated to female Balb/C mice via the drinking water before and after the inoculation of F3II tumor cells. In conclusion, we described a potential beneficial effect of Yerba Mate on breast cancer models.

ONCOLOGÍA / ONCOLOGY 4

158. (449) **SUPERANTIGEN INDUCE APOPTOSIS IN HUMAN NEOPLASTIC T CELLS.**
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Superantigens (Sags) are bacterial and virus protein that share the ability to activate large number of T-cells. Sags bind to major histocompatibility complex (MHC) class II molecules as unprocessed proteins and interact with T cells expressing particular T-cell receptor (TCR) Vβ chains. We have previously shown that bacterial and mouse mammary tumor virus (MMTV)-encoded Sags induced apoptosis of different murine-cognate lymphoma T cells both in vitro and in vivo. Moreover, bacterial T Sags increased the survival of lymphoma-bearing mice. Furthermore, we have recently described that Sags were also able to induce apoptosis of the Jurkat-established human cell line from an acute T-carrier leukemia of the Vβ8 region in TCR. Thus, we now aimed to evaluate the effect of Sags on xenografts of Jurkat Cells in mice. For this propose we subcutaneously inoculated Jurkat cells (5x106) on Scid/Nod mice and then we treated them intraperitoneally with specific Sag SEE (50μg). Afterward, we analyzed the tumor growth and Vβ8+ cells in blood weekly for 6 weeks when final weight and histology were evaluated. Results: While SEE treatment did not affect immunological parameters or general fitness of mice, the tumor growth as well as lymphocyte number in blood, were significantly reduced at week 5 and 4 respectively ($p<0.04$). Conclusion: in vivo treatment with specific Sag SEE, strongly reduce subcutaneous xenografts growth and leukemia T cells counts in blood, leading us to discuss the possibility of consider Sags as therapy for lymphoma/leukemia T cell malignancies.

159. (545) **FILAMIN A MODULATES AUTOPHAGY INDUCED BY HYPOXIA AND SPHINGOSINE-1-PHOSPHATE IN MELANOMA CELLS**
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Melanoma is the most aggressive type of skin cancer and constitutes one of the most common causes of cancer death in individuals between 20 and 35 years of age, which generates a socioeconomic impact. Although recent therapies have shown an impressive success, unfortunately patients develop resistance after a short period of disease control. Autophagy has been indicated as a possible mechanism of resistance in tumor cells, which favors their survival and metastasis. Autophagy is a process regulated by numerous factors, including hypoxia, a distinctive feature of the tumor microenvironment, and sphingosine-1-phosphate (S1P), a bioactive lipid with important functions in cancer and inflammation. Recently, our group has shown that Filamin-A (FLNa), an actin binding protein, reduces the activation of the PI3K / Akt pathway and the migration of melanoma cells in the presence of S1P, depending on the expression and activation of its receptors (S1PR), mainly S1PR1, S1PR2 and

S1PR3. Our hypothesis is that FLNa positively regulates autophagy and tumor survival induced by S1P and hypoxia. To this end, we explored some biological actions triggered by culturing two melanoma cells, M2 cells (FLNa-) and A7 cells (FLNa+), in hypoxia (1% O2) or normoxia (21% O2) and treated them with or without extracellular S1P (100 nM). We observed that, compared to normoxia, hypoxia significantly increased the LC3II levels only in A7 cells. Mechanistically, FLNa expression is required for activation and nuclear translocation of HIF-1α, which in turn support the autophagic process. Furthermore, S1P showed a synergistic effect with hypoxia to induce autophagy only on FLNA+ cells, suggesting that FLNa could regulate this process through different pathways. These results suggest that FLNa is a key protein in the autophagy induced by hypoxia and S1P present on the tumoral microenvironment.

160. (665) **LIPOFECTION WITH THE CYTOSINE DEAMINASE::URACIL PHOSPHORIBOSYL TRANSFERASE/5-FLUOROCYTOSINE SUICIDE GENE SYSTEM DISPLAYS SIGNIFICANT CYTOTOXICITY IN HUMAN MELANOMA DERIVED CULTURED CELLS**

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Background: The yeast fusion protein cytosine deaminase/uracil phosphoribosyl transferase (CD::UPRT) [plus its associated prodrug: 5-fluorocytosine (5-FC)], was proposed as a suicide gene therapy approach. The fusion enzyme CD::UPRT catalyzes the 5-FC conversion to 5-fluorouracil (5-FU), which interferes with RNA processing and DNA synthesis.
In order to anticipate its potential therapeutic application, we tested in vitro the cytotoxic effect of CD::UPRT/5-FC suicide gene system on four human melanoma derived cell lines. One of them is a commercially available line (A375) and the remaining three were established in our laboratory (hM1, hM4, hM9).
Methods: We performed 5FC dose-response experiments on CD::UPRT lipofected cells cultured as monolayers or spheroids. Cell survival was measured by the acid phosphatase assay (APH). In addition, we explored the mechanisms related to cytotoxicity by colony formation assay and senescence-associated beta-galactosidase (SA-βgal) activity.
Results: The 50% inhibitory concentrations (IC50) of 5-FC for the suicide gene lipofected cells were as follows: 4.4 ± 1.7 μM for A375 (n=4), 3.8 ± 0.8 μM for hM1 (n=3), 11.4 ± 4.6 μM for hM4 (n=4) and 12.1 ± 3.1 μM for hM9 (n=4). A remarkable finding was that this suicide gene system strongly reduced the spheroids viability in all cell lines ($p<0.0001$). Preliminary results suggested that CD::UPRT/5-FC reduces colony formation while increasing senescence of treated cells.
Conclusion: The CD::UPRT/5-FC suicide gene therapy system offers a promising approach for human melanoma management. Further studies about the mechanisms underlying the observed cytotoxicity will help in the design of more effective ways of applying this treatment.

161. (672) **1A116 RAC1 INHIBITOR AS A THERAPEUTIC AGENT IN GLIOBLASTOMA: EVALUATION OF IN VITRO AND IN VIVO ANTITUMOR ACTIVITY**

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The small Rho GTPases family is composed by large group of proteins in which Rac1 is one of the most representative members. This protein acts as a molecular switch by cycling between an inactive state bound to GDP and an active state bound to GTP. In this way, Rac1 can regulate various signaling pathways that affect different cellular processes. Aberrant Rac1 GTPase activation is linked to tumor progression, invasion and chemoresistance. This protein is overactivated in glioblastoma cells and is considered an attractive molecular target for the development of targeted therapies. In line with this idea, in this work we propose that the inhibition of Rac1 could have an antitumor effect in models of glioblastoma affecting signaling pathways linked to cell growth, survival and resistance to established therapies.
Here we show the preclinical evaluation of 1A-116, a Rac1 GTPase inhibitor developed by our group, in search of antitumor effects using a glioblastoma cell line panel.
In vitro, 1A-116 showed inhibition of proliferation rate in the cell lines evaluated presenting IC_{50} values between 9 μM and 30 μM. The effect of 1A-116 on Rac1- associated signaling pathways was determined by western blot and qPCR, where a decrease survival and activation signals was observed. Interestingly, the inhibitor significantly increased the overall survival of mice bearing intracranial glioblastoma tumors compared to the control group. These results strongly suggest a potential use of 1A-116 as a therapeutic agent for glioblastoma treatment.

162. (698) **CHARACTERIZATION OF ABERRANT GLYCOSYLATION IN HUMAN GLIOMA CELL LINES**

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Gliomas are the most common and aggressive primary brain tumor in adults. It accounts for 45.6% of primary malignant brain tumors. New therapeutic targets are needed for this indication since patient´s survival after current therapy is 2 years. Glycosylation reflects the coordinated effort of a complex array of glycosyltransferases (GST) to successfully generate carbohydrate-associated posttranslational modifications. The aberrant glycosylation is a key actor in crucial processes for cancer cells survival and it is a direct consequence of the deregulation of GST´s expression. Even when gliomas represent the intracranial neoplasm of greater incidence and aggressiveness, little is known about their profile of glycosylation and its participation in the malignant phenotype. The objective of this work is the characterization of the glycophenotype of four human glioma cell lines by studying cancer associated glycans expression, glycans ramifications, enzymes expression and their impact in cell behaviour. High and medium expression of terminal glycans SleX and LeY were found in LN229, U87MG, U251 and U373 cell lines measured by FACS.The evaluation of glycan branching structures by lectin binding showed higher expression of β-1,6 ramifications and bisected N-glycans in U373 and LN229 in relation to U87MG and U251. These structures are associated with an overexpression of the glycosyltransferases MGAT5 and MGAT3, measured by qRT-PCR. Tunicamycin treatment suggested that described glycans are part of N-linked structures. Furthermore, Tunicamycin treatment provoked a significant decrease in cell adhesion and migration *in vitro*. In conclusion, the four cell lines evaluated present high expression of Lewis family glycans -mainly SLeX and LeY- with N-glycan type ramifications based on the activity of the enzymes MGAT5 and MGAT3. The glycophenotype study in cellular models constitute a value tool for the identification of novel therapeutic targets in Glioma.

163. (710) **MITOCHONDRIAL PEPTIDE HUMANIN ENHANCES CHEMORESISTANCE OF GLIOBLASTOMA CELLS**

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The mitochondrial-derived peptide Humanin (HN) exerts potent cytoprotective effects in several normal and tumoral cells. However, the role of this peptide in tumor pathogenesis is not well understood. Glioblastoma multiforme (GBM) is the most common and aggressive primary brain cancer. We aimed to evaluate whether HN affects the sensitivity of GBM cells to chemotherapy. We observed expression of HN in rat C6 GBM cells by inmunofluorescence. We determined the effect of HN in the response of GBM cells to cytotoxic stimuli. HN (10 μM) inhibited the effect of serum deprivation, Cisplatin (CP, 2 μM) and Temozolomide (100 μM) on the viability of C6 cells (MTT,

t test, *p<0.05). We next evaluated the role of endogenous HN in the apoptotic response of GBM cells, using a baculoviral vector encoding a shRNA that inhibits the expression of endogenous HN (BV. shRNA). To readily assess transduction efficiency, the construct also encodes the red fluorescent protein dTomato as a reporter gene. BV.shRNA decreased the viability of C6 GBM cells cultured in presence of 2 μM CP, when compared to cells infected with BV.control (t test, *p<0.05). Both BV.control and BV.shRNA efficiently transduced cells in the mouse naive brain and GL26 GBM tumors inoculated in the brain of immunocompetent mice as detected by dTomato fluorescence. These results suggest that BV.shRNA could be an efficient tool to achieve the silencing of HN in vivo. Blockade of HN expression could constitute a therapeutic strategy to improve the efficacy of chemotherapy in GBM.

164. (734) **COFILIN-1 IMMUNOCONTENT CAN UPGRADE THE PROGNOSTIC VALUE OF BRESLOW INDEX FOR THE PREDICTION OF METASTASIS OCCURRENCE IN MELANOMA PATIENTS**

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Nowadays, histopathological criteria for melanocytic lesions are the mainstay prognostic factors for melanoma. However, there are cases in which these parameters fall short to predict melanoma spread. We aim to estimate the probability of a melanoma to metastasize as a function of both a conventional histopathological parameter (Breslow Index, BI), and a marker that we propose for metastasis prediction: cofilin-1 immunocontent levels since this protein is key for cell migration and tumor invasion.
We performed this analysis using a Bayesian approach. Clinical and cofilin-1 datasets were obtained from a patients cohort diagnosed with malignant melanocytic lesions between 2000 and 2008 with at least 5 years of clinical follow-up.
Low BI values exhibited wide variance to predict metastasis occurrence, while the differential diagnostic value of cofilin-1 confirmed BI diagnosis or resulted more precise to predict outcome. Particularly, the probability of metastasis estimation improved when cofilin-1 was combined with BI for specific cases, where BI displayed large uncertainties.
Although further studies are needed in a larger cohort, our Bayesian analysis and the cofilin-1 determination provided statistically significant prognostic value in low BI melanomas, which could improve diagnostic, follow-up and treatment decision-making.

165. (278) **INTERRELATION BETWEEN INTRACELLULAR CALCIUM AND PHI CHANGES UPON VOLTAGE-GATED PROTON CHANNEL HV1 INHIBITION IN TUMOURAL AND NON-TUMOURAL HUMAN T LYMPHOCYTES**

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It is widely known that cytoplasmic calcium dynamics play an important role in cellular processes key for tumour development such as pro-proliferative signaling, migration, proliferation, and tumourigenesis. On the other hand, increased metabolic rates and the pathways rewiring (known as Warburg effect) lead to an intracellular overproduction of acidic species that must be extruded in order to maintain cellular viability. Thus, targeting proton transporters has arisen as a promising strategy in experimental oncology. From those, the voltage-gated proton channel Hv1 represent a singular passive pathway for extrusion being scarcely studied compared with other structures (NHE1, CA, MCTs, etc.). We found that Hv1 inhibitor Cl-GBI induces an immediate pH decrease in leukemic Jurkat T cells independently of extracellular Na+ availability (-0,11±0,01 w/o Na+e p=0,02; -0,05±0,02 w/ Na+e p=0,04). As intracellular acidification may have several consequences, including calcium transporters activity, in this work we tested the effect of Cl-GBI induced acidification on cytoplasmic calcium levels ([Ca2+]c). This derived in a sustained [Ca2+]c increase in both Jurkat and non-tumoural T cells being considerably higher in the former ones (0.116±0.009 p<0.0001 and 0.029±0.008 p=0.0005, respectively). The same effects are observed in absence of extracellular calcium (0,14±0,01 p<0.0001 and 0,030±0,004 p<0.0001, respectively). The differences observed between tumoural and non-tumoural cells correlate well with the calcium increase due to endoplasmic reticulum (ER) depletion induced by CPA (0.160±0.005 vs 0.021±0.002, p<0.0001 unpaired t test). Moreover, Cl-GBI was not able to induce any change in [Ca2+]c when ER is previously depleted (0.01±0,01 p<0,2230; Jurkat T cells). This evidence points out a relationship between Hv1 pH regulation and ER calcium release that might explain the Cl-GBI pro-apoptotic effect that we have previously reported in Jurkat T cells.

166. (194) **EFFECT OF COMBINED TREATMENT OF INTERFERON ALFA-2B (IFN) AND VITAMIN E (E) OR QUERCETIN (Q) ON THE DEVELOPMENT OF LIVER CANCER**

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Our group has postulated IFN as an effective antitumor agent in the treatment of carcinoma hepatocellular (HCC). Besides, E and Q were shown to have inhibitory effects on liver cancer due to its antiangiogenic and antiproliferative activities. We aimed to evaluate whether the combined therapy of IFN with E or Q has a synergistic inhibitory effect on liver cancer development compared with each separate therapy. We treated human liver cancer cells SK-Hep 1 with 10000 U/l of IFN, 25 uM of δ-Tocotrienol, an isomer of E, and 12,5 uM of Q. Treatments were used alone (IFN-group, E-group and Q-group) or combined (IFN-E-group and IFN-Q-group). Duration of the treatments varied according to the experiment that was carried out. We perform the MTT assay to determine cell viability at 72 hours, the wound healing assay to determine migration at 16 hours and invasion, in transwell chambers, at 24 hours. As expected, IFN-E and IFN-Q-groups showed a higher decrease in cell viability (-35 %*& and -52 %*# , respectively) compared to monodrug therapy: IFN-group (-25 %*), E-group (-30 %*) and Q-group (-15 %*). In migration assay IFN-E-group did not show a significant decrease (-35 %*) compared to monodrug therapy: IFN-group (-28 %*) and E-group (-30 %*), but IFN-Q-group showed a significant decrement (-46 %*&) compared with individual therapy: IFN-group (-28 %*) and Q-group (- 25 %*). In Invasion assay IFN-E-group and IFN-Q-group showed a significant diminution (-93 %*#) and (70 %*&) respectively. The monodrug groups showed lower decreases: IFN-group (-25 %*), E-group (-14 %*) and Q-group (- 7 %*), (*p<0.05 vs Control), (#p<0.05 vs IFN-group and E-group) and (&p<0.05 vs IFN-group and Q-group). The results demonstrate an additive effect in the treatments that could be taken into account in the development of antitumor strategies against HCC.

167. (487) **BIOINFORMATIC IDENTIFICATION OF CIP4 AS AN EFFECTOR OF THE HIPPO PATHWAY. IMPACT ON HEPATOCARCINOMA PROGRESSION**

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The CDC42 interacting protein 4 (CIP4) is CDC42 effector involved in the regulation of actin dynamics and membrane deformation, which participates in the development of metastatic properties in breast and lung cancer cells. The Hippo pathway is a signaling pathway conserved from *Drosophila*, which regulates organogenesis

and epithelial tissue homeostasis. The relevance of this pathway in cancer progression has received a surge of attention in the last decade.
The aim of this study was to evaluate the impact of CIP4 overexpression on hepatocarcinoma prognosis and to identify putative transcriptional regulators for this protein in hepatocarcinoma cells. Bioinformatic analysis of CIP4 mRNA expression on Cancer Atlas Hepatocarcinoma dataset (372 patients) showed a significant difference between patients with high CIP4 expression (first quartile, CIP4OE) and the others on overall survival* (CIP4OE: 37.29 months; Others: 60.84 months), disease free survival* (CIP4OE: 13.0 months; others: 23.03 months) and others prognosis markers such as nodule invasion and distant metastasis. Analysis of genetic network interactions using ARACNE algorithm showed that TEAD transcription factors, key members of Hippo signaling pathway, are possible regulators of CIP4 expression. Concomitantly, CHIPsec data in hepatocarcinoma HepG2 cells showed that TEAD4 binds to a region near CIP4 transcription starting site. That sequence contains a predicted JASPAR binding site. GSEA enrichment analysis showed a significant difference in Hippo pathway molecular signature in patients with high CIP4 expression. In situ assessment of CIP4 protein levels in Hepa1-6, MCF7, MDA-MB-231 and A549 cells treated with TEAD4 inhibitor Verteporfin showed that CIP4 expression was significantly reduced by this treatment, indicating that CIP4 expression was indeed regulated by the Hippo pathway.
These findings showed evidence, for the first time, of CIP4 regulation by TEAD transcription factors and supports the idea that CIP4 could link the Hippo pathway to the cytoskeleton remodeling associated to cancer invasion and metastasis.

168. (496) **ALPHA-LIPOIC ACID HAS ANTICANCER EFFECTS IN HEPATOCELLULAR CARCINOMA CELLS PROBABLY MEDIATED BY AN LKB1/AMPK/P53 AXIS**
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Hepatocellular carcinoma (HCC) is the second lethal cancer, which is in part due to both its high metastatic capacity and resistance to current therapies. In these sense we focus in the activation of the kinase AMPK as an antitumor strategy because it has been found to be negatively regulated in HCC, and that its activity is inversely associated to more aggressive forms of the disease. Previously, we showed that α-Lipoic acid (aLA), proposed as an anti-cancer agent associated with AMPK activation, increased cell death and decreased cell migration in HCC cell lines, among other effects. In this stage of the study we were interested in analyzing the impact of aLA treatment in cell invasiveness and deepening into the signaling pathway involved, both upstream and downstream AMPK. To achieve this goal we attempted to determinate if LKB1 participated in the signaling pathway, and also look at p53 due to it is an AMPK target whose stabilization by aLA was previously described in literature. Our results showed that p-AMPK(Thr172) levels were increased in aLA (0.5-1 mM) treated cells, at the same time that total and cytosolic levels of LKB1 were increased. We measured the level of phosphorylation of p53(Ser17) and found it was increased by aLA in HepG2/C3A cells, and this was in accordance with the fact that the same treatment significantly decreased migration in HepG2/C3A (p53 WT) but not in Hep3B (p53 null) hepatocarcinoma cells. On the other hand, the treatment with aLA (0.5 mM) significantly (p=0.00014) decreased by half the invasive capacity of HCC cells, in Matrigel-coated invasion chamber assays. These findings allow us to conclude that aLA not only increases apoptosis but also decreases invasiveness of HCC cells and to propose that an LKB1/AMPK/p53 pathway is a possible axis through which aLA could exert its anti tumor effects.

169. (216) **REXINOIDS FOR THE TREATMENT OF T CELL LYMPHOMA (TCL): IMPLICATIONS OF THYROID HORMONES (TH) IN BEXAROTENE ANTI-LYMPHOMA ACTIVITY**
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Bexarotene (Bex) is a sintetic rexinoid mostly used for the treatment of cutaneous T-cell lymphoma. Currently, Bex is being studied as alternative therapy for other types of cancer including different subtypes of T cell lymphomas (TCL). TCL are a heterogeneous group of aggressive lymphoproliferative disorders. Most TCL patients have poor prognosis, due to the aggressive clinical course and the lack of specific treatments. Tumor growth, including TCL, has a complex relationship with immune and endocrine systems, since cytokines and hormones are involved in tumor progression. We recently found that TH, through the action on its membrane receptor (integrin αVβ3) are required for proliferation of TCL. Paradoxically, Bex is associated with hypothyroidism, being patients candidates for replacement therapy with high doses of TH. The consequences of TH administration on the activity of Bex are unknown. The aim of this work was to evaluate Bex action on different subtypes of TCL, distinct from cutaneous TCL, and how TH could affect the anti-lymphoma activity of this rexinoid.
We first evaluated cell viability after 48 hours treatment on human TCL cells lines representing different subtypes and origins; CUTLL1 (immature) and, OCI-Ly12, OCI-Ly13.2 and MAC2a (mature). We treated them with increasing concentrations of Bex (0-80 μM) and found a significantly decrease in cell viability in all cells tested ($p<.0.01$ vs vehicle). Then, we evaluated on OCI-Ly12 and OCI-Ly13.2 cells Bex activity in the presence or absence of physiological concentrations of TH. After treatment Bex decreased viability and induce apoptosis in all cell lines, but in the presence of TH both effects decreased by 20-40% ($p<0.05$ vs Bex alone). These results provides us the knowledge bases that allow us to continue studying new therapeutic approaches for this pathology; and in the future, may help to improve the response to treatment of TCL patients.

METABOLISMO Y NUTRICIÓN / METABOLISM AND NUTRITION 2

170. (244) **SPARC IS REQUIRED FOR THE MAINTENANCE OF GLUCOSE HOMEOSTASIS AND INSULIN SECRETION IN MICE**
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Obesity, metabolic syndrome and type 2 diabetes, three strongly interrelated diseases, are associated to increased morbidity and mortality worldwide. The pathogenesis of obesity-associated disorders is still under study. SPARC is a matricellular glycoprotein expressed in many cell types including adipocytes, parenchymal and non-parenchymal hepatic cells and pancreatic cells. Studies have demonstrated that SPARC inhibits adipogenesis and promotes insulin resistance; in addition, circulating SPARC levels were positively correlated with BMI in obese individuals. Therefore, SPARC is being proposed as a key factor in the pathogenesis of obesity-associated disorders. The aim of this study is to elucidate the role of SPARC in glucose homeostasis. We studied $SPARC^{+/+}$ and $SPARC^{-/-}$ mice maintained on a normal low-fat (CD) or high fat western diet (WD) diet. Animals in each group were euthanized after 12 or 20 weeks of WD or CD feeding. $SPARC^{+/+}$ and $SPARC^{-/-}$ mice fed with CD were also studies at different time point since weaning (6 animals per group). We assessed glucose levels, glucose tolerance, insulin and c-peptide expression and secretion.
We show here that $SPARC^{-/-}$ mice displayed an abnormal insulin-regulated glucose metabolism. $SPARC^{-/-}$ mice presented an increased adipose tissue deposition and an impaired glucose homeostasis as animals aged. In addition, the absence of SPARC worsens high-fat diet-induced diabetes in mice. Interestingly, although $SPARC^{-/-}$ mice on high-fat diet were sensitive to insulin they showed an impaired insulin secretion capacity. Of note, the expression of glucose trans-

porter 2 (GLUT2) in islets of SPARC$^{-/-}$ mice was dramatically reduced. This study provides the first evidence that deleted SPARC expression causes diabetes in mice. Thus, SPARC deficient mice constitute a valuable model for studies concerning obesity and its related metabolic complications, including diabetes.

171. (352) **INVOLVEMENT OF INFLAMMATION AND APOPTOSIS IN THE ADRENOCORTCIAL DYSFUNCTION IN INSULIN RESISTANT RATS: EFFECT OF HEMIN TREATMENT**

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The worldwide increase in the consumption of sweet beverages has been linked to the generation of insulin resistance (IR). In rats with diet-induced IR, oxidative stress and chronic inflammation have been detected in several tissues. As an adrenocortical dysfunction was previously demonstrated in rats fed 30% sucrose in the drinking water (SRD), we analyzed the effect of HO-1 induction, an antioxidant enzyme, on functional parameters in the adrenal cortex.
Rats were randomly distributed in Control and SRD-groups and treated for 13 weeks. Some animals from both groups received hemin (15 mg/kg i.p every 48 h; H and SRDH groups) and treatments went on for 2 more weeks. Statistical significance of data was analyzed by ANOVA followed by Tukey's test.
Induction of HO-1 was detected in the adrenal cortex of both hemin treated groups ($p<0.01$ vs. C). Immunohistochemical studies revealed an increase in the number of Iba1+ cells ($p<0.01$ vs. C) and higher levels of the inflammasome components ASC and Caspase 1 ($p<0.001$ vs. C) in the SRD-group. Interleukin 1 β and iNOS expression were also elevated ($p<0.05$ vs. C). Hemin treatment prevented these changes (SRDH, $p< 0.01$ vs. SRD). In addition, a higher number of TUNEL+ cells was also detected in the adrenal cortex of SRD-treated rats ($p<0.05$ vs. C) and this effect was also prevented by hemin treatment ($p<0.01$ vs. DRS).
Animals in the SRD group exhibited a lower response in the ACTH stimulation test ($p<0.05$ vs. C). This effect was not observed in the SRDH group ($p<0,001$ vs DRS). A lower basal corticosteronemia was detected in SRD, H and SRDH groups ($p<0.05$ vs. C).
In summary, induction of HO-1 in the adrenal cortex of SRD-treated rats restored the functional capacity of the adrenal gland and blunted the SRD-dependent increases in pro-inflammatory mediators and apoptosis.

172. (365) **ALTERED CHOLESTERYL ESTER TRANSFER PROTEIN AND LIPOPROTEIN ASSOCIATED PHOSPHOLIPASE A2 ACTIVITIES IN CHILDREN AND ADOLESCENTS WITH INSULIN RESISTANCE.**

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Background: Triglyceride (TG)/high density lipoprotein cholesterol (HDL-C) ratio has been proposed as an accessible marker of insulin resistance, which is known to cause alterations in lipoprotein metabolism. Cholesteryl ester transfer protein (CETP) is a plasma enzyme responsible for the exchange of cholesteryl esters and TG between apolipoprotein B-containing lipoproteins and HDL particles. Increases in CETP activity are known to lead to alterations in HDL atheroprotective properties. Lipoprotein associated phospholipase A2 (Lp-PLA2) is an enzyme mainly associated to low density lipoproteins (LDL). This enzyme is capable of hydrolyzing oxidized phospholipids liberating proinflammatory lysophopsholipids and oxidized fatty acids to the subendothelial space. No prior studies have explored the association of TG/HDL-C ratio with CETP and Lp-PLA2 activities in children and adolescents.
Methods: Twenty-five male children and adolescents with TG/HDL-C>3.0 (cut-off point proposed by McLaughlin et al.) and 25 age-matched male healthy controls were recruited from the city of Balcarce, Argentina. Glucose and lipid profile were determined by automatized methods. CETP and Lp-PLA2 activities were determined by radiometric assays.
Results: Children with TG/HDL-C>3.0 displayed higher TG [164(126-186)vs65(48-72)mg/dl;$p<0.01$] and lower HDL-C [41(37-49)vs52(48-62)mg/dl;$p<0.01$] levels in addition to higher CETP [250(232-263)vs223(193-237)%/ml.min;$p<0.01$] and Lp-PLA2 (4.5±1.9vs3.5±1.3;$p<0.05$) activities. In univariate association analysis, CETP activity correlated with TG ($r=0.53;p<0.01$), total cholesterol (TC) ($r=0.4;0.01$), HDL-C ($r=-0.52;p<0.01$), LDL-C ($0.54;p<0.01$), TG/HDL-C ($r=0.63;p<0.01$) and Lp-PLA2 ($0.32;p<0.05$). Lp-PLA2 correlated with TG ($r=0.34;p<0.05$), TC ($r=0.52;p<0.01$), LDL-C ($r=0.6;p<0.01$), and TG/HDL-C ($r=0.32;p<0.05$). Finally, in separate multiple linear regression analysis, adjusted by age and BMI-z, TG/HDL-C was shown to be an independent predictor of CETP ($r2=0.29;beta=0.49;p<0.01$) and Lp-PLA2 ($r2=0.21;beta=0.32;p<0.05$).
Conclusion: The more atherogenic lipid profile observed in children and adolescents with TG/HDL-C>3.0, typical of an insulin resistant state, would be indicative of an abnormal lipoprotein metabolism in which CETP plays a crucial role and Lp-PLA2 provides a proinflammatory environment, a key feature of atherogenesis.

173. (37) **DIETARY SALVIA HISPANICA L (CHIA) SEED IMPROVES INSULIN SENSITIVITY AND THE ALTERED LIPID METABOLISM IN THE SKELETAL MUSCLE OF DYSLIPEMIC, INSULIN-RESISTANT RATS FED A SUCROSE-RICH DIET**

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Previous studies have shown that *Salvia hispanica* L (chia) seed, rich in α-linolenic acid (18:3 n-3, ALA), is able to normalize dyslipidemia, insulin resistance and the increase of lipid storage in the skeletal (gastrocnemius) muscle of rats chronically fed a sucrose-rich diet (SRD) -an experimental model that mimics the phenotype of the human Metabolic Syndrome-. The aim of the present work was to evaluate the possible metabolic pathways that could be involved in these beneficial effects of chia seed. Methods: Male Wistar rats were fed a SRD (% energy: 60 sucrose, 23 corn oil (CO), 17 protein) for 3 months. After that, half of the animals continued with the SRD until month 6 while in the other half CO was replaced by chia seeds for 3 months (SRD+chia). A reference group consumed a control diet all the time. In all groups were analyzed in gastrocnemius muscle: a-triglycerides (TG), long-chain acyl-CoA (LCA-CoA) and diacylglycerol (DAG) content, b- muscle-type carnitine palmitoyltransferase I (M-CPT1), M-CPT2 and total M-CPT enzymatic activities, c. PPARα, total AMPK and phosphorylated AMPK (pAMPK) protein mass levels (Western Blot). Besides plasma levels of glucose, insulin, TG, free fatty acids and whole-body peripheral insulin sensitivity (euglycemic–hyperinsulinemic clamp) were analyzed. Results: The replacement of CO by chia seed in the SRD: a- reduced the levels of intramuscular lipids (TG, LCACoA and DAG) ($P<0.05$), b- normalized the reduced activities of M-CPT1 and total M-CPT ($P<0.05$). M-CPT2 activity was similar in the three dietary groups; c -reverse the decrease in PPARα and pAMPK protein mass levels($P<0.05$). These changes were accompanied by a normalization of hyperglycemia, dislipidemia and the reduced insulin sensitivity. This study provides new information showing the beneficial effects of *Salvia hispanica* L (chia) seed upon the altered lipid metabolism in the skeletal muscle of dyslipemic, insulin-resistant rats fed a SRD.

174. (353) **THE POTASSIUM CHANNEL KIR6.2/K-ATP PARTICIPATES IN LIPID AND CARBOHYDRATE METABOLISMS IN THE MOUSE**

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Obesity is characterized by excessive accumulation of fat. It is related to the metabolic syndrome and its associated pathologies such as diabetes, hypertension, atherosclerosis, dyslipidemia and hyperuricemia. ATP-sensitive potassium channels (K-ATP) are composed of 4 protein subunits Kir6.x (6.1 or 6.2) that form the pore, and 2 regulatory subunits. Kir6.2/K-ATPs couple metabolism with cell membrane potential, and regulate several cellular activities acting as metabolic sensors, especially in response to situations of cellular metabolic stress such as hyper or hypoglycemia, ischemia and hypoxia. Based on this, we asked: does Kir6.2/ATP play a role in lipid metabolism? If so, is it direct or indirect? We worked with male C57/B6, wild-type (WT) and Kir6.2-/- mice (n=7) subjected to a high fat diet (HFD) for 2 months. We measured several metabolic parameters before and after the HFD. Before HFD: Body weight (BW) was similar for both groups (WT: 19.14±0.30g; Kir6.2-/-: 17.90±0.08g). Serum markers of liver function (alanine and aspartate aminotransferases and alkaline phosphatase) did not differ between genotypes. Basal blood glucose levels were considerable different: WT: 103,6±5.7g/L; Kir6.2-/-: 36.0±4.5g/L#). WT mice responded as expected to the oral glucose tolerance test (OGTT); however, Kir6.2-/- showed a marked basal incapacity to lower plasma glucose levels. Plasma triacylglycerol and cholesterol levels were similar for both groups. Upon HFD: Liver damage markers were slightly increased (still no significant) in Kir6.2-/- mice. BW was, as expected, increased in WT mice (31.90±0.74g); however Kir6.2-/- seemed resistant to HFD-induce body weight increase (23.90±0.28g*). OGTT for WT mice showed now a clear incapacity to lower plasma glucose levels; no significant changes were observed in Kir6.2-/- mice respect to the initial time. Plasma triacylglycerol and cholesterol levels for both groups were increased respect to initial time, but not different (*p<0.05; #p<0.01). These results, although preliminary, showed that Kir6.2 is participating in body metabolism.

175. (49) **EFFECTS OF METFORMIN AND LOSARTAN ON THE RELEASE OF VASCULAR PROSTANOIDS IN TWO MODELS OF DIETARY ALTERATION IN THE RAT**

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Fructose overload (F) and high-fat (HF) diet are experimental models that resemble human metabolic syndrome (MS). Mesenteric vascular bed (MVB) is formed by resistance vessels and a source of prostanoids (PR). Metformin (M) and losartan (L) are used for MS and high blood pressure (BP) treatment. We analyze M and L effects on PR release. Nine groups of male Sprague-Dawley rats were studied (9 weeks): Control (C): standard diet (SD) and tap water (W); fructose-overloaded (F): SD and F solution (10% w/v); HF diet (HF): 50% (w/w) bovine fat added to SD and W; C+M (CM): SD + 500 mg/Kg/day M in W; C+L (CL): SD + 30 mg/Kg/day L in W; F+M (FM): SD and M in F solution; F+L (FL): SD and L in F solution; HF+M (HFM): HF + M in W; HF+L (HFL): HF + L in W. Released PR were measured by HPLC (ng PR/mg tissue). HF increased vasoconstrictor prostaglandin (PG) F2α (HF: 155 ± 7 vs. C: 83 ± 3, p <0.01) and thromboxane (TX) B2 (HF: 119 ± 5 vs. C: 62± 2, p <0, 01) release; prevented by M and L, (HFM: 88±9 and HFL: 89±7 vs. HF, p<0.01; HFM: 59±7 and HFL: 71±3 vs. HF, p<0.01, respectively). F decreased vasodilator PG 6 -keto F1α (F: 62 ± 4 vs. C: 103 ± 3, p <0.01) and PGE2 (F: 48 ± 3 vs. C: 94 ± 3, p <0.01), prevented by L (FL: 112±10 vs. F, p<0.05 and FL: 96±10 vs. F, p<0.05 respectively). Meanwhile M only decreased PGF2α (FM: 55±4 vs. F, p<0.01) and TXB2 (FM: 45±10 vs. F, p<0.01). In conclusion, a possible mechanism by which M and L prevent BP increase in both models could be the prevention of the imbalance between vasodilator and vasoconstrictor PR release in MVB.

176. (101) **HEPATIC AND METABOLIC EFFECTS OF SIMVASTATIN AND CURCUMIN IN DIET-INDUCED HYPERCHOLESTEROLEMIC RATS**

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Hypercholesterolemia is a risk factor for the development and progression of cardiovascular diseases and nonalcoholic fatty liver disease(NAFLD).A high-fat and high-cholesterol diet (HCD) is associated with the increased prevalence of NAFLD. Simvastatin(SMV) is a commonly used cholesterol-lowering drug to treat hypercholesterolemia. Curcumin(CUR) is a polyphenol extensively investigated for antioxidant, anti-inflammatory, and probably, hypolipidaemic properties. However, the possible therapeutic value of CUR in improving lipid metabolism or NAFLD has not been studied. The objective was to investigate the hepatic and metabolic effects of SMV and CUR on HCD-induced hypercholesterolemic rats. Methods: rats were assigned to 1 of 4 groups: 1.control (C): fed pellets, 2.high-cholesterol diet (HCD), 3.HCD+SMV (orally,5mg/day), 4. HCD+CUR (orally,20mg/day). After 5 weeks, rats were euthanized, blood was drawn for: serum lipids determination (mg/dL) and transaminase activities [AST,ALT (UI/l)].The liver and visceral depots of adipose tissue (VAT) were removed and weighed. The hepato-somatic index (HSI%) and VAT were calculated (organ mass(g)/body mass(g)%). Hepatic samples were processed with H&E staining for evaluation of steatosis. Results (mean±SD, ANOVA-SNK): neither SMV nor CUR decreased cholesterol (p<0,001) and HSI(p< 0,001). However, SMV and CUR showed VAT lower than HCD and similar to control group (HCD:3,60±0,60 > C:2,86±0,46 = HCD+CUR:2,51±0,76 = HCD+SMV:2,17±0,45%; p< 0,01). All groups with HCD showed hepatic steatosis grade 3, but HCD+CUR did not present inflammatory infiltrate. HCD+CUR could decrease AST activity more than HCD+SMV (C:94,00±15,36 < HCD+CUR:116,00±15,31 < HCD+SMV:180,50±19,98 =HCD:196,88±14,08UI/l; p<0,001). HCD+CUR, HCD+SMV and C showed less ALT activity than HCD (p<0,001).
Administration of curcumin, rather than simvastatin exerts effect against lipemic-oxidative injury induced by HCD in rats, possibly through prevention of inflammatory damage to liver through its direct antioxidant effect. Additional studies on human subjects are needed to shed some light on the possibility to make use of curcumin as a part of the hepatoprotective strategies against bad nutritional habits.

177. (471) **STUDY OF SREBP1 AND SERBP2 IN PERIPHERAL BLOOD MONONUCLEAR CELLS FROM HYPERCHOLESTEROLEMIC RABBITS**

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Peripheral blood mononuclear cells are a possible biomarker that could reveal molecular alterations before the development of the disease.
Therefore, the objective of the present investigation is to study molecular and genetic changes that indicate metabolic modifications even with normal biochemical values. SERBP1 and SERBP2 (sterol regulatory element binding protein) are proteins associated with lipogenesis and regulated by the levels of insulinemia and cholesterol respectively. These molecules can expressed in peripheral blood mononuclear cells. This allows study tissue changes without resorting to biopsies.
In this study, one control group of New Zealand rabbits was fed with balanced feed (C) and another group received the same balanced feed supplemented with 17% fat (F). These animals did not receive fructose overload, maintaining constant concentrations of carbohydrates and protein in both groups.
In biochemical tests from both groups were observed similar levels of glucose (C group: 140.7 mg/dl + 28.4 / F group: 118.3 mg/dl

+12.0) and triglyceride (C group: 144.1 mg/dl + 15.5 / F group: 135.6 mg/dl +8.3), while F group showed increased levels of cholesterol (42.8 mg/dl + 21.6) compared with C group (27.1 mg/dl + 4.5).
Insulin levels also present differences between both groups: 20.0 uU/ml + 1.4 (C gruop), 5.3 uU/ml +1.15 (F group). However, there is variability in the cholesterol values because some animals of the F group do not experience significant increment despite the intake of fat.
This interesting finding leads to the hypothesis that changes in lipid metabolism can be examined by the expression of different genes early.
As preliminary results, we observed by immunohistochemistry the presence of SREBP1 and SREBP2 in lymphocytes of the F group, while in C group was not observed immunereaction. This result is indicating an activation of the lipid metabolism before being able to observe changes at a biochemical level.

178. (274) **"POTENTIAL ROLE OF SPEXIN IN MODULATING THE INNATE IMMUNE SYSTEM DURING OBESITY"**
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Spexin (SPX) is a new adipokine involved in many processes, such as energy balance, lipid metabolism, glucose homeostasis and weight control. SPX plasma levels were decreased in obesity/metabolic syndrome (MS) cohorts. Therefore, the hallmarks of obesity and MS are low chronic inflammation and adipocyte hypertrophy. Thus, the aim of this work was to evaluate SPX as able to modulate the innate immune system in obese mice. Swiss mice were supplemented with a fructose rich diet (FRD, 20%w/v) or tap water for 10weeks. Ten days prior to the end of protocol, mice were randomly divided and intraperitoneally injected with SPX 29μg/kg (C-SPX and F-SPX) or PBS (CTR and FRD). Caloric intake and body weight were recorded. At the end of protocol, plasma samples were collected and epididymal adipose tissue (EAT) was dissected, weighted and processed for qPCR and flow cytometry analysis. We found a significant negative correlation between body weight and body weight variation during SPX treatment in F-SPX and C-SPX. Glycaemia was unchanged, but plasma triglycerides were decreased only in F-SPX vs. FRD mice and were similar to CTR/C-SPX levels. F-SPX also had significantly less EAT mass, reaching values similar to CTR/C-SPX. mRNA expression of pro-inflammatory markers in EAT decreased in both groups treated with SPX vs. their counterparts. IL10, an anti-inflammatory marker, increased in F-SPX mice reaching similar values to CTR/C-SPX. Flow cytometry of stromal vascular fraction from EAT showed that ly6C+ monocytes and M1 macrophages were significantly decreased in SPX treated animals vs. their counterparts. M2 macrophages were increased in F-SPX, restoring the population to normal values. In conclusion, SPX induced a decrease in the inflammatory markers and restored the anti-inflammatory profile in EAT upon FRD. Furthermore, the negative correlation between body weight and weight variation indicates a possible use of SPX as a therapy for obesity/MS. PICT2015-2352.

179. (470) **NUTRITIONAL STATUS AND BIOCHEMICAL PROFILE DETERMINE A HIGHER-METABOLIC RISK IN AN ADULT POPULATION OF SAN LUIS CITY- ARGENTINA**
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Overweight (OW) and obesity (OB) are risk factors for the metabolic syndrome; and other frequent, serious and expensive chronic-diseases. There is not data regarding how OW and OB (i.e., body mass index, BMI≥ 25 Kg/m2) affect the biochemical profile in a population of patients from San Luis capital, Argentina. Because of the particular nutritional habits in San Luis, we hypothesize that the nutritional status in our population may cause a particular biochemical profile associated with a higher-metabolic risk. In this study, the relationship between nutritional status and biochemical profile is assessed throughout a population-transversal study in 316 adult patients (18-80 years-old) residing in San Luis City, Argentina, during the period 2015-2017. Inclusion and exclusion criteria were stated in an approved IRB-protocol and informed consent was signed. Anthropometric data including weight and height were acquired and the BMI was calculated. Biochemical profile was measured using a fasting-blood sample (n = 79) in which the following parameters were measured in plasma: triglycerides (TG, Normal Value (NV) <150 mg / dL), total cholesterol (TC, NV<200 mg/dL), HDL-C (>40 in men and >50 in women mg/dL), LDL-C (NV 70 to 130 mg/dL), glycemia (GL, <100 mg/dL), glutamine-oxaloacetic transaminase (GOT, men <38 and women <32 IU/L) and glutamate-pyruvate transaminase (GPT, NV<50 IU/L). This population included 37% women, 38% were overweight or obese (average BMI 25.2+5.21), 17.7% had high TG, 5% high TC, 2.5% high LDL-C, 13.9% high-blood glucose; 49% had HDL-C values below the recommended, and 2-3% of the patients had high GOT and GPT values. Correlation and association studies show that only patients with a BMI>25 had hypertriglyceridemia (X2 p = 0.000). GOT positively-correlated with BMI (CS p=0.04). In our population-based study, the nutritional status positively-associates with a biochemical profile consistent with and increased metabolic risk. PICT-2014-3369 / PROICO 10-0218 / PROICO 02-3418/ PIP916

180. (398) **BODY COMPOSITION AND PHYSICAL PERFORMANCE IN AN ELDERLY GROUP FROM ARGENTINA: PRELIMINARY STUDY**
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Low muscle mass and physical performances occur with advancing age. The aim of this study was to analyze body composition and physical performance in older subjects from Argentina. A descriptive study was conducted in 64 women (74.9±9.9y) and 23 men (75.1±6.7y) who attended community centers or nursing homes for elderly in Buenos Aires province, previous signature of the informed consent. The protocol´s study was approved by the Ethics and Research Committee of the Hospital Interzonal General de Agudos San Roque/Gonnet/Province of Buenos Aires. Body weight (BW,kg) and height (H,m) were determined to calculate Body Mass Index (BMI= BW/H2,kg/m^2). Fat-free mass (FFM,kg) was evaluated by deuterium dilution technique and fat mass (FM,kg) was obtained as FM= BW-FFM. The FMI (FM/H^2,kg/m^2) and FFMI (FFMI/H^2,kg/m^2) indexes were calculated. Handgrip strength (HS,kg) was measured using a Jamar® Hydraulic Hand Dynamometer. Physical performance was evaluated by Gait speed (GS,m/s) and Timed get up and go test (TGUG,s). HS, GS and TGUG were evaluated according to cut-off points defined by European Working Group on Sarcopenia in Older People (2010). 39% of women and men were overweight while 33% of women and 30% of men were obese. FMI was statistically higher in women than in men (11.4±4.7 vs 8.2±3.1, p<0.0005). 40% of women and 39% of men presented GS<1m/s, while TGUG>10s was observed in 36% of women and 30% of men. On the other hand, 42% of women and 41% of men presented decreased HS (<20kg and <30kg, respectively). Physical performance tests decreased significantly with age (0.001>p<0.05), in both sexes. In this preliminary study, it was found a high obesity degree in the elderly population and a significant decrease in physical performance tests, being these conditions risk factors for adverse events associated with sarcopenia such as falls, fractures, disabilities of daily life and loss of independence.

181. (419) **SEXUAL DIMORPHISM IN THE RESPONSE OF HUMAN NEUTROPHILS TO LDL**
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Hyperlipidemia milieu, characterized mainly by elevated low-density lipoprotein (LDL) cholesterol levels, activates endothelial and circulating cells which promotes leukocyte infiltration to large arteries and atherosclerosis development. In addition to the well-studied role of monocytes/macrophages, emergent evidence indicates a pathogenic role for neutrophils during various stages of atherosclerosis. Although there is a atherosclerosis-associated male:female mortality ratio 2:1, it remains to be elucidated if sexual differences occur in neutrophil response to atherogenic conditions. Therefore, our objective was to determine the sexual differences in the LDL-induced neutrophil activation. Human neutrophils were isolated from peripheral blood of healthy young men and women (18–40 years old) using Polymorphprep™, resuspended at 1-5x10^{5}cells/ml and incubated at 37°C, 5% CO2 with LDL (100 ug/ml) or vehicle for 2 to 8 hs to evaluate the surface expresion of CD11b, CD16, CD11a, CD29, CXCR2, CXCR4, and CD62L by FACS, functional static adhesion assay to endothelial adhesion molecules (P-selectin, ICAM1 and VCAM1), and phagocytosis of FITC-labeled latex beads by FACS. Statistical analysis: Paired t test, $p<0.05$ (at least 3 independent protocols). LDL induced up-regulation of CD11b ($p<0.05$) and increased the phagocytic ability ($p<0.05$) in both men and women. However, male neutrophils had a higher CD62L shedding and static adhesion in response to the LDL stimulus ($p<0.05$).
All together, the higher activation of neutrophils from men, as indicated by a stronger loss of CD62L and increased adhesion to endothelial molecules in atherosclerotic conditions, renders neutrophils as potential effector cells involved in the higher prevalence of atherosclerosis and cardiovascular diseases in men.

INFECTOLOGÍA / INFECTOLOGY 1

182. (128) **ESTABLISHMENT OF AN EXPERIMENTAL MURINE MODEL OF HEPATIC CYSTIC ECHINOCOCCOSIS**

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The search for therapeutic alternatives to optimize the treatment of cystic echinococcosis (CE) is performed at two levels: in vitro on the larval stage and in vivo in mice infected intraperitoneally with *Echinoccocus granulosus* protoscoleces. In the current murine model of CE, the cysts are located in the peritoneal cavity. Human CE is characterized by cystic lesions in the liver. The aim of the present work was to establish an experimental murine model of hepatic CE. CF-1 mice were injected via the portal vein. We used two different concentrations of protoscoleces [group A (n=9): 1000 protoscoleces/100 µL saline and group B (n=8): 500 protoscoleces/100 µL saline]. The diameter of cysts was periodically measured by ultrasound. Seven months post-infection (p.i.), mice were euthanized and samples of liver were taken for histopathological examination. During ultrasound evaluation, cystic lesion could be detected 4 month p.i. After 7 month, the infection rates of groups A and B were 44,54% (4/9) and 75% (6/8), respectively. These results coincided with the macroscopic examination during the necropsy. Although the number and diameter of cysts recovered from group B (3,83 ± 2,92 cysts; 5,28 ± 4,39 g) were higher than those from group A (3 ± 1,41 cysts; 4,56 ± 3,03 g), no significant differences were found ($P > 0,05$). Moreover, the development of cysts in the liver of mice did not show preference for any lobe. Histopathological studies revealed the typical features of *E. granulosus* metacestodes, surrounded by the adventitial layer with chronic inflammation and histiocytic reaction. We established an experimental model of hepatic CE. This model will be useful for pharmacokinetic and efficacy studies of drugs in mice infected with cysts located in the orthotopic and primary infection organ.

183. (179) **PHENOTYPIC PROFILE OF CIRCULATING T FOLLICULAR HELPER CELLS IN HUMANS CHRONICALLY INFECTED WITH TRYPANOSOMA CRUZI**

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Previous studies have identified that the population of IFN-γ-secreting T cells specific for *T. cruzi* consists mainly of early differentiated (CD27$^+$CD28$^+$) memory cells, which decline as chronic Chagas disease becomes more severe (Albareda *et al*, 2009). The contraction of CD4$^+$CD27$^+$ T cells correlated with the decline of circulating CD19$^+$CD27$^+$ cells, which comprised IgG$^+$, IgM$^+$ and isotype-switched memory B cells and terminally differentiated plasma cells (Fernandez *et al*, 2014).
To advance our understanding of the impact of *T. cruzi* infection on human immune responses, we now measured circulating T follicular helper cells of various phenotypes in chronically infected individuals and age-matched controls.The frequencies of lymphocyte subsets were estimated in freshly isolated PBMC using mAb against CD4-FITC, CD45RO-PERCyP Cy5.5, CXCR5-APC, CCR7-PECy7, PD-1 PacBlue, CCR6-PE, and CXCR3-PECy7 by Flow Cytometry and analyzed with FlowJo software.
We first delineated circulating Tfh cell subsets based on the expression of CD4, CXCR5, CCR7 and PD-1 markers and found no differences in the proportion of total (CD4$^+$CXCR5$^+$), central memory (CD4$^+$CXCR5$^+$CCR7$^+$), effector memory (CD4$^+$CXCR5$^+$CCR7$^-$), resting memory (CD4$^+$CXCR5$^+$PD1$^-$), activated (CD4$^+$CXCR5$^+$PD-1$^+$) and precursor (CD4$^+$CXCR5$^+$CCR7^{lo}PD1^{hi}) cells between infected patients and controls. Afterwards, we used CXCR3 and CCR6 markers to delineate Tfh subsets associated with IFN-γ (CXCR5$^+$CXCR3$^+$), IL-4 and IL-5 (CXCR5$^+$CXCR3$^-$CCR6$^-$) and IL-17A and IL-22 (CXCR5$^+$CXCR3$^-$CCR6$^+$) production, and detected a significant contraction of CD4$^+$CXCR5$^+$CXCR3$^+$, CD4$^+$CXCR5$^+$CXCR3$^+$PD-1$^-$ and CD4$^+$CXCR5$^+$CXCR3$^+$CCR6$^+$ phenotypes in infected patients ($p<0.05$). Interestingly, non-follicular T cells (CD4$^+$CXCR5$^-$) coexpressing CXCR3$^+$ and CCR6$^+$ markers were reported to produce IFN-γ, IL-17 and IL-22 in healthy subjects (Morita *et al*, 2011). No correlation between the size of the altered subsets and serum anti- *T.cruzi* IgG levels was found. These observations raise a question as to whether the declining of the particular circulating Tfh subsets plays a critical role in the immune deregulation process provoked by the parasite in humans.
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184. (276) **ROLE OF FENOFIBRATE ON THE IMMUNE RESPONSE IN AN EXPERIMENTAL MODEL OF CHAGAS DISEASE: MODULATION TO A REPARATIVE PROFILE IN THE HEART**

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Chagas disease, the main cause of dilated cardiomyopathy in Latin America, causes an inflammatory response that is critical for the control of acute infection. Since benznidazole eliminates parasites while PPARα ligands modulate inflammation and restores ventricular function, we evaluated the effect of fenofibrate (fen), a synthetic ligand, on the modulation of the immune response in an experimental model of T. cruzi (Tc) infection.
BALB/c mice were sequentially infected with a non-lethal Tc strain. After 6 weeks (w) mice were reinfected with a lethal strain for additional 4w. Thereafter, mice were treated with fen for 4w. Then, the expression of several genes involved in the modulation of the immune response to Tc infection was analyzed in the heart.
IL-6, TNFα and NOS2 of Tc-infected mice (Tc), Tc-infected and treated mice (Tc+fen) and uninfected mice were analyzed in the heart using RTq-PCR. Infection increased the expression of IL-6, TNFα

and NOS2 (p<0.05), while fen inhibited the expression of these mediators (p<0.05). The expression of TGFβ increased in Tc mice (p<0.05), while fen reduced it (p<0.05). Besides, IL-10 expression increased in Tc mice (p<0.05). This increment was higher upon treatment with fen (p<0.05). To study whether fen changes the profile of the myeloid population, we evaluated the expression of M2 profile markers in the heart. We observed that fen increased the expression of Mannose Receptor, FIZZ and YMI (RTq-PCR p<0.05). While Tc infection induced the liberation of IL-17 to serum (ELISA p<0.05), fen treatment inhibited its release (ELISA, p<0.05). Moreover, neither the infection nor the fen treatment induced changes in the expression of FOXP3, a typical marker of Treg cells (RTq-PCR, p= NS). We suggest that fenofibrate modulates the immune response to Tc infection, leading towards a reparative profile in the heart of infected mice, independently of the Treg involvement.

185. (283) **HOST GENOTYPE-PARASITE INTERACTIONS IN THE CHRONIC STAGE OF INFECTION, IN MICE OF THE CBI-IGE MODEL, AFTER CHALLENGE WITH INCREASING DOSES OF TRICHINELLA SPIRALIS (TS) OR TRICHINELLA PATAGONIENSIS (TP)**

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Trichinellosis is a zoonosis caused by the nematode Trichinella spp. Parasites of this genus show a high genetic variability. The prevalent species worldwide is Ts. In 2004, Krivokapich et al. isolated a new species, Tp, in cougars (Puma concolor) from Patagonia; this species, like Ts, is capable of infecting humans. Clinical signs and infection severity differ according to the Trichinella species and host species involved; clinical signs are also dose-dependent. This work aimed to compare the effect of the host genotype in the response to infection with increasing doses of Tp or Ts in two mouse lines (CBi and CBi/L) of the CBi-IGE murine model. Adult males (n=8 per group) infected orally with one (GI), two (GII), or four (GIII) L1 infective larvae/g bw were used. In the chronic period (42±2 days post-infection), the relative parasitic burden (rPB, number of parasites/g of tissue) was determined in the tongue. Significant differences between Tp and Ts (P<0.05) were observed in rPB in the three doses, for each genotype (Mean±SE, CBi/L GI: Tp 15±6.8 – Ts 82±21.0; GII: Tp 30±7.1 – Ts 66±14.6; GIII Tp 47±13.7 – Ts 110±21.1; CBi GI: Tp 62±14.6 – Ts 472±109.9; GII: Tp 70±17.2 – Ts 555±124.4; GIII: Tp 127±13.9 – Ts 1068±160.0). Genotype CBi/L was more resistant than CBi, presenting lower rPB with both species. In each line, Ts showed greater infectivity than Tp with a ratio rPBTs/rPBTp of 5.4 (GI), 2.2 (GII) and 2.3 (GIII) for CBi/L, and of 7.6 (GI), 7.9 (GII) and 8.4 (GIII) for CBi. The effects genotype of the host, Trichinella species, and infective dose, as well as all the interactions between them, were significant, revealing differences in the biology of the two Trichinella species as well as particular responses of the two genotypes for the parasite-dose combinations used.

186. (512) **COULD THE AXIN-BASED LYSOSOMAL PATHWAY BE INVOLVED IN THE ANTI-ECHINOCOCCAL EFFECTS OF METFORMIN?**

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Echinococcosis is a neglected zoonotic disease caused by infection with the larval stage of tapeworms within the genus *Echinococcus*. Chemotherapy treatment for this disease has had limited effectiveness thus far, which is why it is a dire need to find new drugs for its treatment. We have previously shown that Metformin (Met), an anti-hyperglycemic and anti-proliferative drug, exhibits considerable *in vitro* and *in vivo* activity against *E. granulosus* larval stage (the causative agent of cystic echinococcosis). Here, we extended the study and evaluated the effect of the drug on *E. multilocularis*, the causative agent of alveolar echinococcosis (AE), a more aggressive variant of the disease. Metformin exerted a dose-dependent effect on the viability of totipotent parasite stem cells. By *in toto* immunolocalization assays, the expression and cellular localization of Em-AMPKα, Em-TOR, and Em-Atg8 were detected in the germinal layer of *in vitro* obtained metacestodes vesicles. The drug induced the activation of AMPK (Em-AMPKα-P176) as well as the reduction of the Ser3122-phosphorylated form of Em-TOR and the overexpression of the autophagic marker Em-Atg8. The induction of the autophagic process was concomitant with the nuclear localization of Em-foxO, which could be correlated with the transcriptional regulation of this pathway. Based on our *in vitro* results, we then examined the *in vivo* chemopreventive effect of Met on the growth of *E. multilocularis* larval stage in the murine AE infection model. Oral administration of Met (50 mg/kg/day) exhibited remarkable chemoprotective properties against secondary alveolar echinococcosis in mice. These results raise the question of whether Met controls the development of *Echinococcus* through the indirect inhibition of TOR, as a consequence of the ATP synthesis inhibition, and/or through the direct inhibition of TOR, by the Lysosomal pathway, which involve LAMPTOR and AXIN, identified in the parasite.

187. (519) **ECHINOCOCCUS GRANULOSUS EXOSOME-LIKE VESICLES CONTAIN IMMUNOMODULATORY AND LAMINATED LAYER-INTERACTING PROTEINS**

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The secretion of extracellular vesicles (EVs) in helminth parasites is a constitutivemechanism that promotes survival by improving their colonization and adaptation in thehost tissue. In this study, we isolated and characterized the EVs produced in cultures of *E. granulosus* protoscoleces and metacestodes and analyzed their biological function after contact with host cells. Similarly to that observed by TEM in protoscolex cultures, the supernatants of metacestode cultures were enriched in exosome-like vesicles. It is known that a true exchange of macromolecules across of the laminated layer occurs between the host and the parasite, with constant vesicular trafficking through the tegument. This movement may depend on signature organellar targeting motifs within the proteins and on their interactions with certain components of the laminated layer. Interestingly, the laminated layer of *E. granulosus* possesses deposits of the calcium salt of inositol hexakisphosphate which have been reported to bind to numerous proteins present in *E. granulosus* EVs (such as synaptogmins, ATP-dependent RNA helicases, pleckstrin, ezrin/radixin/moesin, gelsolin and galectin) acting as a “dynamic anchorage” that promotes their passage across the laminated layer. Also, it is widely known that EVs derived from helminthparasites, mediate the immune modulation through their protein-, lipid- and RNAs-cargo. We strikingly found that the exposure of dendritic cells (DCs) to EVs induced an unconventional activation profile with MHCII decrease. Based on mass spectrometry analysis and *in silico* functional categorization, we identified: a putative immunomodulatory protein similar to the human B-Cell Receptor Associated Protein 29 (Bp29); basigin (EMMPRIN- or CD147); a maspardin ortholog (MAST or ACP33 protein) and annexins, which could explain the type 2 immune response observed in patients with cystic echinococcosis. In conclusion, our study demonstratedan important role of EVs in the maturation process of DCs which are essential for the coordination of specific immune responses.

188. (263) **EFFECTIVE SEPARATION OF TRITRICHOMONAS FOETUS MEMBRANE LIPIDS IN ONE DIMENSIONAL THIN LAYER CHROMATOGRAPHY**

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Gangliosides are a family of sialic-containing glycosphingolipid present in cell membranes. They play biological functions such as cell recognition, signal transduction, adaptation of plasma membrane to environmental variations. Glycolipids are also related to immunological factors associated with cell surfaces. Phospholipids make up a group of compounds whose basic structure presents a phosphate

radical that is linked to different alcohol's, and may hold specific substituting molecule groups such as choline, serine and ethanolamine. These membrane compounds carry out structural functions and take part in cellular signaling. Cholesterol, chemically derived from cyclopentanoperydrophenanthrene, is necessary for viability and cellular proliferation. *Tritrichomonas foetus* is parasitic protozoa that affect the urogenital tract of cattle, where it interacts mainly with epithelial cells. But, the cellular mechanisms by which *T. foetus* colonizes mucosal surfaces and causes tissue injury are not well understood. Studies have demonstrated the involvement of membrane lipids in the interaction between host cells and pathogenic protozoa. However, to study the lipids from different living beings the investigator ought to select precise conditions for effective separation of the lipids. The present work is aimed to obtain a separation of membrane lipids of *T. foetus* on the same plate and in one dimension for further identify and quantifying lipid spots using a scanning device. The lipids were extracted according to the method of Folch et al., (1957). A method for effective separation of lipids of *T. foetus* in one dimensional thin layer chromatography on ready-made plates has been developed. The result demonstrates that *T. foetus* present phospholipids and cholesterol. It was also observed the presence of glycosphingolipids, which are associated to cell recognition and cell surface markers. Based on these results, it is intended to determine if these lipids are involved in the interaction of this parasite with host cells *in vitro.*

189. (682) **CHARACTERIZATION OF HISTONE DEACETYLASE 8 ISOTYPE FROM CESTODES AS NEW POTENTIAL DRUG TARGETS OF NEGLECTED DISEASES**

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Echinococcosis and cysticercosis, tropical diseases caused by cestode parasites, represent a significant problem in human and animal health and are considered neglected and a priority matter for the WHO. Histone deacetylases (HDACs) have been validated as drug targets for the treatment of several diseases, including parasitic infections. HDACs remove acetyl groups from histones and other cellular effectors, thus directly influencing the chromatin structure and thereby regulating gene transcription and other cellular processes. Previously, we have shown the presence of HDAC genes of class I and II on cestode genomes and showed that they have an essential role in parasite development and survival. Specifically, HDAC8 was one of the more promising drug targets since it is expressed in different parasite stages of the genus *Echinococcus* and is up-regulated in the metacestode, the clinical relevant stage. Furthermore, HDAC8s showed only 40% amino acid identities with human HDAC8. In this work, we characterized HDAC8 from cestodes for the treatment of diseases they cause. We have cloned, sequenced and performed homology modelling and protein expression of HDAC8 from *Echinococcus canadensis* and *Mesocestoides corti*, a parasite used as a laboratory model of cestodes. Cestode HADC8s showed HDAC domain conservation with specific insertions compared to their human ortholog. Homology models adopted canonical α/β HDAC fold, the structures suggested that the catalytic pocket are conserved, only an amino acid is substituted and distinguished of human HDAC8, human M274 by histidine. Also, important residues implicated in binding to pan HDAC inhibitor "Trichostatin A" (TSA) are conserved. *Escherichia coli* (BL21) was used as expression system to produce recombinant HDAC8 from both cestodes; the obtained proteins were of the expected molecular weight. Future studies will include HDAC8 activity inhibition assays with specific inhibitors. This work is the first step to study HDAC8 from cestodes as a possible new drug target.

190. (506) **ROLE OF ATP HOMEOSTASIS IN THE UNINFECTED ERYTHROCYTES FROM PLASMODIUM FALCIPARUM CULTURES**

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During the course of its complex life cycle, the malaria parasite *Plasmodium falciparum* invades red blood cells (RBCs). The parasite grows and multiplies within the RBCs modifying the membrane structure and biochemical properties of the host cell. Activation of ATP release by RBCs can control vasodilatation and malaria patients show microcirculatory impairments which might be associated altered extracellular ATP (ATPe) homeostasis. Patients with uncomplicated malaria show low parasitemia (4%), so that most circulating RBCs are uninfected RBCs (ui-RBCs).
Here, we tested if ui-RBCs undergo a deregulation of ATP homeostasis, with consequences on rheological properties of ui-RBCs.
Two methods for isolating ui-RBCs were developed: 1- culture of infected RBCs and ui-RBCs, which were later separated by magnetic columns, and 2- culture of RBCs and ui-RBCs in separated chambers which were connected by a mesh, allowing solute exchange but no physical contact.
When stimulated with an adrenergic cocktail, ui-RBCs showed a 4-fold increase of [ATPe], while control RBCs exhibits a 2-fold increase. In both cases, ATPe kinetics was compatible with acute and transient activation of ATP release. There were no differences between ui-RBCs obtained by either of the two isolation methods. When mechanical deformation was imposed to RBCs, ATPe was similar in ui- and control RBCs, displaying a 10-fold increase over basal values. ATPe hydrolysis by ectonucleotidases was upregulated 4-fold in ui-RBCs, when compared to control RBCs, so that ATPe by-products are assumed to accumulate and signal in the extracellular milieu.
In short, ui-RBCs showed an altered metabolic balance affecting ATPe regulation with potential consequences on vasodilatation. No physical contact with infected cells was necessary for ui-RBCs to exhibit a deregulation of ATPe homeostasis.
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191. (489) **IDENTIFICATION OF HOST PROTEINS IN ECHINOCOCCUS GRANULOSUS GERMINAL CELLS CULTURE**

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The parasite *Echinococcus granulosus* (Eg) is the causative agent of cystic echinococcosis, an important zoonosis that affects humans and ungulate animals. Platyhelminthes have a unique population of undifferentiated stem cells, known as 'germinal cells' which represent an interesting source for studying tissue turnover, growth and regeneration. Recently important advances have been introduced for *in vitro* cultivation of Eg germinal cells which were mantained in culture for at least 24 month. This provides a useful tool for investigating host–parasite interactions. The host–parasite relationship in cestode infections is complex. One characteristic of this bidirectional molecular communication is the uptake of host proteins by the parasite. Using a proteomic strategy, we describe the presence of several host proteins in Eg germinal cell culture and discusse the potential roles for some of these proteins. Eg primary cell culture was obtained from hydatid cysts and mantained with weekly splitting during 1-4 month. Then, 50 μl of cells were homogenized and the peptide content was estimated. Aliquots of 100 μg protein per sample were used for filter-aided sample preparation and the resulting peptides were subjected to nano-LC-MS/MS-analysis. A combined library was set-up by combining the different runs using the Protein Pilot-software and Uniprot database. We identified 319 host proteins. There were 250 proteins that were assigned to GO terms for molecular function, 242 for cellular component and 226 for biological process. Regarding cellular localization we identified 31.6% secreted proteins, 28.3% cytoplasmic proteins and 14.8% mitochondrial proteins. Some of the most abundant proteins were serum albumin and several proteins related with the immune response such as Ig mu chain C region and complement component C9. Our study provides valuable data on the biological role of the host proteins incor-

porated by the parasite, allowing novel insights into the molecular mechanisms involved in host–parasite interactions.

192. (305) **STX2 TRANSLOCATION PATHWAYS ACROSS HUMAN INTESTINAL EPITHELIAL CELLS ARE DIFFERENTIALLY STIMULATED BY AN HYPERVIRULENT E. COLI O157:H7**

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Gastrointestinal infection with Shiga toxin (Stx2)-producing Escherichia coli causes bloody diarrhea, hemorrhagic colitis and hemolytic uremic syndrome (HUS). E. coli O157:H7 is the most prevalent serotype associated with HUS and Stx2 is its major virulence factor. However, mechanisms involved in pathogenesis mediated by Stx2 and how toxins cross the intestinal epithelium are unknown. Our aim was to study the effects of E. coli O157:H7 on human colonic epithelial cells to better understand the means by which Stx2 induces diarrhea and translocate the intestinal barrier.
We have evaluated translocation of Stx2 across HCT-8 cells cultured as monolayers on Millicell inserts in presence of a O157:H7 mutant lacking stx2 gene (O157:H7Δstx2) or its filtered culture supernatant (SNO157:H7Δstx2). Additionally, O157:H7Δstx2 effects were evaluated after a 30 min pre-incubation with pathway-specific endocytic inhibitors: Amiloride (1mM), Dynasore (80 μM) and Methyl-β-Cyclodextrin (MβCD, 4mM). Transepithelial electric resistance was monitored before and after treatments. Dextran-FITC was used as an indicator of paracellular translocation and EDTA (0.1 mM) as a tight junction disruptor. Collected basal media cytotoxicity was evaluated on Vero cells and Dextran-FITC was measured by fluorometry.
Maximum Stx2 translocation was observed after treatment with O157:H7Δstx2 supplemented with Stx2 compared to EDTA and, lastly, bacterial SN treatment. Additionally, maximum Dextran-FITC passage was achieved with EDTA. MβCD showed the highest Stx2 translocation inhibition, followed by significant inhibition by Amiloride and Dynasore ($p<0.05$).
The cytotoxic effects induced by Stx2 measured on HCT-8 as neutral red uptake at 72 h were significantly inhibited ($p<0.05$) when cells were pre-incubated with MβCD, in comparison to Amiloride and Dynasore, which showed reduced or no cytotoxic activity inhibition at 4 and 72h respectively.
These results indicate that a direct contact between O157:H7 and the intestinal barrier stimulate Stx2 translocation mainly across a transcellular caveolin-dependent pathway, but also across the paracellular pathway.

193. (310) **CROSSTALK BETWEEN HUMAN MICROVASCULAR ENDOTHELIAL CELLS AND TUBULAR EPITHELIAL CELLS MODULATES PRO-INFLAMMATORY RESPONSE INDUCED BY SHIGA TYPE 2 AND SUBTILASE TOXINS**

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Shiga toxin (Stx) is considered the main virulence factor associated to the renal damage of the hemolytic uremic syndrome (HUS). However, Subtilase (SubAB) cytotoxin has also been related to the HUS pathogenesis. To clarify the involvement of endothelial-epithelial crosstalk in the kidney toxins damages, previously, we developed an in vitro model of renal proximal tubule by microvascular endothelial cells (HGEC) and tubular epithelial cells (HK-2) in coculture (HGEC/HK-2). After 24h of incubation with Stx2 (0.01ng/ml), SubAB (1ng/ml) or Stx2+SubAB, we had found a differential release of IL-6, IL-8 and TNF-α between monocultures and cocultures, having considered both compartments together. While IL-6, IL-8 and TNF-α release was increased on the coculture and HGEC monoculture by all treatments and Stx2, respectively, it was not modulated on HK-2 monoculture by the toxins. In this work, we compared the release of these soluble mediators, measured in supernatants by ELISA, between HGEC and HK-2 monocultures respect to HGEC or HK-2 coculture compartments. On the HGEC side, only Stx2 increased IL-6 and IL-8 secretion. Surprisingly, on the HK-2 side, all treatments caused a significant increase in IL-6 and IL-8 release and statistically different from HK-2 monoculture. TNF-α was increased on both HGEC and HK-2 side by all conditions ($p<0.05$, n=4). When, cocultures cell viability was analyzed by neutral red uptake, no cytotoxic effects were observed at these toxins concentrations ($p<0.05$, n=3). Then, we evaluated by flow cytometry the presence of toxins inside the cells. Both, cells from HGEC and HK-2 side were positive (50%) for Stx2 or SubAB, respectively. After Stx2+SubAB treatment, 40% of cells were double positive and only 10% were single positive for Stx2 and SubAB (n=1). Endothelium-epithelium crosstalk modulates the inflammatory responses caused by bacterial toxins on renal cells. A cooperative effect between Stx2 and SubAB could increase their uptake by target cells.

194. (520) **THE ROLE OF IMMUNE COMPLEXES AND THEIR INTERACTION WITH COMPLEMENT IN THE INFECTIONS PRODUCED BY DENGUE AND ZIKA VIRUSES IN HUMAN IMMUNE CELL LINES**

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The re-emergence of the 4 serotypes of dengue virus (DENV) and emergence of Zika virus (ZIKV) in recent years have greatly affected the panorama of public health in South America. Infections with these flaviviruses cause a range of manifestations; from asymptomatic infection or mild febrile illness to severe disease, characterized by increased vascular permeability and hemorrhages in DENV infections and neurological complications in ZIKV infections. Several hypotheses have been advanced to explain the mechanism of DENV severe disease, including antibody dependent enhancement of infection (ADE) in Fc-receptor expressing cells, serotype cross-reactive memory T cell responses, and viral virulence. It is postulated that these three non-mutually exclusive processes trigger a cytokine cascade and the activation of the complement system, resulting in severe disease. Recent publications have shown that cross-reactivity between anti-DENV antibodies and ZIKV promotes enhanced infection of ZIKV in vitro. Our hypothesis is that the complement system, C1q in particular, modulates DENV and ZIKV infection in the presence of anti-DENV antibodies in human immune cells and that this modulation promotes protection against infection with these viruses. In this study, we characterize cross-reactivity and the neutralizing activity of dengue-immune human sera (Institute of Tropical Medicine, Asunción, Paraguay) and commercial anti-DENV antibodies for DENV serotypes and ZIKV using ELISA and plaque reduction neutralization tests (PRNT). Using the human cell lines K-562, THP-1 and U-937, which express FcgRI (CD64) and FcgRII (CD32) differentially, we show that ADE modulation in the presence and absence of human C1q is both Fc-receptor and isotype- dependent. Future studies will focus on the determination of the mechanisms of this modulation and the study of the effects of C1q on viral entry processes. The results of this research will contribute to understanding of the mechanisms of protection versus pathogenesis of flavivirus infections in humans.

TOXICOLOGÍA / TOXICOLOGY 1

195. (217) **SURFACE MODIFICATION OF METALLIC GOLD NANOPARTICLES WITH CYSTEINE DECREASES CELL TOXICITY**

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Metal nanoparticles -NP- (10-100 nm) are applied in pharmaceutical products, and the modification with anti-oxidants can improve their

properties. The aim of the present work was to investigate differences in the toxicity of metallic gold NP (AuNP) and those modified with cysteine (AuCysNP), on a human cell line.
The chemical synthesis of AuNP and AuCysNP was carried out using CTAB (ligand) and sodium borohydride (NaBH4) as a reducing agent: 0.01M AuHCl4; CTAB 0.01M; 10mM NaBH4 and 0.025mM Cysteine. The NPs were characterized by UV/VIS spectroscopy and transmission electronic microscopy (TEM). The HTR-8 / SVneo cell line was incubated for 6 and 24 h at different NPs concentrations (0.01 - 0.5 mM). Cell viability was evaluated by MTT, the production of reactive oxygen species (ROS) by NBT, the levels of nitric oxide by Griess, and the activity of antioxidant defense enzyme, superoxide dismutase (SOD) by riboflavin-NBT method.
Exposure for 6 h to AuNP significantly decreased cell viability at 0.2 and 0.5 mM whereas AuCysNP diminished cell viability at 0.5 mM. After 24 h incubation, both NPs significantly modified cell viability at different extent at 0.2 and 0.5 mM, AuNP decreased cell viability a 29 and 68%, while AuCysNP a 19 and 49%, respectively. ROS levels were investigated after 24 h incubation, ROS increased after AuNP (0.1-0.5 mM) incubation, while AuCysNP only increased ROS at 0.5 mM. No changes in nitric oxide levels were observed in the conditions tested. SOD activity was determined after 24 h incubation, an increase in enzyme activity was determined at all the concentrations tested for AuNP and in 0.1; 0.2 and 0.5 mM for AuCysNP, compared to controls.
These results suggest that the AuNP modification with the aminoacid cysteine decreases the cell toxicity of AuNP and it may favors an antioxidant response.

196. (484) **BRAIN CORTEX AND CEREBELLUM BIOENERGETICS AND MITOCHONDRIAL PATHWAYS IN ACUTE ENDOTOXEMIA**

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The mitochondrial role as cellular energy source and its participation in multiple signaling pathways is largely known. Alterations in mitochondrial function have been linked to inflammatory, metabolic, cardiovascular and neurological pathologies. The aim of this study is to analyze mitochondrial bioenergetics in two CNS areas: cortex and cerebellum. Female Sprague-Dawley rats (45 days old) were intraperitoneally injected with a single dose of LPS (8 mg/kg body weight) or vehicle. After 6 h of treatment, mitochondria of cerebral cortex and cerebellum were isolated by differential centrifugation. Mitochondrial function was analyzed by different approaches, oxygen consumption was measured in state 4 (rest respiration state) and in state 3 (active respiration state) by oximetry, and no statistics differences were observed in both areas (control: 145 and 136 n ng-at O min-1 mg protein-1 for cortex and cerebellum respectively). Same results were found in mitochondrial inner membrane potential, analyzed by flow cytometry. ATP production rate was assessed by luciferin-luciferase assay, and a 45% decrease was observed only in cortex (control: 181 nmol ATP min-1 mg protein-1, $p<0.05$). Regarding oxidative stress, mitochondrial superoxide anion level in as analyzed by flow cytometry, showing an 85% and 71% increase in cortex and cerebellum respectively and compared to control animals ($p<0.05$). Moreover, cardiolipin content was found decreased, probably due to the direct effect of the oxidation of superoxide anion in mitochondria. These results showed an increase in the production of reactive oxygen species in the mitochondria in both sections of the CNS, accompanied by a decrease in ATP availability in the cortex. In this context, regulation of CNS mitochondrial bioenergetics could play an important role role in endotoxemia, placing mitochondria as a clinical target in this syndrome.

197. (510) **THE UV FILTER BENZOPHENONE 3 PROVOKES LOSS OF OOCYTES IN RAT OVARY CULTURES**

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Benzophenone 3 (BP3) is frequently used as an organic UV filter in sunscreens, as photoinitiator, indirect food additive, or fragrance enhancer. Given the increased use of sunscreens in the general population because of the growing concern about UV radiation and skin cancer, as well as the high prevalence of BP3 in water supplies, humans are highly exposed to BP3 by both dermal and oral routes. Several in vitro and in vivo studies have evidenced the ability of BP3 to act like an endocrine disrupting chemical. Our goal was to study the effect of BP3 in the follicular assembly and the potential involvement of Foxl2 pathway using whole ovary cultures. Ovaries were collected from Wistar rats at birth, treated in vitro with vehicle (0.01 % DMSO), BP3 (5.8 nM and 876 nM) or ESR2 inhibitor (0.1 nM), and cultured for 7 days. Nest breakdown, follicular assembly and the expression of several regulators of these processes (p27, Foxl2, Sox9, Bmp2, Cyp19 and Fst) were evaluated. In vitro exposure to BP3 (5.8 nM) decreased the population of total oocytes, the number of nests per ovary and early primary follicles population. In addition, BP3 (5.8 nM) induced overexpression of Foxl2 mRNA levels through ERS2 but increased Fst mRNA levels independently from ERS2 or Foxl2. We also observed that the number of p27-positive oocytes was decreased after BP3. Taken together, our results show that exposure to BP3 (5.8 nM) perturb nest breakdown, causes a decrease of total oocytes reserve, and induces an overexpression of Foxl2 mRNA through ESR2. In conclusion, exposure to an environmentally relevant dose of BP3 is enough to perturb the early events of germ cell development as showed here in whole ovary cultures.

198. (662) **ENVIRONMENTAL CONCENTRATIONS OF CHLORPYRIFOS AFFECTS THE NORMAL FEMALE CYCLICITY IN RATS MODIFYING THE HISTOLOGICAL CHARACTERISTICS OF THE UTERUS.**

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Previously, we demonstrated that the pesticide chlorpyrifos (CPF) acts as an endocrine disruptor in human mammary adenocarcinoma cells MCF-7 and in rat mammary gland.We investigated whether environmental concentrations of CPF induce changes in the uterus, and whether this effect depends on the accumulation of CPF in serum or fat in rats.Female 40-day-old Sprague-Dawley rats were exposed to CPF 0,01 y 1 mg/Kg/día (CPF 0,01 y CPF 1) or vehicle (C) orally for 100 d. We determined the estrous cycle phase daily. The rats were euthanized in estrus. The uterus, abdominal fat and blood were removed. Serum and fat CPF-levels were determined by gaseous chromatography. We performed the anatomopathological study of the uterus. Immunohistochemical expression of the proliferating cell nuclear antigen (PCNA) was determined. Fractal dimension (FD) analysis, a measure of the irregularity and complexity of the tissue was used.Serum levels of CPF were 0.107 +/- 0.10 ng/mL and 0.62+/- 0.22 ng/mL in the animals of CPF 0.01 y CPF 1 groups, respectively and undetectable in group C. In fat we found a significant accumulation of the pesticide in the CPF 1 group (14.16+/-9.48 ng/gr fat; $p<0.001$ vs C). Both doses of CPF increase the frequency of irregulars cycles in the animals ($p<0.05$). However, the uterus histology were in meteestrus or diestrus phases. The histopathology of the uterus indicated that both doses of CPF increase the FD ($p<0.001$ vs C). Finally, CPF 0.01 produces an increase of PCNA in glandular epithelium and stroma ($p<0.01$ and 0.05 vs C,

respectively) whereas CPF 1 increases this marker also in the luminal epithelium (p<0.05 vs C).We conclude that CPF is an endocrine disruptor that affects the normal female cyclicity in rats modifying the histological characteristics of the uterus. These changes are more pronounced with the highest dose tested.

199. (760) **INTOXICATION WITH CADMIUM AND ITS POSSIBLE EFFECTS ON THE HISTOARCHITECTURE OF PLACENTA OF RAT AT 20G. ACTION OF DIFFERENT PROTEIN DIETS.**

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Introduction: The occurrence of intrauterine growth retardation (IUGR) is higher in infants born to mothers exposed to cadmium (Cd) through environmental sources such as smoking and industrial work. A contributing factor of IUGR is improper placentation. It has been postulated that extravillous trophoblasts (EVTs) are central to pregnancy-specific remodeling of spiral arteries by associating the presence of EVTs with the appearance of fibrinoid and degenerative changes within in the vascular wall of decidual and myometrial arteries. Importantly, trophoblast-mediated remodeling provokes conversion of spiral arteries with narrow lumina into larger conduits delivering low-pressure, high blood flow to the growing fetus. These arterial changes were found to be vastly absent in hypertensive pregnancies and intrauterine growth restriction suggesting trophoblastic malfunction. In the other hand, soy protein is becoming increasingly important in the human diet. It was demonstrated that isoflavones (genistein) could cause hypertrophy in the rat endometrium and alter reproductive function in numerous species. Objective: we evaluate a potential involvement of environmental exposure relevant concentrations of Cd exposure on placental function and the possible protective role of the consumption of soy protein. Materials and Methods: 4 lots of female Wistar rats were used: 2 lots received casein (Cas) and 2 lots soybean (Soy) as protein source. Within each group, 1 lot received regular water (control-Co) and the other, 15 ppm of Cd in the drinking water during pregnancy period (20G). Results: we observed several sections of placental tissue from the different experimental groups. The histomorphological study showed the presence of trophoblast invasion in different regions of the tissue. Conclusion: our previous results demonstrated the presence of oxidative and nitrosative environment in the placenta at 20 G. This situation accompanied by morphological alterations iwould suggest an imbalance during the implantation process and consequently an inadequate intrauterine development.

200. (778) **ROLE OF DIFFERENT PROTEIN SOURCES IN THE MORPHOLOGY OF CD INTOXICATED LUNGS**

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Cadmium (Cd) is a toxic metal and an important environmental contaminant. We studied its effects on morphology, bronchoalveolar lavages (BAL) and LDH activity under different diets. 4 lots of female Wistar rats were used: 2 lots received casein (Cas) and 2 lots received soybean (Soy) as protein sources. Within each group: 1 lot received regular water (control-Co) and the other 15ppm of Cd in the drinking water for 60days. BAL was performed, LDH activity was measured and cells obtained were analysed. Lungs were fixed, sectioned, stained, examined for evidence of injury and analyzed morphometricaly. A lung section was mineralized and cadmium concentration was measured by ICP-MS.
Cadmium concentration was higher in intoxicated groups, but only significative in Cas-Cd (p<0,01). Alveolar diameters ratio decreased in Cas-Cd and So-Co vs Cas-Co (p<0,001) and also decreased in So-Cd vs So-Co (p<0,005). Non-functional areas are widely spread in Cas-Cd when compared with Cas-Co (p<0,005); there are also larger non-functional areas in Soy-Co vs Cas-Co and no differences were seen among soy groups. Alveolar macrophages decreased in Soy-Cd vs Soy-Co (p<0,05); neutrophils in BAL increased in Soy-Cd vs Cas-Cd and Soy-Co (p<0,01); lymphocytes increased in Cas-Cd vs Cas-Co (p<0,01) and in Soy-Cd vs Soy-Co (p<0,05). LDH activity increased in Cas-Cd vs Cas-Co (p<0,001) and decreased in Soy-Cd vs Cas-Cd (p<0,0001). Cas-Cd showed the presence of numerous non-functional spaces and advanced pulmonary fibrosis. On the other hand, when we analyzed Soy-Cd group, it also showed evidence of pulmonary fibrosis but the same wasn't homogeneus and the non-funcional spaces (with presence of connective tissue) were in the periphery of lobes. In this study, we report that the protein source in the diet is important when lung is expossed to Cd, because the pulmonary function is compromised.

201. (505) **EFFECTS OF DIFFERENT NUTRITIONAL DIETS ON RAT MAMMARY GLAND RESPONSE TO CADMIUM ENVIRONMENTAL INTOXICATION**

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Cadmium (Cd) is a toxic element and an important environmental contaminant. We studied the effects of this metal at the molecular, biochemical and cellular levels on rat mammary gland (MG) and looked at the potential protective outcome of a Soy based diet. For this purpose, 4 female lots of Wistar rats were used: 2 lots received casein and 2 lots soybean (Soy) as protein source. Within each group, 1 lot received regular water (control) and the other, 15 ppm of Cd in the drinking water for 60 days (n= 6 animals per group, from three independent experiments). Lipids were extracted by Hexano:Isopropanol:BHT (3v:2v:1%). Total cholesterol, phospholipids and fatty acids were determined by colorimetric assay, thin-layer chromatography and gas chromatography-mass spectrometry, respectively. HMG CoA Reductase (HMGCoAR) and NFκB activation were determined by Western blot. The inflammation marker COX2 and the Bax/Bcl2 ratio were measured by RT-PCR. MGs were subjected to hematoxylin-eosin stain for morphological studies. Our results show that Cd alters the lipid profile of the gland and this effect is modulated by Soy. The expression of HMGCoAR is affected by Cd and Soy (*p<0,001), showing some synergism. On the other hand, both Cd and Soy activate NFκB and this effect is accompanied by augmented expression of COX2 and apoptosis according to the Bax/Bcl2 ratio. In addition, Cd and Soy stimulate the development of the gland, but Soy decreases the fat pad. The double treatment of Soy and Cd cause MGs fibrosis and loss of functionality, suggesting a protective mechanism of soy rich diets against developing mammary transformation induced by Cd. In summary, Cd affects the physiology and development of rat MGs and a Soy diet may modulate these effects.

INMUNOLOGÍA / IMMUNOLOGY 2

202. (29) **INFECTION KINETICS AND IMMUNE RESPONSE TO BRUCELLA ABORTUS OF A 3D HUMAN LUNG TISSUE**

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The bronchial tissue contains epithelial cells and fibroblasts in close contact that interact with each other. Therefore, infection studies performed on cultures of a single cell type (epithelial cells or fibroblasts) fail to reproduce what happens in vivo in the lung. This problem can be solved by using a 3-dimensional (3D) air-exposed organotypic human lung tissue model. This model consists of a monolayer of bronchial epithelial cells on top of a collagen matrix with embedded fibroblasts. The 3D model was constructed on Transwell inserts and apically infected with *Brucella abortus*for 4h and then incubated with gentamicin for 1h to kill extracellular bacteria. At different days

post-infection (p.i.) cells were lysed to determine CFU of intracellular bacteria (CFUi) and conditioned media (CM) from the basolateral side were collected to determine CFU and to measure cytokines. Results showed that *B. abortus* infects and replicates in the 3D model since day 1 p.i. until day 16 p.i. (end of follow-up). Whereas no viable bacteria were detected in CM during the first days p.i., a progressive increase of CFU was detected between days 5 and 16 p.i. The presence of viable bacteria in the CMs would not be due to a cytotoxic effect of the infection, since no significant increase in lactate dehydrogenase activity was detected in CMs of the infected models as compared to non-infected controls. In concordance with the presence of bacteria in CM, at day 5 p.i. the highest levels of interleukin-6 (IL-6) ($p<0.001$), IL-8 ($p<0.0001$) and monocyte chemoattractant protein-1 (MCP-1) ($p<0.0001$) were achieved. These results suggest that *B. abortus* persists in the lung, but part of the bacterial progeny leaves the tissue to colonize other organs. In response to the infection the bronchial mucosa secretes cytokines and chemokines that may attract neutrophils and monocytes to the site of infection.

203. (91) **HELMINTH INFECTION ENHANCES ARYL HYDROCARBON RECEPTOR (AHR) PATHWAY IN PATIENTS WITH MULTIPLE SCLEROSIS DAMPENING PATHOGENIC TH17 RESPONSE**

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Multiple Sclerosis (MS), a demyelinating autoimmune condition that is highly disabling, affects over 2.5 million people worldwide and largely depends on pathogenic Th1/17 activity both for the onset and the progress of the disease. In 2007 it has been reported that natural helminth infections improve the clinical course of MS by enhancing immunological regulatory mechanisms. Additionally, microbiota-derived metabolites have been shown to down-regulate inflammation in the central nervous system (CNS) by targeting aryl hydrocarbon receptor (AhR) in a model of experimental autoimmune encephalomyelitis. We hypothesized that helminth infection also enhances the AHR pathway and therefore dampening the development of pathogenic Th17 cells in MS patients. We measured the expression of AHR in monocytes-derived Dendritic Cells (moDCs) and in $CD4^+$ T cells as well as the development of pathogenic Th17 lineage in patients with MS or MS concomitantly infected with helminths (HIMS). Our results indicate that helminth infection increases AHR expression in moDC (> 20 fold) and activated $CD4^+$ T cells (> 10 fold) of patients with MS compared to non-infected ones and healthy controls (HC). Additionally, $CD4^+$ T cells from this infected cohort produce lower amounts of IL-17 compared to non-infected MS patients ($p<0,05$) after 7 and 10 days of polyclonal activation. Finally, $CD4^+$ T cells from HIMS induced lower levels of CD86, HLA-DR and CD40 activation markers on moDC in a MLR assay compared with $CD4^+$ T cells from MS patients. Interesting we have also observed increased levels of the Growth arrest-specific 6 (GAS6), a TAM tyrosine-kinases subfamily ligand in $CD11b^{high}$ and $CD1c^+$circulating populations of MS patients with helminthic infection. In vitro supplementation with hGAS6 or IFNβ potentiates AHR expression in $CD4^+$ T cells. Altogether, our data suggest that helminth infection enhances the immune-regulatory AHR pathway dampening innate and adaptive immune response in patients with MS.

204. (113) **UP REGULATION OF TYRO3 TYROSINE KINASE RECEPTOR AND PROS1 IN THE MYELOID COMPARTMENT OF PATIENTS WITH LANGERHANS CELL HISTIOCYTOSIS**

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TYRO3, AXL and MERTK (TAM) tyrosine kinase receptors and their agonist Protein S (PROS1) have been identified as negative regulators of the immune response, as well as non-classical proto-oncogenes aberrantly expressed in multiple haematological and epithelial malignancies. Langerhans Cell (LC) Histiocytosis (LCH) is a disorder characterized by an abnormal accumulation of CD207+CD1a+ myeloid cells in almost any tissue. The etiology of this disease is still under scientific discussion and it is not clear if LCH results from malignant transformation or unbalanced immune response that leads to the proliferation of pathogenic LC-like cells. Our aim is to explore the role of the TAM axis in the pathogenesis of pediatric LCH. We analyzed the expression of TAM receptors and PROS1 in peripheral blood mononuclear cells of pediatric patients with confirmed diagnosis of LCH with active disease (AD) or non-active disease (NAD) and adult controls. The expression levels of PROS1 and TAM receptors were determined by flow cytometry and expressed as fold increase of mean fluorescence intensity (MFI) compared to the isotype control. Circulating total CD11b+ fraction was significantly expanded in AD (36.4 ± 3.7 %) vs NAD (18.9 ± 1.7 %) and adult controls (24.9 ± 1.3 %). Interestingly, this fraction that is considered the main source of inflammatory myeloid cells, showed higher levels of PROS1 in AD (14.2-fold) compared with NAD (6-fold) and adult controls (6.7-fold). TYRO3 was also up regulated in circulating CD11b+ cells in AD (10.6-fold) compared with NAD (5-fold) and adult controls (4.5-fold). Our results show that higher levels of TYRO3 and PROS1 are associated with active and multisystem LCH suggesting that this axis could be involved in the expansion of precursor and pathological LC-like cells.

205. (132) **HETEROLOGOUS CHIMERIC IMMUNOGEN COMPRISING A TRYPANOSOMA CRUZI ANTIGEN AND A MUTANT NON-TOXIC BACTERIAL SUPERANTIGEN ELICITS STRONG AND EFFICIENT IMMUNE RESPONSE WHICH PROTECTS AGAINST PARASITIC CHALLENGE**

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In search of new strategies for vaccines against Chagas disease, we hypothesize that changing the way antigens are presented and processed is essential to confer protection against parasitic challenge. To this effect, we developed a chimera comprising a T. cruzi antigen and a mutant non-toxic superantigen (CruziSAg) and evaluated its capacity to elicit effective immune responses and protection against challenge.

CruziSAg was genetically engineered and produced as recombinant protein. C3H mice were immunized with: I- CruziSAg+ODN-CpG by intramuscular route; II- an attenuated bacterial vector delivering the CruziSAg gene by oral route; III- a priming with II and boosting with I; IV- control with empty vector. We had previously described significant humoral responses evoked after immunization in groups I and III, with Th1-bias.

Here, specific cell-mediated immunity was assessed by in-vivo DTH and ex-vivo proliferation of spleen cells: in both cases, groups II ($p<0.01$) and III ($p<0.05$) showed significant antigen-specific cellular responses compared with control. Furthermore, splenocytes significantly secreted Th1-cytokines such as IFN-γ and IL-12 in the same groups (II: $p<0.05$; III: $p<0.001$). Only group III was able to secrete IL-10 ($p<0.001$). By Flow Cytometry we detected IFN-γ and TNF-α producing CD4+ T cells in immunized groups, being group II where more antigen-specific polyfunctional cells were found (45%). Moreover, in group II we measured a significant production of IFN-γ by CD8+ T cells ($p<0.01$).

To evaluate protection, animals were challenged with CA-I clone K98 blood trypomastigotes of T. cruzi. All groups significantly reduced parasite loads throughout the acute phase of infection, compared with control ($p<0.0001$), and this was reflected in more than

10-fold reductions of the areas under the parasitemia curve.
Our results show that these strategies elicited efficient Th1-directed immune responses able to confer protection against parasitic challenge. Thus, chimeric heterologous immunogens using superantigens represent promising strategies against chronic infections.

206. (137) **EVIDENCE OF APOPTOSIS AND PYROPTOSIS AS MECHANISMS OF CELL DEATH IN DUODENAL MUCOSA IN ACTIVE CELIAC DISEASE**

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Celiac disease (CD) is a chronic enteropathy characterized by massive loss of enterocytes from proximal small intestine. Apoptosis has been considered as the main pathway of enterocytes death, however this mechanism has been only partially studied.
The aim of this work was to characterize the cell death pathways in intestinal mucosa in CD enteropathy.
Duodenal biopsies were collected from pediatric and adult patients during the routine procedure for CD diagnosis. Ethic committees from Public Health Institutions approved this work. Expression of caspase 1, 3, 8, 4 and Gasdermine D (GSDMD) were assessed by Western blot (WB) in protein extracts from whole biopsy. TUNEL reaction and Immunofluorescence microscopy (IFI) analysis were performed on sections of paraffin-embedded tissues.
TUNEL reaction, which accounts for an advanced stage in cell death, showed increased number of TUNEL+ cells of duodenal lamina propria of CD patients compared with healthy controls ($p < 0.001$).
WB analysis of protein extracts from biopsies of CD patients showed higher expression of Caspase 3 ($p<0,001$). In addition, IFI analysis showed significative increment in caspase 3 and 8 in epithelium and lamina propria of duodenal samples of CD patients.
Moreover, expression of Caspase 1 ($p <0,005$) and GSDMD ($p < 0,001$) determined by WB were also significantly increased in active CD patients. Higher levels of Caspase 4 were observed by IFI in epithelium and lamina propria of duodenal samples of CD patients ($p<0,01$).
In conclusion, we found that multiple pathways associated with cell death and inflammation are activated in the small intestine of CD patients at diagnosis. These findings suggest that apoptosis, as well as pyroptosis, may occur jointly in the enteropathy.

207. (145) **HUMORAL AND CELLULAR PARAMETERS EVALUATION IN CHILDREN AND ADOLESCENTS WITH CYSTIC FIBROSIS**

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Lung disease in cystic fibrosis (CF) is a multi-factorial process. Although neutrophils play a central role in the active inflammatory response, the involvement of T and B cells in the systemic lymphocyte immune response is not clearly defined.
Aim: To evaluate different immunological parameters in a pediatric population with CF.
Material and methods: Twenty CF patients, aged 1-17 years, were included in this cross-sectional study. Immunoglobulin values, T-cell subsets: naive (N), central (CM), and effector memory (EM), CD4N recent thymic emigrants (RTE), senescent CD8 T-cells, (S), B and NK, peripheral blood percentage levels, were determined. Patients were evaluated clinically, microbiological data were also obtained. For comparison, 10 healthy controls were included.
Results: In CF patients, an increase in the average of ratios (Patient´s value/Age-reference value) for immunoglobulin A, M and G (1.99, 2.16 and 1.42), was found. When patients were divided according to presence (Group A) or lack (Group B) of respiratory exacerbations, a significant decrease in CD4NRTE T-cells levels respect Co group, (A:43±15 vs Co:67.5±7, B:48±13 vs Co:67.5 ± 7, $p<0.05$), was recorded. Decreased values of CD4NRA+T-cells, with increase in CD4MRO+T-cells, in children older than 6 years, respect Co group of similar age, (NCF:50±13 vs Co:63± 6; MCF:56 ±12 vs Co:46 ± 5, $p<0.05$),were observed. In children under 6 years no significant differences in these subsets, were detected. N and CMCD8 T-cells were significantly decreased ($p<0.05$) in patients with CF versus Co (CD8NCF:42.8 ±3.2 vs NCo:53.3 ± 2.39; CD8C-MCF:11.5 ±1.27 vs CMCo: 16.1±1.77). Patients with CF presented substantial increase ($p<0.01$) in EMCD8 T-cells respect Co group, (CD8EMCF:40.2 ±2.8 vs EMCo:6.9 ±0.76).A positive correlation between CD8EM T-cells and CD28-CD57+CD8S T-cells, (r: 0.84, $p<0.05$), was also recorded.
Conclusion: changes in immunoglobulin levels and peripheral blood lymphocyte populations represent different features of the chronic immune activation present in patients with CF.

208. (174) **CD4 AND CD8 T-CELLS SUBSETS: LONGITUDINAL STUDY IN CHILDREN INFECTED WITH HIV AND DIFFERENT ANTIRETROVIRAL TREATMENT ADHERENCE LEVELS**

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Persistent viral replication and continuous immune activation during HIV infection are associated with alterations in T-cells subsets. Adequate homeostasis between them is conditioned to a correct antiretroviral treatment (ART) adherence.
Aim: To study changes in CD4 and CD8 T-cells subsets in HIV-infected children with different ART adherence.
Material and Methods: twenty two mother-to child HIV-infected patients, age: 5-15 years, were evaluated longitudinally in two moments (t0, t1) separated 12 months. Twelve of the patients were receiving ART with high adherence rate (group A), the remaining 10 (group B), presented deficient adherence. Naive (N), central memory (CM) and effector (EM) CD4 and CD8 T-cells subsets percentage levels, clinical status and viral load (log VL), at both points of follow-up, were studied. A control group of 10 healthy children (Co) was also studied.
Results: After follow-up period, substantial changes in tested parameters in the twelve group A patients, were not observed. Five children of group B improved treatment adherence (group B1), with significant increase, $p<0.05$, in CD4 levels (t0:19±7, t1:29±7), and decrease in VL (t0:3.73±1.78, t1:<1.70) ($p<0.05$). The remaining five, (group B2), decreased CD4 levels (p:NS), (t0:18±7, t1: 14±9), and increasing VL (t0:2.42±0.86, t1:4.06±1.03) ($p<0.05$).At t1,the three groups presented a significant decrease respect to Co ($p<0.05$) in CD4 N, (Co:67±7 vs A:47±15 vs B1:44±17 vs B2:40±15), and CD8 N levels (Co:53±8 vs A:30±15 vs B1:27±18 vs B2:13± 8).CD4 CM of B2 group were found to be increased ($p<0.05$) respect to Co (55±21 vs 31±6). A significant increase ($p<0.05$) in the groups respect to Co, in CD8 CM (Co:16±6 vs A:40±18 vs B1:41±23 vs B2:56±20) and CD8 EM (Co:7±2 vs A:29±16 vs B1:25±16 vs B2:27±19), was also recorded.
Conclusion: Quantitative improvement of N and M CD4 and CD8 T-cells subsets would require high and continuous ART adherence rates.

209. (188) **CHANGES IN THE AVAILABILITY OF VACCINE COMPONENTS IN DRAINING LYMPH NODE ARE OBSERVED WHEN A LIQUID CRYSTAL NANOSTRUCTURE IS USED AS VACCINE PLATFORM**

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Commonly subunit-based vaccine requires the addition of an adjuvant. To overcome this challenge, new adjuvant strategies are being developed worldwide in experimental models or in human clinical

trials. In this context, we formulated a TLR-9 agonist, CpG-ODN and a model antigen, OVA, with a nanostructure (Coa-ASC16) formed by self-assembly of 6-O-ascorbyl palmitate (OCC). This nanoformulation elicited superior adaptive immune responses than soluble formulation of OVA/CpG-ODN (OC). In addition, Coa-ASC16 creates a depot of antigen and CpG-ODN at the injection site. However, details about in vivo mechanisms that dictate the priming of vaccine-induced immunity are lacking. Here, we investigate the early events in the proximal-draining lymph nodes (LN) of the vaccine injection site. Mice were subcutaneously immunized with formulations containing fluorescent-dye labeled OVA and CpG-ODN. Then, the availability of both molecules contained in the whole LN was measured with Odyssey® CLx at several time points post-immunization (p.i.). In addition, we determined, by flow cytometry, the uptake of both molecules from LN by dendritic cells (DC). OVA signal was OC>OCC ($p<0.001$) and OCC>OC ($p<0.0001$) at 20 min and 24h p.i. respectively; CpG-ODN signal was OC>OCC ($p<0.0001$) and OCC>OC ($p<0.0001$) at 20 min and 24h p.i. respectively. 72 hs after immunization, we analyzed single cell suspension of LN and observed that Coa-ASC16 impacts on antigen uptake, mice immunized with OCC showed higher numbers of OVA+CD11c+DC, CpG-ODN+CD11c+DC and OVA+CpG-ODN+CD11c+DC compared with those mice immunized with OC ($p<0.001$). Conclusion: The nanoformulation of vaccine modifies the kinetics of antigen and immunostimulant availability in the LN and improves co-uptake of both molecules by DCs despite the fact that our system does not chemically link OVA to CpG-ODN. Understanding of the impact of vaccine formulation on early response might have a significant impact for rational vaccine design.

210. (193) **THE TREATMENT OF THE GRAFT AND THE RECIPIENT WITH A-LIPOIC ACID IMPROVE THE PATIENT CONDITIONS BY REDUCING THE DIALYSIS REQUIREMENTS.**

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A previous pilot clinic trial had showed that treatment of the donor and recipient with α-lipoc acid (ALA), protects against the ischemia reperfusion injury in simultaneous kidney-pancreas transplantation. Therefore, the aim of this work was to evaluate whether the administration of ALA could improve early clinical outcomes of kidney transplant patients.
The study included 68 kidney transplant patients. They were divided in two groups. In the group 1, recipients were treated with placebo (24 patients) and received a graft perfused with placebo. In the group 2, recipients received 600 mg of ALA i.v immediately previous to the surgical procedure and the graft was perfused with 600 mg of ALA 1 hour before the surgery (44 patients). Blood samples were obtained at different moments, before and after the transplant and early clinical outcomes parameters were analyzed.
At first day after transplantation, ALA-treated group had lower plasmatic urea levels ($p=0.001$) and a glomerular filtration rate with a trend to be higher than control group, however, these results were not maintained in the time. Concurrently, the presence of delay graft function (DGF), was not different between both groups. Remarkably, when the number of patients requiring dialysis was analyzed day by day in the first week post-transplant, a lower percentage of dialyzed patients per day could be observed in the group treated with ALA with respect to the control group. Finally, there was a tendency to have fewer episodes of rejection in the treated group vs control group ($p = 0.07$). Overall, this study shows that even though ALA treatment could not avoid DGF, it can improve the patient conditions by reducing the dialysis requirements.

211. (197) **VITAMIN D SERUM LEVELS COMPARISON BETWEEN A COHORT OF MYASTHENIA GRAVIS PATIENTS AND HEALTHY SUBJECTS IN ARGENTINA**

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Myasthenia Gravis (MG) is an autoimmune disease mediated generally by anti-acetylcholine receptor autoantibodies (ACRA), whose production is T cell dependent. Regulation of autoreactive T cells depends on regulatory T lymphocytes (Treg), which are diminished in MG patients. Vitamin D (VitD) has immunomodulatory effects, stimulating Treg and inhibiting effector T cells. As VitD synthesis depends on sunlight exposure, therefore on the country latitude, a controversial association between VitD deficiency and MG development and severity has been established.
We aimed to compare 25-OH-VitD serum levels in 61 diagnosed MG patients (19 during MG exacerbation) and 30 healthy volunteers (hv), and to relate them with the severity of the disease (ADL and MGCscales)and other biomarkers in an Argentinian cohort. We collected samples equally distributed through the year. We measured: 25-OH-VitD (chemiluminescence), C3 and C4 (RID), C5a (ELISA), ACRA (RIA).
There were no statistically significant (ss) differences in 25-OH-VitD mean serum levels between patients and hv, neither between basal and exacerbated patients. When we analyzed 25-OH-VitD mean serum levels of subjects grouped according to the season of sampling, we found a ss difference in spring ($p=0,043$) between patients (13,42 ng/ml) and hv (18,11 ng/ml). Deficient levels of 25-OH-VitD (<25 ng/ml) were found in 83,5% of MG patients vs 57,1% of hv, being this association ss ($p=0,001$). No ss association was found between basal and exacerbated patients using the same cut-off value. We found a strong association between VitD deficiency and illness, though no correlation with the severity of MG or other biomarkers could be established.We were not able to find general differences between VitD levels in MG patients and hv in our population. We aim to increase the number of samples and extent the analysis through the summer, to deepen in the analysis of this serum biomarker in our local population .

212. (204) **THE CANONICAL WNT/B-CATENIN SIGNALING PATHWAY IS INVOLVED IN THE DEVELOPMENT OF INNATE T CELLS IN THE THYMUS UNDER INFECTIOUS/ INFLAMMATORY SYSTEMIC CONDITIONS**

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Our previous work demonstrated that during the acute stage of Th1 inflammatory or infectious processes, SP CD8 thymocytes alter their differentiation from “conventional” to “innate” lineage due to thymic production of IL-4 and IL15. Innate $CD8^+$ cells express a particular phenotype ($CD44^{hi}CD122^{hi}$ $EOMES^{hi}$), produce high levels of IFNγ and have high cytotoxic activity. We demonstrated that the innate phenotype gives rise as early as the double positive (DP) stage in the thymus. The aim of our work was to determinate the molecular mechanisms that are involved in this differentiation.
The canonical Wnt/β-catenin signaling pathway participates both in the differentiation of $CD8^+$ T cells and the generation of functional $CD8^+$ memory T cell. To investigate whether this pathway is involved in the generation of innate $CD8^+$ cells, $CD45.1^+$ control thymocytes were co-cultured with $CD45.2^+$ thymocytes from control or T. cruzi-infected mice (the source of thymic IL-4/IL-15) in the presence or absence of the β-catenin activator lithium chloride (LiCl) or the inhibitor of β-catenin signaling, iCRT14. Activation of β-catenin induced up-regulation of CD44, CD122 and EOMES in the $CD45.1^+$ SP CD8 control thymocytes ($p<0,05$), while iCRT down-regulated those markers in the same population ($p<0,05$). Moreover, LiCl or iCRT were able to respectively up- or down-regulate the expression of CD44, CD122 and EOMES induced in OT-I thymocytes by the culture supernatant of PMA/ionomycin-stimulated WT thymocytes ($p<0.05$). Importantly, the adding of IWP-L6, a Wnt proteins secretion inhibitor was able to revert the innate differentiation induced by the culture supernatant of PMA/ionomycin-stimulated WT thymocytes.
Together these results indicated that β-catenin activation promotes

innate differentiation in an Ag-independent and cytokine-driven system. Additionally, Wnt protein secretion is critically involved in the activation of β-catenin that promotes the differentiation of innate $CD8^+$ T cells in the thymus under Th1 systemic processes .

213. (210) **ZIKA VIRUS NS4B PROTEIN IMPAIRS TRANSLOCATION OF INTERFERON REGULATORY FACTOR 3 TO THE NUCLEUS**

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Type I interferons (IFN I) play an essential role in innate immunity against viral infections. When a cell is infected with a virus, microbial components can be sensed by intracellular pattern recognition receptors, activating interferon regulatory factor 3 (IRF3) and inducing the production of IFN I. However, many pathogens have evolved strategies to evade immune sensing favoring their survival. Among them, flaviviral non-structural proteins, needed for viral replication, are involved in host immune evasion. Here, we aimed to broaden the understanding of the role of Zika virus (ZIKV) NS4b protein in the inhibition of IFN I induction.
For this purpose, we performed transfection assays with plasmid encoding recombinant ZIKV NS4b. Using RAW-Lucia ISG cells, an IFN reporter cell-line, we showed that cells with NS4b were able to reduce luciferase signals in a dose dependent manner compared to empty vectors (two-way ANOVA, $p<0.05$). This reduction was maintained after treatment with TLR ligand, LPS, and STING agonist, c-diAMP (two-way ANOVA+Tukey´s, $p<0.05$). We also conducted immunoprecipitation assays, confirming that NS4b interacts with TANK-binding kinase1 (TBK1), as recently reported. Furthermore, by confocal microscopy we were able to identify that ZIKV NS4b alone impairs IRF3 translocation to the nucleus in Hela cells.
ZIKV is still an international concern. At present, there is no approved vaccine or treatment against ZIKV infection, hence a better understanding of molecular interactions is needed. Our results add evidence that ZIKV NS4b can be involved in disrupting TBK1/IRF3 cascade. Because of this and the role of ZIKV NS4b in assembling the replication complex, we believe that it may be a promising target for antiviral drug development.

214. (214) **POTENTIAL OF MALDI-TOF AS A DIAGNOSTIC TOOL IN SEPSIS**

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Sepsis has a bi-phasic nature with a hyper-inflammatory phase followed by an anti-inflammatory response. Globally, it is one of the main causes of death in ICUs. However, clinically, is difficult to define a therapeutic approach due to the lack of tools to determine the phase that the patient is going through. Our aim was to investigate the usefulness of plasma “fingerprints” using MADI-TOF MS technology as rapid instrument for determination of pro-inflammatory or anti-inflammatory states in sepsis. For this, LPS-challenged murine models emulating the different phases of sepsis (proinflammatory (PRT) and anti-inflammatory (ANT) phases) versus untreated controls (Ctrl) were evaluated. To better characterize the different phases, in parallel, cytokines profiles, hematologic, metabolic and immunologic parameters were determined in plasma. Peripheral blood leukocytes count was decreased in PRT whereas in the ANT phase was increased mainly associated with an increase in neutrophils (Leukocytes (106/ml): Ctrl= 6.6±0.5; PRT= 1.9±0.2*; ANT= 11.2 ±0.7&; *&p<0.001). Elevated levels of proinflammatory cytokines such as TNF-α in PRT phase was a hallmark of this phase (TNF-α (pg/ml): Ctrl=nd (nondetectable); PRT= 1705±297*; ANT= nd&; *&p<0.0001). Furthermore, increased plasma corticosterone levels as well diminished immune response in ANT phase were observed (corticosterone (pg/ml) Ctrl= 260±40; ANT= 1725 ±218&; &p<0.001; antibody title (Ctrl=100±20%;ANT=0.4±0.2%; p<0.05). As an exploratory approach, plasma spectra of PRT and Ctrl mice obtained by MALDI-TOF were studied by the principal components analysis (PCA). The PCA was performed over the pre-processed MALDI-TOF spectra to evaluate whether the clusters obtained could be correlated with the inflammatory state. The first two principal components explained more than 78% variance of the spectral data, which allowed a clear discrimination among to PRT and Ctrl samples. These results show the potential of plasma fingerprints obtained by MALDI-TOF for the rapid determination of the inflammatory state during sepsis.

215. (251) **CHRONIC PROSTATE INFLAMMATION AS A RISK FACTOR FOR PROSTATE CANCER DEVELOPMENT. STUDY OF AN ANIMAL MODEL**

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Prostate cancer (CaP) is the second most common type of cancer in men. It is a multifactorial disease and environmental and genetic factors are involved in his development. Epidemiological studies have suggested chronic inflammation as a major risk factor. Herein, we analyzed if chronic inflammation may have a role in prostate carcinogenesis using an animal model of chronic bacterial prostatitis. C57BL/6 male mice were transurethrally inoculated with $2x10^8$ CFU of uropathogenic *Escherichia coli* 1677 (infected) or saline (controls), euthanized at 5 days (dpi), 12 or 26 weeks (wpi) post infection, and prostate infiltrating leukocytes, histopathology and immunohistochemistry/immunofluorescence for different PCa associated lesions were performed/analyzed. Infected mice developed an acute prostate infection (5 dpi) that evolved chronic (12 and 26 wpi). The infection induced prostate inflammation shown by higher counts of tissue infiltrating CD45+ leukocytes at 5 dpi ($p<0.05$), 12 ($p<0.05$) and 26 wpi. Acute inflammation was shown by increased $CD11b^+Gr1^+Ly6G^+$ cell infiltration, hemorrhage, cellular debris, epithelial shedding and tissue disorganization at 5 dpi. On the other hand, cell infiltrates were mostly composed of $CD3^+$ and $CD11c^+$ cells, accompanied with epithelial cell shedding, papillomatosis, atypical hyperplasia and dysplastic changes mimicking prostate intraepithelial neoplasia (PIN) and high grade PIN (HG-PIN) at 12 and 26 wpi ($p<0.05$). Early after the infection, there were not changes on the expression of VEGF (neoangiogenesis), 8-OHdG (DNA oxidation) or α-SMA13 (periacinar smooth muscle cells). However, the expression of these markers, which are associated to PCa development, was altered in infected mice either at 12 or 26 wpi. Strikingly, these lesions were observed close to areas with PIN and HG-PIN.
Our results point out that chronic inflammation induces tissue lesions, DNA oxidative damage and neo-angiogenesis associated to pre- and neoplastic tissue changes. In conclusion, chronic inflammation could be considered as a triggering factor for PCa development.

216. (257) **IMMUNE RESPONSE INDUCED BY NEW ADJUVANT STRATEGY TO PRODUCE CROTALIC ANTIVENOM**

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Snake envenomation is a serious medical problem in tropical devel-

oping countries and antivenoms are the main treatment. Antisera are produced by immunization of horses with snake venom using complete Freund's adjuvant and incomplete (booster) but it causes severe local reactions. A new adjuvant strategy is required to increase efficiency in antisera production under much less morbilty to immunized animals. CpG-ODN formulated in a 6-O-ascorbyl palmitate nanostructure (Coa-ASC16) was more efficient as adjuvant than CpG-ODN alone using ovalbumin (OVA) as an antigen model. Particularly, immunization of mice with OVA/CpG-ODN/Coa-ASC16 resulted in high OVA-specific antibody titers and IFN-γ and IL-17 secretion compared to immunization with OVA/CpG-ODN. In order to evaluate the immune response induced by this adjuvant strategy using Crotalus durissus terrificus (C.d.t) venom, we determined the titer of antibodies (IgG, IgG1 and IgG2) and their specificity. Balb/c mice were subcutaneously immunized on days 0, 15 and 30 with C.d.t venom/CpG-ODN/Coa-ASC16 or C.d.t venom/Freund's Adyuvant (complete first and incomplete-booster) (dose/mice: C.d.t venom: 10-15µg, CpG-ODN: 30 µg). On day 50 mice were sacrificed. In both immunized group mice, the antibody titers in plasma were high (1x105), with a similar IgG1/IgG2a ratio. The antibodies recognized phospholipase A2 and thrombine like protein but not all C.d.t venom components. Macroscopic and microscopic analysis at the site of injection of mice injected with Freund's adjuvant showed local damage (with non-infectious abscesses) and hypertrophy of inguinal lymph nodes, whereas mice injected with CpG-ODN/Coa-ASC16 did not. Our results show that CpG-ODN/Coa-ASC16 produces a humoral response as strong and specific as Freund´sadjuvant, with minor or null local deleterious effect, demonstrating the potentiality and advisability of this complex as a new adjuvant option for future immunizations to produce C.d.t antivenom.

217. (265) **P31-43 GLIADIN PEPTIDE ACTIVATES IN VIVO NLRP3 INFLAMMASOME**

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Celiac disease (CD) is a chronic enteropathy elicited by gluten peptides which produce a specific and strong Th1 response in small intestine . Using an in vivo murine model aimed to assess the initial stages in CD pathogenesis, we demonstrated that innate mechanisms are critical to drive mucosal changes (increase number of intraepithelial lymphocytes -IELs, and reduction in villus height /crypt depth (V/C) ratio) in proximal small intestine. Innate response was elicited by p31-43 gliadin peptide and our structural studies showed that this peptide forms oligomers. NLRP3 inflammasome may sense a broad range of stimuli including endogenous signals derived from damaged mitochondria by nanostructures.

The aim of this work was to evaluate the role of NLRP3 inflammasome in the enteropathy induced by p31-43.

C57BL/6 wild type, NLRP3 KO or CASP 1 KO mice were treated by intragastric administration of p31-43 or vehicle. Inhibition of caspase 1 activity was evaluated in C57BL/6 by i.p. administration of Ac-YVAD-cmk or vehicle. After 16h, intestinal samples were collected, and sections were H&E stained for histological evaluation. Protein extracts were used for westernblot (WB) analysis.

WB analysis showed activation of caspase-1 and increased levels of IL-1β in small intestine of C57BL/6 mice intragastrically treated with p31-43 ($p<0,01$). Assessment of histology (V/C ratio and number of IELs) in NLRP3 KO or CASP1 KO p31-43 treated mice compared with control groups showed no differences.

Specific inhibition of caspase 1 in C57BL/6 mice abolished the small intestinal damage induced by p31-43. ASC speck formation induced by p31-43 was observed by in vitro analysis.

In conclusion, we demonstrated that NLRP3-ASC-caspase 1 and IL-1β are required for the enteropathy induced by p31-43.

218. (267) **IMMUNOMODULATORY PROPERTIES OF HEAT-KILLED RHODOCOCCUS COPROPHILUS CONTROL THE ALLERGIC REACTION IN A MOUSE MODEL OF FOOD ALLERGY**

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Actinomyces are soil bacteria with immunomodulatory properties that exert biological effects on intestinal epithelial cells in different inflammatory contexts. Our goal is to study the inhibitory effect of dead Rhodococcus coprophilus-Rc on activated epithelial cells exposed to pro-inflammatory stimuli, and in an experimental food allergy model.

Colon cell lines (Caco-2 and Caco-luc) were cultured with flagellin (FliC) and the induction of cytokines (IL-1b, IL-6, TNFa) and chemokines (CCL20, IL-8 and MCP-1) were studied by qPCR, while Nf-κB was analyzed by immunoblotting. In addition, Balb/c mice were sensitized with cow´s milk proteins (CMP) plus cholera toxin by gavage, and orally challenged with CMP to induce intestinal inflammation and hypersensitivity symptoms. Activated cell lines were exposed to Rc before or during activation. On the other hand, mice received Rc by gavage during one week, and then they were sensitized.. The therapeutic effect of Rc was monitored in vivo (clinical score and cutaneous test) and in vitro (serum specific antibodies and cytokines by ELISA, and cell analysis by flow cytometry).

We found that Ccaco cells were unresponsive to dead bacteria; however, in FliC-activated cells Rc suppressed the induction of pro-inflammatory cytokines ($p<0.05$) and chemokines ($p<0.05$), with a reduced traslocation of p65 onto the nucleus. This inhibitory effect was also observed in experimental food allergy with the intragastric administration of Rc.Symptoms and serum specific IgE levels were lower in Rc-treated mice compared with sensitized mice ($p<0.05$), with a concomitant reduction of IL-5 ($p<0.05$) and intestinal CCL20 ($p<0.05$).

In conclusion, we found that Rhodoccocus coprophilus modulated the NF-KB pathway, abrogated the production of pro-inflammatory cytokines and chemokines in intestinal epithelial cells and ameliorated hypersensitivity and the Th2-mediated immune response in the food allergy mouse model.

219. (268) **ANTIBODY AND CD8+ T-CELL RESPONSES IN IMMUNIZED MICE WITH DIFFERENT VACCINATION STRATEGIES**

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Subunit-vaccines require the development of new adjuvant strategies. Recently, CpG-ODN was approved by FDA for a human vaccine. However, the free CpG-ODN present some limitations that restrict its bioavailability. In this context, we nanoformulated CpG-ODN with a nanostructure (Coa-ASC16) formed by self-assembly of 6-O-ascorbyl palmitate. Here, we investigated the effect of various formulations on antigen-specific response. Mice were subcutaneously immunized with a single-dose of OVA and CpG-ODN in solution (OC), OVA and CpG-ODN nanoformulated with Coa-ASC16 (OCC), OVA and CpG-ODN in solution heated at 80ºC for 15 min and then cooled down to room temperature to simulate the conditions of preparation of the nanostructured formulation (OCø), and OVA formulated with Montanide Gel 01 PR (reference adjuvant) (OM). OVA-specific IgG and IgG2a titers were evaluated by ELISA in all groups and SIINFEKL-Kb tetramer+ CD8+ T-cells by flow cytometry in OCC and OCø groups. Antibody response: OCø developed IgG response faster than other groups (day 8: 2.40±0.17), OCC and OC seroconverted on day 10 (2.00±0.66 and 1.35±0.19), and OM on day 13 (1.43±0.54). OCC induced higher IgG titers than OC and OM ($p<0.05$) but comparable to that induced by OCø on day 13, 16, 20, 28 and 35. IgG2c was only induced by OCC and OCø, and titers were comparable between both groups (day 16: 1.7±0.26 and 2.03±0.26; day 20: 2.2±0.1 and 2.1±0.25). CD8+ T-cell response:

OCC elicited a higher expansion of SIINFEKL-Kb tetramer+ CD8+ T-cells than mice immunized with OCø (3.95±0.93 vs 0.82±0.23, p<0.05). Conclusions: OCC and OCø induced better titers and quality of antibody response than OC but OCC is superior to OCø in inducing CD8+ T-cell response. These results showed how various antigen-adjuvant formulations impact the resultant adaptive immune response. Further investigations are required to understand the precise underlying mechanisms.
Keywords: vaccine, adjuvant, nanostructure, CpG-ODN

220. (275) **γδ T LYMPHOCYTES MODULATE NEUTROPHIL FUNCTION**
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We have previously reported that neutrophils acquired different profiles of activation when they are cultured in presence of γδ T cells activated by phosphoantigens or through CD3. Now, we aim to investigate the mechanism involved in the modulation of neutrophils activity by γδ T lymphocytes. γδ T cells were purified from human peripheral blood mononuclear cells by using an anti-TCR γδ MicroBead isolation kit; and neutrophils were isolated by dextran sedimentation. After purification, γδ T cells were stimulated with anti-CD3 antibodies (250 ng/ml) or phosphoantigens (HMBPP, 10 μM) and cultured with or without neutrophils. After that, we analyzed in γδ T cells, IFN-γ and TNF-α production by ELISA, and CD69 expression by flow cytometry. Also, we analyzed the activation state of neutrophils and the expression of perforin by flow cytometry. Treatment of γδ T cells with HMBPP or anti-CD3 antibodies increased the expression of CD69 and the production of IFN-γ and TNF-α (p<0.05, n= 10). In presence of HMBPP- or anti-CD3-stimulated γδ T cells, neutrophils released elastase (p<0.05, n= 4) showing that they were activated. We previously reported that neutrophils were unable to produce reactive oxygen species when they were cultured with anti-CD3-activated γδ T cells in contrast to those cultured with HMBPP-stimulated ones. In these co-cultures, we also observed that γδ T cells degranulated more efficiently when they were activated through CD3 compared to HMBPP stimulation, releasing perforin and granzymes to the extracellular medium. Based on these results, now we evaluated the expression of perforin on neutrophil cell surfaces. Our results showed that when neutrophils were co-culture with anti-CD3-stimulated γδ T cells, they expressed high levels of perforin compared to neutrophils cultured with HMBPP-activated γδ T cells (p<0.05, n= 4). These results suggest that perforin might affect the integrity of neutrophil plasma membrane altering its functionality.

221. (299) **NEONATAL INCREASED ASTHMA RISK CAUSED BY MATERNAL STRESS PERSISTS THROUGH ADULTHOOD AND PROPAGATES TO THE SECOND GENERATION**
María de los Ángeles Aldirico, Florencia Giorgio, Ariadna Soto, Matias Perrone Sibilia, Nadia Arcon, Mariano Picchio, Vanesa Sanchez, Guido Rattay, Rosalia Moretta, Valentina Martin, Alejandra Goldman, Ignacio M. Fenoy
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RATIONALE:We have shown that maternal stress during pregnancy results in an increase of litter susceptibility to develop allergic lung inflammation. In this work we aim to study how stable is this phenomenon.METHODS:To see if the increased asthma risk continues through adulthood, pregnant BALB/c mice(day 15) were subjected to restraint stress exposures. Female pups were treated with two i.p. injection of ovalbumin (OVA)/alum(day 47), challenged with antigen aerosol(days 50-52) and euthanized(day 54) (S). Negative controls included pups of non-stressed dams, subjected to the same protocol (C).To evaluate the intergenerational susceptibility, 6-weeks female pups (F1) from stressed and non-stressed dams were mated. Their pups (F2) were treated with an i.p. injection of OVA/alum(day 4) before antigen aerosol challenge(days 12-14) and euthanized(day 16) (S). Negative controls included F2 from non-stressed F0, subjected to the same protocol (C).RESULTS:In adult F1, bronchoalveolar lavage (BAL) of stressed-dams female progeny had increased eosinophils percentage compared to non-stressed dams progeny (S=15.53±2.942/C=1.825±0.648)(p<0.05). This result correlates with lung histopathological alterations and no differences in OVA-specific antibodies levels.F2 from stressed, but not control F0, showed higher BAL eosinophils percentage (S=5.65±0.90/C:1.7±0.6)(p<0.01) and pathological changes of lung allergic inflammation. There were no differences in serum IgE levels but an increase in anti-OVA IgG1 (S=1.219±0.381/C=0.177±0.092) (p<0.05) and IgG2a antibodies (S=0.044±0,014 /C=0.012±0,002) (p<0.01) were detected in S group compared to C group. These changes were accompanied by a trend to higher levels of IL-4 in BAL (S=40.6±10.49/C=14.94±5.19)(p=0.0525) while IFN-γ levels did not change.CONCLUSIONS:These results show that the increased litter susceptibility to develop allergic lung inflammation continues through adulthood in female mice and it is also transmitted to the next generation.

222. (300) **TOXOPLASMA GONDII SERINE PROTEASE INHIBITOR-1 (TGPI-1) MODULATES DENDRITIC CELL MATURATION**
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We previously showed that treatment with a recombinant T. gondii serine protease inhibitor-1 (rTgPI-1) significantly reduced experimental asthma. Co-administration of rTgPI-1 with the allergen showed an improvement compared with the administration of rTgPI-1 alone, suggesting that this inhibitor may function as a tolerogenic adjuvant.
To further study the mechanism behind TgPI-1 immunomodulatory activity, we aimed to study the effect of this protease inhibitor on dendritic cells (DCs). Murine DCs were obtained from BALB/c mice bone-marrow precursors, cultured for nine days with GM-CSF and then stimulated with LPS. rTgPI-1 (depleted from endotoxin) was added following three different protocols: rTgPI-1 was added a) during differentiation (day 3-9), b) to immature DCs or c) LPS fully-matured DCs.
A diminished percentage of CD11+MHCII+ DCs were obtained when rTgPI-1 was added during differentiation (p<0.001). No differences in the expression of activation markers, CD80 and CD86, measured by flow cytometry, were detected. While no changes were observed in culture supernatant IL-6 and IL-10 cytokines, a significantly diminished IL-12 production was detected (p<0.01). When we analyzed the effect of rTgPI-1 on immature-DCs, surprisingly, we observed a different outcome than the expected. Indeed, rTgPI-1 was able to mature DCs (p<0.01) and promote IL-12 secretion (p<0.01), similar to responses induced by LPS. Finally, addition of rTgPI-1 to LPS-activated dendritic cells showed no differences in cell-surface expression of CD80 and CD86, however a significantly less secretion of IL-12 (p<0.05) was registered. Furthermore and interestingly, an increased ratio of IL-10 vs. IL-12 was observed (p<0.01).
Altogether these results show that rTgPI-1 could interfere with DCs differentiation and, on the other hand, induce different maturation profiles depending on the cell status. Although further analyses are necessary, data obtained from the effect of rTgPI-1 on matured DCs may account for the *in vivo* previous results.

223. (432) **THE ROLE OF SPHINGOSINE KINASES ON CHRONIC LYMPHOCYTIC LEUKEMIA.**
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IMEX-CONICET, Academia Nacional de Medicina

Sphingosine kinases (SK1 and SK2) produce sphingosine-1-phosphate which mediates cell growth,survival and differentiation, among other functions. We previously reported that inhibition of SK1by the commercial inhibitor SKI-II, reduces leukemic cell survival, activation

and proliferation inCLL patients (Almejún, Haematologica-2017). The aims of this study were: 1) to evaluate the effectof SKI-II on non-leukemic lymphocytes, and 2) to determine whether SK2 regulates leukemic cellsurvival. Then, peripheral blood mononuclear cells (PBMC) from CLL patients were cultured withdifferent doses of SKI-II, and NK and T cell viability was evaluated by flow cytometric alterationsof light-scattering properties at 24hs. We found that SKI-II reduces T and NK cell survival in a dosedependent way (n=15, p<0.0001 for 30 and 50μM vs control). Similar results were obtained at 48hs(n=15, p<0.0001). To induce T cell activation, PBMC from CLL patients were cultured onimmobilized anti-CD3 moAbs (αCD3) for 24hs. αCD3 upregulated the expression of the activationmarker CD69 on T cells at 24hs, which was reduced by non-apoptotic doses of SKI-II (n=6,p<0.03). Finally, we employed SK2-selective inhibitor ABC294640, which is being tested inclinical trials for the treatment of different tumors. PBMC were cultured with 1-30μM ofABC294640 and only 30 μM of ABC294640 was able to reduce leukemic cell survival (n=6,p=0,019). Given that up to 22μM of ABC294640 can be found in plasma of treated patients, weevaluated the combination of ABC294640 22μM and SKI-II (15 and 30μM). We found thatABC294640 alone did not modify leukemic cell survival at 24hs, but it enhanced SKI-II inducedcell death (n=6, p<0.0001). Altogether our results suggest that: a) SKI-II not only acts on theleukemic clone but also might affect the tumor microenvironment, and b) both SK1 and SK2 mightregulate in leukemic cell survival in CLL patients.

224. (504) **TUMOR CELL CONDITIONED MEDIUM INDUCE B-CATENIN ACTIVATION IN MURINE AND HUMAN MONOCYTES AND MACROPHAGES.**

Agustina Brajin, Ximena Volpini, Laura Fernanda Ambrosio, Laura Fozzatti, Carolina Lucia Montes, Cristina Motrán
Universidad Nacional de Córdoba, Argentina

Wnt/β-catenin pathway is intricately involved in pathogenesis of several cancers and many therapeutic strategies have been developed with the aim to inhibit Wnt-pathway. During tumor progression, circulating monocytes and macrophages are actively recruited into tumor microenvironment (TME) where they can promote tumor initiation, angiogenesis, metastasis and suppression of adaptive immunity. Tumor associated macrophages (TAM) closely resemble the M2-polarized macrophages and its accumulation in tumors correlates with a poor clinical outcome. We have recently reported that pharmacological inhibition of Wnt signaling, inhibits arginase-1 activity and induces an M1-like phenotype in T. cruzi infected macrophages. Because evidences show that TAM shift their functional phenotypes in response to various microenvironmental signals generated from TME, Wnt proteins secreted by tumor and TME cells could be important for regulate the activation state of TAM and consequently their role in tumor progression. While the role of β-catenin activation pathway is controversial in the metastasic spread of melanoma, nuclear β-catenin correlates with a higher proliferation and a loss of differentiation in anaplastic thyroid carcinoma (ATC). Here, we evaluated the ability of conditioned media from melanoma (B16F10-OVA) and ATC cells (8505C) to up-regulate β-catenin expression and activation in bone marrow derived macrophages (BMM) from B6 mice and THP-1 human monocytes/macrophages, respectively. Conditioned medium obtained from B16F10-OVA cells as well as from ATC cells induced significant accumulation of β-catenin in BMM (p=0.009) and THP-1 monocytes (p=0.05) and macrophages (p=0.02) after 24 h of culture. In addition, conditioned medium from B6 mice derived-tumors alone or in the presence of LPS induced significant secretion of IL-10 (p<0.05) from BMM, while β-catenin accumulation was only induced in these cells by the presence of LPS (p=0.0005). TME can modulate β-catenin activation in human and murine monocytes and macrophages. Experiments to evaluate whether Wnt proteins are involved in the β-catenin activation are underway.

225. (518) **LSP1 KO TUMOR-BEARING MICE HAVE A HIGHER FREQUENCY OF FUNCTIONAL CROSS-PRESENTING DENDRITIC CELLS IN THE SPLEEN**

María Mercedes Pascual, Nicolas Daniel Dho, Rachel Acland Strack, Inés Crespo, Maria Cristina Pistoresi Palencia, Belkys Angelica Maletto, Gabriel Moron
Facultad de Ciencias Químicas-Universidad Nacional de Córdoba

All leukocytes and endothelial cells from human and mice express a 52kDa cytoplasmic F-actin binding phosphoprotein, called Leukocyte-specific protein 1 (LSP1). This protein is known as an important actin cytoskeleton-regulator. LSP1 polymorphisms or downregulation are considered risk factors for some types of cancer. Using the MO5 melanoma model, our group has shown that LSP1 deficient mice have an impaired control of melanoma growth. In order to study the role of LSP1 in antitumor immune response, WT and *Lsp1*$^{-/-}$ mice were subcutaneously-injected with 10^5MO5 cells and followed-up until day 15 or 17 depending on the experiment. At these time points, tumors in *Lsp1*$^{-/-}$ mice were bigger than in WT controls (p<0.01). Taking into account that the MO5 cell line expresses OVA, tumoral-antigen presentation was assessed in vitro using the H2-Kb-restricted OVA257-264-specific CD8+ T cell hybridoma (B3Z). We observed a higher tumoral-antigen presentation when B3Z cells were incubated with CD11c$^+$ splenocytes harvested from LSP1 KO tumor-bearing mice than from WT tumor-bearing mice (p<0.0001). This could be a result of a higher proportion of cross-presenting DCs in LSP1 KO spleen compared to WT (CD8α$^+$ DCs p<0.0001; CD103$^+$ DCs p<0.01). However, no difference in tumor-antigen presentation was found after tumor draining lymph node (dLN) analysis, despite the lower proportion of CD8α$^+$ DCs and higher proportion of CD8α$^-$ CD103$^-$ DCs in LSP1 KO dLN compared to WT (p<0.01). Flow cytometry analysis also revealed a higher frequency of monocytes and a lower frequency of CD11c^{hi} cells in LSP1 KO spleen (p<0.001 and p<0.01 respectively). We hypothesize that cross-presenting DCs accumulate in spleen of *lsp1*$^{-/-}$ mice hindering their action into tumors and resulting in an impaired control of melanoma growth.

226. (608) **STUDY OF THE INTESTINAL MUCOSAL INNATE IMMUNE RESPONSE IN A MOUSE MODEL OF HEMOLYTIC UREMIC SYNDROME (HUS) BY INTRAGASTRICAL INOCULATION OF SHIGA TOXIN-PRODUCING ESCHERICHIA COLI (STEC)**

Romina Jimena Fernandez-Brando, Andrea Bruballa, Gonzalo Pineda, María Victoria Ramos, Marina Palermo
Instituto de Medicina Experimental. Academia Nacional de Medicina (IMEX-CONICET)

Hemolytic Uremic Syndrome (HUS) is the most serious, life-threatening complication following Shiga toxin-producing *Escherichia coli* (STEC) infections and is characterized by microangiopathic hemolytic anemia, thrombocytopenia and renal dysfunction. Given that the innate immune response in the gut is the first barrier encountered by bacteria in these infections, in the present work we aimed at characterizing it in a mouse model of HUS. C57Bl/6 mice at weaning were intragastrically inoculated with a human-isolated STEC strain (125/99) or its isogenic strain defective in Stx production (ΔStx). As a control mice were inoculated with PBS (C). Four hours later mice were killed and leukocytes from mesenteric lymph nodes (MLN) and lamina propria (LP) were isolated. We assessed cytokine production (IL-4,-5, -6, -10, -17, TGF-β) from leukocytes stimulated in vitro with anti-CD3 and -CD28 antibodies, and the relative and absolute number of CD45, CD11b, Ly6G and Siglec-F positive cells from LP by flow cytometry. IL-6 was the only increased cytokine on culture supernatants from MLN leukocytes of mice infected with both bacterial strains ([mean±SEM pg/ml]=C:16±2, ΔStx:41±3, 125/99: 44±7, ANOVA p<0.01, SNK p<0.01). Besides, we observed an increased absolute number of CD45+CD11b+ leukocytes from the LP from infected mice compared to control ([10^4 leukocytes]=C:1.15, ΔStx:1.80, 125/99: 2.05; two-way ANOVA p<0.05, Bonferroni p<0.05), and also an increased absolute and relative number of CD45+CD11b+Ly6G+ leukocytes (([10^3 cells]=C:0.24, ΔStx:0.69, 125/99: 1.36; [%]= C:0.18, ΔStx:0.65, 125/99:1.17) and CD45+CD11b+Siglec-F+ leukocytes (([10^3 cells]=C:2.00, ΔStx:6.20, 125/99: 7.08; [%]= C:46, ΔStx:76, 125/99:71). In conclusion, both bacterial strains lead to an increased IL-6 production which could account for the influx of CD11b+ cells, Ly6G+ myeloid cells and Siglec-F+

eosinophils into the LP of infected mice. Shiga toxin may not induce a differential innate immune response in the gut, at least on the leukocyte cell subsets studied in the present work.

227. (654) **PARAPROBIOTICS AS IMMUNOPOTENTIATING AGENTS OF MYELOPOIESIS**

Gutiérrez Florencia[1], Andrés Daniel Gramajo Lopez[1], Jorge Armando Jimenez Avalos[1], Héctor Maximiliano Ivir[2], Julio Cesar Villena[1], Susana Alvarez[1, 2], Susana Salva[1]
[1]*CERELA, CONICET* , [2]*Facultad de Bioquímica, Química y Farmacia-Universidad Nacional de Tucumán*

Myelosuppression is the major dose-limiting toxicity of systemic cancer chemotherapy. At present, hematological rescue techniques are applied to reduce the chemotherapy-induced neutropenia that includes new adverse effects. Probiotic acid lactic bacteria (BL) has shown to be promising safe agents to reduce myelosuppression. We demonstrated that the dietary supplementation with probiotic *Lactobacillus rhamnosus* CRL 1505 (Lr05) improved steady-state and emergency granulopoiesis, the respiratory innate immune response and the resistance against respiratory pathogens in immunosupressed hosts. While the viability of the BL is an important factor to achieve optimal protective effects, it is also possible to stimulate immunity using non-viable BL. The aim of this work is to study the ability of paraprobiotics (cell fractions) from Lr05 to minimize myelosuppressive effects derived from chemotherapy. Adult Swiss-mice were orally treated with paraprobiotis, peptidoglycan (PG group) and cellular wall (CW group), during 15 consecutive days (8 μg/mice/day). On day 6, paraprobiotic-treated and untreated control mice received one intraperitoneal dose of cyclophosphamide (Cy) (150 mg/kg). Before Cy-injection, both PG and CW groups increased the peroxidase (Px) score in blood and bone marrow (BM) myeloid cells compared with control ($p<0.05$). Cy impaired steady-state myelopoiesis However, the paraprobiotic treatments were able to significantly increase BM hematopoietic stem cells ($Lin^-Sca\text{-}1^+c\text{-}Kit^+$), myeloid multipotent precursors (Lin^+CD34^+), myeloid cells ($Gr\text{-}1^+Ly6G^+Ly6C^-$) and Px+ cells with respect to the control group ($p<0.05$). Besides, the CW treatment was more effective than the PG to allow an early recovery of these parameters. This, in turn, led to an early increase blood neutrophils and Px score. In conclusion, both PG and CW obtain from *L. rhamnosus* CRL1505 were able to improve BM steady-state myelopiesis in mice undergoing chemotherapy

ONCOLOGÍA / ONCOLOGY ORAL SESSION 1

228. (86) **MUSCARINIC RECEPTORS EXPRESSED IN NON-TUMORIGENIC MCF-10A CELLS INDUCED A MALIGNANT PHENOTYPE THAT ACTIVATES HUMAN ENDOTHELIAL CELLS**

Martinez Paola, Maria Gabriela Lombardi, Francisco Sanchez, Gabriel Fiszman, María Elena Sales
CEFYBO-CONICET-UBA

Our laboratory reported the participation of muscarinic acetylcholine receptors (mAChR) in different steps of tumor progression in murine and human breast cancer. To confirm the contribution of mAChR to malignant transformation, we developed a new cell line by stably transfecting the non-tumorigenic human mammary cell line MCF-10A with mAChR, subtypes 3 and/or 4. Transfected cells acquired the ability to generate three-dimensional structures (spheroids) that mimic the first stages of tumor growth in vivo. Tumor microenvironment has a crucial role in tumorigenesis, particularly endothelial cells, as they form the tumor microvasculature. Considering the later, we analyzed the effect of spheroid-derived conditioned media (S-DCM) from different days of culture on human endothelial cells-1 (HMEC-1) viability, vascular endothelial growth factor-A (VEGF-A) expression and tubulogenesis. S-DCM from 14 days significantly increased HMEC-1 cell viability (mAChR3:153.8%+11,7; mAChR4: 211.2+13.3%; mAChR3/4:171.9+13.5%; n=3) in comparison with conditioned media from MCF-10A (100+18.6%), and in a similar manner than MCF-7 tumor cells (155.6+16.7%; n=3). VEGF-A expression was up-regulated when HMEC-1 cells were treated with S-DCM of 14 days (mAChR3:167.4+5.6%; mAChR4:61.5+10.7%; mAChR3R4:126.7+7.7%; n=3) comparably to S-DCM from MCF-7 tumor cell spheroids (109.5+23.0; n=3). Finally, we investigate the effect of S-DCM of 14 days on HMEC-1 cells in a tube formation assay (mAChR3:119.4+9.9; mAChR4: 117.5+9.8; mAChR3R4:102.2+7.2; MCF-7:130.6+6.33) vs. HMEC-1 cells without treatment. In conclusion, the transfection of non-tumorigenic breast cells MCF-10A with mAChR induces a malignant phenotype that triggers three-dimensional growth and the liberation of pro-angiogenic mediators stimulating HMEC-1 cell viability, VEGF-A expression and tubulogenesis in a similar manner to that observed in breast tumors.

229. (139) **RSUME INHIBITS TYPE 2 VHL MUTANTS FUNCTION LEADING TO TUMORAL ANGIOGENESIS BY INHIBITING THE ASSEMBLY OF THE VHL FUNCTIONAL COMPLEX**

David Gonilski Pacin[1], Tedesco Lucas[1], Sergio Senin[1], Mariana Fuertes[1], Belén Elguero[1], Eduardo Arzt[1, 2]
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von Hippel-Lindau (VHL) disease is associated with the development of high vascularized tumors due to Hypoxia Inducible Factors (HIF) deregulation caused by mutations in VHL gene. VHL is the substrate recognition component of the E3 ligase complex, composed of Cullin2, Elongin B, Elongin C, and pVHL, that participates in the oxygen-sensing system that drives HIF degradation.Certain mutations retain a partial function on HIF downregulation implying additional mechanisms involved in VHL mutants loss of function. We have already demonstrated that RSUME interacts with VHL, and inhibits its function, leading to HIFs-α stabilization. Even more, we also found the same action of RSUME on representative Type 2 mutants (VHLY112H; VHLR167Q; VHLL188V) and we found this mechanism was independent of VHL sumoylation status.The aim of this work is to reveal the molecular mechanism behind RSUME potentiation of Type 2 VHL phenotype and its functional impact. COS-7 cells were cotransfected with Flag-VHLY112H-GFP or Flag-VHLR167Q-GFP or Flag-VHLL188V-GFP, the ECV complex components (Cullin2, Elongin B, Elongin C) and/or V5-RSUME. By VHL inmunoprecipitation we observed that RSUME interaction with VHL type 2 mutants impairs the ECV complex assembly, which inhibits its function.
In RCC-786-O clones expressing VHL, VHLK171R, VHLL188V or VHLL188V/K171R, co-expression of shRNA against RSUME resulted in a decrease of VEGF mRNA. EA.hy926 endothelial cells cultured in conditioned media of these clones, in which RSUME was silenced, decrease the capillary-like structures formation. Mice injected with these stable clones presented new vessels around the injection area, but those clones in which RSUME expression was knocked-down showed a decrease in vessel density. This confirms that in absence of RSUME, VHL Type 2 mutant become more potent and might limit early tumoral angiogenesis.RSUME is critical in VHL mutants deregulation that leads to VHL disease onset.Supported by ANPCyT, CONICET, UBA and FOCEM (COF 03/11).

230. (195) **DETECTION OF CIRCULATING MIRNAS AS POSSIBLE BIOMARKERS OF PROSTATE CANCER ASSOCIATED TO METABOLIC SYNDROME**

Guillermo Nicolás Dalton, Paula Lucia Farre, Cintia Massillo, Flavia Piccioni, Georgina Daniela Scalise, Paola De Luca, Adriana De Siervi
IBYME-CONICET

Prostate cancer (PCa) is the most common type of cancer and is the third cause of death by cancer among males in Argentina. Metabolic syndrome (MeS) is a cluster of pathophysiological disorders whose diagnose requires the detection of, at least, three of the following factors: visceral adiposity, high triglycerides, low High Density Lipoprotein (HDL) cholesterol levels, high blood pressure and elevated fasting glucose levels. A recent meta-analysis found a significant correlation associating MeS with more aggressive PCa tumors

and biochemical recurrence. C-terminal binding protein (CTBP1) is a transcriptional co-repressor of many tumor suppressor genes. Binding either NAD+ or NADH is necessary for CTBP1 activation; however, CTBP1 affinity is 100-fold higher for NADH making it a molecular sensor of the metabolic state of the cell and an interesting link between PCa and MeS. Recent years have seen an overflow of reports regarding miRNAs role in cancer. Many reviews have been published on miRNAs deregulation in cancer, both as cause and consequence, and as possible biomarkers or therapeutic molecules. In this work our aim was the identification of circulating miRNAs to be used in the near future as biomarkers of PCa associated to MeS. To this end, we analyzed serum samples collected from mice bearing xenotransplants and detected 4 miRNAs by RT-qPCR. Among them miR-30b-5p was significantly down regulated in the circulation of MeS mice that were inoculated with control CTBP1 expression cells compared to the mice inoculated with CTBP1 depleted cells. We also analyzed circulating miRNA levels on PCa patient serum samples that were clustered depending on Gleason Score and parameters associated to MeS. In addition, we analyzed serum samples from benign prostatic hyperplasia (BPH) patients and healthy donors, which were clustered according to MeS parameters. We identified many candidates for further analysis as possible biomarkers of PCa associated to MeS.

231. (271) **THE HUMAN ADIPOSE TISSUE SECRETE COMPONENTS THAT REGULATE THE TUMOR AND NON TUMOR RENAL EPITHELIAL CELLS BEHAVIOR**

Flavia Bruna[1, 4], Leonardo R Romeo[2], Silvina E Gómez[1], Flavia E Santiano[1], Corina V Sasso[1], Leila E Zyla[1], Constanza Lopez-Fontana[1], Rubén W Carón[1], Virginia Pistone Creydt[1, 3]
[1]*Instituto de Medicina y Biología Experimental de Cuyo (IMBECU)*, [2]*Hospital Español de Mendoza*, [3]*Facultad de Ciencias Médicas, Universidad Nacional de Cuyo*, [4]*Facultad de Medicina, Universidad del Desarrollo, Santiago de Chile*

An essential information exchange is established between renal epithelial cells and adipose/fibroblastic stroma. In the present work, we evaluated the conditioned media (CMs) effects of human adipose tissue explants from normal (hRAN) and tumor (hRAT) kidney on: proliferation, adhesion and migration of tumor (786-O, ACHN, Caki-1) and non tumor (HK-2) renal epithelial cells. We also evaluated pERK/ERK y pPI3K/PI3K changes in different cell lines incubated by 2 and 24 h with hRAN-, hRAT, or control-CMs.Human renal adipose tissues were obtained from patients with renal cell carcinoma (hRAT, n=9) and kidney donors (hRAN, n=10). The CMs of hRAN and hRAT were collected 24 h post incubation and cells were treated with CMs. Proliferation (MTT assay), adhesion and migration (wound-healing and transwells assay) were evaluated in 786-O, ACHN, Caki-1 and HK-2 cell lines incubated with different CMs. The expression of pERK/ERK and pPI3K/PI3K on cell lines incubated with CMs (WB assay). Statistical differences among experimental conditions were evaluated by one-way ANOVA with Tukey´s post hoc tests. All cell lines showed a significant decrease in cell adhesion ($p<0.05$) and increase in cell migration ($p<0.05$) after incubation with hRAT-CMs vs. hRAN- or control-CMs. Surprisingly, HK-2, 786-O and ACHN cells showed a significant decrease in cell migration ($p<0.05$) after incubation with hRAN-CMs vs. control-CMs. The expression of pERK/ERK was found decreased ($p<0.05$), and pPI3K/PI3K increased ($p<0.05$), in HK-2 and ACHN incubated with hRAT-CMs vs. hRAN- or control-CMs ($p<0.05$). No differences on proliferation of cell lines were found after 24 or 48 h of treatment with CMs.In conclusion, the adipose microenvironment could be regulating the behavior of tumor and non tumor human renal epithelial cells. The tumor stroma should be taken into consideration when dealing with a malignancy.

232. (517) **EPIGENETIC INHIBITORS ELIMINATE MELANOMA BRAF V600E CELLS THAT PERSIST AFTER BRAF INHIBITION**

Florencia Madorsky Rowdo, Antonela Baron, María Marcela Barrio, Jose Mordoh
Fundación Instituto Leloir IIBBA-CONICET

Approximately one-half of melanoma patients harbor the BRAFV600 driver mutation, the most common being BRAFV600E, which leads to the activation of MAPK proliferative and survival pathway. BRAF inhibitors are extensively used to treat BRAF-mutated metastatic melanoma but unfortunately acquired resistance occurs in the majority of patients. Resistance mechanisms involve mutations or changes in gene expression that result in the reactivation of MAPK signaling or activation of other proliferative and survival pathways. We studied the effect of PLX4032 (BRAFV600 inhibitor) long-term treatment on sensitive V600E BRAF-mutated melanoma cell lines. After several weeks of long-term in vitro treatment with PLX4032 the majority of the melanoma cells died whereas some remained viable and quiescent. We named this population SUR cells. Discontinuing treatment of SUR cells with MAPK inhibitors allowed the population to regrow and these cells retained drug sensitivity equal to that of parental cells. We performed RNA-seq in order to determine differences between parental cells and this persistent quiescent population, finding that SUR cells presented changes in the expression of 1509 genes ($p<0.05$). These results suggest that the SUR phenotype may be determined by epigenetic changes. We found that SUR cells changed the expression of epigenetic enzymes. We analyzed the sensitivity to different epigenetic inhibitors and we found that both parental and SUR cells were sensitive to the HDAC (SAHA and mocetinostat) and CDK9 (CDKI-73) inhibitors. These epigenetic inhibitors induced apoptosis and reduced proliferation and invasion in a 3D model both in the parental and SUR populations. We propose the combination of PLX4032 with epigenetic inhibitors in order to achieve a complete elimination of SUR cells that persist after BRAF inhibitor treatment.

233. (79) **ANTI-TUMOR ACTIONS OF CYTOTOXIC DRUGS PLUS MUSCARINIC AGONISTS ON HUMAN TRIPLE NEGATIVE BREAST CANCER CELLS**

Agustina Salem, Yamila Sanchez, Alejandro Español, María Elena Sales
CEFYBO-CONICET-UBA

The administration of low doses of cytotoxic drugs alone or combined with repurposing drugs scheduled with short inter-dose intervals is called metronomic therapy (MT). MT is a new strategy in cancer treatment, since it exhibits high effectiveness and low incidence of side effects. We previously demonstrated that the activation of muscarinic receptors (M) can modulate breast cancer cell viability. Triple negative (TN) breast tumors are highly aggressive, and the effectiveness of pharmacological treatment is low probably due to the absence of a specific target. Here, we analyzed the effect of a combination of subthreshold concentrations of a muscarinic agonist, carbachol (CARB) or arecaidine propargyl ester (APE) (non-selective or selective for M2 subtype respectively) with paclitaxel (PX) or doxorubicin (DX), two cytotoxic drugs used in breast cancer treatment, on MDA-MB231 or MDA-MB468 TN tumor-derived cell lines. By MTT assay we observed on MDA-MB231 cells that the combination of PX+CARB or PX+APE reduced cell viability (23.9±2.5%; 23.5±7.1% respect to control; $p<0.05$). When we combined these muscarinic agonists with another cytotoxic drug, DX, we also observed a reduction in cell viability (DX+CARB: 27.4±4.2%; DX+APE: 30.7±1.6%; $p<0.001$ vs. control). To confirm these results we analyzed the effect of these drugs on another TN cell line, MDA-MB468, obtaining similar results (PX+CARB: 50.3±2.4%; PX+APE: 26.9±3.6%; $p<0.001$ vs. control). When we combined CARB or APE with DX we observed the same effect (DX+CARB: 21.1±0.7%; DX+APE: 31.2±0.9%; $p<0.05$ vs. control). None of the combined treatments had effect on the non tumorigenic cell line, MCF-10A. These results suggest that the combination of low doses of cytotoxic drugs with muscarinic agonists can reduce cell viability and could be used as a new strategy to treat TN breast tumors in humans.

REPRODUCCIÓN / REPRODUCTION ORAL SESSION

234. (152) **POSSIBLE EFFECT OF ROUNDUP ON SERTOLI CELL FUNCTION**

Agostina Gorga, Gustavo Rindone, Cristian Sobarzo, María del Carmen Camberos, Eliana Pellizzari, María Fernanda Riera, María Noel Galardo, Silvina Meroni

Centro de Investigaciones Endocrinológicas "Dr. Cesar Bergadá" (CEDIE)

During the last 50 years a progressive decrease in male reproductive function has been observed. Epidemiological and experimental studies suggest that one of the main causes is exposure to environmental toxicants. Some studies suggest that herbicides such as glyphosate (G) and/or its commercial formulation Roundup (R) alter reproductive function; however direct actions at testicular level and the possible molecular mechanisms involved are unknown. The objective of this work was to analyze the effect of G and R on Sertoli cell (SC) physiology. SC from 20-day old rats were used. Cultures were treated with G or R (100ppm). Results are expressed as mean±SD, n=3,*p<0.05. We observed that neither G nor R modified lactate production and fatty acid oxidation. Transepithelial Electrical Resistance (TER), which indicates integrity of the Blood-testis-barrier (BTB), was also evaluated. G and R decreased TER (C:60.1±2.4; G:37.9±3.1*; R:38.9±5.2*, Ω.cm2). Neither G nor R modified the expression of Claudin11, ZO1 and occludin, proteins that constitute the BTB. Analysis of cellular distribution of Claudin11 by immunofluorescence showed that G and R induced a delocalization of the signal from membrane to the cytoplasm. Finally, in vivo experiments were performed. 14-day old male rats were treated orally with R (50 mg/kg/day) for 16 days, period in which the BTB is assembled and finally established. The integrity of the BTB was evaluated on day 30 using a biotinylated tracer. It was observed that R induced an increase in the permeability of the BTB that enables the passage of the tracer to the adluminal compartment of the seminiferous tubule (p<0.05). In addition, histological analysis showed alterations in the organization of the seminiferous epithelium. In summary, the results suggest that R could alter an important function of SC such as the establishment the BTB and thus it could compromise the normal development of spermatogenesis.

235. (200) **NEONATAL EXPOSURE TO METFORMIN: EFFECTS ON RAT SERTOLI CELL PROLIFERATION**

Gustavo Rindone, Agostina Gorga, Eliana Pellizzari, María del Carmen Camberos, María Noel Galardo, Vanina Da Ros, Mariano Buffone, Silvina Meroni, María Fernanda Riera
Centro de Investigaciones Endocrinológicas "Dr. Cesar Bergadá" (CEDIE)

Sertoli cell (SC) proliferation, a factor defining SC population size, occurs during a restricted period of time. In rats, fetal and postnatal periods up to 15 days of age constitute the time period for SC mitosis. Metformin (MET), a first line anti-hyperglycemic agent, has started to be used in pediatric population at ages when SCs are still proliferating. In addition to its strong anti-diabetic properties, numerous studies have demonstrated that MET has anti-proliferative activity. Additionally, we have previously demonstrated in SC cultures obtained from 8-day old rats that MET decreases basal and FSH-stimulated SC proliferation. The aim of this study was to analyze the in vivo relevance of MET treatment on SC proliferation. At postnatal day 3 (Pnd3), rat pups were randomly assigned to the following groups: MET (receiving daily 200 mg/kg MET i.p., day 3-7) and control group (receiving daily sterile saline solution i.p.). At Pnd8, a set of animals were injected with BrdU (50 mg/kg i.p.) before sacrifice to evaluate cell proliferation. In another set of animals, testes were utilized to perform SC isolation for gene expression analysis. Our results showed that MET group exhibited a significant decrease in BrdU incorporation in SC compared to controls (n=6 p<0.05). In addition, MET group showed a reduction in Cyclin D1 and E2 and an increase in p21Cip mRNA levels in isolated SC (n=6 p<0.05). Considering that a single SC can support a limited number of germ cells, we studied the impact of neonatal treatment with MET on sperm parameters in adult animals. Epididymal sperm parameters were evaluated at Pnd90. No differences in sperm motility, morphology or number between groups were observed. Thus, our results showed that MET exerted a mild but significant suppressive effect on Sertoli cell proliferation with no further impact on spermatogenesis and epididymal maturation. (PICT2015-0228).

236. (254) **EFFECT OF ACTH ON THE PREOVULATORY PERIOD IN CATTLE AND ITS INFLUENCE ON THE EXPRESSION OF TGFB AND ITS RECEPTORS IN THE OVARY.**

Carla Sofia Peust, Juliano Barale, Eduardo Matias Belotti, Hugo Héctor Ortega, Natalia Raquel Salvetti, Gustavo Juan Hein, Valentina Matiller
ICiVet

The components of the transforming growth factor-beta (TGFB) superfamily exert functions related to follicular development, ovulation and corpus luteum formation. We found an altered expression of these components in the ovaries of animals with cystic ovarian disease (COD). In addition, dairy cattle are exposed daily to stressors that can affect reproductive function. Therefore, the aim of this study was to determine the protein expression of the TGFB isoforms (TGFB1, 2, 3) and its receptors (TGFBR1, 2, 3) by immunohistochemistry in different follicular categories in granulosa and theca interna cells from the ovaries during the preovulatory period of cattle. Animals were treated with adrenocorticotropic hormone (ACTH) every 12 hours for five days before the ovulation. We detected a higher expression of TGFB1 in granulosa cells of large preantral follicles of the ACTH-treated group compared to those of the control group. TGFB2 and TGFB3 showed a higher expression in theca cells of dominant follicles of the ACTH-treated group compared to antral follicles of control group (p<0.05). TGFBR1 was more expressed in the granulosa and theca cells of the dominant follicles of the ACTH-treated group than in the antral follicles of the control group. Furthermore, its expression was higher in granulosa cells of primary and primordial follicles of the ACTH-treated group than those of the control group. A lower expression of TGFBR3 was detected in theca interna cells of dominant follicles of the ACTH-treated group compared to dominant and antral follicles of the control group (p<0.05). These results contribute to that previously published for animals with COD. The changes detected incipiently in animals stimulated with ACTH could contribute to a failure in ovulation and, consequently, with the pathogenesis of diseases occurring with anovulation as one of its signs.

237. (264) **ANALYSIS OF THE OVARIAN ESTROGEN RECEPTOR A AND B IN COWS GESTATED UNDER HEAT STRESS CONDITIONS.**

Ulises Notaro, Emilia Huber, Sebastián Recce, Pablo Díaz, Hugo Héctor Ortega, Florencia Rey, Natalia Raquel Salvetti, Fernanda Mariel Rodríguez
ICiVet

The effects of adverse ambient conditions (number of days with temperature-humidity index (THI) exceeding the comfort threshold: >72) on dairy cows can affect the reproduction not only in a direct way, also in the progeny of the animals that were gestated under stress conditions. In fact, the ovarian follicular reserve is established during the first half of the fetal life (0-150 days) and many factors can affect the future fertility of the offspring. Within these factors, Estrogen Receptors (ER) α and β are the most important regulators which gene expression could be modulated. The aim of this study was to evaluate the gene and protein expression of ERα and ERβ in preovulatory follicles of animals gestated under heat stress in different stages of pregnancy. Holstein cows (n=20) were divided in two groups: group 1 (cows gestated under heat stress between 0-150 days of pregnancy) and group 2 (cows gestated under heat stress between 151days-end of pregnancy). Similar levels of ERα and β mRNA were detected by real time PCR, in follicular wall of different groups analyzed (p>0.05). For quantitative methylation analysis of the 5´UTR sequences of ERα and β, an AGENA's MassARRAY platform was used. No differences were detected for any CpG analyzed for both receptors (p>0.05). On the other hand, the protein expression evaluated by immunohistochemistry show similar levels of ERα in granulosa and theca interna of preovulatory follicles of the groups analyzed (p>0,05) and a higher expression of ERβ in granulosa and theca cells of preovulatory follicles of cows of Group 2 related to Group 1 (p<0.05). These data suggest that changes in the expression of estrogen receptors, mainly ERβ, can lead to an altered response of steroid hormones, and thus contribute to ovarian alterations in animals gestated under heat stress.

238. (293) **EVALUATION OF OVARIAN GLUCOCORTICOID RECEPTOR A EXPRESSION IN COWS GESTATED UNDER HEAT STRESS CONDITIONS**

Emilia Huber, Ulises Notaro, Sebastián Recce, Hugo Héctor Ortega, Natalia Raquel Salvetti, Florencia Rey, Fernanda Mariel Rodríguez
ICIVET Litoral - UNL CONICET

Adverse ambient conditions (temperature-humidity index (THI) exceeding the comfort threshold (72) for 3 or more days) on dairy cows can affect reproduction not only in a direct way, also in the progeny of animals that are gestated under these stress conditions. In fact, the ovarian follicular reserve is established during the first half of the fetal life (0-150 days) and many factors can affect the future fertility of the offspring. Under stress situations, nervous system triggers a signaling cascade with a final secretion of glucocorticoids (GCs). It is known that GCs exert actions in the ovary, mainly through the GC receptor α (GRα). In this study, we evaluated gene expression of GRα in preovulatory follicles of cows gestated under heat stress in different stages of gestation, and epigenetic mechanisms that could regulate gene expression. Holstein cows (n=20) were divided in two groups: group 1 (cows gestated under heat stress between 0-150 days of gestation) and group 2 (cows gestated under heat stress between 151 days-end of gestation). Higher levels of GRα were detected by Real Time PCR in follicular wall of preovulatory follicles of group 1 relative to group 2 ($p<0,05$). For quantitative methylation analysis of the 5' UTR region of the GR gene, an AGENA's MassARRAY platform was used. Similar levels of methylation were found in both groups for every CpG analyzed. These data suggest that differential expression of GRα could be related to an altered ovarian response to GCs in cows gestated under heat stress in first half of gestation. However, we could not confirm that this variation is due to differential methylation of the GR gene.

239. (754) **ALTERED EXPRESSION OF MATRIX METALLOPROTEINASE 14 IN THE OVARY OF COWS WITH ACTH-INDUCED STRESS AND WITH CYSTIC OVARIAN DISEASE (COD).**

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Dairy cattle are daily subjected to stressful situations some of which have been associated to reproductive disorders. Under these conditions, the microenvironment of the ovary is modified, affecting directly their function. One of the important functions influenced by external stressors is the extracellular remodeling, necessary to ovulation. The aim of this study was to evaluate the expression of matrix metalloproteinase (MMP) 14, a protein involved in tissue remodeling during ovulation. MMP14 expression was analyzed by immunohistochemistry on ovarian samples of cows treated with 100 IU of adreconcorticotropin (ACTH) every 12 hours, for four days before ovulation time, and cows with cystic ovarian disease (COD). Granulosa and theca interna cells of primary, small preantral, large preantral, antral, atretic and preovulatory follicles of treated animals (n=7), control group (n=5) and from cows with spontaneous COD (n=7) were evaluated. We detected that granulosa cells of all follicular structures from spontaneous COD, presented higher expression than those of ACTH-treated and control groups in all categories ($p<0.05$). Moreover, the theca interna cells of all follicular structures of COD group showed higher expression compared to those from all categories of ACTH-treated and control groups ($p<0.01$). These results might indicate that exist an increased expression of MMP14 in spontaneous COD ovaries, which could affect the follicular remodeling at follicles of all categories and extracellular matrix. MMP14 is a crucial regulator of gelatinases, acting as a complex with pro-MMP2 and TIMP2 to activate MMP2, one of most important gelatinase enzyme. Follicular wall degradation process, necessary for ovulation, considered as a focal acute inflammatory response, depends on an equilibrated and complex mechanism that could be affected by tissue metalloproteinases and their inhibitors.

INMUNOLOGÍA / IMMUNOLOGY ORAL SESSION 3

240. (434) **EXTRACELLULAR ACIDOSIS DRIVES THE DIFFERENTIATION OF HUMAN MONOCYTES INTO DENDRITIC CELLS: ROLE OF THE ARYL HYDROCARBON RECEPTOR AND MTOR**

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Little is known about the identity of the factors that in vivo control the differentiation of monocytes into macrophages and dendritic cells (DCs). Local acidosis is a common feature of inflammation and the tumor microenvironment. We have previously shown that low pH (pH 6.5) promotes monocyte differentiation into DCs, in peripheral blood mononuclear cells (PBMCs) or isolated monocytes cultured for 5-7 days with PHA. Here, we further characterize the influence exerted by low pH on the differentiation of monocytes into DCs. Previous studies showed that when human monocytes were cultured with M-CSF (100 ng/ml), IL-4 (30 ng/ml) and TNF-alfa (5ng/ml) a minor fraction of cells acquire an inflammatory DC phenotype. Here, we found that low pH markedly promoted the differentiation of monocytes into inflammatory DCs induced by M-CSF, IL-4 and TNF-alpha: % iDCs = 26 ± 4 vs 72 ± 2% of CD1a+/CD14-/CD16- cells (mean ± SE, n=7, $p<0.001$, pH 7.3 vs 6.5, respectively). Interestingly, promotion of DC differentiation by low pH was also observed in conditions strongly associated with macrophage differentiation. In fact, we found that monocytes cultured in serum-free medium with M-CSF (50ng/ml) for 5 days at pH 7.3 or pH 6.5, resulted in 5 ± 5 vs 60 ± 10% of CD1a+ CD14- cells (mean ± SE, n=7, $p<0.001$). Mechanistic studies showed that DC differentiation induced by acidosis was prevented by the inhibitor of the of the aryl hydrocarbon receptor (AHR) stemregenin-1 (8 μM) (% inhibition >75%, n=7). Interestingly, the inhibitor of mTORC1 temsirolimus (1-100 nM) promotes the differentiation of monocytes into iDCs in a similar fashion that low pH. Because extracellular acidosis has shown to inhibit mTORC1 activity, our observations suggest that low pH promotes DC differentiation through a mechanism that involves the interplay between AHR and mTORC1 pathways.

241. (522) **ENDOMETRIAL INFLAMMATORY CYTOKINES: THE EFFECTS OF MILK PRODUCTION LEVELS AND HORMONAL CONTEXT ON LOCAL EXPRESSION OF THEM DURING EARLY LACTATION OF DAIRY CATTLE.**

Maria Belén Peralta, Emmanuel Angeli, Antonela Florencia Stassi, Sofía Cainelli, Leandro Durante, Hugo Héctor Ortega, Florencia Rey, Natalia Raquel Salvetti, Melisa María del Luján Velázquez
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Dairy cattle undergo metabolic and hormonal changes that impact in the immunological system during peripartum. Previous reports suggest that cows are immunosuppressed during postpartum and immune components of the reproductive tract are altered. We analyze the impact of milk production and the hormonal context on the expression of endometrial cytokines. The animals were divided in high production group (HP: > 4800 L, n = 11) and low production group (LP: < 4400 L, n = 13) considering the average of the dairy production up to 150 days in lactation. Blood samples and endometrial biopsies were obtained at 45 and 60 days postpartum (dpp). The mRNA expression of interleukin IL1α, TNFα and IL6 was evaluated by real time PCR. The protein expression was analyzed by immunohistochemistry in the endometrial luminal epithelium (LE), glandular epithelium (GE) and stroma (S). Plasmatic concentrations of cortisol, estrogen and progesterone were measured by ECLIA. A general linear model was used to analyze the results. The results

showed that mRNA levels of IL1α were lower at 60 dpp than 45 dpp, and LP group showed a decrease of IL1α mRNA expression in relation to HP group (p<0,05). Also, the estradiol levels had significant effect on the increase of IL1α mRNA expression. The mRNA levels of TNFα showed no differences between analyzed groups and the 75% of the samples showed no expression of IL6 mRNA. The protein expression of IL1α was significantly increased in LE under progesterone effect (p<0,05). TNFα showed no differences in LE, GE and S between HP and LP groups (p>0,05). However, the protein levels of IL6 in GE were significantly increased under the progesterone effect. These results suggest that milk production and the hormonal context affect the uterine cytokine expression during evaluated postpartum period, especially under estradiol and progesterone effects.

242. (558) **HYPOXIA MODULATES TISSUE TRANSGLUTAMINASE EXPRESSION AND PROINFLAMMATORY MEDIATORS PRODUCTION IN SWAN-71 TROPHOBLAST CELLS**

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Tissue transglutaminase (TG2) is a ubiquitous and multifunctional protein with four C-terminal truncated splice variants described. It contributes to several processes such as apoptosis/survival, efferocytosis, inflammation and tissue repairing under physiological and pathological conditions. TG2 is expressed in trophoblast cells but there is limited information concerning its role at maternal-fetal interface and the relation between its modulation and placenta dysfunction. Hypoxia or persistent inflammation in placenta are reported in some pregnancy pathologies linked to problems in placenta functions and fetal development and, as described in other cellular types, these conditions could modulate TG2 expression and/or activity at placenta level.
We analyzed hypoxia effects on trophoblast cells concerning: TG2 protein expression, TG2 splice variants mRNA expression and proinflammatory cytokine production.
Swan-71 cell line was treated for 24 h with cobalt chloride (CoCl2) - a hypoxia-mimetic agent that stabilizes HIF-1α-. Cell viability was studied by flow cytometry using FITC-labeled annexin-V and propidium iodide double staining. HIF-1α and TG2 protein were assessed by immunoblot and its splice variants by RT-qPCR. Cytokine production was evaluated by ELISA.
Incubation with 200 µM CoCl2 did not affect cell viability but affected apoptotic/necrotic cells ratio (0.4 vs 1.9, control vs CoCl2). Treatment with 200 µM CoCl2 significantly increased HIF-1α expression (p<0.001, n=3). CoCl2 treatment decreased IL-6 production (p<0.001, 0 vs 200 µM, n=4) and increased IL-8 production (p<0.001, 0 vs 200 µM, n=4), both were dose dependent effects (0, 50, 100 and 200 µM). In these conditions full-length TG2 decreased its expression at protein (p<0.05, n=4) and mRNA level (p<0.05, n=3) and preliminary results showed some changes in TG2 splice variants expression.
Results support that hypoxia alters full-length TG2 and TG2 splice variants expression and affects cytokine production by trophoblast cells. Further work is currently underway to decipher molecular mechanisms underlying this regulation, and its pathophysiological implications.

243. (577) **IFNB EXPRESSION IN THE NERVOUS AND RESPIRATORY SYSTEM OF BOVINE ALPHA-HERPESVIRUSES-INFECTED CALVES**

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Induction of interferon (IFN) β is an important feature of bovine herpesviruses (BoHV) infections. The aim of this research was to determine the variations in IFNβ expression in the respiratory tract and nervous system of calves during BoHV-1 and BoHV-5 experimental acute infection [6 days post-infection (dpi), n=4] and reactivation (dexamethasone treatment, 25 dpi, n=4) in comparison to mock-infected animals (n=2). RNA was extracted from nervous system [olfactory, frontal and posterior cerebral cortex, cervical medulla and trigeminal ganglia (TG)] and respiratory tract (retropharyngeal lymph nodes, nasal mucosa, trachea and lung) to determinate the gene expression of IFNβ1 by RT-qPCR. Relative expression analysis was performed with REST software (P <0.05). During acute BoHV-1 and BoHV-5 infection, a slight but statistically significant increase in IFNβ mRNA levels in posterior cortex was observed, while this cytokine was down-regulated in cervical medulla during BoHV-5 acute infection. The main differences were observed during reactivation. For both viruses, there was a decrease in the IFNβ mRNA levels in the frontal cortex between 0.4- and 0.5-fold. However, a marked increase was determined for BoHV-1 (25- and 26.5-fold) and BoHV-5 (14.4- and 133.8-fold) in posterior cortex and cervical medulla. Moreover, contrary to mock-infected animals, upon viral reactivation this cytokine was detected in TG. It was also detected in all bovine respiratory samples from control and infected animals. IFNβ mRNA levels were lower in retropharyngeal lymph nodes (0.02-fold) and higher in the nasal mucosa (6.7-fold) from BoHV-5 and BoHV-1 acutely infected calves, respectively. IFNβ expression was upregulated in retropharyngeal lymph nodes and lungs (2.2- and 3.2-fold) from BoHV-5-infected calves during reactivation. This work showed that IFNβ plays a crucial role in the innate antiviral immune response that mediates the containment of herpesvirus infections in respiratory and neural tissue. These findings seem particularly relevant during virus reactivation.

244. (590) **A-HELICAL CATHELICIDINS ARE MODULATED IN THE PLACENTA AND FETAL SPLEEN OF NEOSPORA CANINUM EXPERIMENTALLY INFECTED CATTLE**

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Innate immune responses at the maternal-fetal interface are key in the pathogenesis of Neospora caninum, an obligate parasite that causes abortion in cattle. Among antimicrobial peptides, cathelicidins are small cationic peptides which have generated great interest as multifunctional molecules that mediate various host responses, including microbicidal action, chemotaxis and activation of cytokine secretion. Their functions are poorly characterized during the immunological response against N. caninum. Herein, we determined the gene expression of two α-helical cathelicidins in the placenta and fetuses from both non-infected pregnant heifers and pregnant heifers intravenously challenged with live tachyzoites of NC-1 strain of N. caninum on day 70 of gestation. On day 104 of pregnancy, mRNA expression of bovine cathelicidins BMAP27 and BMAP28 was determined by RT-qPCR. Relative expression analysis was performed with REST software (P <0.05). Basal gene expression of both cathelicidins were detectable in the placenta, maternal caruncle and spleens of fetuses from non-infected control animals, mostly in fetal spleens. Basal BMAP27 and BMAP28 levels were 285- and 225-fold higher in fetal spleen than in placental tissues. N. caninum infection induced the expression of BMAP27 in both placenta and maternal caruncle (between 6- and 7-fold) and BMAP28 in placenta (10-fold) regarding non-infected pregnant heifers. However, BMAP27 and BMAP28 relative expression was inhibited in the spleen of fetuses (0.06 and 0.18-fold) from N. caninum-infected heifers. These studies demonstrate that upon homeostasis, cathelicidins expression is reduced in placental tissues. However, infection during pregnancy

induce key antimicrobial peptides at the maternal-fetal interface, probably triggering a strong inflammatory immune responses associated with abortion. Contrary, infection with N. caninum seems to attenuate the innate immune response and reduce it at the fetal side. This finding represents novel information on how innate antimicrobial peptides responses are induced at the maternal-fetal interface of cattle in response to N. caninum experimental infection.

GASTROENTEROLOGÍA / GASTROENTEROLOGY

245. (31) **EFFECTS OF YERBA MATE (ILEX PARAGUARIENSIS) ON GASTRIC MUCOSA**

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The consumption of Yerba Mate (YM) is very common in South America. There is a popular belief that its consumption causes heartburn, gastric irritation or gastritis. However, there are poor scientific evidence and the information is contradictory. The gastric mucosa is exposed to harmful substances and, if its protection mechanisms are exceeded, a gastric injury occurs. Gastritis is an inflammatory disease that involves multiple factors such Helicobacter pylori infection, NSAIDs, alcohol and tobacco use, genetic predisposition, etc. The damage of the mucosa depends on the time of injury exposure, the intensity of it and the previous state of the gastric tissue. The aim of this work was to evaluate the effect of different YM concentration on gastric mucosa without concomitant injurious. For this, 30-day Sprague Dawley rats were divided into 3 groups (n=9/group) and they were given ad libitum: A. water, B. YM infusion (25 g/500 ml, prepared at 70°C),C.YM concentrated infusion (50 g/500 ml, prepared at 90°C). After 90 days, the animals were sacrificed, the stomachs were excised and digital images were taken for macroscopic assessment. For microscopic examination, samples were taken from different zones, paraffin embedded and stained with H&E, in order to determine parameters of injury such as congestion, edema, hemorrhage, leucocyte infiltration, etc. Results: No macroscopic and microscopic mucosa differences between groups were found. The gastric mucosa showed preserved morphology in the four layers and without significant signs of injury, both in control and treated animals (only few animals of all groups [n=1, n=1, n=2 respectively] showed mild mucosal edema, congestion and mild chronic inflammatory infiltrate). We can conclude that YM infusions would not produce deleterious changes in gastric mucosa. The effect of YM on damage mucosa or in the presence of concomitant injurious factors should be assess for a completed evaluation.

246. (446) **ENDOTOXEMIA INDUCES MITOCHONDRIAL DYSFUNCTION AND MODIFIES MITOCHONDRIAL DYNAMICS IN PANCREAS**

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Pancreas has been described to be affected in early-endotoxemic process. Since mitochondria are the main source of ATP it is essential to maintain a suitable mitochondrial bioenergetics to recover from cellular, tissue and pancreatic organic damage in this syndrome. The aim was to analyze the status of pancreatic mitochondrial function and dynamics in a rat model of low grade and severe endotoxemia. Female Sprague Dawley rats (45 days) were ip injected by 0.5 mg/kg (LPS 0,5), by 8 mg/kg (LPS 8), or vehicle (control), and after 6 h assays were performed in pancreas. To evaluate mitochondrial function, mitochondrial oxygen consumption, ATP production and mitochondrial complex activities were measured. ATP production was found significantly decreased (30%) in both LPS-treatments (control value: 70.4 ± 5 nmoles ATP/min mg protein, $p < 0.05$). While the activity of mitochondrial complexes II (control: 22.3 ± 1.2 nmoles/min mg protein), and IV (control: 3.45 ± 0.22 min-1/mg protein) decreased in both endotoxemic models, complex I (control 42.2 ± 3.5 min-1/mg protein) was found to be decreased (32%) only in the LPS 8 group. In addition, a decreased respiratory control rate was observed in animals treated with LPS. DRP1 (mitochondrial fission protein) and OPA1 (mitochondrial fusion protein) expression were evaluated to study changes in mitochondrial dynamics. DRP1 expression were increased by 3-fold in mitochondrial fraction in both endotoxemia model, while OPA1 increased its expression only in severe endotoxemia. Finally, oxidative and nitrosative stress markers were determined, observing an increase in $O_2^{\bullet-}$ production and a decrease in nitrites in the cytoplasmic fraction. Our results suggest that during endotoxemia ROS production increases by the activation of cytosolic NADPH oxidase, which may induce damage in pancreatic mitochondria. Changes are triggered at mitochondrial dynamics, probably as a cellular protective mechanism to recover mitochondrial function and cellular bioenergetics to restore the organic function.

247. (614) **RETROSPECTIVE DESCRIPTIVE STUDY ABOUT HEPATOCELLULAR CARCINOMA IN MAR DEL PLATA**

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INTRODUCTION Hepatocellular carcinoma (HCC) is the seventh most common neoplasia worldwide and the third mortality cause related to cancer. In Argentina, and particularly in Mar del Plata, few data are available related to this disease. OBJECTIVE: To describe the characteristics of HCC cases previously diagnosed in the city. MATERIALS AND METHODS: Patients attended and diagnosed at a public health institution (years 2005-2016), were included in this study. Epidemiologic and clinical information were collected, loaded into a database and statistically analyzed using RStudio software. RESULTS: Fifty seven HCC patients diagnosed during the period were analyzed. A 77.19% (44/57) were males and 22.81% (13/57) females. The mean age of the cohort was 58.66±7.21 years (no differences between males 58.83±7.63 and females 58.75±5.09). Etiology and risk factors for the development of the disease were attributed to: alcohol 36.84% (21/57), HCV & alcohol 28.07% (16/57), HCV 21.05% (12/57), HBV 5.26% (3/57), HBV & alcohol 3.5% (2/57). A lowest mean age was detected for the HCV & alcohol group, maybe related to the main way of HCV transmission in the region (IV drugs). A majority of male patients were observed in alcohol intake groups [alone 80.95% (17/21); & HCV 87.5 % (14/16)]. Regarding characteristics of tumors, 60% (33/54) exhibited a single-nodule, 7.7% (3/54) two-nodules and 32.3% (18/54) presented multinodular images. Independently of its cause, cirrhosis was the main risk factor for the development of HCC in this cohort, exhibiting cirrhotic liver at diagnosis a 98.43% (56/57) of the patients. CONCLUSIONS: The characterization of HCC cases in the region enables public health actions related to prevention, following and treatment of disease. In particular, the fact that HCV (associated or not with alcohol) is one of the main risk factors for the development of HCC, highlights the importance of diagnosis and treatment of this infection locally.

248. (686) **FUNCTION OF USP9X AS A NEW PLAYER OF AUTOPHAGOSOME BIOGENESIS IN VMP1-DEPENDENT AUTOPHAGY.**

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Intracellular activation of zymogen granules leads to acute pancreatitis by pancreas self-digestion. Most of acute pancreatitis cases are self-limited, suggesting the importance of protective mechanisms of acinar cells. VMP1 is an autophagy-related protein whose expression is induced by pancreatitis and essential for autophagosome biogenesis. VMP1 mediates zymophagy, a selective type of autophagy-mediated degradation of zymogen granules that prevents acute pancreatitis. Previously we demonstrated that the deubiquitinase USP9x interacts with VMP1 during zymophagy. The aim of this work

is to elucidate the function of USP9x in autophagosome formation. By immunofluorescence, USP9x presents a cytoplasmic diffuse pattern at basal conditions, and it relocates after 15 min of starvation to a perinuclear area (48.0% ± 1.2 vs 40.5% ± 1.9 p<0.01). This response is not accompanied by an increase of USP9x protein levels. In the relocalized pattern, USP9x co-localizes with autophagy-related proteins such as Wipi1 (a marker of isolation membrane) and Beclin 1 (a marker of PI3k complex). Moreover, we demonstrate that VMP1 is necessary for USP9x relocation since USP9x relocation is reduced in cells treated with a shVMP1 (41.3%±2.2 vs 48.0%± 1.2 p<0.01) and it is increased in cells overexpressing VMP1 (47.5%± 2.2 vs 40.5%± 1.0 p<0.01). Finally, shRNA-mediated depletion of USP9x significantly reduces autophagy evaluated by LC3-RFP dots per cell in starved HeLa cells (6.8±4.2 vs 33.6±2.4 p<0.05), indicating that USP9x is required for autophagosome formation.
In conclusion, we demonstrate for the first time that USP9x is a novel autophagy-related protein. In response to starvation, USP9x relocates to a perinuclear area and participates of the initial steps in the autophagosome biogenesis. Hierarchially, USP9x acts downstream of VMP1, since VMP1 expression induces USP9x relocation during autophagosome formation. Finally, USP9x plays a relevant role in the cell response to acute pancreatitis, being part in the early nucleation steps of the VMP1-mediated autophagy.

249. (60) **AQUAPORIN-1 KNOCKOUT INCREASES PROLIFERATION AND MIGRATION IN HUMAN CHOLANGIOCARCINOMA CELLS**

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Introduction: Cholangiocarcinoma (CCA) is a highly aggressive liver tumor arising from cholangiocytes, the epithelial cells lining the biliary tree. Aquaporin-1 (AQP1) is expressed in cholangiocytes where facilitates the osmotic transport of water during ductal bile formation. Recent studies in non-hepatic AQP1-expressing tumoral cells have suggested the involvement of AQP1 in migration and proliferation processes. Our aim was to begin to study whether the lack of AQP1 expression affects proliferation and migration in CCA cells. We made use of the established human CCA cell line HuCCT1. By using confocal immunofluorescence microscopy as well as subcellular fractionation followed by immunoblotting, we confirmed that HuCCT1 cells express AQP1 protein which was largely located in intracellular compartments. Then, we generated an AQP1 knockout HuCCT1 cell line (AQP1 KO) by using the CRISPR/Cas9 genome editing technique. A wild type HuCCT1 cell line (WT) was used as control. Cell proliferation and migration were assessed using IncuCyte live-cell imaging system, which allows cell growth and wound healing to be tracked and quantified over time. Results: AQP1 silencing induced a significant increase in both cell proliferation (WT: 58.0 ± 3.4% vs. AQP1 KO: 76.0 ± 2.6% of confluence at 24h; P<0.005) and migration (WT: 60,4 ± 4,4% vs. AQP1 KO: 97.1 ± 0,8%, of relative wound density at 16 h; P<0.0001) rates. Conclusion: In contrast with results reported in other tumoral cells, we found that the lack of AQP1 expression in human CCA cells promoted proliferation and migration, which may suggest a novel tumor suppressor function for AQP1. Understanding the mechanisms through which AQP1 could modify tumor cell phenotype might help improve therapies against CCA.

250. (260) **EFFECT OF STEVIA REBAUDIANA BERTONI INFUSION ON SALIVARY ALPHA-AMYLASE**

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Salivary alpha-amylase (sAA) is one of the most abundant proteins in saliva. The enzymatic activity of sAA undoubtedly plays a role in carbohydrate digestion. Binding of sAA to bacteria and teeth may have important implications for dental plaque and caries formation. The purpose of the present study was to investigate the inhibitory effect of *Stevia rebaudiana* Bertoni infusion on the activities of sAA, in order to offer an effective strategy to lower the levels of additional glucose for metabolism by plaque microorganisms in close proximity to the tooth surface. Unstimulated saliva were obtained from young male subjects (20-33 years) who ingested mineral water (G1, n = 15), infusion stevia (G2, n = 15) and orange juice (G3, n = 15). Whole saliva samples were collected, immediately before beverage intake (T0), and 60 (T1), and 120 min (T2) later. Those subjects who presented some oral/systemic pathology or drug/ substance consumption related to flow alterations or salivary composition were excluded. The sAA activity was determined by a commercial kit (Amilokit, Wiener lab, Rosario, Argentine) and spectrophotometric method with wavelength 640 nm (Spectrum, USA). The values were expressed as mean ± SD of amylolytic units (UA/dl). The results obtained were: G1: T0 = 417.66 ± 56.97, T1 = 408.15 ± 58.71, T2 = 296.87 ± 86.38; G2: T0 = 415.06 ± 59.88, T1 = 409.55 ± 88.79, T2 = 346.28 ± 109.77; G3: T0 = 375.39 ± 56.02, T1 = 393.07 ± 48.83, T2 = 428.65 ± 57.06. UA/dl decreased from T0 to T2, in G1 (p = 0.003) and G2 (p = 0.007). UA/dl increased from T0 to T2, in G3 (p = 0.009). The intake of mineral water and the stevia infusion does not stimulate the activity of sAA.

251. (292) **LIVER REGENERATION (LR) AFTER PARTIAL HEPATECTOMY (PH) IS IMPAIRED IN KIR 6.2 KNOCKOUT (KIR6.2-/-) MICE**

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ATP sensitive potassium (K-ATP) channels are composed of pore-forming Kir6.x (6.1 or 6.2) subunits and SUR1 or SUR2 regulatory subunits. Kir6.2/K-ATP channels are expressed in the liver and, besides coupling cell metabolism to cell membrane potential, they play critical roles in the cellular responses for tissue protection under stress. It was reported that activation of liver mitochondrial Kir6.1 channels improves LR after PH by keeping a higher ATP content. On the other hand, Kir6.2-/- mice showed aggravated LPS-induced liver injury, indicating the involvement of Kir6.2 in the pathology of liver disease. The aim of the present study was to evaluate the role of Kir6.2 in LR after PH. Male C57/B6 wild type (WT) and Kir6.2-/- mice (n=4) were subjected to 2/3 PH. Forty-eight hours post-PH, mice were euthanized and serum and liver samples were obtained. Serum markers of liver function (alanine and aspartate aminotransferases and alkaline phosphatase) did not present differences between WT and Kir6.2-/-. Liver to body weight ratio showed a tendency to be reduced in Kir6.2-/- mice (WT: 0.0306±0.0001; Kir6.2-/-: 0.0234±0.007). Western blot studies of Proliferating Cell Nuclear Antigen (PCNA) and Cyclin D1, markers of cell proliferation, showed significant differences between WT and Kir6.2-/-, with a diminution of the expression of both proteins in Kir6.2-/- mice (PCNA (arbitrary units): WT: 328±43, Kir6.2-/-: 77±20*; Cyclin D1 (arbitrary units): WT: 149±11, Kir6.2-/-: 95±6*; *p<0.05). The present results, despite being preliminary, show the participation of Kir6.2 channel in LR after PH. Kir6.2 deletion impairs the regenerative phenomenon possibly due to the reduction of ATP hepatic content. Future studies at different times post-PH are necessary for delineating the role of Kir6.2 in LR after PH.

252. (327) **FRUCTOSE-ENRICHED DIET DECREASES PROTEIN EXPRESSION AND ACTIVITY OF INTESTINAL MRP2**

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Increased intake of fructose in humans and laboratory rodents is known to be a risk factor for the development of metabolic disorders

as insulin resistance, metabolic syndrome (MS) and type 2 diabetes as well as cardiovascular diseases. Mrp2 and phase II biotransformation enzymes, acting as intestinal biochemical barriers, strongly influence the bioavailability, and hence the efficacy and safety, of orally administered drugs. Aim: to evaluate the effect of the chronic administration of 10% fructose (F) in drinking water to male Wistar rats for 8 weeks on jejunal Mrp2 expression, by western blot, and on its activity, by using the everted intestinal sacs model. Control rats (C) received tap water. Results: fructose consumption led to high plasma triglycerides and insulin levels, as well as insulin resistance and glucose intolerance in F vs C ($p<0.05$). A significant decrease in Mrp2 protein expression was observed in F (-80%) respect to C ($p<0.05$). This result correlated with the decrease of serosal-to-mucosal transport of the Mrp2 substrate dinitrophenyl-S-glutathione (DNP-SG), generated from its precursor 1-chloro-2,4-dinitrobenzene (CDNB) (F:-30% respect to C, $p<0.05$). Cytosolic GST activity involved in the conversion of CDNB to DNP-SG, was also reduced by the treatment (F:-14% respect to C, $p<0.05$). This result correlated with a significant decreased in GSTα expression in F rats (-43%) respect to C. Moreover, fructose administration induced alterations in parameters of oxidative stress by decreasing both the activity of antioxidant enzyme superoxide dismutase (-34%, $p<0.05$) and the GSH/GSSG ratio (-39% $p<0.05$), as well as an increased of lipid peroxidation end products (+130% $p<0.05$). Conclusion: Chronic fructose feeding caused severe alteration on the Mrp2-dependent intestinal biochemical barrier, thus increasing the bioavailability of its substrates. This suggests that toxicity of food contaminants, as well as availability of therapeutic drugs, substrates of GST/Mrp2, may be exacerbated in patients with MS.

253. (349) **ACUTE MODULATION OF INTESTINAL MRP2 ACTIVITY BY NUTRIENTS. INVOLVEMENT OF GLP-2**

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Multidrug resistance-associated protein 2 (MRP2) is an ATP-dependent transporter expressed at the brush border membrane (BBM) of the enterocyte. It confers protection against absorption of toxicants from foods or bile. We recently demonstrated that MRP2 localization is subjected to a dynamic equilibrium between BBM and intracellular domains, thus allowing for rapid regulation of its function. Now we hypothesize that this acute MRP2 modulation takes place after intake of certain nutrients. The aims of this work were i) to evaluate whether this regulation takes place in vivo by effect of nutrients incorporated into the intestinal lumen and ii) to identify the underlying mechanism. Nutrient-containing preparations were incorporated into distal jejunum of fasted rats. Thirty min later, MRP2 activity was evaluated by quantifying efflux of the model substrate dinitrophenyl-S-glutathione into the apical compartment of proximal jejunum along a 30-min period. As result, we found a selective effect of nutrients, with oleic acid (10% V/V) and glucose (100 mM) leading to significant increases in MRP2 activity (+202% ± 22% and +188% ± 45%, respectively; $p<0.05$ vs control preparation) and no effect for glucose at a lower concentration (10 mM). The participation of the intestinotrophic hormone GLP-2 was demonstrated since intraperitoneal treatment with anti-GLP-2 (20 μg) prevented oleic acid effect. By confocal microscopy we confirmed that increases in MRP2 activity were associated with increased sorting of MRP2 from the intracellular domain to BBM. Additionally, intravenous administration of GLP-2 (125 μg/kg b.w.) mimicked the effect of oleic acid on MRP2 activity and localization. In conclusion, rat intestinal MRP2 is subject to rapid regulation of its localization and activity in response to certain nutrients, with GLP-2 being a mediator of this effect. This may constitute an on-demand regulation of transcellular barrier to prevent absorption and toxicity of food contaminants during absorptive periods.

254. (514) **PROLACTIN INCREASES TRANSPORT ACTIVITY AND EXPRESSION OF P-GLYCOPROTEIN IN RAT LIVER**

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Prolactin (PRL) receptor is expressed in liver suggesting a role for this hormone in regulating liver function. Previously we demonstrated that PRL up-regulates the expression of P-glycoprotein (Pgp), a canalicular transporter responsible for endo- and xenobiotic excretion. Here we evaluate the effect of PRL on hepatic Pgp activity in vivo using Rhodamine 123 (Rh 123) as substrate in a single bolus 0.52 μmol/kg b.w., i.v. followed by fluorimetric detection in bile samples collected at set time periods during 90 min. In vivo experimental groups: 1- Lactating rats (21 days post-partum, PP) vs virgin females (VF) and 2- Ovariectomized rats (OVX) treated with PRL (OVX+PRL: 300 μg/day for 7 days at a constant rate via osmotic minipumps, reaching PRL plasma levels observed in PP rats) vs OVX treated with vehicle (controls). To further evaluate the mechanism involved in protein induction we quantified Mdr1a and Mdr1b mRNA by qRT-PCR (since Pgp is encoded by both genes) in primary hepatocytes culture exposed to 0.10 μg/mL of PRL (concentration mimicking plasma levels in PP rats) or vehicle for 4 and 12 h. RESULTS (% change in comparison with respective control, n=3, $p<0.05$): Pgp activity was similar between PP and VF, and resulted significantly higher in OVX+PRL when compared to OVX (AUC +43%). A significant increase in Mdr1a mRNA by PRL was observed after 12 h of culture (+87%). CONCLUSIONS: 1- PRL increased Pgp activity in OVX rats livers correlating with the higher protein levels previously reported, suggesting altered pharmacokinetics of Pgp substrates, including therapeutic agents in situations of elevated PRL levels. 2- The absence of changes in Pgp activity in PP could be due to a competitive action on Rh123 biliary excretion by any endogenous compound exhibiting increased plasma levels during lactation. 3- Increased Mdr1a levels suggest a transcriptional up-regulation of Pgp by PRL.

255. (692) **REDUCTION OF INTESTINAL MRP2 AND P-GP ACTIVITIES IN A HIGH FAT DIET MODEL**

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Metabolic syndrome (MetS) is a grave health disorder that increases the risk for cardiovascular complications and type 2 diabetes. Some drugs used in patients with MetS are substrates of intestinal multidrug resistance-associated protein 2 (Mrp2) and P-glycoprotein (P-gp), two important efflux pumps that limit the absorption of xenobiotics. Thus, their bioavailability could be affected by changes in these transporters. Because one of the major causes of MetS in humans is diet induced obesity, the aim of this study was to evaluate the effect of a high fat diet on intestinal Mrp2 and P-gp activities.C57BL/6J male mice of 5 weeks old were randomly treated for 16 weeks, with standard diet, control group (C) or were fed with standard diet enriched with 40% kcal from bovine fat, high fat diet group (HFD) (n=10/group). Results: Mice receiving HFD showed a significant lower percentage of maximum glucose clearance in the insulin test tolerance (7.14±2.94) than C (16.18±7.53) $p<0.05$; and significantly increased the weight of epididimal fat (% relativized to body weight) (3.14±0.71) respect to C (1.26±0.35) $p<0.0001$. Biochemical parameters (mean±SD, mg/dL) of HFD showed higher values of glycemia (133.4±32.9), triglycerolemia (104.5±39.05) and cholesterolemia (203.1±61.92) than control animals: (114.7±27.63)

p<0.05; (77.73±39.54) p<0.05; (110±25.32) p<0.0001, respectively. The activity of both transporters was evaluated using the in vitro model of everted intestinal sacs, using non therapeutic substrates. Efflux of the Mrp2 substrate DNP-SG was decreased in HFD 54% respect to C, p<0.0001. In the same way, transport rate of the substrate rhodamine 123 by P-gp decreased of about 55% respect to C, p<0.05. The present study demonstrated that MetS-like conditions generated by a high fat diet decreased activities of both ABC-transporters, jejunal Mrp2 and ileal P-gp, altering the intestinal biochemical barrier against the external environment.

NEUROCIENCIAS / NEUROSCIENCE 1

256. (59) **RESVERATROL EXERTS ANTI-INFLAMMATORY EFFECTS IN MICROGLIAL CELLS AND PROTECTS THE FETAL CENTRAL NERVOUS SYSTEM IN A MURINE MODEL OF MATERNAL INFLAMMATION**

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Introduction: Fetal exposure to an inflammatory environment during the central nervous system (CNS) development produces a series of structural, metabolic and epigenetic changes resulting in long-term neurological and neuropsychiatric consequences in adulthood. Therefore, pharmacological interventions aimed at preventing maternal inflammation could reduce these changes frequently observed in premature infants. Importantly, microglia plays a central role in the CNS innate immune response.
Reveratrol, a polyphenol with anti-inflammatory and antioxidant properties, has demonstrated neuroprotective effects in several murine models.
Objectives: To study the possible neuroprotective effects of resveratrol in the fetal CNS in a model of maternal inflammation induced by bacterial lipopolysaccharide (LPS) and to characterize the signaling pathways involved in the modulation of the microglial response.
Experimental design: Resveratrol (30 mg/kg) was administered to Balb/c females on day 15 of pregnancy, exposed or not to two doses of LPS (0.17 mg/kg and 0.5 mg/kg, three hours apart). Five hours after the last administration of LPS, fetal brains were removed and frozen at -80°C until use. Additionally, primary cultures of microglial cells were carried out for in vitro signaling experiments.
Results: Our results show that the LPS-triggered maternal inflammation induces the mRNA expression of the chemokines Rantes and Mcp-1 (p<0.05 respectively), as well as the proinflammatory cytokines Il-1β and Il-6 (p <0.05 respectively) in the fetal CNS, with maternal administration of resveratrol preventing this induction (p<0.05).
Since microglia is the main effector of the CNS innate immune response, we studied whether resveratrol targeted these cells to counteract LPS-induced neuroinflammation. We observed that the in vitro treatment with resveratrol interfered with the activation of ERK1/2 MAPK, p38 MAPK (p<0.05 respectively) and NF-κB (p<0.05) signaling pathways.
Conclusion: Resveratrol exerts anti-inflammatory effects that protect the fetal CNS against maternal inflammation induced by LPS by inhibiting the signaling pathways triggered by the endotoxin in microglial cells

257. (66) **GALECTIN-3 EXERTS A PRO-DIFFERENTIATING AND PRO-MYELINATING EFFECT WITHIN A TEMPORAL WINDOW SPANNING PRECURSORS AND PRE-OLIGODENDROCYTES: INSIGHTS INTO THE ACTION MECHANISM**

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Galectin-3 (Gal-3) is a chimeric protein whose structure contains unusual tandem repeats of proline and short glycine-rich segments fused onto a carbohydrate recognition domain. Our previous studies using treatment of oligodendroglial precursors (OPC) with recombinant Gal-3 (rGal-3) during 5 days with renewal every two days showed enhanced differentiation and myelin integrity and function through signaling pathway and actin cytoskeleton modulation. The cytoskeleton is key in OLG maturation, as early OLG process extension requires dynamic actin assembly, while subsequent myelin wrapping correlates with actin disassembly, dependent on MPB expression. The aim of the present work was to evaluate rGal-3 time of action and myelination capacity in rGal-3-treated OLG. rGal-3 was administered as a single pulse at treatment day (TD) 0, 2 or 4. OLG treated with rGal-3 at TD0 or TD2 showed higher MBP area and IOD, Erk1/2 deactivation, an increase in pAkt and β-catenin and no changes in PDGFRα. Furthermore, rGal-3 increased the polymerized actin area, activated 4EB-P1 and increased gelsolin expression in both treatments. No significant changes were observed in MBP, Akt or 4EB-P1 in OLG treated at TD4, although Erk1/2 and 4EB-P1 phosphorylation levels slightly decreased and gelsolin expression increased. These results indicate rGal-3 exerts a pro-differentiating effect within a relatively short time window along OLG maturation, acting on OPC and pre-OLG. Axons simulated through aligned poly-lactic fibers were used to evaluate rGal-3-treated OLG myelination capacity. An increase in MBP IOD was observed upon rGal-3 treatment at TD5 and TD10, followed by a reduction in MBP area and F-actin area and IOD at TD15, indicating myelin compaction. Myelinated fibers were detected at TD15 with vehicle treatment but as early as TD10 with rGal-3 treatment, supporting our previous findings that rGal-3 accelerates OLG differentiation. Altogether, these findings indicate that rGal-3 enhances differentiation and myelination acting on OPC and pre-OLG.

258. (246) **OXYTOCIN INHIBITS INFLAMMATION IN LIPOPOLYSACCHARIDE-TREATED SPRAGUE DAWLEY MALE ADULT RATS**

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The hypothalamo-neurohypophysial system emerged as a component of neuroendocrine–immune network, wherein the oxytocin (OXT)-secreting system, plays an essential role. Recent data indicate that OXT regulates the immune response and have anti-inflammatory properties. Our previous studies provided evidence for the enhancement of OXT production and release following immune challenge. Also, we found that OXT reduced TNF-alpha release from glial cells cultured with an endotoxin.
In the present work, a systemic lipopolysaccharide (LPS)-treated acute inflammation rat model was used to study the suppressive effects of OXT against neuroinflammation. A peripheral injection of LPS was administered to evoke neuroinflammation in adult male Sprague Dawley rats. LPS was dissolved in sterile 0.9% saline vehicle. The LPS groups were i.p. injected with LPS (5 mg/kg). In the control group, rats were injected i.p. with vehicle. OXT groups were injected with OXT (10, 100 and 1000 ug/kg, sc) 15 min previous to vehicle or LPS administration. OXT 100 and 1000 ug/kg significantly (p<0.05) decreased TNF-alpha (ELISA) plasma levels 4 hours post LPS. Atosiban (3mg/kg, sc) an inhibitor of OXT receptors, administered 5 min prior to OXT significantly increased basal TNF-alpha plasma levels, exacerbates the LPS inflammatory effect and reversed the anti-inflammatory effect of OXT.
To evaluate neuroinflammation the hypothalami were removed from rats immediately after sacrifice and processed for PCR. The hypothalamic mRNA expression of TNF-alpha, IL1 beta, IL-6 and OXT receptor were determined. We showed that mRNA expression of all cytokines studied was increased after LPS (p<0.05) and that OXT (1000ugr/kg, sc) completely blocked this neuroinflammatory effect. Furthermore, atosiban induced a slight increase in basal hypothalamic TNF-alpha and IL-1 mRNA expression and blocked OXT anti-inflammatory effect.
Our study demonstrates an anti-inflammatory role of OXT since the hormone reduces systemic levels of TNF-alpha and the hypotha-

lamic expression of pro-inflammatory cytokines probably attenuating overactivation of glial cells.

259. (325) **GLIAL RESPONSE TO NUTRIENT DEPRIVATION IN EXPERIMENTAL MODELS OF ALZHEIMER'S DISEASE.**
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Alzheimer's disease is a neurodegenerative pathology clinically characterized by progressive and irreversible cognitive decline. Dietary restriction (DR) has been proposed as a possible neuroprotective strategy able to increase neurotrophic factors levels, enhance life span, promote autophagy and reduce oxidative stress. Our previous results demonstrated that transgenic animals modelling Alzheimer's disease and exposed to DR reverted cognitive impairment, showed a reduction of amyloid beta plaque load in the hippocampus and presented an increase in hippocampal neurogenesis. Our present objective was to evaluate the glial response to nutrient restriction in adult PDAPP-J20 transgenic (Tg) mice and in an in vitro approach. The in vivo experiment consisted in a periodic DR protocol alternating ad libitum periods (9 days) with DR periods (5 days, 60% daily intake) for 1.5 months, from 6.5 to 8 months of age. We found that microglial soma size, morphological parameter compatible with cell activation, was increased in Tg mice ($p<0.05$ vs. Control) and prevented by the exposure to DR. In vitro experiments showed that astrocytes exposed to fibrillar amyloid beta (fAb, 0.5 μM, 2 h) were activated (NFkB nuclear translocation, $p<0.05$) but not when pre-exposed to nutrient restriction (2% FBS, 6 h). Microglia incubated with conditioned medium proceeding from fAb-treated astrocytes showed activation (NFkB, $p<0.01$ vs. Control) but this effect was prevented if astrocytes where previously cultured in a nutrient-restricted condition. Finally, microglia did not show activation upon direct exposure to fAb. We propose that astrocytes would be able to sense amyloid and nutrient levels and to further elicit a response in microglia. Under this hypothesis, microglia would be indirectly responding to astrocyte signalling but not after direct exposure to fAb or nutrient restriction. Our results suggest that glial cells could play a role in the protective effects of nutrient deprivation in the context of Alzheimer's disease.

260. (345) **MYELINOGENESIS IS ACCOMPANIED BY AN INCREASE IN THE MRNA OF NEUROSTEROIDOGENIC MACHINERY IN THE CEREBELLUM OF POSTNATAL MICE**
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The novo synthesized steroids influence various important physiologic and neurotropic functions that occur in the brain during early postnatal development. Progesterone and its reduced metabolite allopregnanolone promote the dendritic growth, spinogenesis, and synaptogenesis of cerebellar Purkinje cells. In order to assess the influence of locally synthetized steroids on physiologic myelination, we studied the expression of the protein involved in the neurosteroidogenic rate-limiting step of cholesterol entry to the mitochondria, the steroid acute regulatory protein (StAR), the enzymes P450scc (cholesterol side-chain cleavage) and 5-alpha-reductase and myelin basic protein (MBP) mRNAs in the mouse cerebellum at postnatal day (P) 5, 18 and 35 by quantitative PCR. We found a pronounced increased in the expression of MBP mRNA at P18 and P35 compared to P5 ($p<0.001$ for both ages) confirming an important postnatal myelination period. A parallel change in the neurosteroidogenic proteins were observed, which showed a significant increase in StAR and 5-alpha-reductase mRNAs at P18 compared to P5 ($p<0.05$ for both parameters) and remained elevated at P35. An increase in the expression of P450scc mRNA was also observed postnatally reaching significance at P35 vs P5 ($p<0.05$). Our results suggest an influence of steroid hormones on white matter development in the cerebellum of postnatal mice. Further analysis of the expression of different steroid receptors will help to understand the role of steroids on the myelination process.

261. (429) **BDNF EFFECT ON MITOCHONDRIAL DYSFUNCTION INDUCED BY 3-NITROPROPIONIC ACID IN STRIATAL ASTROCYTES**
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Huntington disease (HD) promotes oxidative stress, mitochondrial dysfunction and neurotoxicity that primarily affect the striatum. 3-nitropropionic acid (3-NP), generates mitochondrial dysfunction and oxidative stress as it occurs in HD. High levels of reactive oxygen species (ROS) produced in the mitochondrial matrix generate oxidative stress which is associated with neuronal death. Uncoupling proteins (UCP) are proton transporters of the inner mitochondrial membrane that uncouple the electron transport chain from oxidative phosphorylation. UCP4, which is expressed in astrocytes, seems to be involved in the reduction of mitochondrial ROS levels and UCP4 overexpression protects neurons from mitochondrial dysfunction. We have previously shown that brain-derived neurotrophic factor (BDNF) reduces ROS levels and prevents cell death induced by 3-NP in cortical astrocytes. Now, we studied BDNF effect on striatal astrocyte viability, ROS production and UCP4 expression. We found that BDNF had a significant protective effect on 3-NP-induced death of striatal astrocytes determined by trypan blue exclusion ($p<0.01$). Also, BDNF reduces the increase in ROS levels induced by 3-NP in striatal astrocytes using a DCFH-DA assay ($p<0.001$). UCP4 protein levels were determined in cortical and striatal astrocytes by western blot. We show that, in both astrocyte populations, BDNF per se increased UCP4 expression ($p<0.05$) whilst 3-NP alone reduced it ($p<0.05$). In the presence of BDNF, 3-NP inhibitory effect on UCP4 expression is not observed. In conclusion, we show that BDNF protects striatal astrocytes from 3-NP toxicity reducing ROS levels and increasing UCP4 expression. These actions could represent new mechanisms of action of BDNF protection.

262. (755) **CHARACTERIZATION OF MÜLLER GLIA RESPONSE AFTER NITRO-OLEIC ACID TREATMENT**
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Proliferative retinopathies are among leading causes of irreversible blindness. Inflammation, oxidative and nitrosative stress are involved in the pathogenesis of diabetic complications, including retinopathy. Although, vascular endothelial growth factor (VEGF) inhibitors have been established as the mainstay of current treatment, the clinical benefits have not always been successful in preserving retinal function. In this regard, we hypothesized that Keap1/Nrf2 pathway can modulate the antioxidant response in neovascular retinopathies. For this reason, we used the activating Keap1/Nrf2 nitro-fatty acids (NO2-FA), which are important electrophilic signaling mediators with anti-inflammatory and cytoprotective activities. Due to Müller Glial cells (MGCs) are commanding survival and death in retina, we studied the effect of nitro-oleic acid (NO2-OA) in the human MGC line, MIO-M1. Initially, cell viability was assessed by MTT assays. MIO-M1 cells exposed to 0,1, 1, 2,5, 5 or 10 µM NO2-OA during 24 to 72 h showed no significant reduction in cell viability ($p<0.05$). Results are expressed as percentage of cell viability relative to 0.1% v/v vehicle (metanol). On the other hand, the ability of MIO-M1 cells to respond to a NO2-OA stimulus was evaluated through the increase of antioxidant enzymes such as hemo-oxygenase 1 (HO-1). MIO-M1 cells were stimulated with NO2-OA, and the expression of HO-1 was measured by WB at 8 and 16h post-stimulus. The results showed a significant increase of HO-1 with 5 µM of NO2-OA at 8h post-stimulus ($p<0.05$). To determine whether NO2-

OA could be beneficial for retinal cells against oxidative stress, we treated MIO-M1 cells with H2O2 (50-200 μmol/l). Preliminary results showed that NO2-OA reduced H2O2 damage. Thus, NO2-OA may act as an antioxidant protecting retinal cells from oxidative damage. At present, to corroborate this effect in an in vivo model, we are evaluating NO2-OA in an oxygen-induced retinopathy mouse model.

263. (731) **LONG-TERM ADAPTATION TO HYPOTONICITY IN HUMAN MÜLLER CELLS INVOLVES A DECREASE IN AQP4 EXPRESSION AND CELL PROLIFERATION**

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During neuronal activity, Müller cells are surrounded by a hypotonic environment, leading to cell swelling and consequently to a regulatory volume decrease (RVD) response. We previously demonstrated in human Müller cells (MIO-M1) that RVD depends on the efflux of KCl, Taurine and Glutamate, as well as water outflow through Aquaporin-4 (AQP4). However, adaptive changes to long-term hypotonicity (HT) are largely unknown. It was reported that AQP4 was also involved in cell growth in astrocytes, although the mechanisms were not described yet. The aim of this study was to correlate HT-related changes in gene expression triggered by MIO-M1 cell swelling with physiological processes necessary for long-term HT adaptation. Gene transcription of cells exposed to control or HT (ΔOsm:100 mOsm) for 30 minutes was compared using Affymetrix microarray for 10000 genes. Microarray data analysis was performed with GenArise software and DAVID Bioinformatics Resources Database. AQP4 expression was evaluated by Western Blot and cell proliferation by cell count in both experimental conditions. HT induced the upregulation (Z-score>2SD) of 241 genes and downregulation (Z-score<-2SD) of 264 genes. AQP4 gene expression was decreased after HT, which was also evidenced as a 40% decrease in AQP4 protein levels at 30 minutes and maintained for 60 minutes following HT. Genes participating in biological processes such as cell proliferation and differentiation, MAPK signaling (which participates in cell growth regulation) and cell adhesion were decreased. In line with this, MIO-M1 cell proliferation was decreased during adaptation to HT (doubling time in hours, control vs. HS: 39±2 vs. 50±4, n=3 experiments, p<0.05). We propose that the reduction of AQP4, the downregulation of genes involved in cell growth and the consequent impairment of cell proliferation induced by HT support the involvement of AQP4 in retinal physiological processes, such as water transport and cell plasticity, for maintaining retinal homeostasis under hypotonic conditions.

264. (409) **PARTICIPATION OF HIPPOCAMPUS IN THE NEUROPEPTIDE W REGULATION OF FOOD INTAKE**

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Neuropeptide W (NPW) is involved in the regulation of feeding behavior and energy homeostasis. The peptide and its receptor NPBWR1 are expressed in several regions of the brain, including the hypothalamus and hippocampus (Hi). Intracerebroventricular (ICV) administration of NPW can produce anorectic/orexigenic effect when is performed in the dark/light phase, respectively, and the hypothalamic injection stimulates food intake. The Hi is classically associated with learning and memory processes; however, recent studies implicate this structure in the regulation of food intake. Objective: to investigate the hippocampal role of NPW30 on feeding. Material and methods: male adult free-feeding rats were infused in the hippocampus with 0.5 µl per side, 1h after the onset of dark phase (feeding phase), with artificial cerebrospinal fluid (Control) and 24h later, with a single dose of NPW30 (0.3 or 3 nmol/µl), n≈13/dose. Food intake was registered 1, 4, 12 and 24 h after the infusions. Statistics: repeated-measures ANOVA and LSD *post hoc* test (significance: $P<0.05$). Results: the highest dose of NPW30 increased cumulative food intake at the 12h (NPW30, 3 nmol/µl: 20.73±1.48g vs. Control: 16.74±1.38g; $P<0.05$) and the 24h time points (NPW30, 3 nmol/µl: 26.82±1.00g vs. Control: 23.61±0.68g; $P<0.05$). We have previously found that intrahippocampal infusion of NPW30 in the light phase is anorectic. These results show an opposite behavior respect those obtained from the ICV or hypothalamic administration. Both anorectic and orexigenic effects are possible after hippocampal infusion of the peptide, depending on the time of the day that it is performed. This suggests that the influence of NPW30 on hippocampal feeding regulation could be modulated by circadian variable factors. Further investigations on the biochemical and physiological functions of NPW will help us to better understand the hippocampal role on feeding and energy homeostasis.

265. (605) **IGF1 GENE THERAPY ASSOCIATED WITH NANOTECHNOLOGY REVERSED OXIDATIVE STRESS IN AN ANIMAL MODEL OF TRAUMATIC BRAIN INJURY**

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Traumatic Brain Injury (TBI) is the major cause of morbidity and mortality in individuals under 40 years old. After the injury, neuroinflamation and oxidative stress (OS) are induced, leading to the development of many neurological deficits as well as reduction in the survival rate of critical trauma patients. Despite the efforts focused to develop anti-inflamatory and neuroprotective treatments, many of the pre-clinical studies failed to show significant effects, probably because the access to central nervous system of no steroidal anti-inflammatory drugs (NSAID) or steroids is limited. A therapeutic alternative of increasing clinical interest in the treatment of neurological deficits, is the use of neurotrophic factors such as Insulin-like growth factor 1 (IGF1), since they are neuromodulators associated with neuroprotection and anti-inflamatory effects. To highlight the pathophysiological effects of OS in rats with TBI, specific biomarkers have been studied such as advanced oxidation protein products (AOPP)-to identify the protein oxidation damage and malondialdehyde (MDA) -as a final product of lipid peroxidation. The aim of the present investigation is to elucidate the temporal course of OS related to neuroinflammation, and to test IGF1 therapy. For this purpose, magnetic nanoparticles-adenoviral vectors complexes (over-expressing IGF1) were administrated 15 min after TBI, via Cisterna Magna and magnetically redirected to TBI regions. The results showed an increase of AOPP and MDA at 60 min, 24 h and 7 days after TBI in the motor cortex, the prefrontal cortex, and hippocampus. Gene therapy significantly reduced AOPP and MDA levels in the studied brain areas, leading to similar values as control animals. In conclusion, IGF1 gene therapy associated with nanotechnology could be a valuable therapeutic approach for neuroinflammatory processes related to TBI. Further experiments will be performed in order to determine a correlation between OS parameters and behavioral deficits associated to TBI.

266. (802) **LOCOMOTOR SENSITIZATION TO COCAINE AND NICOTINE ENHANCED NICOTINE-CPP IN THE ADULT ZEBRAFISH**

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Locomotor sensitization is a convenient behavioral test to evaluate the effects of psychostimulants. Conditioning place preference (CPP) is an associative learning procedure to examine the rewarding properties of drugs. Since sensitization was not previously evaluated for nicotine and cocaine reward in zebrafish, we first analyzed sensitization to both drugs by exposing zebrafish five days (20 min-day) to either drug followed by five days of abstinence before a challenge dose to evaluate these drug effects on zebrafish

locomotor activity. Then, we selected the lower dose of nicotine that induced sensitization which interestingly cannot induce CPP. Locomotor sensitization was increased by 103% with nicotine and 166% with cocaine. Following this, sensitized zebrafish were trained using a two-chamber nicotine-driven CPP protocol. Cocaine-sensitized animals showed the highest score for the establishment of nicotine-CPP compared to previously nicotine-sensitized fish. Furthermore, detailed behavioral and molecular analyses confirmed these findings. The levels of nicotinic receptor subunits α7 and α6, but not β2, mRNA were increased in both nicotine- and cocaine-sensitized zebrafish. Only cocaine-sensitized zebrafish showed significant increases of the dopamine transporter (DAT). Nicotine-CPP but not control CPP showed similar values compared to sensitized animals for practically all the markers measured, suggesting that some specific markers are sensible to both processes. These findings suggest that doses of nicotine that can induce sensitization might be not enough to induce nicotine-place conditioning. On the other hand, suggest that previous exposure to low doses of drugs of abuse can increase subjects' sensitivity to the rewarding properties of drugs of abuse.

267. (143) **EPIGENETIC TRANSGENERATIONAL BEHAVIOURAL EFFECTS INDUCED BY TELLURIUM (TE) ADMINISTRATION IN MATURING RATS.**

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Previous evidence from our laboratory, showed that after systemic administration of K2TeO3 in non-toxic doses to pregnant mother and its litter rats, several behavioural parameters related to motivated and lateralized exploration in the offspring (F1) were affected. The objective of the present work was to determine if F2 generation still might be influenced by the previous Te administration to its parents. For this purpose, parent rats and its litters were exposed to K2TeO3 1.55 nM in drinking water (n=10;F1). Te treatment ended at 35 day-old of litters. After that, animals remained at rest until 90 day-old, when female rats were mated with normal males. Behavioural tests were performed when offspring reached 30 days of age (F2, n=10). Tap water administration was considered control (n=9). Behavioural tests performed at 30 day-old (F1 andF2) were: Double Lateral Hole-Board Labyrinth (LDHB) to register motivated lateralized exploration; Resident-Intruder Challenge (RIC), to register social behavioural parameters, and Forced Swimming (FS), to register survival motivation. Experiments were videotaped and behavioural activity recorded by a digital automatic counter in Counts/3min (C/3m). Results showed in F2 generation: loss of lateralized exploration in a similar way that in F1 parents (34±5.1, left Vs. 36±3.4, right C/3m, F2, n.s.; 50,5±4.7, left Vs 46,5±7.1 right C/3m, F1, n.s.; LDHB), increased latency to confront the intruder animal (45±13 Vs 15.5±1.5 C/3m, F2 Vs Control, p<0.01, RIC), and decreased active swimming (158±7.1 Vs 188±16 C/3m, F2 Vs Control, p<0.01, FS). In conclusion, Te treatment on F1 animals extended to its progeny, supporting the epigenetic action of Te.

ONCOLOGÍA / ONCOLOGY 5

268. (272) **HEMATOLOGIC TOXICITY ANALYSIS OF METRONOMIC CHEMOTHERAPY IN PEDIATRIC PATIENTS WITH ADVANCED SOLID TUMORS**

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Metronomic chemotherapy (MCT) is a novel approach for treating cancer; it consists in the chronic administration of low doses of conventional chemotherapy drugs, without prolonged drug-free periods. It was originally conceived to overcome drug resistance, targeting the tumor blood vessels rather than the tumor cells. Recent clinical studies have demonstrated the ability of MCT to control disease and improve life quality in children with different types of advanced cancer that do not have other therapeutic options available. Our objective was to study hematologic variables in children with relapsed/resistant/high risk solid tumors treated with MCT in order to test our hypothesis of lack of severe hematologic toxicity caused by the treatment. Complete Blood Count (CBC) results from ten patients (6 boys and 4 girls, mean age 10,9 years, SD: 5,7) were analyzed. Samples were obtained before, and every 8 weeks during 6 months of treatment with 1) cyclophosphamide (25mg/m^2/day, PO) / vinblastine(3mg/m^2/week, IV) or 2) cyclophosphamide (25mg/m^2/day, PO) / vinorelbine (25mg/m^2 day 1-8-15, IV) or 3) etoposide (50mg/day, 28 days cycles; 21 days YES, 7 days NO, PO). Data was compared with the CTCAE v. 5. CBC values showed no statistical differences during treatment in RBC (P=0.99), hemoglobin (P=0.87), hematocrit (P=0.87), Mean Corpuscular Volume (P=0.89), platelets (P=0.97), WBC (P=0.99) and neutrophils (P=0.99), after six months of treatment. Interestingly, a high percentage of patients had normal hematologic values; moreover, there were no grade 3 toxicities. We conclude that MCT with cyclophosphamide/vinblastine, cyclophosphamide/vinorelbine or etoposid administered to pediatric patients with advanced oncological diseases is safe, from the hematologic point of view. The lack of toxicity, which leads to a good quality of life and avoids additional treatments, together with the expected therapeutic effect, supports its use in pediatric cancer.

269. (212) **ANTITUMOR ROLE OF HEME OXYGENASE-1 IN BREAST CANCER**

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It has been reported that HO-1 can translocate to multiple subcellular compartments and can have non-enzymatic signaling roles. Thus, in the nucleus the protein may act as a transcriptional co-regulator protein and may bind and modulate other important proteins. HO-1 is an enzyme involved in cellular responses to oxidative stress and has also been shown to regulate processes related to cancer progression. In this regard, HO-1 has been shown to display a dual effect with either antitumor or protumor activity, being this also true for breast cancer (BC). In this work we intended to address this discrepancy regarding the role of HO-1 in BC. HO-1 was detected in human BC tissues, and its protein levels correlated with reduced tumor size (p=0.046) and longer overall survival time of patients (p=0.004). Contrariwise, nuclear localization of HO-1 correlated with higher tumor grade (p=0.05). However, nuclear HO-1 was not significantly associated to patient overall survival time (p = 0.13). In vivo experiments showed that both pharmacological activation and genetic overexpression of HO-1 reduced the tumor burden in two different animal models of BC. Furthermore, the activation of HO-1 in several BC cell lines reduced cellular viability by inducing apoptosis (p<0.05) and cell cycle arrest (p<0.003) and decreased the cellular migration, invasion and adhesion rates by modulating pathways involved in the epithelial-mesenchymal transition. Furthermore, HO-1 activation impaired in vivo the metastatic dissemination (p=0.020). In concordance, HO-1 expression associated with reduced number of lymph node metastases (p=0.0243) and higher levels of E-cadherin (p=0.0026) in human BC. In addition, the enzymatic activity of HO-1 in nuclear and cytoplasmic fraction was studied by ICP-AES. In conclusion, we demonstrate that HO-1 displays antitumor activities in BC. Furthermore, our studies suggest that HO-1 subcellular localization may explain the differential effects observed for the protein in different tumor types.

270. (355) **ROLE OF HEME-OXYGENASE 1 IN THE CELLULAR METABOLISM OF PROSTATE CANCER**

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Deregulation of cellular energetics has become one of the hallmarks of cancer evidenced by the numerous connections between signaling pathways that include oncoproteins and key metabolic enzymes. Heme Oxygenase-1 (HO-1) is a cellular homeostatic regulator counteracting oxidative and inflammatory damage. We previously showed that HO-1 has an antitumoral activity in prostate cancer cells. It inhibits cell proliferation, migration, tumor growth and angiogenesis. The aim of this project was to further study the role of HO-1 on the energetic metabolism of prostate cancer cells.
In earlier studies, we demonstrated a significant reduction in ATP production and oxygen consumption rate in PC3 cells (derived from a metastatic prostate tumor) after treatment with hemin (inducer of HO-1 expression and activity) 80 μM for 24h. These results confirmed a negative regulation on the metabolic rate.
In this work, in order to further analyze the regulation of cell metabolism by HO-1, we studied glucose uptake in PC3 cells. We found lower glucose uptake in cells treated with hemin (20.32 vs 3.52 fmol/cell/min; $p<0.0001$). We also inferred the number of mitochondrias by the quantification of mtDNA by qPCR and analyzed mitochondria integrity by flow cytometry using the TMRE dye. Neither the number nor integrity showed significant changes as a result of the hemin treatment. In addition, we analyzed the expression of key genes involved in metabolic pathways and cancer progression. HO-1 induction downregulated *LDHA* (FC=0.5; $p<0.05$) while it did not alter the expression of *PKM2*, *ACO2* and *PDHB*.
In conclusion, our results showed that HO-1 might be involved, at least in part, in the reprogramming of the metabolic state of PC3 cells, which might favor the establishment of a less aggressive phenotype of the disease.

271. (289) **STUDY OF THE ROLE OF P300 IN THE DEVELOPMENT AND PROGRESSION OF BREAST CANCER**

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Breast cancer (BC) is a heterogeneous disease with many subtypes that has different treatment responses and clinical outcomes, suggesting the need to find new molecular markers. We have previously shown that pharmacological inhibition of p300 displays antitumor activity in LM3 and MDA-MB-231 BC cell lines and in their respective murine models. Through genetic silencing of p300 in MDA-MB-231 cells we also demonstrated a decrease in cellular viability, migration, invasion and adhesion. We also showed that p300 silencing decreases cellular viability in LM3 cells and reduces the primary tumor growth in its syngeneic murine model. However, the role it plays in the metastatic process remained unknown. Therefore, in this work we aimed to study the effect of genetic silencing of p300 on tumor progression and invasion in LM3 cells and its syngeneic murine model. We obtained LM3 cells stably-overexpressing a shRNA for p300 (LM3-p300NEG) or its control plasmid (LM3-CTRL). The reduction in p300 levels was confirmed by RT-qPCR. We observed reduced cellular migration (wound healing), invasion (transwell with matrigel), adhesion (adhesion to the substrate) and an increase in the levels of E-cadherin and ß-catenin (WB) in LM3-p300NEG compared to LM3-CTRL ($p<0.05$). In the murine model we observed significant reduction in the tumor burden and in the number of lung metastases in mice injected with LM3-p300NEG compared to mice injected with LM3-CTRL ($p<0.05$). In the primary tumors belonging to LM3-p300NEG-inoculated mice, an increase in the expression of E-cadherin, Elf5, ß-catenin and a decrease in p300 were detected when compared to LM3-CTRL-inoculated mice (IHQ, $p<0.05$). We also observed nuclear and cytoplasmic localization of ß-catenin in LM3-CTRL tumors compared with LM3-p300NEG tumors in which only cytoplasmic localization was observed. In conclusion, these results show a protumor activity of p300 in BC, carried out at least in part by modulating tumor invasion, migration and adhesion.

272. (303) **INTEGRIN-SPECIFIC ACTIVATION OF RHO GTPASES, THEIR ROLES IN MECHANOSIGNALLING AND CANCER**

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Mechanotransduction is mediated by the integrin family of cell adhesion receptors. Integrins bind cell extracellular matrix proteins and connect to the F-actin cytoskeleton and non-muscle-myosin inside the cells. Using genetically engineered cells, biochemical assays, in combination with mass spectroscopy (MS), traction force microscopy and micropatterns, we observed that α5β1-integrin expressing cells promote the formation of small nascent adhesions, low RhoA activation and high force, while αVβ3-integrin expressing cells showed large focal adhesions connected to contractile stress fibers (SFs), resulting in high RhoA but low force. To further analyze pKO-cells phenotypes, we looked for specific RhoA activators (GEFs). We performed a MS-proteomic analysis and amongst the interesting hits was GEF-H1, together with biochemical assays we observed that GEF-H1 activation is dependent on a specific integrin-class suggesting that integrins may activate specific GEFs during adhesion, migration and invasion. Recent studies have also shown that an increase in GEF-H1 expression correlates with an increase in tumor progression and metástasis. In addition, GEF-H1 is involved in the cross-talk between microtubules and the actin cytoskeleton. Our data shows that GEF-H1 is localized in the cytoplasm and more active in αVβ3-cells when compared to α5β1-cells, where GEF-H1 is in an inactive state bounded to the microtubules. Similar results we observed in breast cancer cells depending their invasiveness and the integrin-class-expression. These results could explain the increase in SFs formation in αVβ3-cells and RhoA activation. GEF-H1 can be released to the cytoplasm either by microtubule depolymerization or by protein phosphorylation. A phosphoenrichment-label-free MS analysis revealed that GEF-H1 is highly phosphorylated in αVβ3-cells. Furthermore, using integrin-tail pull-down and MS assay, we observed that GEF-H1 binds to β3-integrin tail. These results show for the first time that GEF-H1-RhoA activation is αVβ3-integrin dependent and it can mediate the signaling involved in controlling cell structure, force generation, migration and invasion.

273. (284) **TNF CONTRIBUTES WITH RAC3-INDUCED MALIGNANT TRANSFORMATION**

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RAC3 is a coactivator of steroid receptors and transcription factors and an important oncogene in tumor development. We have previously demonstrated that inflammatory cytokines increase the RAC3 expression and that high levels of this molecule could transform non-tumor cells into cancer stem cells. The aim of this work was to investigate if the inflammatory cytokine TNF could contribute to RAC3 transforming effect, maintaining or increasing stem prop-

erties. HEK293 cells (human embryonic kidney) overexpressing RAC3 (tumor) or not (non-tumoral) and other tumor cell lines (HeLa and T47D, silencing or not RAC3) were stimulated with TNF (10 ng/ml) or vehicle and analyzed for their mesenchymal properties, migratory, invasive behavior and signals that contribute to the stem phenotype. We found that TNF potentiated the RAC3 overexpression effects, increasing the mesenchymal phenotype, through the decrease of E-cadherin ($p<0,05$), increase of Vimentin ($p<0,05$) (both by WB) and SNAIL (qPCR) ($p<0,05$) respect to cells overexpressing RAC3 without TNF stimulus. It also increased migration capacity (wound assay) ($p<0,05$) and metalloprotease production (zymography) ($p<0,05$). In addition, TNF induced the nuclear translocation of β-Catenin (IF), and also, the transcriptional activity of TCF-β-Catenin (Luciferase reporter assay) ($p<0,05$) under high expression of RAC3. All these actions were significantly decreased by sulfasalazine, an inhibitor of IKK. Our results demonstrate that TNF potentiates the transforming action of RAC3 overexpression, contributing to increase the mesenchymal phenotype and transduction signals of NF-κB and β-Catenin- dependent, both involved in the preservation of cancer stem cells. Therefore, our results suggest that inflammatory microenvironment could contribute to the initiation and propagation of tumors, increasing the expression of RAC3 and then enhancing its biological action.

274. (183) **EFFECTS OF H4 HISTAMINE RECEPTOR LIGANDS ON THE MIGRATORY CAPACITY AND MAMMOSPHERE FORMATION IN BREAST CANCER CELLS: PHOSHO-SRC INVOLVEMENT.**

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The inhibitory effect on cell proliferation exerted by the selective H4 histamine receptor (H4R) agonists in breast cancer is well documented. However, we reported that histamine (HA) in low doses as well as the HR4 agonist VUF8430 enhances the gelatinolytic activity, cell migration and invasion in MDA-MB-231 breast cancer cells. Recently, we also demonstrated that HA dose-dependently modulates the radio-induced epithelial to mesenchymal transition (EMT) events in mammary tumor cells via Src phosphrorylation. Evidence shows that cells undergoing EMT acquire characteristics of cancer stem cells (CSCs) which may be involved in radio- and chemoresistance, tumor recurrence and cell spreading.
We proposed to investigate the action of H4R ligands on the migratory capacity and mammosphere formation in MCF-7 and MDA-MB-231 cells, the Src involvement and a potential link between EMT and CSCs.
MCF-7 and MDA-MB-231 cells were treated with H4R agonists (10 μM), the H4R antagonist JNJ7777120 (10 μM) and the selective Src inhibitor PP2 (2 μM). We performed cell migration assays using transwell units, Western blot to evaluate phospho-Src levels, indirect immunofluorescence for the EMT transcription factor Slug and one of the Stem cell markers (CD44), and a functional assay for CSC (mammosphere formation). Results showed that H4R agonists significantly increase MCF-7 and MDA cell migration ($p<0.05$), phospho Src levels ($p<0.05$ and $p<0.01$) and mammosphere formation $p<0.05$). These effects were blocked by JNJ7777120 or PP2 ($p<0.01$). H4R agonists increased the number of cells with nuclear Slug, with CD44 expression ($p<0.01$) and with simultaneous expression of Slug and CD44 ($p<0.05$) suggesting a possible link between EMT and CSCs.
In summary, our results confirm the stimulatory action of HR4 agonists on breast cancer cells migration and CSCs. It is a challenge for oncologists to improve cancer treatments finding out drugs that target not only tumor bulk populations but also CSCs

275. (182) **SOLUBLE GUANYLYL CYCLASE ALPHA1 SUBUNIT MEDIATES CELL PROLIFERATION, SURVIVAL AND MIGRATION IN HUMAN CERVICAL TUMOR CELL LINE HELA**

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Soluble guanylyl cyclase is an heterodimeric enzyme composed by two subunits, alpha1 (a1) and beta1 (b1). Previously we have shown that a1 increased levels strongly correlate with E2-induced proliferation in E2-depentent tissues and a1 knock-down decreased cell proliferation in estrogen-responsive endometrial and breast cancer cell lines. a1 role in tumor progression is widely unknown, however it was shown that a1 blocked p53 in some tumor prostate cell lines. The aim of the present study was to investigate the role of a1 in proliferation, survival and migration in the E2-unresponsive, p53-defective human cervical tumor cell line HeLa.
a1 expression was silenced through siRNA specific sequences using scramble sequences as control. Protein levels were measured by western blot. Apoptosis and mitosis were assessed by nuclear morphology (Hoechst). Apoptosis were measured by Annexin-V/PI staining. Cell cycle was studied by flow cytometry. Migration was determined through scratch motility and transwell assays.
a1 knock-down (a1KD) significantly reduced PCNA expression (20% decrease vs control, $p<0.05$). a1KD did not modify cell cycle distribution but increased subG1 cell population (+41% vs. control). Nuclear staining confirmed that a1KD decreased mitotic index (-66% of control, $p<0.05$) and increased apoptotic index (+275% of control, $p<0.05$), further confirmed by Annexin-V/PI positive cells (control: 3.35%; a1KD: 6.16%). Additionally, a1KD inhibited cell migration measured by wound closure (-33% of control, $p<0.05$) and transwell assay (-31.03% of control, $p<0.05$).
Our results show for the first time that a1 promotes tumor cell proliferation and migration in HeLa cells through mechanisms independent of estrogen signalling pathway and p53. The role of a1 in E2-independent cancers or those with acquired hormone-resistance or early loss of p53 need to be exhaustively studied.

276. (403) **CONCORDANCE BETWEEN THE PROGESTERONE RECEPTOR ISOFORM RATIO IN PRIMARY BREAST CANCER AND IN MATCHED AXILLARY METASTASIS**

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Two thirds of breast cancer (BC) patients express estrogen receptors alpha (ERα) and progesterone receptors (PR). Antiprogestins may become a therapeutic approach to treat patients with PR+ tumors.
The PR is expressed as two isoforms, isoform A (PRA) and B (PRB). Our lab demonstrated that the antiprogestin mifepristone (MFP) induced tumor regression in experimental models with PRA levels higher than those of PRB but it may increase the growth of those with the opposite profile.
In our ongoing clinical trial (MIPRA NCT02651844), the inclusion criteria to select patients to receive MFP treatment is based on the PR isoform ratio evaluated in the biopsy. Since endocrine treatment is administered as an adjuvant treatment to target undetectable metastatic foci, we decided to evaluate the concordance of PR isoform ratio between primary tumors and axillar metastases.
Matched primary tumors and axillary metastasis were obtained at surgery from BC patients from the Magdalena V Martinez Hospital, Buenos Aires (22 pairs). Samples were categorized by Western Blot (WB) as PRA-H (PRA high) when PRA/PRB ≥ 1.2, PRB-H (PRB high) when PRA/PRB ≤ 0.83, equimolar (EQUI) when ratios were between 1.2-0.83, and PR negative (NEG). The percentage of PR+ cells was determined by Immunohistochemistry (IHC).
When comparing the PR isoform ratio of the 22 pairs a 91% of concordance and a Kappa Coefficient of 0,85 ($N= 22$, $p< 0.01$) was found. The primary tumors of the two discordant pairs were categorized as PRB-H while the metastasis was PRA-H or NEG.
In 43/44 samples PR status correlated in WB and IHC studies (Kappa= 0,91; $p< 0.01$).

We conclude that similar PR isoform ratios are observed in primary tumors and in their axillary metastases. MFP might be used as an effective adjuvant treatment in PRA-H BC patients together with standard endocrine therapy.

277. (494) **DECIPHERING HEME OXYGENASE-1 ROLE IN HEAD AND NECK CANCER**

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Head and neck squamous cell carcinoma (HNSCC) is a remarkably heterogeneous disease due to phenotypic, aetiological, biological and clinical characteristics with around 50% mortality, which has propelled research for new approaches to improve patient survival. We previously reported that heme oxygenase-1 (HO-1) protein is up-regulated in human HNSCC samples and that it is localized in the cytoplasmic and nuclear compartments; additionally, we demonstrated that HO-1 nuclear localization is associated with malignant progression. In this study, we aim 1) to further evaluate the prognostic utility of HO-1 expression as well as 2) to begin to elucidate which role does HO-1 play in HNSCC pathophysiology. To address our aims we used bioinformatics analysis as well as in culture assays using human HNSCC primary cultures and cell lines. Using in silico analysis from TCGA (N=588) we found that in early stages of HNSCC high expression of HO-1 was associated to decreased overall survival (p<0,05) and relapse free survival (p<0,01) whereas in late stages high expression of HO-1 was associated to longer overall survival times. Also, association analysis between HO-1 and HNSCC risk factors showed lower HO-1 expression in HPV (-) tumors than HPV (+) ones (p<0,05). We subsequently evaluated HO-1 pharmacological activation (hemin) on cell viability at different doses (20-80 μM) and at 48 h and 72 h, by manual cell count and crystal violet staining. We first studied hemin effect on the human SCC cell lines HN12 and HN13 and then studied HO-1 activation on a human skin SCC (stage IV) mixed primary culture, composed by tumor epithelial cells and fibroblasts. In both cell systems, our preliminary findings showed that hemin treatment increases cell viability (p<0,01). Although more studies are being carried out, our results suggest an association between HO-1 expression and patient survival time that depends on disease stage.

278. (100) **INTRATUMOR HETEROGENEITY INDEX OF BREAST CARCINOMAS BASED ON DNA METHYLATION PROFILES**

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Background. Cancer cells evolve and constitute heterogeneous populations that fluctuate in space and time and are subjected to selection generating intratumor heterogeneity. This phenomenon is determined by the acquisition of genetic/epigenetic alterations and their selection over time which has clinical implications on drug resistance.

Methods. DNA extracted from different tumor cell populations (breast carcinomas, cancer cell lines and cellular sub-clones) were analyzed by MS-MLPA. Methylation profiles were used to generate a heterogeneity index to quantify the magnitude of epigenetic heterogeneity in these populations.

Results. The study of methylation profiles of 23 fresh breast carcinomas revealed heterogeneous allele populations in these tumor pieces. With the purpose to measure the magnitude of epigenetic heterogeneity, we develop a heterogeneity index based on methylation information and observed that all tumors present their own heterogeneity level. Applying the index calculation in pure cancer cell populations such as cancer cell lines (MDA-MB 231, MCF-7, T47D, HeLa and K-562), we also observed epigenetic heterogeneity. In addition, we detected that sub-clones obtained from MDA-MB 231 cancer cell line diverged from the initial population over time and generated their own new heterogeneity without selective pressure. Using epigenetic information derived from TCGA tumors, we determined that the heterogeneity index correlated with prognostic and predictive factors like tumor size (p=0.0088), number of affected axillary nodes (p=0.007), estrogen receptor expression (p<0.0001) and HER2 positivity (p=0.0007). When we analyzed molecular subtypes we found that they presented different heterogeneity levels. Interestingly, we also observed that all mentioned tumor cell populations shared a similar HI mean.ConclusionsEach tumor presents a unique epigenetic heterogeneity level, which is associated with prognostic and predictive factors. We also conclude that tumor subtype's behavior could be described in terms of epigenetic heterogeneity, which could serve as a new contribution to understand the different prognosis of these groups.

279. (745) **ANTIOXIDANT EFFECTS OF FIBRE MICROPARTICLES OBTAINED FROM JAPANESE PLUM (PRUNUS SALICINA) IN COLORECTAL EPITELIUM HUMAN CELLS**

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After human consumption, certain polyphenols can exert cytoprotective actions in the gastrointestinal tract, interfering with the oxidative stress implicated in inflammatory disorders. Discards obtained from the food industry can be used as important sources of bioactive compounds.In this context,fibremicroparticles (MPCs) obtained fromthe skin of Japanese pulms(Prunussalicina)were evaluatedin the antioxidant effects of theirpolyphenolsonthecolorectal epithelium (Caco-2) humancells before and after a digestive process.Anin vitrodigestion method was carried out with MPCs,accordingto an international consensus standardized static digestion method(Minekus et al 2014).Briefly, 1g MCPs was treated withbuffers resemble to gastric andintestinal pH and digestive enzymes (alpha amylase, pepsin y pancreatin).The polyphenoliccomposition(mg/1g)determined in MCPsby HPLCbefore digestionwascyanidin3-galactoside 0.085; cyanidin3-rutinoside 0.036, quercetin3-galactoside + quercetin3-rutinoside 0.048; quercetin3-xyloside + quercetin3-rhamnoside 0.036 and proanthocyanidns2,000 , while after digestionquercetin3-galactoside + quercetin3-rutinoside 0.30; quercetin3-xyloside + quercetin3-rhamnoside 0.26; and proanthocyanidins0.33 were determined. Caco-2cell viability was evaluated through MTT assay. Cells treated for 24 h with 3 to 100 μg/mlof extract presented a CC50>100 μg/mL.In order to assesst-BOOH induced cellular oxidation through a DCF assay, caco-2 cells were incubated with an ethanolicextract obtained from MCPs or an ethanolicextract fromdigestedMCPs. When Caco-2 cells were co-incubated with 0.5 to 10μg/mL of polyphenols obtained from MCPs or digestedMCPs, the t-BOOH induced oxidative stress was significantly reduced (p<0.001), showing a concentrationdependence, reaching 100% of protection with 10 μg/mL polyphenols. Therefore, polyphenol extracts showed a protective effect against the oxidative stress before and after the digestion process. Phenolics present in plum MPCs can be useful as antioxidantsagainst the oxidative stress implicated in inflammatory bowel diseases.Financialsupport: UBA , PICT 2015-1603

FARMACOLOGÍA / PHARMACOLOGY 2

280. (464) **CROSSTALK BETWEEN GLUCOCORTICOID AND HISTAMINERGIC SIGNALING SYSTEMS IN NEUROINFLAMMATORY CONTEXTS.**

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UBA-CONICET

The relationship between neuroinflammation and neurodegenerative disease has been extensively documented, pointing to glial cells

as crucial players in neuroinflammation. We have shown before a crosstalk between histamine H1 receptor (H1R) and glucocorticoid receptor (GR) signaling that could have specific impact on (neuro) inflammatory conditions. The objective of the present work was to evaluate this signaling interaction in a neuroinflammatory context. In a first stage, we used the BV2 murine glial cell line. Using this system, we aimed to set an in vitro neuroinflammatory model by treating the cells with 1μg/μl of lipopolysaccharide (LPS) and evaluating the induction of the inducible nitric oxide synthase (iNOS) gene expression. Pretreatment with 1nM of the synthetic glucocorticoid dexamethasone (DEX) reduced LPS-induced iNOS response to 50%, while co-incubation with the antihistamines chlorpheniramine and diphenhydramine enhanced DEX-induced iNOS reduction to 65 and 90%. To extend these results to a human glial cell model, we used hiPSC-derived astrocytes. In this system we modeled neuroinflammation using synthetic beta-amyloid oligomers (Aβ). Treatment with 1μM of Aβ resulted in an induction of TNF-α gene expression. Pretreatment with 10nM DEX showed a significant reduction of TNF-α response to Aβ. Co-incubation with 10μM of the antihistamines mepyramine and triprolidine resulted in a 2 and 3-fold enhancement of DEX effect respectively. This enhancement was not induced by a different antihistamine, chlorpheniramine. We conclude there is a ligand specific interaction between H1R signaling and GR transcriptional activity that can have pharmacological impact on neuroinflammatory contexts where glial cells play a central role. This work was partially supported by a Boehringer Ingelheim Fonds travel grant awarded to CDZ.

281. (507) **MODULATION OF GLUCOCORTICOID RECEPTOR ACTIVITY BY HISTAMINE H2 RECEPTOR SIGNALING. INVOLVEMENT OF PI3K AND MTOR.**

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There are reports describing the interaction between membrane G-protein coupled receptors (GPCRs) signaling and glucocorticoid receptor (GR) transcriptional activity. The aim of the present work was to study the modulation of GR activity by histamine H2 receptor (H2r) signaling. HEK293T cells were co-transfected with plasmids coding for H2r, GR and a GR-driven reporter gene TAT3-Luc. In this system, 10 minutes pretreatment with 10μM H2r agonist (amthamine) significantly increased GR activity, duplicating dexamethasone-induced signal ($p<0.05$). To study the mechanism of such interaction, we co-incubated the cells with different signaling inhibitors. When cells were exposed to G-protein βγ inhibitor gallein, PI3K inhibitor wortmannin or mTOR inhibitor rapamycin, dexamethasone response was increased to levels achieved in presence of amthamine. Interestingly, co-treatment of amthamine with dexamethasone and any of the inhibitors mentioned above did not enhanced dexamethasone-induced GR activity. Consistently, as previously described in HEK293T cells, H2r agonist reduced the levels of phosphorylation of the PI3K substrate AKT, phospho-mTOR and its target phospho-S6K. The whole of these results shows that PI3K/AKT/mTOR pathway has an inhibitory effect on dexamethasone-induced GR activity, and that H2r agonist amthamine potentiates GR transcriptional activity inhibiting this signaling path. Considering the co-expression of H2r and GR in several physiological systems and the widespread use of their ligands, the interaction described herein could have an impact in glucocorticoid based therapy and grants further research.

282. (541) **BIASED AGONISM AT HISTAMINE H1 RECEPTORS**

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GPCRs (G-protein coupled receptors) exist as conformational collections in which different conformations lead to differential downstream behaviours such as G-protein activation, receptor phosphorylation or internalization. In this context, a ligand may cause differential activation of some, but not all, of the signaling events associated to a particular receptor and would lead to biased agonism. On the other hand, antihistamines used clinically as antiallergics rank among the most widely prescribed and over-the-counter drugs in the world. The aim of the present study was to investigate whether widely used histamine H1 receptor (H1R) ligands that exert therapeutic actions by blocking the effects of histamine, due to null or negative efficacy towards Gαq-phospholipase C (PLC)-inositol triphosphates (IP3) and Nuclear Factor-kB cascades, could display positive efficacy concerning receptor desensitization or internalization. We used A549 cells, derived from human lung epithelium, endogenously expressing the H1R. Pretreatment of A549 cells during 10 minutes with 1, 3, 10 and 33 μM of chlorpheniramine and triprolidine prevented the increase of cytosolic Ca2+ levels evoked by 100 μM of histamine suggesting that both ligands may promote H1R desensitization. On the contrary, pretreatment with diphenhydramine did not modify the H1R response to the agonist. To examine the mechanisms involved in these desensitizations we transfected A549 cells with GRK2 and dynamin dominant-negative mutants. Our results indicate that although these mutants potentiate calcium response to histamine and partially impaired histamine induced H1R desensitization they did not revert chlorpheniramine nor triprolidine induced desensitization. Finally, preliminary results of saturation-binding assays suggest that some of these ligands may also lead to receptor internalization. In conclusion, H1R desensitization and/or internalization promoted by these ligands demonstrate their biased nature and could explain their undesired effects. Accordingly, this study contributes to a correct classification, providing evidence for a more rational and safe use of antihistamines.

283. (546) **PRECLINICAL PHARMACOLOGY STUDY OF RATIONALLY DESIGNED GRK2 INHIBITORS**

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GPCR kinase 2 (GRK2) plays a major role in GPCRs desensitization and has been extensively validated as an effective target for heart failure (HF) treatment. Its overexpression is associated to disease progression due to the lack of cardiac BAR responsiveness. Accordingly, we have previously obtained four compounds (C2, C3, C4, C5) that exert significant in vitro GRK2 inhibitory activity, postulating them as suitable candidates for in vivo testing. However, there are several risks inherent in preclinical drug discovery that might lead to drugs attrition in late stages. Therefore, the objective of this work was to identify potential early failures of our hits before reaching in vivo phases.

To achieve this, we evaluated their ADMET (Absorption-Distribution-Metabolism-Excretion-Toxicity) properties. As a lipophilicity descriptor, experimental logP was obtained by RP-HPLC. Hits cytotoxicity was assessed in U937 and HepG2 cells by trypan blue exclusion test after 48hs treatment. Even though all compounds exhibited an appropriate lipophilicity, with logP values ranging from 1 to 3, compounds C3 and C5 stood out as they did not affect cellular viability, while C2 presented an EC50=10,5μM and C4 an EC50=17,2μM. Moreover, GRK2 desensitizes GPCRs that couple to different G-proteins. Since compounds that specifically potentiate cAMP could be of interest for HF treatment, we compared their ability to increase responsiveness of GPCRs that couple to different G-proteins. Initial cell-based screening assays proved that the hits increased cAMP response of H2R (histamine type 2 receptor). Nonetheless we observed that compound C5 also increased histamine-stimulated intracellular calcium release in A549 cell line endogenously expressing H1R (histamine type 1 receptor), revealing an undesirable promiscuous behavior.

In conclusion, we applied strategies to mitigate the risks of drug attrition in late phases of clinical trials, increasing the confidence in our candidate compound C3 for proceeding to HF animal models research.

284. (130) **OPIOID-ASSOCIATED QT-INTERVAL PROLONGATION: EFFECTIVENESS OF DATA MINING TECHNIQUES OF THE PUBLIC VERSION OF THE FDA ADVERSE EVENT REPORTING SYSTEM (FAERS) FOR EARLY ADVERSE DRUG REACTION SIGNAL IDENTIFICATION.**

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INTRODUCTION: the prolongation of the QT interval is a serious and potentially fatal adverse reaction that has led to the discontinuation of many drugs (including some opioids). Data mining on pharmacovigilance databases can detect signals that identify early the risk associated with some drugs.
OBJECTIVE: To examine the association between opioids and risk of QT prolongation in reports submitted to the US Food and Drug Administration (FDA) Adverse Event Reporting System (FAERS) between 2004 and 2017.
METHODS: Relevant reports in the FAERS were identified and analyzed. The reporting odds ratio (ROR±IC95), Proportional ADR reporting ratio (PRR±IC95), Chi square (Yates' correction), and Yule's Q (Q±IC95) was used to detect spontaneous report signals, calculated using the case/non-case method. Cases were identified using Standard Medical Querry (SMQ) for QT Prolongation defined by MedDRA 21.0 in which opiods (Meperidine, Tramadol, Dextropropoxyphene, Methadone, Fentanyl, Morphine, Hydromorphone, Oxycodone, Buprenorphine, and/or Nalbuphine) were the suspected drug.
RESULTS: A total of 25885 drug-reaction pairs was found in 445627 opioid reports through 36.389.458 total reports. Significant Signal (ROR, PRR, Chi2, Q) were found for whole opioid group: ROR 1.30 (1.28-1.31), PPR 1.28 (1.27-1.30), Yule's Q 0.13 (0.12-0.14) and Chi2 With Yate's Correction: <0.000001. Analysis of individual opioids show significant signals for QT prolongation for each drug. The temporal evolution of the different signals according to the number of reports included from 2004 to 2017 shows early significant positivization of signals in the first 6 to 12 months.
CONCLUSIONS: Analysis of the FAERS database showed significant signals for QT prolongation with opioid treatments. Underlying mechanism is unknown, but it seems to be linked to hERG channel blocking. Proposed mining shows that statistical signals could warn of this risk in some drugs between 5 to 10 years before data from specific clinical studies, proposing an early tool to minimize adverse reactions.

285. (131) **IN-VITRO CARDIOVASCULAR SAFETY PHARMACOLOGY PROFILE OF TWO GRANULOCYTE COLONY STIMULATING FACTORS (G-CSF).**

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Guideline ICH S7B asks for in vivo and in vitro non-clinical evaluation to determine the arrhythmogenic potential prior marketing authorization of new compounds. For products in the market, such as some recombinant G-CSF, this evaluation is usually incomplete. Consequently, the aim of this work was to evaluate the effects of recombinant G-CSFs for cardiac risk in vitro. Using Langendorff technique, guinea pigs hearts were isolated and arterially perfused. The ECG and the left ventricular developed pressure (LVDP) were constantly recorded. After 50min-stabilization, hearts were exposed to increasing and cumulatively doses of filgrastim and lenograstim (10-30-100-400-800ng/ml), 10 min each. The corrected QT interval was calculated with Bazzet formula and expressed as value in milliseconds against the control (ΔQTc). The LVDP, maximum contraction and relaxation rates (+dP/dT and -dP/dT) were presented as percentage value respect to perfusion without drug. The inhibition of IKr was assessed using an automated patch clamp platform (SyncroPatch 384PE) on CHO cells that stably expressed hERG channels. All cells were recorded in the voltage clamp whole cell mode, in control condition and after the exposure to 800ng/ml of filgrastim. The hERG current amplitude was quantify at the peak tail current elicited by a +40mV pulse followed by a -40mV pulse. The inhibition of hERG current was expressed as percentage of block against the prior control condition. In isolated hearts, only filgrastim showed a significant increase in LVDP (+35 ± 13.3 %, $p<0.05$) and diminished of ΔQTc (-55 ± 6.5 ms, $p<0.05$) at 800 ng/ml. No significant differences regarding control were found in +dP/dT and -dP/dT for both drugs. The SyncroPatch study showed up a non-significant hERG current block after the exposure of a single dose of 800ng/ml of filgrastim. In conclusion, different electrical and mechanical heart behavior between the glycosylated and non-glycosylated form of the G-CSF were observed.

286. (297) **STUDY OF THE EFFECT OF SIRTUINS 1 AND 2 INHIBITORS ON THE SURVIVAL AND MIGRATION OF HEPATOCELLULAR CARCINOMA CELLS UPON SORAFENIB TREATMENT CONDITIONS**

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Sorafenib (SFB) is the only approved drug for hepatocellular carcinoma (HCC) treatment but it only prolongs patients' median survival by nearly 3 months. The main reason underlying the impaired sensitivity to SFB is the multidrug resistance (MDR). Sirtuins 1 and 2 (SIRTs1/2) are overexpressed in HCC and are associated with tumoral progression and MDR. Nowadays there is no second-line treatment for patients who fail to respond to SFB therapy. Aim: to analyze whether SIRTs1/2 activities blockage overcomes MDR during SFB treatment. Methods: HepG2 and Huh7 cells were treated for 72 h with SFB (2 μM) in presence or absence of the SIRTs1/2 inhibitors cambinol (Camb, 50 μM) or EX-527 (EX, 20 μM). Cell survival (2D culture: MTT, clonogenic assay, Annexin V-IP; 3D culture: spheroid growth delay and acid phosphatase assays) and migration (2D: wound healing; 3D: spheroid migration assay) were assayed. Results: In 2D cultures, cells treated with SFB and SIRTs1/2 inhibitors showed a greater fall in cellular viability (Huh7: SBF -24%*, SFB+CAMB -62%*#, SBF+EX -52%*#; **), number of colonies and cellular migration compared to cells treated with SFB alone. In the same way, cells treated with SFB and SIRTs1/2 inhibitors presented with more apoptosis than cells treated with SFB alone (Huh7: SBF +202%*, SFB+CAMB +337%*#, SBF+EX +306%*#; **). In 3D cultures, treatment with SFB and SIRTs1/2 inhibitors significantly diminished spheroid growth (volume), viability and migration compared to SFB treatment. 3D culture was less sensitive to drugs than 2D culture. *$p<0.05$ vs. control; #$p<0.05$ vs. SFB. **HepG2 cells behaved in a similar fashion manner. Conclusions: cambinol and EX-527 exacerbated the effects of sorafenib on cellular survival and migration supporting the potential application of SIRTs1/2 inhibitors in combination with SFB. Results from 3D cultures, that mimic tumor features in vivo, reinforce the clinical relevance of the current data.

287. (334) **ANTI-INFLAMMATORY EFFECT OF MICROPARTICLES CARRYING INDOMETHACIN ON HUMAN RESPIRATORY EPITHELIUM LINE CULTURES**

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Introduction: High levels of IL-1β, IL-6 and IL-8 have been described

in airways of asthmatic patients. It has been shown that stimulation with IL1-β induces the release of IL-6 and IL-8 in the human bronchial cell line Calu-3. This effect decreases with budesonide (BUD-topical steroid), the most used drug for asthma therapy. Previous studies described that nebulized Indomethacin (IN a non-steroidal anti-inflammatory drug) has antiasthmatic properties in humans. Recently microparticles (MPs) carrying IN were developed to deliver this drug to airways as a Dry Powder Inhaler system (DPI).
Objetive: The aim of this work was to evaluate if MPs containing IN have effect on the release of IL-6 and IL-8 by stimulated Calu-3 cells and to compare it with raw IN (to assess if MPs excipients influence the IN effect) and with BUD.
Materials and Methods: Acidic groups on IN structure were combined with cationic groups of the polyelectrolyte polylysine at 50% neutralization degree and processed by spray-drying to obtain MPs. Calu-3 cells were stimulated by adding IL-1β/H2O2 (50ng-ml-1/100μM, pro-inflammatory mediators) during 4 hours. Then, cells were divided into control and experimental groups and treated with MPs (20 and 50μM of IN), raw IN (20 and 50μM) and BUD (30μM). After 20 hours treatment, IL-6 and IL-8 released to the supernatant were quantified using a human IL-6 and IL-8 ELISA set.
Results: IL-1β/H2O2 exposure increased IL-6 production by 200% and IL-8 by 500%. IL-6 and IL-8 expression was reduced in around 40% when cells were treated with IN MPs, raw IN and BUD. No statistically significant difference was found between materials and concentrations applied (p-value >0.05).
Conclusion: IN contained in MPs has the same effect as pure IN and BUD on IL-6 and IL-8 release by inflamed Calu-3 cells.

288. (427) **IMIQUIMOD INDUCES DIFFERENTIAL OXIDATIVE STRESS AND ANTIOXIDANT RESPONSES IN MURINE NORMAL AND HEMANGIOMA TRANSFORMED ENDOTHELIAL CELLS.**

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Infantile hemangiomas (IH) are the most common benign tumours of infancy, however intervention may be required when major complications are developed. Previous results have shown that Imiquimod (IQ) induces apoptosis at lower concentrations in murine hemangioma (H5V) than in normal endothelial (1G11) cells. To understand whether oxidative stress was involved in cell death, we compare the ability of IQ to trigger ROS generation and its influence on antioxidant systems in H5V and 1G11 cells.
Cells were treated with IQ (0, 5, 10 and 50 μg/mL) during 2, 4 or 12 hours and analyzed for ROS generation, mitochondrial stability, catalase (CAT) and superoxide dismutase (SOD) activities. Redox status was assessed by measuring reduced and oxidized glutathione levels (GSH/GSSG).
After 2 hours of treatment H5V cells suffered an abrupt drop (70+/-12%; p<0.05) in CAT activity, accompanied by a (40+/-11)%-increase in ROS levels (p<0.05) with no changes in SOD activity and GSH/GSSG. On the contrary, 1G11 cells didn't show major changes in any of the measured parameters.
When treating H5V cells for 4 hs, both antioxidant enzymes were inhibited (circa 40%) along with (50+/-15)% loss of mitochondrial membrane potential. 1G11 cells, showed decreased activity of CAT (40+/-20)% and SOD (50+/-15)% without loss of mitochondrial stability.
After 12 hs, H5V cells showed restored or enhanced (198+/-30%; 50 μg/mL) CAT activity. SOD showed a 25% increase and mitochondrial stability remained impaired (40+/-10)% for ≥ 5 μg/mL IQ. 1G11 exhibited a pronounced induction of CAT (157-200%) for ≥ 10 μg/mL IQ and restoration of SOD (70-80%) activities. Interestingly, H5V cells suffered a 40-65% reduction in the GSH/GSSG ratio while in 1G11 cells this ratio raised up to 15 times.
In conclusion, treatment with IQ would produce oxidative stress in hemangioma cells but not in normal endothelial cells explaining H5V higher sensitivity than 1G11 to IQ.

289. (537) **ECHINOCOCCUS SIRTUINS: EXPRESSION AND LINK TO METFORMIN-TREATMENT**

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AMPK and Sirtuin 1 are involved in a positive amplification loop which acts to initiate autophagy in nutrient deprivation conditions. We have reported that during *Echinococcus* pharmacological treatment with metformin were promoted the AMPK activation, glycogenolysis, homolactic fermentation and autophagy (through the transcripcinal activity of FoxO), attacking the glucose-dependent metabolism and thus, the parasite survival. Since metformin is well-established to activate both AMPK and sirtuin 1, and sirtuin 1 could modify the acetylation status of FoxO, we infer the presence and activity of *Echinococcus* sirtuin class I. Also, we described the presence and levels of differential expression of the different sirtuins and to link them with functions that would have in relation to the metabolic characteristics of the parasite stages. Sirtuins form a superfamily of conserved NAD+-dependent protein deacylases with roles in metabolism and cell survival. Of the seven types of sirtuins (Sirt1-7) present in metazoans, *Echinococcus* sp. express Sirt1-3 and Sirt5-7, but lack Sirt4in accordance with the chromosomal instability and high rate of cell division that shown the cestode. *Echinococcus* sirtuins have a conserved structure of the catalytic domain (composed of a NAD+-binding Rossmann fold domain and a smaller Zn2+-binding domain with four conserved Cys residues) as do those of other helminthes, albeit large insertions are identified in their sequences, probably involved in protein-protein interactions. Our results showed that Eg-sirt1 and Eg-sirt2 genes have a high expression level inlarval forms, and that Eg- sirt3 and Eg- sirt6 has a low expression level in metacestodes. This issue is accordance tothe Warburg effect promotion of this larval form,a strategy acquired by *Echinococcus* germinal cells to cope with the high demand of energy and intermediate metabolites under limited oxygen supply, which could confer cells susceptibility to metformin, as it has been described for tumor cells.

290. (159) **PHARMACOKINETIC PARAMETERS AND THERAPEUTIC EFFICACY OF ALBENDAZOLE (ABZ) MICROCRYSTAL FORMULATIONS IN SUSCEPTIBLE MICE OF THE CBI-IGE MURINE MODEL OF TRICHINELLOSIS**

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ABZ is commonly used in oral chemotherapy against trichinellosis, its main disadvantage being poor bioavailability due to low solubility in water. Microcrystalline ABZ formulations were developed using a bottom-up methodology to optimize oral absorption. After physicochemical characterization and in vitro anthelmintic assays, hydroxyethyl-cellulose (S4A) and chitosan (S10A) based formulations were selected to analyze whether ABZ pharmacokinetic parameters and in vivo efficacy against the encysted T. spiralis larvae improved, in mice susceptible to the parasite. CBi+ mice of both sexes were given a single oral dose of ABZ, S4A, or S10A (30 mg ABZ/kg bw) and afterward, blood samples were collected at different times to quantify plasma concentration of albendazole sulphoxide, the main ABZ metabolite, by HPLC analysis. Mice given S4A and S10A showed an increased Cmax (peak plasma concentration) compared to those receiving pure ABZ (♂ P=0.01, ♀ P=0.009); also, AUC (area under the concentration-time curve) increased significantly (P<0.05) (S4A: ♂84%, ♀104%; S10A: ♂95%, ♀126%). The in vivo therapeutic efficacy was studied in mice orally infected with 2 L1 T. spiralis larvae/g bw (n=5/group). Control and treated mice given a daily oral dose of ABZ, S4A or S10A on days 27, 28 and 29 post-infection were euthanized seven days after the last dose. Muscle worm burden (MWB, number of L1 larvae/g muscle weight) and number of dead larvae (NDL) were examined. The formulations did not decrease MWB. NDL differed between males and females; while treated males tended to increase NDL percentage (C 24%; ABZ 30%; S4A 38%; S10A 54%), females did not differ from the controls without treatment (C 7%; ABZ 7%; S4A 13%; S10A 12%). Though the formulations showed higher Cmax and systemic exposure (AUC), only a slight improvement in therapeutic efficacy

was observed in males with the treatment protocol used during the chronic phase of the infection.

291. (186) **THERAPEUTIC EFFICACY OF NOVEL MICROCRYSTALLINE FORMULATIONS OF ALBENDAZOLE (ABZ) IN THE ACUTE STAGE OF INFECTION WITH TRICHINELLA SPIRALIS (TS), IN MICE WITH DIFFERENT SUSCEPTIBILITY TO THE PARASITE**

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ABZ, the preferred therapeutic agent to treat trichinellosis, has a very low and erratic bioavailability, which limits its oral absorption and increases its therapeutic failure. To modify that property, microcrystalline ABZ formulations were developed by a bottom-up methodology, using stabilizing polymers. After physicochemical and in vitro assays, hydroxyethyl-cellulose (S4A) and chitosan (S10A) based formulations were selected as those which most probably improve ABZ in vivo efficiency. This work aimed to compare the in vivo antiparasitic effectiveness of ABZ, S4A, and S10A in the acute stage of Ts infection, in two mouse lines differing in susceptibility to the parasite. CBi+ (susceptible) and CBi/L (resistant) adult mice of both sexes were infected orally with 2 Ts larvae/g bw and treated with ABZ, S4A, or S10A (30 mg ABZ/kg bw) on days 5, 6 and 7 post-infection (p-i), or non-treated, controls. Half the animals were sacrificed two days after the last administration to estimate intestinal parasitic load (number of adult parasites recovered, nAP) and Ts females fecundity (Ff). The remaining animals were sacrificed 37 days p-i to assess the effect of the treatment on muscular worm burden (MWB, number of parasites/g of tissue). nAP from treated mice did not differ significantly from controls. Ff could only be determined in Ts females recovered from control mice since female parasites found in treated mice were dead or with an altered morphology. In both lines MWB was significantly lower in treated animals (CBi+, ♂ P=0.0002, ♀ P=0.02; CBi/L, ♂ P=0.01, ♀ P=0.0001,). These results indicate that ABZ, S4A, and S10A were effective when administered in the acute stage of the infection. No significant differences were observed among the treatments. The alteration of the female internal morphology, caused by the treatments, would lead to a modification in Ff with the consequent reduction of muscle encysted larvae.

NEFROLOGÍA / NEPHROLOGY

292. (62) **RENAL EXPRESSION AND FUNCTION OF SODIUM-DICARBOXYLATE COTRANSPORTER 1 (NADC1) IN RATS WITH BILATERAL URETERAL OBSTRUCTION**

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NaDC1 is a transporter expressed in the apical membrane of proximal tubule cells. Its main function is to reabsorb the filtered Krebs cycle intermediates. NaDC1 is an important modulator of urinary citrate concentrations. Citrate is involved in the regulation of renal oxidative metabolism and moreover, is a calcium chelator that inhibits the formation of kidney stones. The aim of this study was to evaluate the renal expression and function (as urinary citrate excretion) of NaDC1 in male Wistar rats with bilateral ureteral obstruction (B) of a different time evolution. B was induced by occlusion of both ureters for 1 h (B1, n=5), 2 h (B2, n=7), 5 h (B5, n=6) and 24 h (B24, n=6). The studies were carried out after 24 h of ureteral releasing. Parallel to each group, a Sham one (Sh, n=11) was processed. The creatinine clearance (ClCr) was determined by conventional clearance techniques. Renal expression of NaDC1 in homogenates (H) and in apical membranes (M) was evaluated by Western blotting. Fractional excretion of citrate was determined (FE%Cit). Results: Mean±SEM. Data were analyzed with ANOVA plus Newman Keuls, P<0.05: (a)vsSh, (b)vsB1, (c)vsB2, (d)vsB5, (e)vsB24. ClCr (mL/min/100g): Sh=0.47±0.03, B1=0.44±0.02, B2=0.34±0.03a, B5=0.37±0.03a, B24=0.08±0.02a,b,c,d; NaDC1H (%): Sh=100±4; B1=91±3; B2=90±3; B5=84±3a; B24=72±4a,b,c,d; NaDC1M (%): Sh=100±3; B1=97±3; B2=102±4; B5=79±5a,b,c; B24=71±3 a,b,c; FE%Cit: Sh=8.1±0.5; B1=10.5±1.5; B2=11.3±1.5; B5=17.9±2.6a,b,c,e; B24=45.3±2.2a,b,c,d. The treated rats showed a significant decrease in ClCr after 2, 5 and 24 h of ureteral ligation confirming the establishment of the pathology. A decreased expression of NaDC1 in H and M was detected in B5 and B24 groups. These two experimental groups also showed a significant increase in FE%Cit which could be a consequence of the decrease observed in NaDC1M.The modifications in NaDC1 expression and function induced after B could be an adaptive mechanism to prevent the formation of renal stones.

293. (88) **RENAL EXPRESSION AND URINARY EXCRETION OF CAVEOLIN 1 IN RATS WITH OBSTRUCTIVE CHOLESTASIS**

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Caveolin 1 (Cav1) is an integral membrane protein constituent of caveolae, a subset of lipid rafts. These structures are platforms where proteins accumulate and transmit rapidly amplified signalling cascades. Renal expression of Cav1 in obstructive cholestasis remains poorly understood. The aim of this work was to evaluate the renal expression of Cav1 in rats with obstructive cholestasis by means of in vivo and in vitro studies. Urinary excretion of Cav1 (Cav1u) was also evaluated. Male Wistar rats were subjected to bile duct ligation of 21 h (BDL, n=4). Sham-operated rats served as controls (S, n=4). Renal homogenates (H) were obtained. In addition, isolated renal cells from control animals were incubated with S (n=6) or BDL (n=8) serum for 1 h 30 min (t1) and 3 h (t2). All incubations were performed at 37°C with constant agitation and exposition to 95% O2–5% CO2. Cell homogenates (CH) were obtained from respective incubations. Cell viability, tested by Trypan Blue exclusion, was maintained independently of the incubation achieved. Cav1 expression and Cav1u (%) were determined by immunoblotting. H: S=100±4 BDL=66±2*; CH, t1: S=100±3 BDL=62±5*; CH, t2: S=100±3 BDL=74±7*; Cav1u: S=100±5 BDL=120±6* (*p<0.05). Cav1 expression was lower in H from BDL rats. This decrease is reflected in an increase of Cav1u, probably due to the dumping of the protein into the urine. A decrease in CH was also observed in cells incubated with BDL serum demonstrating that components, present in BDL serum, are modifying Cav1 expression. Changes in the expression of Cav1 could be influencing various mechanisms in renal cells in the presence of obstructive cholestasis, for instance, vesicular trafficking. This is the first work to detect Cav1 protein in urine of rats. These preliminary data suggest that Cav1 in urine could be a potential noninvasive biomarker of obstructive cholestasis.

294. (425) **A GLOBOTRIAOSYLCRAMIDE INHIBITOR PREVENTED THE EFFECT OF SHIGA TOXIN 2 ON CELL PROLIFERATION AND MIGRATION IN RENAL TUBULAR EPITHELIAL CELLS**

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In Argentina, post-diarrhea Hemolytic Uremic Syndrome (HUS) due to Shiga toxin-producing Escherichia Coli (STEC) is a common cause of acute renal failure in children. Shiga toxin type 2 (Stx2) binds to the globotriaosylceramide (Gb3) receptor on the surface of renal cells. We have previously shown that the compound C-9 (Genzyme), an inhibitor of glucosylceramide synthase, decreased Gb3 expression in renal epithelial cells. The aim of this work was to study whether the C-9 prevents the cytotoxic actions of Stx2 on cell proliferation and cell migration in Vero cultures. Cell proliferation was measured by bromodeoxyuridine (BrdU) uptake, and cell migration was evaluated as the percentage of wound closure in wound healing assay. Incubation of Vero cultures with Stx2 (10 nM, 24 h) signifi-

cantly decreased the BrdU uptake to 48 ± 18 % (p<0.05), compared to control cells. Pre-treatment with C-9 (5 uM, 24 h) totally prevented the effect of Stx2 on cell proliferation in Vero cells, without having effect per se. The treatment of Vero cells with Stx2 significantly decreased the wound closure by 50 ± 21 % (p<0.05), compared to control cells. Despite the fact that C-9 decreased Vero cells migration, it partially prevented the effect of Stx2 on cell migration. In conclusion, the inhibition of Gb3 expression by compounds like C-9 could be a novel substrate inhibition therapy to neutralize Stx2 action on intracellular mechanisms in renal epithelial cells.

295. (426) **STUDIES OF CELL VIABILITY AND PROLIFERATION IN VERO CELLS TREATED WITH SHIGA TOXIN TYPE 2 IN THE PRESENCE OF L-NAME.**

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Post-diarrhea Hemolytic Uremic Syndrome (HUS) due to Shiga toxin-producing Escherichia Coli (STEC) is a common cause of acute renal failure in children in Argentina. It was reported that the inhibition of nitric oxide (NO) by Stx2 enhanced renal damage in mice and baboon models of Stx-mediated HUS. Furthermore, it was found that Stx reduced the NO synthesis, and therefore, increased Stx effects in human renal mesangial and endothelial cells. Therefore, the aim of the present work was to evaluate whether the inhibition of NO synthesis potentiates the cytotoxic effect of Stx2 in renal epithelial cells. Hence, the direct effect of L-NAME (N(G)-Nitro-L-arginine methyl ester), was evaluated in Vero cells, used as model of renal tubular epithelial cells. Cell viability was measured by neutral red uptake, 24 h after pre-treatment with different doses of L-NAME (1μM-1mM) followed by co-treatment with L-NAME and Stx2 (10 ng/ml) for 48 h. Cell proliferation was evaluated by bromodeoxyuridine uptake using the same experimental protocol. The treatment of Vero cells with L-NAME did not significantly modify the cell viability (N=4, n.s.) and the cell proliferation (N=3, n.s.), indicating that L-NAME was not toxic at any dose assayed. Incubation of Vero cells with Stx2 significantly inhibited cell viability (p<0.05). However, L-NAME did not modify the action of Stx2 on cell viability (N=4, n.s.). These results appear to indicate that Stx2 action is not sensitive to the inhibition of NO generation in Vero cells. Further experiments will be necessary to test NO production under these experimental conditions.

296. (436) **EFFECTS OF LOSARTAN ON THE APELIN/APJ SYSTEM AND ON NF-KB PATHWAY ELEMENTS IN A RENAL ISCHEMIA REPERFUSION INJURY (IRI) MODEL**

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Apelin is a selective endogenous ligand of APJ receptor, which has closest identity to the angiotensin II type 1 (AT-1) receptor. Nevertheless, the apelin/APJ system has been found to exert opposing actions to angiotensin II/AT-1.
Apelin administration improved renal function in an IRI rat model. In previous studies, we found a decreased renal expression of apelin mRNA in IRI. Other work from our laboratory, demonstrated that the renoprotective effects of losartan, in the same model, would be mainly mediated by its anti-inflammatory actions, since the AT-1 blocker treatment decreased TNF-α, IL-6 and IL-1β mRNA levels.
The aim of this work was to evaluate the effects of losartan in renal IRI on the expression of apelin, APJ, pNF-kB-p65, A20 (TNF-α-induced protein 3), which functions as NF-kB signaling inhibitor, and Toll-like receptor 2 (TLR2), a major NF-kB activator in IRI.
Wistar rats underwent sham-surgery (C) or renal 40min-ischemia followed by 24h-reperfusion (IRI). Losartan 80 mg/Kg/day, i.p. was administrated during 3 days prior to IRI (IRI+LOS) or sham-surgery (C+LOS). In cortical tissue, apelin and APJ mRNA expression was analysed by qRT-PCR; and NF-kB, A20 and TLR2 protein expression, by Western blot.
The decrease in Apelin mRNA observed in IRI was prevented by losartan (fold changes: C=1; IRI=0.08*; IRI+LOS=0.49#). APJ mRNA levels were diminished in IRI, while losartan increased its expression in C and IRI (C=1; C+LOS=6.68*; IRI=0.08*; IRI+LOS=7.83*#). Both pNF-kB-p65 and TLR2 expression augmented in IRI (*) and decreased in IRI+LOS group (#). IRI-induced increase in A20 expression (*) was inhibited by losartan (#). *p<0.05 vs. C; #p<0.05 vs. IRI.
Renal IRI inhibited the expression of apelin/APJ system, which was described to mediate renoprotective actions. Losartan treatment prevented this inhibition. AT-1 antagonism also decreased NF-kB activation, probably by reduction in TLR2 expression and through an A20-independent pathway.

297. (298) **RENAL PROTEIN HANDLING IN A RAT MODEL OF DIABETES MELLITUS**

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Diabetic nephropathy is the major cause of end stage renal disease worldwide. Renal protein handling is altered in diabetes mellitus and has been classically associated with glomerular alterations. Megalin and Neonatal Fc Receptor (FcRn) are two proteins located in the brush border of the proximal tubule which reabsorb filtered protein. Its possible role in the development of proteinuria has never been clearly addressed. In this work we studied Megalin and FcRn expression at two different stages of diabetic kidney disease, and its relation with proteinuria.
Diabetes was induced in male Sprague-Dawley rats after intraperitoneal injection of 65 mg/kg Streptozotocin (STZ). Studies were carried out at 15 days and 5 months post injection (n=6 for each group). 15 days post STZ injection rats presented normal glomerular filtration rate and a decrease in urinary protein excretion (p<0.05). Histological observation revealed no morphological alterations. We considered 15 days post STZ injection representative of early diabetes where hyperglycemia without renal damage is found. In these rats Megalin and FcRn expression were significantly increased in the diabetic group (p<0.05 for both).
Functional studies at 5 months after STZ injection revealed that diabetic rats showed an increase in glomerular filtration rate (p<0.01) and they presented proteinuria (p<0.05) and histological alterations. Here, Megalin and FcRn expression were significantly decreased (p<0.05).
Our results suggest that altered renal protein handling, a classical feature of diabetic kidney disease, could be caused not only by glomerular alterations, but also by changes in Megalin and FcRn expression. Further work is need to clarify the role of Megalin and FcRn in renal protein handling during diabetes mellitus.

298. (758) **NEPHROTIC SYNDROME INDUCED BY DOXORUBICIN IN RATS. PRELIMINARY STUDY OF THE EFFECTS ON PROXIMAL TUBULES**

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Introduction. Nephrotic syndrome is a consequence of renal damage, with proteinuria > of 3 g/d, a reduced concentration of blood proteins, high levels of cholesterol and triglycerides, with a higher risk of blood clotting.Doxorubicin is an anthracycline used for the treatment of solid and hematological tumors. The mechanisms of

action proposed for the anthracyclines are the interposition between base pairs of nucleic acids with inhibition of DNA and RNA synthesis, DNA alkylation, cellular apoptosis and free radicals synthesis. At this moment, it is not known the acute physiopathological mechanisms involved in tubular damage caused by doxorubicin.
The aim of our work was to study the response of proximal tubules under the effects of doxorubicin in rats.Materials and methods. We used 10 Sprague Dawley male rats (230-240g) divided into two groups. Experimental group was endovenously inoculated with 7.5 mg/Kg of doxorubicin. Control group was inoculated with saline solution. The animals were maintained in controlled conditions of light, temperature, free access to water and food for 1 week. 24 hours prior to sacrifice, rats were kept in metabolic cages to obtain urine samples to determine proteinuria. Histological and immunohistochemical studies were performed to detect megalin and FcRn receptors in proximal tubules.Results. Results showed an increase in proteinuria in experimental rats ($p<0.05$) with no differences in the excreted volume. We detected proximal tubules cytoplasmatic granuls, cystic dilation with intraluminal proteins. Glomeruli showed mesangial proliferation and segmental necrosis. The immunohistochemistry expression of megalin and FcRn endocitotic receptors in proximal tubules were increased ($p< 0.01$).Conclusion. The develop of significant proteinuria and the increase in the expression megalin and FcRn, could be an adaptive mechanism involved in the early stages of nephrotic syndrome induced by doxorrubicin. More studies are necessary to clarify the doxorrubicin effects.

299. (538) **POSTNATAL INHIBITION OF ENDOTHELIN (ET) SYSTEM AND SALT SENSITIVITY GENERATION IN ADULTHOOD: MOLECULAR MECHANISMS INVOLVED**
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Previously we have shown that postnatal inhibition of ET system generates salt sensitivity in male adult rats. These animals have an impaired ability to eliminate water and sodium overload and have an increased blood pressure. ET-1 produced in the renal medulla is a chief regulator of water and sodium excretion. It was shown that KO mice for ET-1 and for ETB receptor at collecting duct (CD) level have an increased blood pressure that is exacerbated when they are fed with hypersodic diet. Besides, ET-1 is a positive upstream regulator of NOS1 and consequently of NO production.
The aim of this work was to evaluate: pre-pro ET-1 production by real time PCR; ETA, ETB, NOS1 and NOS3 expression by western blot and nitric oxide metabolites (NOx) by the Verdon technique, in the renal medulla of adult male Sprague-Dawley rats fed with a normosodic (NS) or hypersodic (HS) diet (the animals had been treated during their postnatal period with a dual ET receptor antagonist [ERA]: bosentan 20 mg/kg/day). Four experimental groups were studied: control males with NS diet (CmNS), control males with HS diet (CmHS), ERA males with NS diet (ERAmNS) and ERA males with HS diet (ERAmHS). Two-way ANOVA was used for statistics.
Pre-pro ET-1 increased in CmHS vs CmNS ($p<0.05$) but failed to increase in ERAmHS. ; ETA expression didn't show significant changes between groups but ETB significantly decreased in both ERAmNS ($p<0.01$) and ERAmHS ($p<0.05$) vs CmNS and CmHS respectively. NOS3 expression didn´t show significant changes between groups while NOS1 expression significantly increased in CmHS vs CmNS ($p<0.05$) but failed to increase in ERAmHS. NOx had a tendence to increase only in CmHS.
The salt sensitivity of adult male ERA rats may be consequence of decreased renal medullary ET-1 production, altered ratio of ETA/ETB receptors and decreased NOS1 expression.

300. (759) **URINARY CYCLIC ADENOSINE MONOPHOSPHATE (CAMP) AND KALLIKREIN ACTIVITY (UKA) AS PROGRESSION MARKERS IN AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE (ADPKD)**
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ADPKD is characterized by both hypertension and cyst growth that deteriorate kidney function over decades of disease progression. The kallikrein-kinin system is involved in blood pressure regulation and UKa is a marker of its renal activity. In addition, vasopressin-renal V_2 receptor axis promote cystic expansion through intracellular cAMP pathway activation. Our preliminary data has shown altered urine UKa compared to controls as well as that plasma vasopressin levels correlated with total kidney volume (TKV) and GFR.
In order to evaluate UKa behavior over time and urine cAMP levels, daily UKa (U/d) was measured by amidolytic method in a 3-years follow-up of 12 ADPKD (35 ± 1 years, 5 women) and urine cAMP (nmol/g Cr) was determined by radioligand binding assay in a cohort of 9 patients and 5 controls. The data were compared with TKV, GFR, albuminuria, daily osmolal excretion, blood pressure and antihypertensive treatment.
Daily UKa increased over time (1.9 ± 0.7 U per year, $p = 0.02$ paired-T test) and correlated with daily osmolal excretion (spearman $r = 0.7$, $p = 0.02$).
Urinary cAMP was higher in ADPKD as compared to controls (4.1 ± 0.4 vs 0.8 ± 0.3, $p = 0.002$). No clear association with clinical variables was found, except on patients with GFR > 70 ml/min/1.73 m^2 that showed cAMP levels <3.4.
Taken together, the results showed that UKa timely varies in ADPKD, probably in association with changes in osmolal excretion, whereas the enriched cAMP urine content could reflect the hyperactivation of vasopressin- renal V_2 receptor axis in cyst expansion.
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301. (591) **STUDY OF MEGALIN EXPRESSION AND DETERMINATION OF PODOCYTURIA IN PREECLAMPTIC PREGNANT WOMEN AS AN EARLY MARKER OF KIDNEY DAMAGE.**
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Preeclampsia is a hypertensive disorder with multisystem involvement in pregnant women with more than 20 weeks of gestational age, of unknown cause. It is characterized by anomalous placentation, with presence of hypoxia/ ischemia placental, dysfunction of the maternal endothelium, imbalance between pro and anti angiogenic factors and marked expression of proinflammatory cytokines. Within the clinical manifestations of renal involvement, it can be associated with the presence of proteinuria together with elevated creatinine levels. During glomerular injury, podocytes can detach from the basement membrane, as well as alter the endocytosis mechanism of proximal tubule proteins. The pathophysiological mechanisms of preeclampsia and renal involvement are still unknown.
Our objective was to determine the podocituria in normal and preeclamptic pregnants and to correlate the results with the development of proteinuria.
Urine samples from pregnant women of the Fernández Hospital were used before and after 20 weeks of gestation and preeclamptic renal biopsy tissue from CEPEA between 1970 and 1980.
Indirect immunofluorescence was performed for synaptopodin (1/100) and the expression of megalin (1/100) in histological samples was studied. We carry out the association of the findings with the clinical data of the patients, relating it to the presence of proteinuria.
Our results showed an increase in podocyturia as the gestational age progressed in the patients who developed preeclampsia before the presence of proteinuria, being more representative at gestational ages> 30 weeks.
In renal biopsies, megalin expression decreased in membrane and cytoplasm of proximal tubules.
The increase in podocyturia with gestational age in these patients

could be an early marker of kidney involvement compared to proteinuria.
The diminished expression of megalin at the tubular level in renal biopsies demonstrates the involvement of tubular damage in the genesis of proteinuria during preeclampsia.

302. (718) **CELLS DERIVED FROM RENAL PROXIMAL EPITHELIA ARE AFFECTED BY EXTRACELLULAR PH IN A DIFFERENT WAY THAN CELLS DERIVED FROM CLEAR RENAL CELL CARCINOMA: EFFECT OF NHE1**

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IFIBIO-HOUSSAY UBA-CONICET

The association between cell death and intracellular pH elicits the possibility that extracellular pH (pHe) may modify cell survival. Moreover, as tumor extracellular acidity is a hallmark of cancers, is probable that pHe affects differently cancer or normal cells. The aim of this study was to investigate whether exposure to acidic or alkaline media altered cell survival in two renal cell models: HK2, derived from normal human proximal epithelial cells and 786-O, derived from human renal clear cell carcinoma. Cells were exposed for 24h to media with different pHe and then cell survival was estimated by acrydin orange–ethidium bromide experiments. Our results show that HK2 cell survival decays with an acidification of 0.1 pH units (Δ Cell death at pH 7.3 = 14.30 ± 3.00, p< 0.05 n=5) while 786-O cells can survive even at pH 6.4 (Δ Cell death at pH 6.4 = 0.24 ± 0,50 ns n=5). On the other hand 786-O cells were more sensible to alkalinization than HK2 cells. While an alkalinization of 0.1 affected only malignant cells (Δ Cell death at pH 7.5 HK2= 0.03 ± 0.50 vs 786-O= 10.57 ± 2.00 p< 0.01 n=10) higher alkalinization affected both cell lines. As control of pHi is regulated in part by the isoform 1 of Na/H exchanger (NHE1) we inhibited the transporter at different pHe. At pH=7.4 cell death is greater in both cell lines. However at pH=7.5 cell death rises in normal cells and drops in malignant ones (NHE1 Cell death HK2= 16 ± 1 vs 786-O= -9 ± 2 p< 0.01 n=12). Thus, NHE1 regulation is altered in malignant cells. In summary while tumor acidification might allow normal tissue destruction and tumor overgrowth, countering the tumor acidification with mild alkalosis might improve tumor control without normal tissue damage.

303. (727) **ROLE OF THE NA+/H+ EXCHANGER NHE1 IN THE AQP2-DEPENDENT RENAL CELL MIGRATION**

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Cell migration is the basis for many physiological and pathophysiological processes such us wound healing or metastasis. We have previously shown that Aquaporin 2 (AQP2) promotes renal cell migration. Moreover, we have already demonstrated the participation of NHE1 in the increment observed in the % of migration of AQP2-expressing cells. However, it is well known that NHE1 may contribute to cell migration in several different ways: affecting cell volume, regulating intracellular pH (pHi) and controlling cell adhesion, anchoring the cytoskeleton proteins to plasma membrane. The aim of the present work was to perform pHi measurements to evaluate if the activity of NHE1 exchanger influences the microenvironment of the lamellipodia in migrating cells. For experimental procedures two renal cell lines were used: WT-$RCCD_1$ (not expressing AQPs) and AQP2-$RCCD_1$ (stably transfected with AQP2). Fluorescence videomicroscopy measurements were performed in scratched monolayers: for ratiometric pHi measurements cells were loaded with the pH-sensitive probe BCECF/AM. Fluorescence data were acquired every 10s using a charge coupled-device camera connected to a computer and the Metafluor acquisition program. Changes in pHi were inferred from the ratio 490/440 of the fluorescence emitted from the lamellipodia of dye-loaded cells localized in the migrating front. Cells were incubated in control isosmotic solution or with HOE-694 (1uM) to inhibit NHE1 activity. Results showed that after incubation with HOE only AQP2-expressing cells present an acidification of their lamellipodia (ΔControl-HOE; AQP2-$RCCD_1$: 0.70±0.24 pH-units, n=40 cells in N=7 experiments, p<0.05). This result let us to propose that, in the presence of AQP2, an enhanced NHE1 exchanger activity exists that make a significant contribution to the migratory behavior observed in AQP2-$RCCD_1$ cells.

304. (793) **EFFECT OF HYPERAMMONEMIA ON THE PODOCYTIC MITOCHONDIA.**

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The alteration of the ammonia metabolism leads to hyperammonemia. The kidney plays a key role as a secondary metabolizing organ. During the initial stages of hyperammonemia it has been described mitochondrial dysfunction in central nervous system and skeletal muscle but the effects on the kidney were not evaluated until now.
Our objective was to characterize the effects of hyperammonemia on the mitochondria induced by incubation with ammonium chloride (NH4Cl) in-vitro in human immortalized podocytes (HIP).
HIP were incubated with 2 mM and 5 mM of NH4Cl at 37°C for 15 days. For analyzing the mitochondrial oxygen consumption rate (OCR), a mitochondrial stress test (Seahorse) was performed at day 15. Western Blot was performed for analyzing the mitochondrial cell content and the amount of the respiratory chain complexes using specific markers: TOM20 subunit of the TOM and subunits of the complexes.
Seahorse showed a conserved OCR at 2 mM of NH4Cl compared to control with a trend of its enhancement after the uncoupling of the oxidative phosphorylation with FCCP. In cells exposed to 5 mM of NH4Cl a significant increase in the OCR after the addition of FCCP was observed. At higher concentrations, ammonia increase the maximal respiration and the spare respiratory capacity. The mitochondrial content and the complexes of respiratory chainshowed conserved levels of TOM20 as well as of the subunits of the complexes with a trend towards an increase compared to control.
This work shows for the first time an enhancement of the mitochondrial function in podocytes as an initial effect of chronic hypermmonemia at its lowest levels. This could be related to an improvement of the cell fitness and the response to an increment of the energy demand. These could be an adaptative response which could explain the late onset of kidney alterations in patients with hyperammonemia.

305. (795) **EFFECT OF HYPERAMMONEMIA ON THE SLIT DIAPHRAGM AND THE ENDOCYTOSIS OF THE NEPHROCYTES.**

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Alterations in ammonia metabolism leads to hyperammonemia. The kidney plays a key role as a secondary metabolizing organ. The effects on the kidneys during chronic and severe hyperammonemia involved glomerulopathies and tubulopathies. The effects of the initial stages of chronic hyperammonemia on the glomeruli have not been yet characterized.
The objective is to characterize the initial effects of hyperammonemia on the slit diaphragm and endocytotic components of nephrocytes induced by administration of ammonium chloride in-vivo during developmental stages of Drosophila melanogaster.
A novel model of chronic hyperammonemia on the kidney was developed. Drosophila melanogaster developmental stages were ex-

posed to 0.20 M, 0.25 M and 0.30 M. The L3 nephrocytes' were dissected and the function of the slit diaphragm and endolysosomal compartment were analyzed using the Pulsed-Albumin uptake assay. Nephrocytes of those exposed to 0.30 M of NH4Cl were used for the ultrastructural analysis performing the cell surface analysis. Finally, transmitted electron microscopy was used.
The efficiency of filtration and endocytosis was significantly increased in the nephrocytes exposed to 0.25 M and 0.30 M NH4Cl compared to control and to the 0.2 M exposed population. This last population showed no significant differences compared to control.
The pattern of expression of the protein Kirre and the ultrastructure of the slit diaphragm were conserved after exposure to 0.30 M NH4Cl and showed an increase in the content of intracellular vesicles.
Ammonia is one of the first substances which at toxic concentrations, led to an increase of the filtration and endocytotic function on nephrocytes. These findings were correlated with a conserved structure of the slit diaphragm and an increase on the content of the intracellular vesicles. These results could be an adaptative response which could explain, in part, the late onset of the kidney component observed in patients with end stage hyperammonemia.

306. (420) **ESTRADIOL STIMULATES CELL PROLIFERATION IN PRIMARY CULTURES OF HUMAN RENAL TUBULAR EPITHELIAL CELLS**

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We have previously demonstrated that 17β-Estradiol (E2) stimulates cell proliferation through classic estrogen receptors (ER) and the G protein-coupled estrogen receptor 1 (GPER-1), in primary cultures of human cortical renal tubular epithelial cells (HRTEC). The aim of the present work was to study the effects of E2 on cell proliferation and to evaluate the estrogen receptor and intracellular signals involved in this mechanism in HRTEC. Primary cultures were developed from nephrectomies performed in pediatric patients at the Hospital Nacional Prof. A. Posadas. HRTEC were treated with E2 (10 nM for 24 h) with or without classic ER antagonist, ICI 182,780, and GPER-1 agonist and antagonist, G-1 and G-15, respectively. Cell proliferation rate was measured by incorporation of 5-bromo-2-deoxyuridine (BrdU) in nuclei of HRTEC primary cultures, and by counting the cell number at 24 and 48 h. ERalpha, ERbeta, β-catenin, and cyclin D1 expression and localization were assayed by western blot analysis and immunofluorescence, respectively. Treatment with any of both E2 and G-1 (10 nM, 24 h) stimulated significantly the BrdU uptake in HRTEC primary cultures, and increased the number of cells at 48 h. Co-incubation of HRTEC with E2 and G-1 increased BrdU uptake as occurred by E2 alone. The treatment of HRTEC with E2 significantly increased CyD1 and β-catenin protein expression, and increased the number of cells that translocated ERalpha and β-catenin into their nucleus. However, E2 did not modified ERbeta expression and localization, compared to control HRTEC. Both G-15 and ICI 182,780 totally abrogated E2- stimulation of BrdU uptake, and β-catenin expression and translocation to the nucleus in HRTEC. GPER-1 expression and localization was not modified by E2 treatment. In conclusion, E2 regulates HRTEC proliferation by activating both estrogens receptors, ERα and GPER-1, possibly by stimulating β-catenin pathway, in normal human renal tubular epithelium.

MEDICINA REGENERATIVA / REGENERATIVE MEDICINE 2

307. (54) **MIR-210 IS REGULATED BY HIF-1A IN HUMAN PLURIPOTENT STEM CELLS CULTURED UNDER HYPOXIC CONDITIONS**

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FLENI

Human pluripotent stem cells (hPSCs), obtained from embryos at the blastocyst stage (embryonic stem cells (hESCs)), or from reprogrammed somatic cells (induced pluripotent stem cells (hiPSCs)) are self-renewing cells that can be differentiated into a wide range of specialized cells. hPSCs are routinely cultured under atmospheric air enriched with 5% CO2 (20% O2). However, first stages of embryogenesis evolve in a hypoxic environment. Response to low oxygen conditions is mediated primarily by gene expression changes induced by hypoxia-inducible transcriptional factors (HIFs). Prolyl-hydroxylation of HIFs at normal oxygen tension results in HIFs degradation by the proteasome. When oxygen levels drop, HIFs are no longer hydroxylated, resulting in protein accumulation. Recently several studies have shown that the expression of a specific group of microRNAs is induced under hypoxic conditions. On this sense, we previously demonstrated that miR-210 is up-regulated by chemical and physical hypoxia in hPSCs. This finding prompted us to deepen our knowledge about how miR-210 is regulated in hPSCs under hypoxia and to describe some of its mRNA targets. First, we studied the role of HIF-1α and HIF-2α in miR-210 up-regulation in hESCs (H9 line) and hiPSCs (FN2.1 line) under hypoxia. To this end, the effect of siRNAs against HIF-1α and HIF-2α on miR-210 expression levels regulation by chemical (CoCl2, deferroxamine and DMOG) and physical (1% O2) hypoxia in hPSCs was analyzed by RT-qPCR. Importantly, we observed that miR-210 up-regulation by hypoxia was reverted by HIF-1α siRNA but not by HIF-2α siRNA. Similar behavior was found with a known HIF-1α target like bnip-3. Finally, we performed RT-qPCR analysis of several miR-210 predicted target mRNAs in hypoxic hPSCs and observed a significantly downregulation of gpd1l. Thus, we can conclude that miR-210 is regulated by HIF-1α and that gpd1l could be a miR-210 target in hPSCs cultured under chemical and physical hypoxia.

308. (209) **DECREASED ANGIOGENIC ACTIVITY OF COXSACKIEVIRUS B3-INFECTED ENDOTHELIAL COLONY FORMING CELLS**

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Coxsackievirus B3 (CVB3) is a globally prevalent Enterovirus frequently associated with viral myocarditis that may lead to dilated cardiomyopathy and heart failure. Local or homed progenitor cells are involved in myocardial repair, especially endothelial progenitor cells which have a lead role in new blood vessel formation and tissue revascularization. We have previously demonstrated that CVB3 infects and replicates in ECFC and although it failed to affect ECFC survival, it significantly reduced proliferation and tubulogenesis. Here, we aimed to study the effect of CVB3 on other functional responses of late outgrowth endothelial colony forming cells (ECFC) involved in new vessel formation. Cord blood-derived CD34+ cells were seeded in EGM2 and ECFC colonies were obtained after 14-21 days. ECFC were incubated with CVB3 at a multiplicity of infection (MOI) 0.1 or 1 for 1 hour and then washed to remove the remaining virus. N=3-5. *p<0.05 vs Mock (M) or untreated control (C), one-way ANOVA. Our results indicated that CVB3 infection significantly decreased ECFC chemotaxis (M: 98.9±0.7; MOI 1: 62.9±14.9*, % of C) and the release of the proangiogenic molecules TGFβ (C: 316.3±62.2; M: 269.8±45.7; MOI 1: 40.4±15.7*, pg/ml) and bFGF (C: 108.5±3.2; M: 79.9±1.0*; MOI 1: 57.3±3.9*, pg/ml). ECFC adhesion to untreated HUVEC was not affected by CVB3. However, adhesion to TNFα-treated HUVEC was inhibited by this infection (M: 100.7±1.3; MOI 0.1: 73.0±1.2*; MOI 1: 57.4±4.1* % of C). Similar results were obtained when HUVEC were infected with MOI 1 CVB3. Our results show that CVB3 infection affects ECFC functionality through an inhibition of their chemotactic and adherent ability and their capability of pro-angiogenic cytokine release, which could possibly lead to an impaired myocardial repair.

309. (237) **DESIGN OF A LENTIVIRAL VECTOR FOR HUMAN FOXP3 REGULATION IN PRIMARY T CELLS.**

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Foxp3 is a transcriptional factor involved in the differentiation of regulatory T cells (T-reg). Alteration of human T-reg cell function is associated with disorders, including cancer and autoimmune diseases. It was demonstrated that E3 ubiquitin-protein ligase STUB1 can negatively modulate T-reg suppressive activity by promoting degradation of Foxp3.
The aim of this work is to design an efficient tool for STUB1 gene delivery that could be used for modulating FoxP3 and studying Foxp3-dependent T cell functions.
We constructed a specific lentiviral plasmid by cloning RTPCR-derived human STUB1 cDNA under the control of the ubiquitous EF1alfa promoter and cloning GFP reporter gene after STUB1 ORF following an IRES sequence. We generated lentiviral particles by transient transfection of 293T producer cells using PEI system, by co-transfectiion of SIN-lentiviral codon-optimized STUB1-IRES-GFP expression plasmid, PPAX packaging plasmid and envelope plasmid carrying vesicular stomatitis virus (VSVG) glycoprotein. We transduced an immortalized cell line of human T lymphocytes at different multiplicities of infection (MOIs) and measured GFP by Flow Cytometry 72 hs post-transduction. LV-STUB1 transduced over 30% of cells at an MOI of 0.5 vector/cell, meaning that we could expect higher levels of expression at higher MOIs. At this moment STUB1 vector is being tested in an in vitro model of immune suppression and we are generating vector pseudotypes with different glycoproteins in order to ensure high level transgene expression in primary human T cells, for which VSVG pseudo typed lentiviral vectors are inefficient.

310. (313) **IN VITRO AND PRELIMINARY IN VIVO ASSESSMENT OF ANODIZED MG-BASED ALLOY AS A POTENTIAL MATERIAL FOR BIOMEDICAL APPLICATIONS**

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Magnesium (Mg)-based alloys are promising candidates for the development of new biodegradable materials for fracture repair implants. However, the high degradation rate of Mg in aqueous media releases hydrogen gas, which causes pain and local swelling. Superficial treatments, such as anodization, emerge as a potential solution to this limitation. The aims of this study were to evaluate the 1) in vitro citocompatibility of anodized Mg-based material on fibroblasts, and 2) the in vivo osteoconductivity of anodized Mg-based implants after 7 days of implantation in a rat model. Anodization of Mg alloy (AZ91) pieces was performed at low voltage for 40 min in potassium hydroxide solution. For the objective 1, bovine fibroblasts adhesion and proliferation were evaluated after 24 h in culture on the materials (anodized or control -without anodization- AZ91 pieces). As cellular control, cells growth was evaluated in culture without material (on a plastic dish). For objective 2, bone formation stimulation of anodized AZ91 or control –polylactic acid, PLA; a biodegradable material used clinically- was evaluated in adult male rats; two pins of the same material were placed transversely one in each femur. At 1 and 6 days after surgery, different fluorochromes were administered intraperitoneally, to be incorporated at mineralization sites of the new bone. Rats were sacrificed 7 days after implantation and femurs were retrieved, cleaned, fixed and embedded in polymethyl methacrylate. Fluorescence and Toluidine blue staining were observed on thin sections (150 μm) of the implanted area. In the in vitro test, an increase in bovine fibroblasts adhesion and proliferation was detected over the anodized surfaces pieces, compared with controls. The in vivo preliminary results suggest a higher osteoinductivity of the Mg-based pins, compared with PLA pins. In conclusion, anodization at low voltage is a promising superficial treatment for the development of Mg- based biomedical applications.

311. (396) **EXTRACELLULAR VESICLES FROM PLURIPOTENT STEM CELL-DERIVED MESENCHYMAL STEM CELLS ACQUIRE A STROMAL MODULATORY PROTEOMIC PATTERN DURING DIFFERENTIATION**

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Mesenchymal stem/stromal cells (MSC) obtained from pluripotent stem cells (PSC) constitute an interesting alternative to classical MSC in regenerative medicine. Among their many mechanisms of action, MSC extracellular vesicles (EV) are a potential substitute for MSC in future cell-free-based therapeutic approaches. Unlike cells, EV do not elicit acute immune rejection, and they can be produced in large quantities and stored until ready to use. Although the therapeutic potential of MSC-EV has already been proven, a thorough characterization of MSC-EV is lacking. In this work, we used a label-free liquid chromatography tandem mass spectrometry (LC-MS/MS) proteomic approach to identify the most abundant proteins in EV that are secreted from MSC derived from PSC (pdMSC) and from their parental iPSC. Initial analysis of samples by PCA showed consistent aggregation of iPSC samples with their EV and pdMSC samples with their EV. We found that while iPSC-EV enclose proteins that modulate RNA and miRNA stability and protein sorting, pdMSC-EV are rich in proteins that organize extracellular matrix, regulate locomotion and influence cell-substrate adhesion. Moreover, compared to their originating cells, iPSC-EV share ~76% of proteins with a spearman correlation value of r=0.5 ($p<2.2e-16$), while pdMSC-EV share only ~37% of proteins with a spearman correlation value of r=0.12, ($p<0.0094$) and functional analysis demonstrated that protein content from pdMSC-EV relate to biological processes such as angiogenesis and modulation of immune response. Finally, wound healing assays proved that exposure of HMEC-1 cells to EV originated from two types of MSC (pd- and umbilical cord) improved closure significantly (vs without EV: $p < 0.05$) in contrast to exposure to iPSC-EV, indicating that MSC microvesicles could potentially regenerate injured endothelial tissue. Altogether, these findings suggest that during differentiation, compared with their parental iPSC-EV, pdMSC-EV deliver a more specific set of proteins; arguably, this difference might confer their therapeutic properties.

312. (502) **CRISPR-ON SYSTEM FOR THE ACTIVATION OF THE ENDOGENOUS HUMAN LNCRNA PLUTO**

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In order to improve the quality life of patients with Type 1 Diabetes some strategies are becoming to take part in alternative treatments. One of these is the development of cell therapies based on cellular reprogramming from stem cells or adult cells into insulin-producing cells. Our group has been working with CRISPR-ON system to switch on relevant human beta pancreatic cell genes. On the other hand non coding RNAs, microRNAs (miRs) and long non coding RNAs (lncRNAs), are starting to be a focus for this purpose. For example, the lncRNA PLUTO (HI-LNC71) influences interactions between an enhancer cluster and *PDX1* (a key transcription factor in pancreas development). The objective of our work was to activate lncRNA PLUTO using CRISPR-ON system to improve future reprogramming protocols. For this, we designed four sgRNAs to target the PLUTO proximal promoter (-135/-50 pb respect to TSS) and cloned them into sgRNA expression vector (Addgene #47108).

HEK293T cell line was transfected with dCas9-VP160 expression vector (Addgene #48226) and the 4 sgRNAs using Lipofectamine 2000® (Invitrogen). Six days after, RNA was extracted using miRNeasy Mini Kit (Qiagen). By RT-PCR and specific primers, our results showed that PLUTO was activated in CRISPR-ON group and no activation was observed in control group. In comparison with the positive control, human insulinoma, the product size suggests that an alternative splicing form was induced in HEK293T group. In conclusion, we could activate the lncRNA PLUTO using CRISPR-ON system in a model cell line. Further studies are needed to evaluate if this molecule has the capability of modulate *PDX1* expression and become a new molecular tool in beta pancreatic cell differentiation protocols.

313. (775) **TGFBR2–SE, A NEW SOLUBLE HUMAN TGF-B TYPE II RECEPTOR ISOFORM, IS SUMOYLATED, INTERACTS WITH CYTOPLASMIC AND NUCLEAR PROTEINS, AND IS SECRETED ON EXOSOMES**

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We have recently described in human cells a new splicing variant of TGF-β type II receptor lacking the last 63 nucleotides of exon II and the first 86 nucleotides of exon III. This deletion of 149 nucleotides causes a frameshift with the appearance of an early stop codon rendering a truncated protein of 80 amino acids lacking the transmembrane domain, known as TGFBR2 soluble endogenous (TGFBR2-SE). Based on a computational sumoylation analysis we found that TGFBR2-SE has three potential non-concensus sumoylation lysines (K76, 77 and 78). To test whether TGFBR2–SE is actually sumoylated, we performed protein immunoprecipitation of 293T cell lysates with a specific α-TGFBR2-SE pAb, followed by immunoblotting with mAbs (α-TGFBR2–SE, and α-SUMO1). In this way we found that the new isoform is posttranslationally modified by sumo addition. For further protein characterization, and to allow increased expression in mammalian cells, we codon optimized the TGFBR2-SE cDNA. Furthermore, to ease protein purification and enhanced in vivo half-life, we fused it in frame with the human IgG1 Fc domain, and expressed it in 293T cells by means of a lentiviral vector (Lv.TGFBR2-SE/Fc). Additionally, we identified the interactome of purified TGFBR2-SE/Fc by screening of the Huprot v3.0 Human Proteome array. This assay indicated that TGFBR2-SE is able to bind to a panel of 155 proteins. This set of proteins was compared with exosomal Vesiclepedia data using FunRich, indicating that TGFBR2–SE interacts with 79 proteins present in exosomes, of which 67 are found either only in the cytoplasm (22), only in the nucleus (11) or both (34). Finally, we confirm exosomal secretion of TGFBR2-SE and the Fc-tag protein by Western blot of 293T cell microvesicles with mAbs (α-TGFBR2-SE and α-CD63). These results show for the first time important TGFBR2-SE characteristics contributing to unravel the function of the newly described isoform.

314. (83) **IN-VITRO MACROPHAGE ASSAY PREDICTS THE IN-VIVO ANTI-INFLAMMATORY POTENTIAL OF HUMAN MESENCHYMAL STEM CELLS-DERIVED EXOSOMES**

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Exosomes play key roles in cell biology and may provide new clinical diagnostics and therapies. However, one of the major barriers in the field has been a lack of convenient assays for their bioactivity. Here, we developed an in vitro assay that quantitates the anti-inflammatory potential of mesenchymal stem cells (MSC)-derived exosomes based on their ability to suppress the acquisition of M1 phenotype in LPS-stimulated RAW264.7 macrophages. We first standardized the LPS dose (10 ng/ml) that induces macrophage cells to secrete a moderate level of IL-6 which can be significantly suppressed by dexamethasone. To test the assay, we purified exosomes from seven independent lots of MSCs-conditioned medium by anion exchange chromatography. All the preparations were positive for human-specific CD63 and CD81, and showed a mode size ranging from 99 to 156 nm by nanoparticle tracking analysis. Dose-response curves indicated that a dose of $0.5x10^9$ exosomes/ml provides reproducible results on categorizing the anti-inflammatory potential. To assess the predictive efficacy of the assay, the same seven exosome preparations were tested in a murine model of LPS-induced systemic inflammation. The inflammatory response was followed by expression of IL-1β and IL-6 in spleen at 2 h after LPS administration (2.5 mg/Kg). The percentage of reduction in secreted IL-6 from the macrophage assay correlated not only with the suppression of mRNA levels for IL-1β (r2=0.812, p=0.006) and IL-6 (r2=0.861, p=0.003), but also with the reduction of IL-1β (r2=0.694, p=0.020) and IL-6 (r2=0.811, p=0.006) contents in spleen of mice receiving exosomes after LPS administration. In conclusion, the macrophage assay allows to rank different preparations of exosomes by their anti-inflammatory activity, and their ranking predicts their efficacy in suppressing inflammation in mice. The assay is convenient for comparing multiple samples and therefore should be useful in developing protocols for the purification and characterization of anti-inflammatory exosomes.

315. (482) **NEURAL STEM CELLS-DERIVED NEURONS OBTAINED FROM HUMAN PLURIPOTENT STEM CELLS AS AN IN VITRO MODEL FOR STUDYING CDK5/P25 MEDIATED NEURODEGENERATIVE PROCESSES.**

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Human embryonic and induced pluripotent stem cells (hESCs and hiPSCs) can differentiate into a wide range of specialized cells, including neural stem cells (NSC). Moreover, NSC-derived neurons are proposed as a model for studying neurodegeneration. CDK5/p35 complex is involved in neuronal homeostasis and development. However, its function is deregulated in neurodegeneration by p35 cleavage into p25, which allows the aberrant phosphorylation of targets through the constitution of a more stable complex CDK5/p25. In this work we aimed to set up an *in vitro*CDK5/p25 neurodegenerative model using NSC-derived neurons obtained from hESCs and hiPSCs subjected to different cellular stressors. For this purpose, we first derived NSC from hESCs (H9 line) and hiPSCs (FN2.1 line), which were further differentiated into neurons using a commercial Neural Induction Kit. NSC and neuron-like phenotype were validated by immunofluorescence and RT-qPCR of cell specific markers (Sox-1, Sox-2, Pax-6 and Nestin for NSC; MAP5, MAP2 and Tuj-1 for neurons). Then, CDK5 and p35 mRNA and protein expression levels were analyzed in hESCs, hiPSCs, NSC and neurons by RT-qPCR and western blot. Interestingly, we observed that although CDK5 was ubiquitous, p35 mRNA and protein were mainly expressed in neurons. We next evaluated how different stress stimuli (rotenone, glutamate and calcium ionophore A23187) affected NSC-derived neurons viability. We determined the percentage of cell viability after 24 hours treatment with increasing concentrations of rotenone and glutamate and 2 hours treatment with A23187 using a XTT vital dye assay. Cell viability felt down significantly in the case of rotenone and A23187 treatments in a concentration dependent manner. However, only a little effect was observed with glutamate treatment. In conclusion, NSC-derived neurons obtained from hESCs and hiPSCs expressed high levels of p35 and responded to rotenone stressor, making them a suitable CDK5/p25 neurodegenerative *in vitro* model.

316. (508) **MESENCHYMAL STROMAL CELLS AS CELLULAR CARRIERS OF THERAPEUTIC GENES: USE OF IN VITRO TRANSCRIBED MRNA**

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The use of mesenchymal stromal cells (MSCs) has been proposed during the last years for therapeutic purposes, especially for regenerative therapies. They are promising candidates as vehicles for delivery of therapeutic agents. The majority of engineering strategies have focused on use of viral vectors for gene transfer. However, the use of *in vitro* transcribed mRNA (IVT mRNA) is gaining attention as a promising tool for the delivery of therapeutic genes by MSCs. The aim of this work was to express the mRNA of insulin growth factor I (IGF-I) and DsRed in MSCs, with the aim to treat liver fibrosis. For this purpose, mRNA (of IGF-I or DsRed) was *in vitro* transcribed, modified to be more stable within the cell, and then transfected into human umbilical cord perivascular cells-derived MSCs with lipofectamine. IGF-I production by MSCs was evaluated by ELISA, and DsRed expression by fluorescence microscopy. Different quantities of mRNA were assayed, and we observed that low amount of mRNA (0.2 *µ*g mRNA / 40.000 cells) were sufficient to express the exogenous proteins up to 15 days. Since the use of MSCs for therapeutic purposes relies on their capacity to migrate to the injured tissues, we also evaluated their migration capacity (using a modified Boyden chamber). We observed that MSCs expressing IGF-I or DsRed have the same migratory capacity compared with unmodified MSCs. Our results demonstrate that IVT mRNA is an efficient method to engineer human MSCs, and suggest that this approach would be useful for therapeutic purposes.

317. (642) **ATTACHED SEGMENTS OF UMBILICAL CORD BLOOD UNITS AS POTENTIAL PREDICTORS OF CRYOPRESERVED HEMATOPOIETIC PROGENITOR CELLS POTENCY.**

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Umbilical cord blood (UCB) is a source of hematopoietic progenitor cells (HPCs) used in hematopoietic transplant. UCB is usually cryopreserved after collection and thawed for transplantation. Each cryopreserved unit consists of a product bag (25 ml) and 3 integrally attached segments (0.3 ml total). Our goal was to evaluate the use of segments as potential predictors of HPCs potency on the cryopreserved unit. We thawed segments and product bags corresponding to 8 UCB units. CD45+/CD34+ cells content and viability (7-AAD staining) were analyzed by flow cytometry (FC). Pearson Correlation coefficient (r) and a range of agreement (RoA) between segments and bags based on limits of agreement from the Bland-Altman method were calculated for each analyzed parameter. Our results showed high correlation coefficients and narrow RoAs for the percentage of CD34+ cells (r=0.98 RoA=-0.085% to 0.083%), and total CD34+ cells count (r=0.95 RoA=-1.35x106 to 1.67x106 CD34+ cells). For viable CD34+ cells count we observed a high correlation coefficient but a wider RoA (r=0.89 RoA=-2.77 x106 to 1.34 x106 viable CD34+ cells). Finally, percentage of CD45+ viable cells (r=0.769 RoA=-24.8% to 14.6%) and percentage of CD34+ viable cells (r=0.62 RoA=-46.2% to 5.6%) showed low correlation coefficients and wide RoAs. According to these findings, data obtained from segments can be useful to predict the percentage and count of CD34+ cells in UCB products. However, they are not good indicators of the viability of those cells. Viability of CD34+ cells is a key factor to consider when analyzing thawed segments. Unfortunately, this parameter showed the lowest correlation. Nevertheless, since viability of CD34+ cells was lower in the segment than in the bag for all the tested samples, segments are still potentially useful to establish a minimum expected value of CD34+ cells viability in the final product.

318. (668) **SLPI IMPROVES THE RECELLULARIZATION OF RENAL SCAFFOLDS**

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To deal with the problematic of the lack of donors for organ transplantation, regenerative medicine proposes the generation of histocompatible bioartificial organs by recellularizing scaffolds. In previous studies, we observed that secretory leukocyte protease inhibitor (SLPI) exhibits nephroprotective activity in vivo. The aim of this work was to evaluate the use of SLPI to improve the recellularization of renal scaffolds with human proximal tubule epithelial(HK-2). First, we evaluated in vitro a protective effect of SLPI on HK-2 cell exposed to FK-506 by the MTT assay and annexin V/propidium iodide. Furthermore, we study the ability of SLPI to induce HK-2 proliferation and the effect of SLPI in the wound healing assay. Finally, we produce rat kidney scaffold with the constant admistration in renal artery of 1% SDS (2ml/min) for 24 h followed by 24 h of 0.1% Triton X-100. After washing with saline solution, scaffolds were seeded through the ureter with 2.106 HK-2 cells previously treated or not with SLPI (4µg/ml). Our results showed that the incubation of HK-2 with SLPI (0,4 µg /ml) for 24 h reduce the cytotoxicity induced by FK-506 ($p < 0.05$). Also, SLPI reduces apoptosis induced by serum deprivation and FK-506 incubation in human tubular epithelial cells from over 90% to 57.3% and 56.7% respectively. In addition, SLPI was able to induce the proliferation of HK-2 cells during 24 h of culture and reaching a better recovery in a wound healing model compared to untreated cell (83,9±4% and 35,3±7% surface covered) ($p=0.0021$). With these results, we proceeded to seed renal scaffolds with HK-2 cells, observing a higher percentage of cells adhered to the matrix when the cells were previously treated with SLPI. Here we propose SLPI as a new factor to improve the recellularization process in renal scaffolds.

319. (362) **THERAPEUTIC EFFECT OF HUMAN UMBILICAL CORD PERIVASCULAR CELLS-DERIVED EXTRACELLULAR VESICLES ENGINEERED TO PRODUCE IGF-I ON LIVER FIBROSIS IN MICE.**

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Introduction: Liver cirrhosis involves chronic damage, wound healing and fibrogenic processes. Human umbilical cord perivascular cells (HUCPVs) are a type of mesenchymal stromal cells (MSCs) obtained from birth-associated tissues. MSCs support tissues repair and modulate the inflammatory process. Particularly, extracellular vesicles (EVs) could mediate MSCs paracrine effects. We previously demonstrated that bone marrow-derived MSCs engineering to produce Insulin Growth Factor like-I (IGF-I) ameliorate liver fibrosis in mice.We aimed to evaluate the therapeutic effect of HUCPVs engineered to produce IGF-I and the role of EVs in experimental liver fibrosis.
Methods: HUCPVs were infected with adenoviruses codifying for human IGF-I (AdIGF I) or green fluorescence protein (AdGFP) and EVs were isolated from supernatants after 3 days of infection by differential centrifugation. EVs characterization was performed by protein quantification, CD63 and internal IGF-I expression. Fibrosis was induced in BALB/c mice by chronic administration of thioacetamide (TAA) during 8 weeks. On week 6, cells (AdIGF-I-HUCPVs or AdGFP-HUCPVs; dose: 5x10E5 cells), EVs (EVs HUCPVs, EVs-HUCPVs-AdIGF-I or EVs-HUCPVs-AdGFP, 3 doses, 15 µg/dose/mice every 5 days) or saline were intravenously administered. Animals were sacrificed at week 8 to collect liver samples.
Results: The application of AdIGF-I-HUCPVs resulted in a further amelioration of liver fibrosis when compared to AdGFP-HUCPVs ($p<0,05$) and saline ($p<0,001$). Similarly, treatment with EVs-AdIGF-I-HUCPVs resulted in a reduction of collagen deposition and gene expression of fibrogenic related factors TGF-β1, α-SMA and Col1a2 ($p<0,05$ vs EVs-AdGFP-HUCPVs and $p<0.01$ vs Saline). Consistently, high levels of IGF-I were observed within of EVs-AdIGF-I-

HUCPVs but not in controls.
Conclusion: Our results support that the observed therapeutic effect achieved with HUCPVs overexpressing IGF-1 could be mediated by a paracrine mechanism exerted by EVs carrying IGF-I. This data provides a novel approach for the experimental treatment of liver fibrosis.

ENDOCRINOLOGÍA / ENDOCRINOLOGY 2

320. (47) **DIETHYLNITROSAMINE (DEN) INCREASE HEPATIC TUMORIGENIC PATHWAYS IN HFD-INDUCED DIABETIC MICE.**

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Type 2 Diabetes Mellitus (T2DM) characterized by a pro-inflammatory state is associated with increased risk of developing cancer. DEN induces hepatocarcinogenesis increasing mitotic hepatocytes and leading to chronic inflammation. Aim: to determine if liver of T2DM induced by HFD is more sensitive to DEN, analyzing both tumorigenic pathways Wnt/β-catenin and TGF-β1/Smads, implicated in the early stage of hepatocellular carcinoma. Five-week-old mice C57BL/6 were divided into 4 experimental groups: Control (C, mice fed with normal diet), C treated with DEN (C+DEN), HFD (mice fed with high-fat diet), and animals fed with high-fat diet and received DEN (HFD+DEN) (n=4 in each group). Mice were euthanized at 25 weeks after DEN-injection. We analyzed by western blotting in nuclear extracts the expression of β-catenin, the levels were significantly increased in both groups HFD (+65%* and +81%#) and HFD+DEN (+57%* and +72%#) in comparison with C and C+DEN highlighting the effect of HFD to promotes the β-catenin accumulation. The amounts determinates in nuclear extracts of pSmad2/3, significantly increased in both groups HFD (+77%* and +73%#) and HFD+DEN (+156%* and +150%#) when comparing with C and C+DEN, it should be noted that the increase is significantly greater in HFD+DEN (+44%†) when compared with HFD, evidencing an additive effect of DEN. Unexpectedly Smad4 only showed a tendency to increase in HFD group, whereas it increased significantly in HFD+DEN (+118%* and +101%#) when compared with C and C+DEN. Both signaling pathways were corroborated in vitro. The exposure to a carcinogen such as DEN in the diabetic state could lead to an early stage in the hepatocarcinogenesis progression. In this regard, this precondition could represent a risk factor to an accelerated DEN-induced process. In this sense, it becomes highly relevant that in liver of HFD both tumorigenic pathways Wnt/β-catenin and TGF-β1/Smads are activated without to be induced by DEN.

321. (110) **COUP-TFI AND TOB1 REGULATE THE INTERACTION OF BMP-4 AND RA ON POMC TRANSCRIPTION**

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Cushing's disease pituitary corticotrophinomas have no definitive pharmacological treatment up-to-date. Studying the Proopiomelanocortin (POMC) gene expression as target, our group described in AtT-20 mouse corticotroph cell line model, the interaction between the signaling pathways of Retinoic Acid (RA) and Bone Morphogenetic Protein-4 (BMP-4) during transcriptional regulation in a putative response element for RA, in the proximal POMC promoter. Here we found that COUP-TFI (RA signaling blocker), disrupted the transcriptional complexes involving Smad proteins (BMP-4 signaling) and nuclear receptors for RA (RXRα, RXRγ and RARβ). We demonstrate that COUP-TFI blocked from 40-80% to 10% the inhibitory effect of 100nM RA on POMC-Luc reporter transcriptional activation and the potentiation of this inhibition in co-treatment with 100ng/ml BMP-4. COUP-TFI blocked the inhibitory effect of 100nM RA on POMC-Luc constructions with deletion or mutation of the BMPRE site, retaining the potentiated inhibition in co-treatment with 100ng/ml BMP-4. Tob1 negatively regulates BMP signaling. We observed by coimmunoprecipitation that Tob1 diminished the interaction between RARβ, RXRα and RXRγ with Smad4. Tob1 expression halted the inhibitory effect of 100ng/ml BMP-4 on POMC-Luc with deletion or mutation of the RARE site, without potentiation effect in co-treatment with 100nM RA. Moreover, the co-expression of COUP-TFI and Tob1 abolished the inhibitory effect of both 100nM RA and 100ng/ml BMP-4 treatments on complete POMC-Luc reporter, backing our observation in AtT-20 cells stably expressing a dominant negative of Smad4, where the effect of RA and BMP-4 is completely nullified. By EMSA with RARE sequence of POMC promoter as a probe and AtT-20 nuclear extracts, we observed a transcriptional complex binding to the RARE element which is displaced by α-Smad4 under RA and BMP-4 co-treatment. This supports our hypothesis about the involvement of BMP-4 pathway elements mediating the RA inhibitory action on POMC transcription.
Supported by ANPCyT, CONICET, UBA and FOCEM (COF 03/11).

322. (219) **LIVER ONCOGENIC POTENTIAL OF PROLONGED GROWTH HORMONE (GH)-ADMINISTRATION TO GROWING MICE**

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Growth hormone (GH) participates in multiple biological processes, including growth and metabolism; it is thus administered to children with growth deficiency and to adults under catabolic states, even if they are not GH-deficient. Due to its mitogenic and antiapoptotic activities, there is growing concern on its pro-tumorigenic potential as an adverse effect of its administration in the long term. To evaluate GH treatment on liver tumor formation, male mice were treated with GH during the growth period. Since GH is not a potent mitogen per se, a hepatic tumor inductor, diethylnitrosamine (DEN), was also administered before weaning.
Livers were removed at 48 weeks of age and were inspected for nodular lesions that differ from the surrounding liver parenchyma regarding size, color and texture. Only groups receiving DEN developed macroscopic tumors; GH-treatment alone did not induce tumor-formation, but given with DEN, increased the number of hepatic lesions. Liver sections were analyzed in search of preneoplastic morphological alterations. DEN-treated groups exhibited microscopically dysplastic foci whereas GH-treatment alone did not generate such alterations. The number of hepatocytes per microscopic field was increased in the dysplastic foci compared to the surrounding tissue, denoting smaller cell size inside the foci. Mice treated with GH exhibited larger cell size inside the dysplastic foci, compared to the non-GH-treated DEN-group. To evaluate hepatocellular proliferation, the expression of proliferating cell nuclear antigen (PCNA) was determined by immunohistochemistry. GH-treated groups exhibited a small although non-significant increase of PCNA positive nuclei. Increased cell proliferation was also observed inside dysplastic foci, although differences were significant only in animals that did not receive GH treatment.
Consequently, GH-treatment to growing mice per se does not promote tumor formation when the hormone is administered during the growth period at a therapeutic dose. However, GH-treatment could facilitate a pro-tumorigenic environment that would promote carcinogenesis.

323. (433) **THE GPCR-GAI REGULATED THE FGF2-INDUCED LACTOTROPH AND SOMATOTROPH CELL PROLIFERATION**

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In a previous study, we demonstrated that the co-incubation of basic fibroblast growth factor (FGF2) with the analog of somatostatin, octreotide (OCT) induced G1-phase arrest in pituitary cells, suggesting that the somatostatin receptors, inhibitory G protein-coupled receptors (GPCR-Gαi), may regulate the FGF2 effects. The aim was to evaluate whether the FGF2 proliferative activity is regulated by GPCR-Gαi specifically in lactotroph and somatotroph cell population. Anterior pituitary cell cultures from female rats were treated with FGF2 (10 ng/mL) or OCT (100mM) alone or co-incubated with or without pertussis toxin (PTX, 500nM), an inhibitor of GPCR-Gαi. The lactotroph and somatotroph cell proliferation was analyzed by double-immunocytochemistry of BrdU and PRL or GH at 24 and 48h. The p-ERK1/2, c-Jun and cell cycle regulation proteins: cyclin D1, CDK4, p21 and p27 were determined by western blot. Statistics: ANOVA-Bonferrony. The percentage of BrdU/lactotroph positive cells was 7.2% and BrdU/somatotroph positive cells was 3.4%. The lactotroph and somatotroph cell proliferation was increased by FGF2 whereas OCT decreased the cell mitosis respect to control group. The FGF2/OCT co-incubation significantly decreased the proliferation in both cells types after 24 and 48 h respect to FGF2 group, effect that was reverted by pre-incubation with PTX. The diminution in the cell proliferation was associated with an increase in the cell cycle inhibitors p27 and p21 expression, and a decrease of cycle D1 while the CDK4 did not show any significant variation. In addition, a remarkable decrease of pERK1/2 and c-Jun expression was observed after combined treatments. These findings show that OCT treatment inhibited the proliferation induced by FGF2 in PRL and GH cell populations regulating the expression of pERK1/2, c-Jun and key proteins controlling the cell cycle progression. This regulatory effect, mediated for GPCR-Gαi, could participate in the homeostasis of lactotroph and somatotroph cells, principal pituitary cell populations.

324. (74) **HISTOPATHOLOGICAL ASSESSMENT OF GONADAL TISSUE IN PATIENTS WITH TESTICULAR AND OVOTESTICULAR 46,XX DISORDERS OF SEX DEVELOPMENT**

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Disorders of sex development (DSD) are congenital conditions in which development of chromosomal, gonadal or anatomical sex is atypical. The aim of this study was to characterize the histology of 46,XX DSD prepubertal gonads.
We studied 25 gonads of fourteen 46,XX DSD patients. The age of biopsy/gonadectomy was 1.17 (0.08-4.17) years (median and range). Molecular studies confirmed the absence of SRY in blood samples of all patients and in 8 patients DNA gonads. Gonadal histology was assessed on H&E stained sections by two double blinded specialists and the findings were classified as testicular, ovarian or ovotesticular parenchyma, undifferentiated gonadal tissue (UGT) and gonadoblastoma (GB). Immunohistochemical (IHC) analysis identified Sertoli cells (SOX9), ovarian follicular cells (FOXL2), somatic cells (INHIBIN B), pluripotent germ cells (OCT3/4) and steroidogenic cells (HSD3B2 and CYP17A1).
Twenty one gonads (12 patients) were classified as ovotesticular and 4 (2 patients)as testicular. Dysgenetic testicular parenchyma was found in all cases.
Regarding the patients with ovotestis, in 2/12 the first biopsy showed only testicular tissue and a second biopsy revealed ovarian tissue as well. Moreover, 3 cases presented UGT and in 2 other patients GB was found.
IHC analysis of SOX9 and FOXL2 confirmed the presence of testicular/ovarian parenchyma, even in apparently undifferentiated structures. OCT3/4 was positive in 6 gonads (3 patients): 4 with UGT features (2 patients) and 2 with GB (1 patient). HSD3B2 and CYP17A1 revealed the presence of active steroidogenic cells. Expression of inhibin B, SOX9 and FOXL2 in UGT and GB was found.
Interestingly, in all cases signs of dysgenesis were only found in testicular parenchyma. It is noteworthy that a second biopsy in 2 former testicular cases revealed the presence of ovarian parenchyma. Considering the histopathological findings in early childhood, a close clinical follow up of patients with a specialized DSD team is suggested.

325. (75) **RELATIONSHIP BETWEEN VITAMIN D SERUM LEVELS AND GONADAL FUNCTION PARAMETERS IN INFERTILE PATIENTS**

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Vitamin D deficiency has been linked with various health disorders. Vitamin D could contribute to optimizing male gamete function and modulating gonadal steroid levels. The aim was to investigate the relationship between serum concentration of vitamin D and gonadal function parameters in male infertile patients and to comparing with results from healthy donors. Blood and semen samples were obtained from infertile patients (n = 29) and healthy normozoospermic donors (n = 27). Serum concentrations of vitamin D, total testosterone, estradiol and sex hormone binding globulin (SHBG) were determined by chemiluminescence assays. Free testosterone was determined by radioimmunoassay. Semen samples were analyzed as suggested by W.H.O. (2010). Statistical analysis was performed using Student t-test, contingency tables, and correlation studies. Significant differences between patients and healthy donors were observed in % progressive sperm motility ($p < 0.001$), % sperm mortality ($p < 0.001$), and % normal sperm morphology ($p < 0.001$). Vitamin D concentrations were lower in infertile patients than in controls (19.74 ± 1.40 ng/ml vs. 32.68 ± 1.52 ng/ml, $p < 0.001$). Serum levels of vitamin D < 20 ng/ml were significantly associated ($p < 0.001$) with infertility. Positive correlations between vitamin D > 30 ng/ml and total testosterone ($r = 0.51$, $p < 0.05$), free testosterone ($r = 0.60$, $p < 0.01$) and estradiol ($r = 0.28$, $p < 0.05$) were found in donors. SHBG levels were significantly lower in infertile patients than in donors ($p < 0.05$). An inverse correlation between vitamin D levels and % of sperm mortality ($r = -0.34$; $p < 0.01$) was detected. A positive correlation between vitamin D concentration and % of progressive sperm motility ($r = 0.32$; $p < 0.05$) was observed. The results suggest that vitamin D levels could affect male reproductive function and its deficiency could be associated with infertility.

326. (248) **HYPOTHALAMIC TRPV1 PARTICIPATES IN THE CONTROL OF THE REPRODUCTIVE AXIS IN MALE RATS**

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It is known that immune challenge inhibits the hypothalamic pituitary gonadal (HPG) axis activity, and we have recently reported that the hypothalamic cannabinoid receptor type 1 (CB1) partially mediates this effect in male rats, favoring the control of inflammatory response in detriment of sexual hormone release. However, no studies evaluating the participation of TRPV1 vanilloid receptor, which also acts as a nonspecific receptor for the endocannabinoid anandamide, were reported. Therefore, we assessed the participation of the hypothalamic TRPV1 on the HPG axis activity in rats submitted or not to immune challenge induced by LPS. Sprague Dawley adult males (n=6/group) were treated via intracerebroventricular (icv) with the TRPV1 antagonist Capsazepine (500ng/5ul), followed by an intraperitoneal (ip) administration of lipopolysaccharide (LPS, 5mg/kg) or saline 15 min later. 180 min post ip injections, hypothalamic pro-inflammatory cytokines and neuropeptides involved in reproductive function were assessed by qPCR. Hypothalamic Tnfα and Il1β mRNA expression was increased with both TRPV1 blockade and by LPS administra-

tion, separately ($p<0.05$). Both experimental conditions also inhibited gonadotropin-releasing hormone (Gnrh), and Kiss1 mRNA expression ($p<0.05$), and increased gonadotropin-inhibitory hormone (Rfrp3) mRNA ($p<0.05$) expression. Inhibitory effects were also observed at pituitary and testicular levels, with decreased plasma Luteinizing Hormone (LH) ($p<0.05$) and Testosterone levels ($p<0.05$) with both hypothalamic TRPV1 blockade and LPS administration. In summary, our results suggest that unlike hypothalamic CB1 receptor, which mediates reproductive axis blockade during the immune challenge to prioritize homeostasis maintenance, TRPV1 receptors could act in basal conditions, allowing the physiological response of the reproductive axis.

327. (33) **MOLECULAR MECHANISMS UNDERLYING INCREASED DE NOVO TRIIODOTHYRONINE FORMATION WITHIN THYROGLOBULIN IN HYPERSTIMULATED THYROCYTES**

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Thyroglobulin (TG), a 330kDa secretory glycoprotein comprising 4 structural regions, is the scaffold for de novo triiodothyronine (T3) formation which requires iodination and coupling of two iodotyrosines within TG. Hyperactivation of thyroidal TSH receptors (TSHRs) favor increased de novo T3 formation in TG as a consequence of other TG post-translational modifications such as its phosphorylation. Fam20C (a secretory serine kinase) is upregulated by TSHR hyperstimulation, accompanied by increased T3 formation in TG. Conversely, suppression of Fam20C decreases de novo T3 synthesis. Interestingly, a phospho-Ser present in hTG occurs at a canonical Fam20C phosphorylation site (equivalent to position S2718 in mTG). We hypothesize that Fam20C-mediated S2718 phosphorylation triggers increased T3 formation in mTG. To study the effects on T3 formation upon disruption of mTG-S2718 and the restitution of the mTG 2718 phospho-environment we bioengineered mTG-S2718A and mTG-S2718E, respectively. We co-expressed the TGs +/- Fam20C, +/- Fam20C inhibitor FL-1607 in 293T cells (lacking endogenous TG expression and with negligible endogenous Fam20C expression), performed enzymatic iodinations of the secreted TGs, and monitored de novo T3 formation by immunoblotting with mAb anti-T3 and polyclonal anti-TG Ab. Co-expression of WT mTG and Fam20C promoted T3 formation in TG and this effect was blocked by Fam20C inhibitor FL-1607. Further, disruption of the established phosphorylation site mTG-S2718A blocked Fam20C-stimulated T3 formation, and this effect was reverted in the phosphomimetic mutant mTG-S2718E. Our data supports the hypothesis that de novo T3 formation in TG is stimulated by Fam20C-mediated phosphorylation of mTG-S2718. Since Fam20C levels are increased in states of TSHR hyperactivation, Fam20C-induced de novo T3 formation may be a contributor to the increased T3 found in hyperstimulated thyroid glands such as occurs in autoimmune hyperthyroidism of Graves´ disease.

328. (563) **GROWTH HORMONE ADMINISTRATION PATTERNS HAVE DIFFERENT MOLECULAR EFFECTS IN FEMALE MICE BREAST TISSUE.**

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Recombinant growth hormone (GH) is used for the treatment of different pathologies, including pediatric and adult GH deficiency, chronic renal insufficiency, Turner syndrome and cachexia secondary to AIDS. The main disadvantages of GH administration are the short half-life of the hormone, its renal toxicity and the necessity of multiple injections which turns the treatment stressing and uncomfortable for patients; thus, GH is a good candidate for depot formulations producing continuous release of the hormone. However, the effects of different GH-administration modes over cell proliferation and tumorigenesis are not known. High GH levels have been associated with the development of tumors in humans, and transgenic mice overexpressing GH show increased tendency to develop cancer. The purpose of the present work was to study the differential effects of GH administration patterns, intermittent and continuous, on the expression of receptors involved in mammary tissue growth and development, and the engagement of signaling pathways involved in cell proliferation and survival. For this purpose, female mice were implanted with osmotic pumps for sustained release of GH or they were given GH by intermittent injections. The effects of different GH administration patterns over the expression of estrogen receptor alpha (ERα), epidermal growth factor receptor (EGFR) and insulin like growth factor-1 receptor (IGF-1R) were assessed by Immunoblotting. Besides, the early genes protein c-fos, c-myc and c-jun, as well the protein content and basal activation of Akt and Src was assessed.
Results showed that intermittent injections of GH induced the up-regulation of EGFR, IGF-IR and c-fos protein content as well as an increase in Akt basal activation. However, continuous GH delivery did not have any significant molecular effect in breast tissue.
Current results suggest sustained GH delivery would have less promitogenic consequences in breast tissue, in accordance to previous observations in mice liver from our research group.

329. (598) **THE FERTILIZING CAPACITY OF HUMAN SPERM DEPENDS ON ITS MEMBRANE POTENTIAL**

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Mammalian sperm cannot fertilize the egg without suffering certain physiological changes triggered during its course through the female reproductive tract, known as capacitation. At the molecular level, capacitation involves plasma membrane reorganization, post translational protein modifications and ion membrane permeability changes, which impact on the plasma membrane potential (Em). Em hyperpolarization associated to capacitation is well described for mouse sperm, and shown to be both necessary and sufficient for sperm to undergo the acrosome reaction. Considering its importance in mouse sperm we hypothesized that it could also be involved in human sperm capacitation.
First, we aimed to set up a fluorimetric population assay that would allow us to analyze Em variations in human sperm. Sperm Em from healthy volunteers was analyzed with the cationic DiSC(3)5 fluorescent probe using a heated stage fluorimeter. Once this assay was optimized, we analyzed Em sperm values from patients attending a reproductive clinic and related these values to their fertilization outcome by in vitro fertilization (IVF). In addition, acrosome responsiveness was evaluated for each sample.
We could determine cell number (3 million) and dye concentration (1 μM) to measure human sperm Em by the mentioned assay. Em changes in samples from patients attending a reproductive clinic correlated to their fertility outcome: samples which hyperpolarized after a 3 hour incubation in capacitating media yielded above 70% fertilized oocytes upon IVF. However, samples where no hyperpolarization was observed gave lower fertilization rates (less than 50%). Accordingly, hyperpolarized samples had a better acrosomal responsiveness when exposed to an inductor as calcium ionophore A23187.
These data strongly suggest that human sperm membrane potential correlates with fertilization rates in IVF, indicating that Em changes have an implication in sperm fertilizing capacity. This study has the potential value to add diagnostic tools to help predict the success of different reproductive techniques.

330. (84) **ANALYSIS OF CONCENTRATION OF PLASMA LIPIDS AND HISTOLOGYCAL LIVER STUDY IN HYPERLIPEMIC WISTAR RATS FED WITH HIGH FAT DIET (HFD) TREATED FOR 7 AND 10 DAYS WITH LIGARIA CUNEIFOLIA (LC)-PROANTOCIANIDIN ENRICHED FRACTION**

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Lc crude extract administrated i.p. decreased plasma cholesterol (Cho) and increased blood viscosity in rats. Now, we analyzed the effect of Lc-proanthocyanidin enriched fraction (PLc) during different times of administration on both Cho and triglycerides (TG), and the content of fat vacuoles in the liver in hyperlipemic rats. Adult male Wistar rats (aged 70 days, n=24) were fed with standard diet added with 40% bovine meat juice during 28 days (HFD). Then, were administered i.p. each 24hr with either physiological solution (controls C, C7 and C10, n=6 each one) or PLc 3mg /100g body weight (treated T, n=12) during 7 (T7, n=6) and 10 (T10, n=6) days. In day 8 and 11 they were anesthesized i.p. with Ketamine/Xylazine (100mg/kg/3mg/kg) to obtain blood samples by cardiac puncture and the liver were removed. In plasma, Cho (total, HDL and LDL) and TG were determined by enzymatic methods. Histological study was performed in liver pieces embedded in paraffin, cut and stained with hematoxylin-eosin, trichromica Masson-Alcian Blue, for semiquantitative evaluation of steatosis. Results: (mean ± SE). C7 and C10 show no significant differences. Plasma: Cho(mg%): C:203.33±21.54; T7:119.50±14.26*; T10:109.83±11.14*; ChoHDL: C:22.40±1.66; T7:21.25±0.85; T10:19.62±0.84; ChoLDL: C:28.30±1.68; T7 :19.25±0.47*; T10:16.27±0.71*; TG: C:274.66±30.66; T7:190.50±21.65*; T10:174.50±16.18* (*p<0.05 vs. C, Student's t Test for unpaired data). Liver: Score (steatosis grade, % of parenchyma involved by steatosis): C:2,0±0,5(33-66%); T7:1,0±0,5(5-33%); T10:0,1±0,01*&(<5%) (*p<0.05 vs. C; & p<0.05 vs. T7); none of the groups presented microvesicular steatosis, and the localization were always in periportal zone. Conclusion: PLc treatment for 7 and 10 days showed an important and similar lipid-lowering effect (Cho, CoLDL and TG) in rats fed with a HFD. The decline in one of the main risk factors in the development of atherosclerosis as ChoLDL allows us to suggest PLc as a possible and safe tool in the prevention of cardiovascular disease.

INMUNOLOGÍA / IMMUNOLOGY 3

331. (190) **SHIGA TOXIN TYPE 2 ACTIVATES AND ALTERS REPAIR FUNCTIONS OF LIPOPOLYSACCHARIDE-SENSITIZED LATE OUTGROWTH ENDOTHELIAL PROGENITOR CELLS.**

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Hemolytic Uremic Syndrome (HUS), the main cause of pediatric acute renal failure, is caused by E. coli producing Shiga toxins (Stx) and characterized by massive endothelial damage, which is worsened by inflammation, especially in the presence of bacterial lipopolysaccharides (LPS). Endogenous regeneration of the vessel wall involves local and bone marrow-derived endothelial progenitor cells (EPC) as well as nearby mature endothelial cells (EC). Although Stx toxic effects on mature EC have been widely studied, its action on EPC and angiogenesis remain to be elucidated. We aimed to analyze the effect of Stx alone or in combination with LPS on survival and angiogenic properties of EPC.
Human cord blood-derived late-outgrowth EPC and umbilical vein EC were cultured in cytokine-rich growth medium EGM2. Cells were sensitized with LPS for 18 h and then Stx type 2 was added for another 18 h.
Nuclear morphology analysis revealed that Stx2, at concentrations that are known to induce mature EC death, had no effect on EPC survival cultured in basic medium (EBM2+ 2% FBS), which undergo apoptosis only after LPS sensitization ($p<0.05$). Moreover, no Stx2 cytotoxicity was observed when EPC were cultured on EGM2 ($p<0.05$), while EPC proliferation was not affected ($p<0.05$).
Stx2 induced activation on LPS-sensitized EPC observed by increased ICAM-1 and E-selectin expression (flow cytometry, ($p<0.05$). In addition, Stx2 altered EPC angiogenic functions. While new vessel formation (matrigel), was augmented by Stx2 ($p<0.05$), self-repair abilities (scratch assay) and adhesion to extracellular matrix proteins were diminished by the toxin when combined with LPS ($p<0.05$).
Stx2 receptor Gb3 was expressed on EPC surface and upregulated by LPS+Stx2 ($p<0.05$, flow cytometry). This could account for increased effects observed when EPC are sensitized with LPS.
Our results showed that EPC not only were more resistant to Stx2-mediated toxicity than mature EC, but also displayed altered angiogenic abilities.

332. (225) **ROLE OF EGFR AND CALPAIN IN THE OSTEOCLASTOGENESIS INDUCED BY STAPHYLOCOCCUS AUREUS.**

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The importance of signaling mediated by the epidermal growth factor receptor (EGFR) during osteoclastogenesis in physiological conditions has been established. *S. aureus* protein A is a critical virulence factor that induces exacerbated osteoclastogenesis and bone damage during the course of staphylococcal osteomyelitis. Considering that protein A signals through EGFR in epithelial cells, macrophages and osteoclast precursors, the aim of this study was to determine the impact of EGFR signaling initiated by *S. aureus* in the cytoskeleton rearrangements that are required for osteoclast formation. *In vitro* differentiation assays using a chemical inhibitor of EGFR demonstrated that EGFR signaling is required for *S. aureus* induced osteoclastogenesis ($p<0.0001$). A dose-dependent blockade in the presence of the inhibitor was observed. Our data also showed that *S. aureus* positively modulates the expression of RANK (receptor activator of NFκB) on the surface of osteoclasts which might have important implications during osteoclastogenesis. E-cadherin surface expression was maximal at 24 hours after the onset of differentiation (MFI: 357.30) and decreased thereafter (MFI: 144.28), suggesting that cell fusion mechanisms similar to those described under physiological conditions take place during *S. aureus* induced osteoclastogenesis. On the contrary, vitronectin receptor expression was not modulated in response to *S. aureus*. The role of calpains in osteoclasts formation was evaluated by using the chemical inhibitor Calpeptin. A dose-response inhibition of osteoclastogenesis was observed in the presence of the inhibitor ($p<0.001$), demonstrating the importance of calpain in the differentiation of mature osteoclasts. Interestingly, calpain inhibition blocked osteoclast differentiation after the first round of fusions and bi-nucleated cells were observed. This process differed from EGFR inhibition which completely abrogated cell fusion. Taken together, these results help to improve our understanding of the mechanisms involved in the osteoclastogenesis induced by *S. aureus* and might contribute to the design of alternative therapies for staphylococcal osteomyelitis.

333. (262) **TIRNAS IN PULMONARY OBSTRUCTIVE DISEASES. STUDY OF THEIR POTENTIAL ROLE AS MODULATORS OF CORTICOSTEROID RESPONSE**

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tiRNAs are 30-40 pb double stranded RNA fragments generated under stress condition by a specific cleavage of mature tRNAs. These molecules have been shown to regulate gene expression by either functioning as novel epigenetic factors or by traslational repression. Different lines of evidence indicate that glucocorticoids (GCs) resis-

tance is the result of significant changes in the cellular microenvironment that occur over time in chronic inflammatory states. Since resistance to corticosteroids in patients with severe asthma and with chronic obstructive pulmonary disease (COPD) is an important barrier to an effective treatment, in this work we studied whether tiRNAs could be playing a role in the corticosteroid response in these diseases. tiRNA-Glu and tiRNA-Gly, two highly abundant tiRNAs in saliva, were successfully quantified in sputum and blood samples (n=20) of healthy individuals and patients with asthma and COPD, both in the intracellular and extracellular fluids. Levels of tiRNA-Glu showed a mild increase in patients with obstructive pulmonary diseases in blood and sputum cells and they positively correlated with cortisol in sputum supernatants ($p<0,05$). Studies on the gene that codifies for the glucocoticoid receptor (GR) in our samples showed that its most abundant transcript is the one that encodes for the GRalpha isoform with a short ~0,7 kb 3'UTR. However, a longer transcript that gives rise to the inactive GRbeta isoform is also expressed in lower extent in all patients. Interestingly, its levels in sputum cells of patients showed a strong association with tiRNA-Glu ($p<0,01$, $R=0,9167$) not observed in healthy individuals, suggesting that this molecule could be involved in the alternative splicing of the transcript in pathological conditions. In summary, these results provide a first approach for the better understanding of the immunoendocrine alterations associated with pulmonary obstructive diseases and the potential role of regulatory mechanisms mediated by tiRNAs in corticosteroid response.

334. (288) **CANDIDA ALBICANS ACTIVATES TYPE I INTERFERON PATHWAY IN CELLS OF THE FEMALE GENITAL TRACT**

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C. albicans (Ca) is an opportunistic fungal pathogen that typically exists as a harmless commensal in the female genital tract; however, approximately 75% of women who are of reproductive-age suffer from vulvovaginal candidiasis(VVC) at least once in their lifetime. Recent studies revealed that, besides viruses and bacteria, fungal pathogens can also induce type I interferons (IFNs-I) production by innate immune cells. The aim was to study whether *Ca* recognition induces IFNs-I in epithelial cells of female genital tract in order to establish a possible role during VVC. For this purpose, human cervical epithelial cell line (HeLa) were stimulated with: *Ca* SC5314 strain as Invasive *Ca* (hypha emission) and Non-invasive *Ca* (pseudomicelio) (0,25:1 fungus:cell ratio); *Ca* DNA complexed with polyethylenimine (*Ca*-DNA), LPS and Poly I:C during 4 and 24h. IFNβ, IRF3, IRF7 and MX1 (IFNAR receptor activation marker) mRNA levels were measured by qPCR. A comparative analysis was made between 4 and 24h. LPS (4h) and Poly I:C (24h) were able to induce a strong IFNβ mRNA expression in HeLa cells($p<0,001$). Interestingly, Invasive *Ca*triggered an early IFNβ expression (4h) ($p<0,001$) involving high levels of IRF7($p<0,01$) and IRF3($p<0,05$). Non-invasive *Ca* induced similar levels of IFNβ and IRF7 at 4h($p<0,001$), but, it was also able to induce IFNβ expression at 24h($p<0,05$). Both stimuli showed a higher MX1 expression at 24h($p<0,05$), however, invasive *Ca* induced a 10-fold increase($p<0,05$). *Ca*-DNA induced IFNβ at 24h($p<0,001$) with high IRF3 expression($p<0,001$). However, it was not possible to observe MX1 induction at the time evaluated. Studies performed in vaginal lavage cells of patients with Recurrent VVC showed that 50% expressed high levels of IFNβ mRNA while Acute VVC patients did not. These results provide important and novel evidence about the ability of *C. albicans* to activate the IFNs-I pathway in epithelial cells of the female genital tract.

335. (308) **THE AQUEOUS EXTRACT OF AN ARGENTINIAN MEDICINAL PLANT (SMILAX CAMPESTRIS) INDUCE OSTEOBLAST DIFFERENTIATION, ACTING AT OESTROGEN RECEPTORS (ER) ALPHA**

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Smilax campestris is a medicinal plant traditionally used as antirheumatic due to its anti-inflammatory properties. We are interested in to evaluate whether Smilax campestris have a bone-protective role during estrogen deprivation. Previously we demonstrated that aqueous extract of Smilax campestris (SM) inhibited osteoclast differentiation and induced osteoclast apoptosis, acting at ERα. The aim of the present work was to evaluate whether SM could regulate the osteoblast differentiation. To elucidate this, we analyzed in vitro the effect of SM on the ability of MC3T3-E1 preosteoblastic cell line to secrete mineral and organic matrix and to compare this effect with the one of estradiol. Cells were cultured with or without SM (10, 100, 1000 ng/ml), estradiol (10^{-8}, 10^{-7}, 10^{-6} M), or with Ascorbic acid (50 µg/ml) and β-glycerophosphate (10 mM) as positive control, during 7 and 14 days. To analyze the role of ER, MC3T3-E1 cells were incubated for 1h with ER antagonists previous to SM or estradiol treatment. The medium and stimuli were replaced every 2 or 3 days. Then we analyzed in these cells the bone specific alkaline phosphatase activity (ALP), using the BCIP/NBT substrate system, as well as calcium and collagen deposition by alizarin red and sirius red staining, respectively. Cells were lysed and staining was measured by spectrophotometry. Statistical analysis was applied and differences were considered significant at a $P<0.05$ ($N=5$ independent experiments). At 14 days, SM 10 ng/ml stimulated ALP activity, calcium and collagen deposition in MC3T3 cells, similar to estradiol (10^{-8} M), acting through ERα. SM 10 ng/ml didn´t affect the osteoblast differentiation induced by ascorbic acid and β-glycerophosphate, measured at 7 and 14 days. We conclude that SM significantly induce osteoblast differentiation, similar to estradiol, and that ERα are involved in this effect. SM doesn´t affect ascorbic acid and β-glycerophosphate- induced osteoblast differentiation.

336. (330) **DUAL ROLE OF THE TLR-AGONIST PROFILIN PROTEIN FROM TOXOPLASMA GONDII: STUDY OF THE ADJUVANT AND IMMUNOGENIC VALUE IN A VACCINE FORMULATION IN TWO MOUSE STRAINS WITH DIFFERENT SUSCEPTIBILITY**

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Toxoplasmosis is a disease that affects 30% of the world's population. At present, there are no pharmacological treatments that eliminate the parasite or vaccines that confer protection to the host. The aim of the present work was to study the immunogenicity of a vaccine formulation containing a recombinant form of the *T. gondii* Profilin protein (rTgPF), a TLR ligand, in combination with the dense granule GRA7 protein (rGRA7), in two mouse strains with different susceptibility to chronic toxoplasmosis. BALB/c and C57BL/6 mice were intradermally immunized 3-times with a 2-week interval with: rGRA7, rTgPF, rGRA7+rTgPF, rGRA7+ACF. Naive mice were used as control. While in BALB/c mice rGRA7+rTgPF vaccination generated an anti-GRA7 humoral response with a Th1 profile and rGRA7+ACF showed a mixed profile, both formulations induced a mixed profile in C57BL/6 mice. *Ex vivo* stimulation of splenocytes with rGRA7 induced significant proliferative responses ($p<0.05$) and IFN-γ production ($p<0.05$) in rGRA7+rTgPF and rGRA7+ACF groups of both mouse strains. In addition, although rTgPF generated very low humoral responses in both mouse strains, significant proliferative responses ($p<0.05$) and IFN-γ production ($p<0.05$) were detected in rTgPF-stimulated splenocytes from both rGRA7+rTgPF and rTgPF groups. After an oral challenge with a non-lethal dose of *T. gondii* cysts, a 62% reduction in brain parasite load compared to control was obtained with rGRA7+rTgPF vaccine formulation in BALB/c ($p<0.05$), similarly to the level obtained with rGRA7+ACF. In contrast, none of the vaccines induced protection in C57BL/6 mice. These results demonstrate a dual role of rTgPF. As an adjuvant with

capacity to enhance the immunogenicity of another antigen, and also as an immunogen given its ability to induce specific-cellular responses. We also showed the importance of the genetic background of experimental animals when evaluating vaccination protocols, therefore highlighting the importance of testing the same vaccine formulation in animals with different haplotypes.

337. (338) **HIV+ SPECIFIC CD8+ T-CELLS ANTIVIRAL ACTIVITY CORRELATES WITH CELL PHENOTYPE AND POLYFUNCTIONALITY IN SUBJECTS WHO INITIATED TREATMENT AT DIFFERENT TIME POINTS AFTER ACUTE INFECTION**

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The success of strategies to achieve HIV functional cure will largely rely on the ability of HIV-specific CD8+ T-cells (CD8TC) to clear reactivated infected cells. AIM: To evaluate the phenotype and function of expanded HIV-specific CD8TCs, from subjects initiating combined antiretroviral therapy (cART) at different times post-infection.
25 HIV+ subjects on one-year cART, that either initiated treatment earlier (ET: Early treatment, 13 subjects) or later (DT: Delayed treatment, 12 subjects) after infection, were recruited. PBMCs were stimulated with peptides pools spanning Nef/p24 proteins during 14 days and, then CD8TC phenotype (CD45RO, CCR7, CD95 and PD1 expression) and function (CD107a/b, IFN-γ, IL-2, MIP-1β and TNF-α) were analyzed. Direct and indirect antiviral activity of expanded CD8TCs against autologous CD4TCs were evaluated by VITAL Assay (VA) and Viral Inhibition Assay (VIA), respectively. Non-parametric statistics were applied.
Expanded cells were highly polyfunctional, regardless of cART initiation timing. Memory/effector phenotype distribution showed a preservation of memory stem-cell ($p \leq 0.005$) and central memory ($p \leq 0.01$) subsets in ET. Contrary, DT showed a fully-differentiated profile ($p \leq 0.005$). PD-1 expression was clustered in HIV-specific terminal effector cells ($p \leq 0.001$). Also, PD-1 directly correlated with CD8TC functionality. Expanded CD8TCs from DTs and ETs were highly capable of mediating antiviral activity, and it correlated with the proportion of fully differentiated effector cells (VIA $p=0.04$, VA $p=0.028$) as well as with CD8TC polyfunctionality and PD-1 expression (VA $p=0.0249$ and $p=0.016$, respectively).
In sum, despite HIV-specific CD8TC response is dampened in subjects under cART, it could be selectively stimulated and expanded in vitro, presenting a high proportion of cells able to carry-out multiple effector functions. cART initiation timing had an impact on the memory phenotype, most likely reflecting different times of antigen persistence. Overall, these results have important implications on the design of strategies for modulating CD8TCs with the objective of reaching an HIV functional cure.

338. (358) **EXPRESSION OF PPARΓ AND PPARA ON A MACROPHAGIC CELL LINE STIMULATED WITH MYCOBACTERIUM TUBERCULOSIS**

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Tuberculosis (TB) is a major health problem characterized by an immune-endocrine imbalance: high plasma levels of cortisol and pro- and anti-inflammatory mediators, as well as lowered Dehydroepiandrosterone levels. The etiologic agent, Mycobacterium tuberculosis (Mtb) is captured by lung macrophages (Mf), whose activation is required to cope with Mtb control, along with some form of bystander tissue damage. Glucocorticoids (GCs) are critical elements to counterbalance the immune-inflammatory reaction, with peroxisome proliferator-activated receptors (PPAR) being also implicated in this regard. The main forms of these receptors are: PPARα, PPARβδ and PPARγ. TB patients showed an increased expression of PPARγ and PPARα transcript in their peripheral blood mononuclear cells, the former being positively associated with circulating cortisol and disease severity. Given this background, we now investigated the expression of PPARγ and PPARα transcripts by real time PCR in Mf (adherent human THP-1 cells, n=8) stimulated with Mtb (strain H37Rv killed by γ radiation -Mtbi-). There was an increased in transcript expression of both receptors after 24 hr stimulation ($p<0.05$). Since PPARγ is the most studied one in terms of its anti-inflammatory effect, receptor agonist-treated cultures were also studied. The stimulation-driven increase of Mf IL-1 production ($p<0.001$) was significantly decreased upon PPARγ agonist ($p<0.05$). As expected, adding GCs (10-6M) to stimulated cultures diminished IL-1 production ($p<0.001$), even more when cells were also exposed to PPARγ agonist ($p<0.01$, vs. cultures with no agonist). Furthermore, we investigated whether this anti-inflammatory effect of PPARγ was evidenced in early stages post-stimulus. Studies in 3 hr stimulated Mf revealed that Mtbi significantly increased the release of IL-1, whereas PPARγ agonist diminished these levels ($p<0.01$) by the same time-point evaluation. These results suggest that PPARγ activation renders Mf cells with a lower inflammatory effect that may further favor mycobacterial development.

339. (366) **POTENTIATION OF THE IMMUNE RESPONSE ELICITED BY A COMMERCIAL VACCINE AGAINST BOVINE RESPIRATORY DISEASE (BRD) EMPLOYING AN IMMUNOMODULATOR IN A MURINE MODEL**

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BRD is a multi-factorial disease that causes high morbidity and mortality in cattle. BRD etiology is composed by viruses and bacteria: Mannheimia haemolytica (MH), Pasteurella multocida (PM), Histophilus somni (HS). Although BRD vaccines are worldwide utilized, there is a need of new therapeutic-adjuvant strategies in order to minimize the disease impact. Propionibacterium acnes (Pa) is a Gram-positive anaerobic bacillus and it is used as a heat-killed suspension for treatment of non-specific respiratory disease in horses because it has beneficial immunomodulatory activity. This work aimed to evaluate the effect of Pa on the humoral and cellular immune response elicited by a commercial BRD vaccine.
BALB/c mice were subcutaneously (sc) injected with P. acnes (350 µg or 500 µg/animal) on days -7, 10 and 21. The BRD immunization was performed on days 0, 15 and 27 by sc injection. Blood samples were obtained on days 0, 10, 27 and day 38 when animals were sacrificed. Control group received the commercial vaccine. Serum specific antibody levels (IgG, IgG1, and IgG2a anti-PM, MH and HS) and cytokines in spleen supernatants, were determined by ELISA.
At final day, BRD immunization induced specific IgG titers and the production of IFNγ, in all immunized mice. Treatment with Pa increased anti-PM IgG levels ($p< 0,001$) and IgG2a for MH, PM and HS ($p< 0,05$). In relation with anti-MH and HS IgG and IgG1, antibody levels in Pa treated-mice were higher than the immunized control group, although without reaching a statistical significance.
These results indicate that the administration of P. acnes was able to enhance the response elicited by a BRD vaccine, so it could be used as an adjuvant strategy to potentiate weak immune responses.

340. (368) **DISSECTING THE IMMUNE STIMULATION PROMOTED BY CSF-470 VACCINE PLUS ADJUVANTS IN CUTANEOUS MELANOMA PTS: SHORT TERM RELEASE OF ACUTE INFLAMMATORY REACTANTS**

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As cutaneous melanoma (CM) remains with a bleak prognosis, thorough investigation of new treatment options are of utmost relevance. In the phase II/III CASVAC-0401 clinical trial, the immunization of

stages IIB-III post-surgery CM patients with the irradiated, allogeneic cellular CSF-470 vaccine plus BCG and GM-CSF as adjuvants, demonstrated a significant benefit over IFN-alpha2B treatment in distant metastasis-free survival, as well as demostrated a successful antitumoral immune response (Mordoh et al., 2017). In order to better understand the efficacious immune response observed, we measured the short-term vaccine-induced cytokine and acute phase protein fluctuations both in in vitro co-cultures and patient serum samples. Time course release experiments using co-cultures of CSF-470 vaccine components and peripheral blood mononuclear cells (PBMC) from healthy donors, showed the release of IL-6, IL-10, and MCP-1. Conversely, patient serum samples analysis at 24 hours following vaccination, revealed increases in IL-6 (5.7 ± 8.7 to 32.0 ± 22.0 pg/ml, Wilcoxon matched-pairs signed rank test, p=0.0005); and C reactive protein (CRP) (2.4 ± 2.4 to 9.4 ± 6.1 mg/l, paired T-test, p=0.0005). As IL-6 is the well-documented stimulator of CRP production we infer that IL-6 likely released at the vaccination site stimulates CRP production from the liver. In order to investigate whether the acute increases in IL-6 and CRP could stimulate an antitumoral immune response in the context of a potential micrometastatic site, PBMC lysis of melanoma cells in the presence of fibroblasts, with or without CRP or IL-6, was assayed in vitro by measuring the number of colonies in clonogenic assays. CRP significantly inhibited tumor cells colony formation by 65%, whereas IL-6 had no effect (ANOVA, p<0.05). Consequently, we start to elucidate the potential mechanism of vaccine-induced immunity thus involving initial pro and anti-inflammatory cytokine fluctuations which could occur at the vaccine site and result in a systemic response.

341. (379) **MYELOID-DERIVED SUPPRESSOR CELLS EXPANDED BY ORAL YERSINIA ENTEROCOLÍTICA INFECTION MEDIATE THEIR IMMUNOSUPPRESSIVE ACTIVITY THROUGH A NITRIC OXIDE-DEPENDENT MECHANISM**

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Myeloid-derived suppressor cells (MDSC) represent a heterogeneous population of largely immature myeloid cells endowed with a robust immunosuppressive activity. *Yersinia enterocolitica* (Ye) are Gram-negative bacteria that cause food-borne gastrointestinal diseases. The MDSCs role in host-pathogen interactions has been poorly defined. In previous studies we demonstrated that oral Ye infection of mice induces MDSCs expansion in intestine and spleen. However, the mechanisms of MDSC immunosuppressive activity in Ye infection has not been defined. The purpose of this work was to analyse the mechanism by which MDSC exert their suppressive activity in this infection. Therefore, C57BL/6 mice were orally infected with Ye WAP-314 serotype O:8. On days 5, 10 and 20 post-infection (p.i), cellular infiltration in mesenteric lymph nodes (MLN), Peyer´s patches (PP) and spleen was analysed. Suppressive activity on cell proliferation was determined by MTT assay, and nitrite levels were measured with Griess reagent. After in vitro iNOS inhibition with Aminoguanidine (AG), arginase-1 activity was indirectly determined by colorimetric quantification of urea. We found that Ye-infected mice increased the frequencies of $CD11b^{+}Gr\text{-}1^{+}$ cells in PP, MLN and spleen on days 5, 10 and 20 p.i ($p<0.05$, compared with uninfected mice). This finding was accompanied with higher serum IL-6 levels on days 5 and 10 p.i ($p<0.01$ and $p<0.05$, respectively). After specific stimulation, splenocytes and MLN cells obtained from MDSC-depleted mice exhibited higher proliferation compared to cells from non-depleted mice ($p<0.001$ and $p<0.05$, respectively). Nitrite production increased significantly in cells of non-depleted mice ($p<0.01$). In addition, immunosuppression was prevented when stimulated splenocytes were treated with AG ($p<0.001$), which also increased IFN-γ production ($p<0.01$). However, stimulated and non-stimulated splenocytes did not show differences in arginase-1 activity. According to the results, we conclude that oral Ye infection induces expansion of MDSCs that mediate their immunosuppressive activity by a nitric oxide-dependent mechanism.

342. (380) **EXTRACELLULAR VESICLES FROM PLASMA OF HIV-1-INFECTED INDIVIDUALS TRIGGER MACROPHAGE-MEDIATED INFLAMMATION AND HIF-1A ACTIVITY**

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Chronic immune activation and inflammation are hallmarks of HIV-1 infection and a major cause of serious non-AIDS events in HIV-1-infected individuals on antiretroviral treatment (ART), such as non-AIDS malignancies, cardiovascular events, renal and hepatic disease, bone disorders and neurocognitive impairment. Activated macrophages are responsible for sustaining chronic inflammation and therefore are key players in the pathogenesis of these diseases. The Hypoxia-Inducible Factor-1α (HIF-1α) is a transcriptional activator factor that plays a central role in cellular metabolism and function, including the pro-inflammatory activity of macrophages. Extracellular vesicles (EVs) comprise a heterogeneous group of membrane-surrounded structures secreted by a wide variety of cells which mediate intercellular communication. The aim of this work was to evaluate the role of circulating EVs from HIV-1-infected individuals in the activation of macrophages and the role of HIF-1α in this process.
EVs from 2 ml of plasma from HIV-1-negative and ART-treated HIV-1-positive individuals with undetectable viral load were isolated by size exclusion chromatography followed by centrifugation. Plasma-derived EVs were incubated with monocyte-derived macrophages for 24 h and the production of pro-inflammatory cytokines in cell culture supernatants was evaluated by enzyme immunoassay and/or cytokine bead array. The ability of EVs to trigger HIF-1α activity was studied in the reporter cell line HeLa HRE-GFP.
Macrophages treated with EVs from HIV-1-infected individuals produced significantly higher levels of IL-6 ($p<0.05$), IL-1β ($p<0.05$) and TNF-α ($p<0.01$) as compared with healthy donors. Remarkably, EVs from HIV-1 infected individuals triggered HIF-1α activity ($p<0.05$), which positively correlated with the secretion of IL-6 and IL-1β ($p<0.05$).
In conclusion, circulating EVs from HIV-1-infected individuals undergoing effective ART exerted a pro-inflammatory effect on macrophages, which was associated with the induction of HIF-1α activity. This EV-mediated inflammatory response could play a role in the induction of non-AIDS-related complications of HIV-1+ individuals.

343. (392) **B. PARAPERTUSSIS SUBVERTS IFN-Γ-INDUCED BACTERICIDAL ACTIVITY IN HUMAN NEUTROPHILS**

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Whooping cough is a human re-emerging disease caused by *Bordetella pertussis* (Bp) and *Bordetella parapertussis* (Bpp). Its resurgence is partially due to an increase in the incidence of Bpp that started with the introduction of acellular vaccine. This vaccine fails to induce opsonic antibodies against Bpp. Our studies showed that in the absence of opsonic antibodies Bpp survives the encounter with resting neutrophils and macrophages by inhibiting cellular bactericidal mechanisms. The presence of IFN-γ in the in vivo infection environment might change the outcome of these interactions. In this study we investigated the eventual role of IFN-γ in the interaction of Bpp with human neutrophils. To this end, neutrophils were treated or not with IFN-γ. Bacterial phagocytosis and intracellular survival were assayed by fluorescence microscopy and polymixin B protection assays, respectively. Statistical differences were analyzed by ANOVA ($p<0.05$). The results showed that the pre-treatment of neutrophils with IFN-γ did not induce significant changes in Bpp phagocytosis (around 4% of total inoculum MOI: 100) or in the intracellular survival (around 20% of the phagocytosed bacteria). These results suggest that Bpp either evade or inhibit the bactericidal activity of IFN-γ-primed neutrophils. We then evaluated neutrophil activation during this interaction. IFN-γ-primed neutrophils incubated with Concanav-

alin A were used as a positive control. Luminometry assays showed that Bpp does not induce respiratory burst in untreated cells but also inhibits ROS production in IFN-γ primed neutrophils. Accordingly, transcription of NADPH oxidase complex subunits gp91phox and p47phox, actively induced in IFN-γ-treated neutrophils, was inhibited upon infection with Bpp, as assessed by RT-qPCR assays. Taken together, these results indicate that in the absence of opsonic antibodies, Bpp can subvert neutrophil killing functions even in the presence of IFN-γ.

344. (405) **ANALYSIS OF CHLAMYDIA TRACHOMATIS INFECTION OF THE MALE GENITAL TRACT FROM FERTILE AND INFERTILE PATIENTS AND ITS CONSEQUENCES IN SEMEN QUALITY.**

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Urogenital infection and inflammation have been implicated in 8-35% of male infertility cases worldwide. *Chlamydia trachomatis* (CT) is the most common bacterial cause of sexually transmitted diseases. Untreated CT infections in women are well known causes of infertility. However, whether CT infection of the male urogenital tract (MUT) is detrimental to sperm quality and male fertility is still controversial. Herein, we analyzed the impact of CT infection of the MUT on semen quality parameters in a population of fertile and infertile patients.
Semen samples were collected from a total of 1345 fertile and infertile men attending a fertility clinic. Infertility was diagnosed as the failure to achieve a clinical pregnancy after one year or more of regular unprotected sexual intercourse. Patients infected with other common uropathogens were excluded. Semen samples were obtained by masturbation and CT infection assessed by PCR. Semen pH and volume, and sperm concentration, viability, motility, morphology and leukocytospermia were analyzed. A multivariate analysis of principal components, conglomerate analysis and non-parametric tests were used for statistical analysis.
By means of the multivariate analysis of the seminal parameters under study, it was observed that non-infected patient groups, both fertile and infertile, stratified differently from CT-infected patient groups. Moreover, CT-infected fertile patients stratified differently from CT-infected infertile patients. Regarding semen quality variables, no major significant differences were detected among groups under analysis. Low sperm viability levels were only found in CT-infected infertile patients with respect to non-infected infertile patients ($p<0.05$). On the other hand, decreased normal sperm morphology was only detected in CT-infected fertile patients with respect to non-infected fertile patients ($p<0.05$).
Our results suggest that male urogenital CT infection does not significantly compromises semen quality. Differences found between fertile and infertile CT-infected patients in the multivariate analysis could not be attributed to the presence of the infection only.

345. (488) **FLUID AND PLASMA LEVELS OF ANTIMICROBIAL PEPTIDES IN PATIENTS WITH PULMONARY OR PLEURAL TUBERCULOSIS AT DIAGNOSIS AND THROUGHOUT SPECIFIC TREATMENT.**

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Tuberculosis (TB) is one of the most important infectious diseases worldwide. Our previous studies in patients with pulmonary TB (PTB) or pleural TB (PLTB) showed an immune-endocrine imbalance less pronounced in the latter group. Based on the role of antimicrobial peptides like cathelicidin (LL37) and human β-defensins type 2 (HBD2) and 3 (HBD3) in the antimycobacterial response, the present study analyzed: 1) the levels of these peptides in plasma of PTB and PLTB patients as well as in the pleural fluid of the latter patient group; 2) the possible correlations of these peptide concentrations with levels of adrenal steroids in both compartments. The sample consisted of 30 healthy controls (HCo), 29 age- and sex-matched TB -HIV negative patients bled at diagnosis (T0) as well as two (T2) and six months (T6) after treatment initiation and 10 untreated PLTB patients (plasma and fluid collection).
Severe PTB patients showed increased HBD2 and HBD3 levels at T0 ($p<0.05$ and $p<0.001$, respectively), further decreasing at T6 (similar to HCo). PTB patients had normal LL37 levels at T0 that were further increased at T6 ($p<0.01$). Plasma from PLTB patients had increased LL37 levels ($p<0.001$ vs. HCo), with HBD2 and HBD3 showing no differences. Pleural fluids from PLTB patients contained increased or decreased amounts of HBD3 and HBD2 ($p<0.00001$ and $p<0.01$; respectively, respect the plasma counterpart), whereas LL37 levels remained unchanged. There was a positive correlation between LL37 and DHEA levels ($r=0.79$, $p<0.01$), as well as cortisol and HDB3 ($r=0.75$, $p<0.05$) in plasma from severe PTB patients.
Systemic changes suggest a divergent immunopathological role of HBD2/HBD3 and LL37 throughout the course of severe PTB, partly related to hormonal variations. Measurements at the *in situ* level – pleural fluid- lead to envisage a possible differential activity of HBD3 and HBD2 in the local response.

346. (536) **MODULATION OF METABOLIC PATHWAYS SIGNALING IN MACROPHAGES INFECTED WITH TRYPANOSOMA CRUZI INDUCE M1-LIKE PHENOTYPES THAT CONTROLS PARASITE SURVIVAL**

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Macrophages (Mo) play a key role in the control of intracellular T. cruzi growth. However, parasite subverts Mo polarization through modulation of several signaling pathways to favor its survival. In this study, we focused in metabolic pathways (mTOR and AMPK) and its role during T. cruzi infection in Mo. We have previously shown that mTOR pathway is activated by T. cruzi, and that its inhibition decreased parasite replication in Mo. We observed that, Rapamycin (Rap) (an mTOR inhibitor) pretreated and T. cruzi infected Mo showed reduced nitric oxide (NO) production and iNOS expression. However, those macrophages could control parasite replication through activation of different inflammatory mechanism. Indeed, those Mo showed a significant increase in NLRP3 inflammasome receptor and IL-1β production. Given that inflammasome could employ the mitochondria as platform of assembly, we found a significantly increase in mitochondrial ROS (mtROS) production (3 and 6h pi). To evaluate the relevance of mtROS and inflammasome activation, Bone Marrow Derived Macrophages (BMDM) were treated with DPI (NADPH oxidase inhibitor), or mitoTEMPO (superoxide species [mtROS] scavenger) or CA-074 (cathepsin B inhibitor) and then infected with T. cruzi. We observed that mtROS and cathepsin B (an inflammasome activator) inhibition induce significantly higher parasite load ($p<0.05$). On the other hand, we found that pretreatment with Metformin (Met) (an AMPK activator) in infected Mo showed a decreased in parasite replication ($p<0.05$). To determinate Mo activation, we evaluate Arginase and iNOS balance in Met pretreated and infected Mo. We found decreased Arginase activity and expression and increased NO production and INOS expression, respect to control Mo. This may indicate that Met induces a M1-like phenotype, capable to control parasite replication. Taking together, these results suggest that T. cruzi replication could be controlled through modulation of metabolic pathways such as mTOR and AMPK, those allows macrophages polarize M1-like phenotypes.

347. (556) **CHARACTERIZATION OF SINGLE DOMAIN ANTIBODIES DERIVED FROM CAMELIDS (NANOBODIES OR VHH) AGAINST THE STRAIN O1/ CAMPOS OF FOOT-AND-MOUTH DISEASE VIRUS FOR DIAGNOSTIC APPLICATION**

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CEVAN - ICT Milstein - CONICET

Foot-and-Mouth Disease (FMD) is a viral disease of livestock with serious economic repercussions. In one hand, development of innovative diagnostic methods for the FMD control is of concern due to the economic importance of susceptible species. In the other hand, camelids present heavy chain only antibodies, so the variable domain consists of a single domain (VHH or nanobody). These antibodies represent a novel tool with unique characteristics conferring great potential in the development of more sensitive diagnostic methods. For this reason, the present work aimed to obtain and characterize VHH specific against FMDV for its application in diagnostic innovation.
For this purpose, 32 specific nanobodies against O1/ Campos (O1C) strain were selected by Phage Display methodology. Two immune VHH libraries constructed previously in the laboratory were used as VHH source. These nanobodies showed no reactivity against other FMDV strains (A24/Cruzeiro, A/Arg/2001 and C3/ Indaial) in ELISA. In order to analyze VHH diversity, the most reactive nanobodies in ELISA (17 out of these 32) were analyzed by fingerprinting, showing 12 different patterns. Moreover, we confirmed that 13 out of these 17 VHH were unique by sequencing. These 13 nanobodies were expressed as soluble protein in *E. coli* WK6 strain. Later, 4 of these VHH were purified from periplasmic extract by IMAC. Reactivity of these soluble antibodies was confirmed by ELISA and Western Blot (WB) against O1C strain and recombinant FMDV structural proteins (SPs). Furthermore, we calculated the IC50 as an indicator of apparent affinity for these nanobodies.
In conclusion, we obtained 13 different VHH that showed a high reactivity against O1C strain in ELISA and only two recognized FMDV SPs in WB. Even more, these nanobodies did not react against other FMDV strains. These VHH represent a potential tool for innovation in diagnostic, typification and vaccine quality control of FMDV.

348. (594) **COMBINATION WITH ANTI-PDL-1 AND A TUMOR-ASSOCIATED ANTIGEN IMPROVES THE OUTCOME OF BLS TREATMENT IN MELANOMA-BEARING MICE.**

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Brucella lumazine synthase (BLS) is a homodecameric protein. It is possible to insert foreign proteins at its 10 N-termini and these chimeras are efficient to elicit immunity without adjuvants. BLS and BLS-OVA, a chimera containing ovalbumin peptide 257-264, have a similar therapeutic effect in B16-OVA-expressing tumor-bearing mice. In this work, mice were s.c. inoculated with B16-OVA cells and at day 2, 200μg of BLS or BLS-OVA were injected s.c.. After 14 days, their splenocytes were co-cultured for 4h with B16-OVA cells. IFNγ was undetectable by ELISA in the supernatants of all groups. Since B16-OVA cells express high levels of PDL-1, anti-PD-1 or anti-PDL-1 antibodies were added to the culture. Interestingly, IFNγ was detected only in co-culture of splenocytes from BLS-OVA treated mice. These results suggest that BLS-OVA induces a specific response against the tumor-associated antigen (TAA) but it is suppressed through the PD-1/PDL-1 pathway. We then evaluated if the administration of BLS-OVA and anti-PD-1 or anti-PDL-1 affected the tumor microenvironment. To that end, mice inoculated with B16-OVA cells were treated with BLS-OVA or BLS at day 2 and with anti-PD-1 or anti-PDL-1 at days 4, 7 and 10. At day 14, tumors were analysed through flow cytometry. Surprisingly, BLS-OVA does not increase the percentage of immune cells into the tumor but combination with anti-PDL-1 increases CD45+ cells (18,28%±10,24 vs 2,91%±0,61, p<0,01) including CD8+, CD4+, dendritic cells and regulatory cells. Tumor growth and survival with these combined strategies were evaluated. Only the combination of BLS-OVA with anti-PDL-1 delays tumor growth compared to BLS-OVA. The presence of the TAA OVA in the chimera improves the outcome of the treatment only in the presence of the checkpoint inhibitor anti-PDL-1. Therefore, BLS can be used as a carrier for a TAA generating a specific response that can be improved with immune checkpoint blockade treatment.

349. (622) **CD8+ T CELLS UP-REGULATE CD39 EXPRESSION AND INHIBITORY RECEPTORS UPON IN VITRO TCR AND CYTOKINES STIMULATION.**

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Previously we have shown that the frequency of exhausted CD39highCD8+ T cells increased with tumor growth but was absent in lymphoid organs. We aimed to evaluate signals that may influence CD39 and inhibitory receptors (IRc) upregulation on CD8+ T cells. Purified CD8+ T cells from draining lymph node of B16F10-OVA tumor bearing mice were stimulated during 48hs with αCD3/αCD28 in presence or absence of IL-6 or conditioned medium (CM) obtained by culturing tumors from B16F10-OVA bearing mice. Then cells were resting in IL-2 (96hs) and re-stimulated with αCD3/αCD28 (24hs). We observed that after TCR stimulation, the frequency of CD39+ and PD-1+TIM-3+ CD8+T cells were (27.3%±8.1% and 50.57%±0.46% respectively), however the frequency of both populations was not increase by neither MC nor IL-6. Next we stimulated the CD8+ T cells reducing the resting with IL-2 (24hs) in presence or absence of IL-6, IL-27 or CM. We found that TCR stimulation increased significantly the % of CD39+CD8+ T cells ($p \leq 0.05$) respect to non-stimulated cells, and the frequency of the CD39+ expressing cells decreased in absence of IL-2 ($p \leq 0.05$). The stimulation with cytokines associated with exhaustion such as IL-27 or a combination of IL-6/IL-27 increased the frequency of CD39+ cells ($p<0.01$ for both), while this frequency was not modified in presence of IL-6 or CM respect to TCR-stimulated control cells. Upon TCR stimulation the % of both PD-1+ and TIM-3+ cells increased significantly respect to non-stimulated cells ($p \leq 0.0001$ and $p \leq 0.01$ respectively), however only IL-6 plus TCR stimulation triggered an increment of PD-1+ T cells frequency ($p \leq 0.05$). We showed that TCR stimulation induce CD39 on murine CD8+ T cells which is more evident when combining IL-6 and IL-27, indicating that CD39 expression may result as a consequence of the integration of different signals.

350. (639) **HUMAN CHORIONIC GONADOTROPIN FACILITATES MELANOMA GROWTH**

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Melanoma is the leading cause of neoplasia in women within reproductive age and the most frequently diagnosed malignancy during pregnancy. However, the pathophysiological mechanisms behind this phenomenon remain unknown.
We aimed to investigate here the role of pregnancy-associated hormone, human chorionic gonadotropin (hCG), in the progression of experimental melanoma.
Mouse metastatic melanoma cell line (B16-F10) was cultured with hCG (100 IU/ml) or PBS for 24, 48 and 72 h. Proliferation rate was evaluated by flow cytometry (FC) using CFSE staining. In addition, the expression levels of PD-L1 and PD-1 on B16-F10 cells were also measured by FC. Each experiment was performed in duplicates and repeated three times.Additionally, virgin 8-10 weeks old C57BL/6 females were subcutaneously injected with 2X105 B16-F10 cells and challenged (IP) every other day with hCG (10 IU) or PBS (n=6 each group) starting on day 1 post-tumor inoculation. Tumor volume was daily monitored and animals were euthanized 22 days after tumor injection. Tumors and spleens were dissected, weighted and mechanically disaggregated using 70-μm nylon filters. Cells were then

stained with specific antibodies against CD45, CD3, CD19, CD4, CD8, Foxp3, NK.1.1, CD11c,PD-1,PD-L1 and analyzed by FC. Mann.Whitney or t-tests were applied to compare groups.
We observed that hCG treatment neither affects proliferation rate nor provoked changes on PD-1/PD-L1 expressionin B16-F10 cells in vitro. However, treatment with hCG accelerated B16 tumor growth in vivo. Furthermore, hCG-treated mice showed significantly lower numbers of tumor infiltrating leukocytes (TIL) andlower percentages of $CD19^+$ B and $CD3^+$ T lymphocytes in the spleen as compared to control animals. Additionally, hCG treatment induced the up-regulation of PD-1 in splenic $CD3^+CD8^+$ T cells as compared to controls.
Our results suggest that pregnancy-associated hormone (hCG) facilitates melanoma growth by mechanisms involving host immune system regulation.

351. (724) **A NANOSTRUCTURED VACCINE AGAINST BOVINE RESPIRATORY DISEASE IMPROVES HUMORAL IMMUNE RESPONSE IN A MURINE MODEL**
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Bovine Respiratory Disease (BRD) is a multi-factorial disease that causes high morbidity and mortality in cattle. Pasteurella multocida (Pm) and Mannheimia haemolytica (Mh) are, among others, pneumonia-causing bacterial agents. Although BRD vaccines are worldwide employed, they do not always provide an adequate control of the pneumonic form of the disease. Consequently, it is recommendable the improvement of antigens and adjuvants to enhance their immunogenicity. Coa-ASC16 or Coagel is a liquid crystal nanostructure formed by self-assembly of 6-O-ascorbyl palmitate that has been shown to improves antigen-specific immune response when is used as vaccine platform. This work aimed to evaluate the action of Coagel as platform in an experimental BRD vaccine, studying the humoral and cellular immune response specific for Pm and Mh. To this end, BALB/c mice (n:5/group) were immunized with heat-killed Pm-Coagel, heat-killed Mh-Coagel or a commercial BRD vaccine. Immunizations were performed on days 0, 15 and 27 by subcutaneous injection. Control group received PBS and Coagel. Blood samples were obtained on days 0, 10, 27 and 38 when animals were sacrificed. Specific Pm and Mh antibody levels (IgG, IgG1, and IgG2a) were measured in sera, and cytokines (IL-2, IL-12) in spleen supernatants (ELISA). All immunized mice generated specific antibodies. Coagel immunized mice presented higher IgG levels for both bacteria compared with commercial vaccine immunized mice ($p<0.05$). Mh-specific IgG1and IgG2a levels were also higher when Coagel was used ($p<0.001$ and $p=0.069$, respectively). For Pm, no differences between groups were found. Cytokine levels were higher in mice immunized with the nanostructured vaccine although statistical significance was not reached. Our results indicate that Coagel would be a good vaccine platform for use in bovines and thus enhance the weak immune response elicited by vaccination for ERB.

ONCOLOGÍA / ONCOLOGY ORAL SESSION 2

352. (339) **EFECT OF THE COMBINED TREATMENT OF METFORMIN (MET) AND PROPRANOLOL (PROP) ON DIFFERENT PHASES OF TUMOR PROGRESSION**
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Drug repositioning refers to the use of drugs for different indications from the original ones. Met is an antidiabetic drug; Prop is a β-blocker. We found, in a triple negative breast cancer (TNBC) model, that Met+Prop treatment induced apoptosis, reduced proliferation and lung metastases. We aimed to test the effect of primary or adjuvant treatment with Met+Prop in intravasation, extravasation and lung colonization processes of 4T1 cells. We quantified circulating epithelial cells (CEC:EpCAM+,CD34-,CD45-) in mice bearing tumors in exponential growth, treated with Met(2g/l)+Prop(25mg/l) (drinking water). The percentage of CECs in Met+Prop group was lower than in controls ($P=0.066$). To study the effect of Met+Prop in post-intravasation steps, BALB/c were i.v challenged with 5x104/100μl 4T1 cells labeled with green cell tracker proceeding as follows: G1)untreated mice injected with control cells; G2)untreated mice injected with in vitro pretreated cells [Met(5mM)+Prop(5μM)]; G3)mice receiving Met+Prop [Met(2g/l)+Prop(25mg/l)] injected with control cells. After two days, blood was collected and lungs excised. G3 group showed lower percentage of circulating green cells (flow cytometry) than G1 or G2 ($P<0.05$). Lungs of mice in G2 and G3 groups had less green cells (confocal microscopy) than G1 mice ($P<0.05$), suggesting an effect of M+P on the ability of cells to extravasate. We evaluated the Met+Prop combination as adjuvant therapy. 4T1 s.c tumors were surgically removed when they reached 100 mm3 and mice were distributed in two groups: Control and Met+Prop. When the first mouse showed signs of metastatic disease, mice were euthanized; lungs were excised and stained to highlight metastases. The percentage of animals with metastases was higher in Control (61.5%;8/13) than in Met+Prop (30%;3/10) ($P=0.067$). Similar data were obtained in another TNBC model (M-406), ($P<0.05$). Our data indicate that Met+Prop treatment: 1) would affect intravasation, extravasation and lung colonization; 2) could be of interest as adjuvant therapy for TNBC.

353. (125) **ROLE OF NITRIC OXIDE IN BREAST TUMOR PROGRESSION**
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In Argentine, breast cancer is the one with highest incidence in women. Nitric Oxide (NO) is generated by a family of NO synthases (NOS), being the inducible isoform (iNOS) which produces higher NO levels. This, often acts as a survival factor, hence inhibition of iNOS has been proposed as a targeted therapy. Fibroblasts, the main cell type in tumor microenvironment, have been described as a heterogenic population and their role in breast cancer associated to NO inhibition has not been yet elucidated.
In this work, using three mouse mammary breast cancer cell lines, the impact of inhibiting NO in tumor progression was evaluated. LM3 and its more aggressive variant LMM3 cell line expressed iNOS. On the other hand, LM2, line did not express iNOS. Consistent with iNOS expression, both LM3 and LMM3 produced NO, which is inhibited by L-NAME. When cell viability was evaluated, NO inhibition on iNOS-positive cells induced a reduction in cell viability. The iNOS-negative cell line, LM2, did not produce NO and its viability was not affected by L-NAME.
In vivo, in parallel with tumor reduction induced by L-NAME, collagen deposition and α-SMA positive stromal cells were identified by histological analyses. These observations take place only when tumor cells express iNOS. (LM3: $p<0.01$; LMM3: $p<0.05$; LM2: NS; L-NAME vs CRL).We have also demonstrated in vitro, that L-NAME induced viability ($p<0.01$) and differentiation on NIH-3T3 fibroblast.
We reveal that NO inhibition induces reduction of tumor growth and contributes to stimulate proliferation and activation of fibroblasts only in iNOS positive breast cancer. These results suggest that NO produced by cancer cells acts a survival factor on tumor cells and blocks fibroblast activation. Understanding the characteristics of this population of activated fibroblasts induced by L-NAME will contribute to the proposal to use pharmacological inhibitors as antitumor therapy.

354. (77) **RAC3 LEVELS ARE CORRELATED WITH METALLOPROTEINASES AND LEPTIN EXPRESSION IN ADIPOSE TISSUE ADJACENT TO BREAST TUMOURS**
María Cecilia Lira, Francisco Damián Rosa, Mileni Soares Machado, Alejandra Palma, Gabriela Inés Marino, María Cecilia Salazar Güemes, Mónica Alejandra Costas, María

Fernanda Rubio
Instituto de Investigaciones Médicas Dr. Alfredo Lanari (IDIM), UBA - CONICET

RAC3 is a transcriptional coactivator that is present in low levels into normal cells, almost undetectable, but it has been found amplified or overexpressed in several types of cancer, including breast tumours. Previously, we demonstrated that RAC3 decreases during adipogenesis, favouring the process through an increase of autophagy and a decrease in the proliferative rate. In addition to the role of the coactivator during adipocyte differentiation, we have started to study the modulation of RAC3 in adipose tissue (AT) in breast cancer context. We collected breast tumour-surrounding AT from IDIM's patients (n=30) and, we have already demonstrated that the higher expression of RAC3 in these samples, not only tumours had molecular characteristics with worse prognosis, but also were in a more advanced clinical stage. Given these results, the aim of this work was to evaluate the correlation between RAC3 expression levels and the expression of adipokines in AT.
We assayed metalloproteinases (MMPs 2 and 9) activity by zymography, from conditioned mediums of AT adjacent to tumours, and determined there is greater activity in AT with higher levels of RAC3. Even more, there is a correlation between MMP2 and RAC3 levels measured by qPCR (y=0.76x + 0.94; R2=0.90). It is known that Leptin induces the expression of MMP2 and 9. Therefore, we confirmed by qPCR that in our samples there is also a correlation between Leptin and MMP2 levels (y=9.12x - 7.93; R2=0.84). Furthermore, we compared RAC3 and Leptin expression in the samples and we found that in those with RAC3 ratios above 5, there is higher Leptin expression (147.0+/-60.0) respect to AT with RAC3 ratios between 5 and 1 (2.3+/-1.1), and below 1 (0.1+/-0.0) ($p<0.001$).
In conclusion, higher expression levels of RAC3 in AT adjacent to breast tumours correlate with different markers of tumoral progression like: MMPs and Leptin.

355. (333) **THE THYROID STATUS REGULATES TRIPLE NEGATIVE BREAST CANCER (TNBC) GROWTH AND METASTASIS THROUGH THE MODULATION OF MESENCHYMAL STROMAL CELL (MSC) MIGRATION AND ANTITUMOR IMMUNE RESPONSE**

Helena A Sterle, Ximena Hildebrandt, Luciana Gutiérrez, María A Paulazo, Florencia Cayrol, María Celeste Díaz-Flaqué, Cinthia Rosemblit, Marcela Bolontrade, Graciela A Cremaschi
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The association between thyroid disease and breast cancer risk remains unclear. Our aim was to evaluate the effect of thyroid status on TNBC development. For this, Balb/c mice were orthotopically inoculated with 4T1 cells after the treatment with thyroxine (12mg/l) for 30 days or propylthiouracil (500mg/l) for 15 days in the drinking water to obtain hyperthyroid (hyper) or hypothyroid (hypo) mice, respectively. An increased tumor growth rate, that became evident at day 21 post-inoculation (p.i.), was seen in hyper mice compared to controls ($p<0.05$). Hypo tumor growth rate was similar to controls and showed a decreased tumor volume only from day 35 p.i. ($p<0.05$), but developed a greater number of lung metastases ($p<0.05$). To further investigate this dual role of thyroid hormones on TNBC development, we evaluated the migration of MSCs to 4T1 tumors. Conditioned medium from hyper tumors induced a reduced migration of murine MSC in vitro, compared to control and hypo tumors ($p<0.05$). Likewise, hyper tumors and lungs showed a decreased presence of MSC ($p<0.05$) when MSC were inoculated in the tail vein of tumor-bearing mice and analyzed by in vivo imaging. Additionally, an increased secretion of MCP-1 was detected in hypo tumors ($p<0.05$), while the level of this cytokine, which is related with tumor aggressiveness and MSC recruitment, was non-significantly decreased in tumors from hyper mice. Hypo tumors also showed increased levels of infiltrating immune cells and of activated CD8+ T lymphocytes ($p<0.05$). However, the infiltration of immune cells was decreased in tumors from hyper mice ($p<0.05$), but they showed increased frequencies of splenic activated T lymphocytes ($p<0.05$) and NK cells ($p<0.05$) but reduced percentages of CD11b+Gr1+ cells ($p<0.05$). Our results suggest that the thyroid status modulates the migration of MSC to the 4T1 tumors and the antitumor immune response, thus regulating tumor growth and metastasis formation.

356. (65) **BACILLUS CALMETTE-GUERIN (BCG) REGULATES THE EXPRESSION OF FIBROBLAST GROWTH FACTOR RECEPTOR 3 (FGFR3) ASSOCIATED WITH CELL VIABILITY IN BLADDER CANCER. DEVELOPMENT OF A RESPONSE BIOASSAY.**

Yanina Langle, Denise Belgorosky, Macarena Zambrano, Marianela Sciacca, Eduardo Imanol Agüero, Catalina Lodillinsky, Hector Malagrino, Mariano Brzezinski, Eduardo Sandes, Ana Maria Eijan
Instituto de Oncologia Angel H. Roffo

Bacillus Calmette-Guerin (BCG) is the standard treatment for high-grade non-muscle invasive (NMI) bladder cancer (BC). Using a murine orthotopic BC model we demonstrated that BCG induces tumor growth inhibition, associated with FGFR3 down-regulation. Objective: a) to study the relationship between FGFR3 expression and cell viability in response to BCG in human BC cell lines; b) to analyze, in human BC samples, FGFR3 modulation by BCG and to evaluate its association with patient outcome.
Mat & Met: a) Human BC cell lines were treated with +/-BCG. Viability was assessed by MTS and FGFR3 expression by qPCR. b) Bioassay: Cells from human bladder tumors (n=41) were divided in two sub-cultures: 1-FGFR3 basal expression; 2-FGFR3 BCG-treated.
Results: a) BCG increased FGFR3 expression and viability in RT4 and 5637 while were reduced in T24 and UMUC6. In J82, BCG diminished viability without modified FGFR3 expression. The reduction in cell viability observed was associated with the activation of pro-apoptotic protein Cathepsin B. b) Bioassay: BCG induced reduction or no variation of FGFR3 expression in 59% of samples (17/41), been 44% low-grade and 64% high-grade NMI tumors; and 75% (9/12) invasive tumors. Five patients were treated with BCG: two reduced FGFR3 expression and remained free of disease for more than 2 years. Three patients presented FGFR3 increased, progressing in their disease ending in radical cystectomy.
Conclusion: FGFR3 variations are related to BC cell viability and could be used as a predictive marker of response to BCG therapy. This modulation occurs in near 60% of patient tumor samples analyzed, appearing to be associated to therapeutic response. A large number of high-grade NMI tumor samples and the follow-up of those patients will be necessary to establish the predictive role of FGFR3 and the usefulness of our bioassay as a marker of response to BCG treatment.

357. (685) **EVALUATION OF MUSCARINIC RECEPTORS SILENCING BY SPECIFIC RNA INTERFERENCE ON MIGRATION AND ANGIOGENESIS IN HUMAN BREAST CANCER CELLS.**

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It has been reported that muscarinic receptors (M) are absent in normal breast cells and are up-regulated in tumor cells. Particularly, MCF-7 cells express M_3 and M_4 subtypes and its activation promotes tumoral progression. We demonstrated that M expression in non-tumorigenic human mammary cell lines triggers malignant transformation. RNA interference (RNAi) is a non-invasive specific technology and in the last years different groups are working in RNAi based therapeutics. Here, we analyzed the effect of RNAi gene silencing of M_3 and/or M_4 receptors in human breast cancer MCF-7 cells (siM_3M_4) on migration activity in vitro by wound healing assay and on induced angiogenesis in vivo. We previously reported that the treatment with the cholinergic agonist carbachol(10^{-8}M) significantly increased the migration capacity of MCF-7 cells (195±4.8%

vs control (cell without treatment considered as 100%) p<0.0001). Here, we show that the pretreatment of cells with the specifics siRNA reverted the stimulating effect of carbachol on MCF-7 cells (siM_3M_4+carbachol:104±0.6% vs MCF-7+carbachol, p<0.0001) in a similar way of non-specific muscarinic antagonist atropine (10^{-8}M). Tumor induced angiogenesis was quantified inoculated $2x10^5$ cells of different experimental groups in female NUDE mice. After 5 days, the animals were sacrificed and angiogenesis was quantified in the sites of inoculation as vessel density. The pretreatment with carbachol increase angiogenic response of inoculated MCF7 cells in comparison to control (6.4±0.7 vs 3.3±0.7, p<0.0001). The specific silencing of M receptors in MCF-7 cells during 3 or 5 days previously to muscarinic agonist stimulation significantly reduced neovascularization capacity of tumoral cells (3 days siM_3M_4+carbachol:4.8±0.6; 5 days siM_3M_4+carbachol:4.7±0.3 vs MCF-7+carbacol p<0.01). In conclusion, the specific M silencing in tumoral MCF-7 cells can effectively reduce the effects of muscarinic activation on migration and angiogenesis. These results could suggest the potential of RNAi-based therapy as a new method of breast cancer therapy in the future.

ENDOCRINOLOGÍA Y REPRODUCCIÓN / ENDOCRINOLOGY AND REPRODUCTION ORAL SESSION

358. (114) **ROLE OF G-PROTEIN COUPLED ESTROGEN RECEPTOR (GPER) IN THE ANTERIOR PITUITARY GLAND FOCUSING ON LACTOTROPH FUNCTION**

Maria Andrea Camilletti, Alejandra Abeledo Machado, Jimena Ferraris, Pablo Pérez, Erika Faraoni, Daniel Pisera, Silvina Gutiérrez, Graciela Díaz-Torga
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Ovarian steroids control a variety of physiological functions. They exert actions through classical nuclear steroid receptors, but rapid non-genomic actions through specific membrane steroid receptors have been also described. In this study, we demonstrate that the G-protein-coupled estrogen receptor (GPER) is expressed in the rat pituitary gland and, at a high level, in the lactotroph population. Our results revealed that ~40% of the anterior pituitary cells are GPER-positive and ~35% of the lactotrophs are GPER-positive. By immunohistochemical and inmuno-electron-microscopy studies we demonstrated that GPER is localized in the plasmatic membrane but is also associated to the endoplasmic reticulum in rat lactotrophs. Moreover, we found that local *Gper* expression is regulated by 17β-estradiol (E2) and progesterone (P4) since *in vivo* treatments with E2 and P4 both negatively regulated pituitary *Gper* expression in female rats. Accordingly, *Gper* mRNA levels were differentially modulated during the estrous cycle. Due to the loss of the control by ovarian steroids, *Gper* expression was significantly increased in the pituitary gland of ovariectomized (OVX) adult female rats. Interestingly, GPER protein levels were found increased specifically in pituitary lactotrophs of OVX rats. The role of GPER in the lactotroph population is unknown. Our results demonstrate a rapid estradiol stimulatory effect on PRL secretion mediated by GPER, both *in vitro* and *ex vivo*, using a GPER agonist G1. This effect was prevented by the GPER antagonist G36, demonstrating a novel role for this receptor in the lactotroph population. Taken together, this data provides the first evidence that GPER is highly expressed in lactotroph population, is regulated by ovarian steroids, and though its pituitary expression increases after OVX. These changes could lead to alterations in the pituitary function and therefore should be taken into consideration in the response of the gland to an eventual hormone replacement therapy.

359. (387) **PROP1 REGULATES EXPRESSION OF PROTEINS INVOLVED IN EMT TO LEAD PITUITARY DIFFERENTIATION**

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Mutations in PROP1, a key transcription factor of pituitary stem cell differentiation, are the most common known cause of hypopituitarism. We determined that PROP1 is essential for stimulating stem cells to undergo an epithelial to mesenchymal transition-like (EMT) process necessary for cell migration and differentiation. The mechanism whereby PROP1 regulates this process is not completely understood. Our goal is to further our understanding of the factors regulating embryonic pituitary progenitor cells. We genetically engineered the GHFT1 mouse pituitary cell line to express biotin-tagged PROP1 and use them to identify *Prop1*-mediated changes in gene expression with RNA-Seq and *Prop1*-interacting proteins with mass spectrometry. Gene expression profiling revealed that *Prop1* upregulates genes that are involved in migration (adhesion/dispersion) and in degrading extracellular matrix proteins, like matrix metalloproteinases, MMP2, 15 and 19, and downregulates tissue inhibitor of metalloproteinases, like TIMP-3. MMPs play key roles in embryonic development and are involved in EMT in the morphogenesis of many tissues. We examined expression of MMP2 during pituitary development in control and $Prop1^{df/df}$ mice. At e12.5, e14.5 and e16.5, MMP2 is normally expressed in the pituitary gland in normal mice and it is almost absent in *Prop1* mutant pituitaries. Furthermore, we observed an increase in cell-cell junctions by TEM in P1 pituitaries from $Prop1^{df/df}$ mice. Using RIME, we identified 93 proteins that specifically interact with *Prop1*. These proteins were tested for GO biological process enrichment, and two predominant themes were identified: Cell-Cell Adherens Junction and Cell-Cell adhesion. Several genes from these groups of proteins are candidates for involvement in migration and EMT that fails in *Prop1* mutants. Together these approaches are uncovering the specific players that *Prop1* regulates or interacts with to direct EMT and differentiation. This basic knowledge could implicate candidate genes to explain cases of hypopituitarism with unknown etiology and thereby, yield better diagnosis.

360. (657) **NOVEL EXPRESSION OF LOCAL GNRH AT SPECIFIC STAGES OF MAMMARY GLAND DEVELOPMENT OF VIZCACHAS SUGGESTS A MODULATORY ROLE IN TISSUE REMODELING (RODENTIA: CHINCHILLIDAE)**

María Clara Corso[1, 2], Sofía Proietto[1, 2], Santiago Andrés Cortasa[1, 2], Alejandro Raúl Schmidt[1, 2], Pablo Ignacio Felipe Inserra[1, 2], Noelia P Di Giorgio[3], Victoria Lux-Lantos[3], Verónica Berta Dorfman[1, 2], Alfredo Vitullo[1, 2], Julia Halperin[1, 2]
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Mammary glands (MG) exhibit extensive tissue remodeling during pregnancy and lactation. Although progesterone, estradiol and prolactin (PRL) are the main regulators of MG development, there are several other factors that also intervene in the dynamics of this gland. The expression of gonadotropin-releasing hormone (GnRH) has been previously reported in reproductive extra-hypothalamic tissues (ovaries, placenta, endometrium, oviducts and in mast cells located in the MG) where it would act by autocrine or paracrine manner. In addition, a GnRH-PRL dependent regulation has been described in both hypothalamus and MG. Herein, we measured GnRH in mammary tissue by RIA and examined the expression of both GnRH and its receptor (GnRHR) by PCR , immunohistochemistry (IHQ) and immunofluorescence (IF) in MG of vizcachas at early-, mid-, term-pregnancy and during lactation. We also examined the expression of Egr-1 as a biomarker of GnRH active signaling. We related these data with the expression of PRL and its receptor (PRLR). Although mammary GnRH content at mid-pregnancy was minimum, MG exhibited the maximum levels of GnRHR and Egr-1 suggesting an active GnRH signaling. On the contrary, MG of lactating females exhibited the highest levels of local GnRH (p<0.05, n=5) and very low levels of both GnRHR and Egr-1 which would point to an eventual storage of the hormone in the glands. The detection of local GnRH by RIA was confirmed by IHQ specifically in the MG epithelium. Moreover, co-localization of PRLR and GnRH was corroborated,,particularly during pregnancy, when tissue remodeling becomes

more evident. This is the first report that describes GnRH expression in the secretory epithelium and raises the possibility of a coordinated action with PRL in the regulation of the MG development. Also, it proposes the accumulation of GnRH in the MG during pregnancy possibly to transfer it to the offspring during the lactation. PIP110/14-PICT1281/2014-FCFF

361. (96) **ALTERATIONS IN THE INSULIN PATHWAY IN THE OVARY OF COWS WITH INDUCED FOLLICULAR PERSISTENCE**

Natalia Gareis, Antonela Florencia Stassi, Fernanda Mariel Rodríguez, María Lucía Cattaneo Moreyra, Natalia Raquel Salvetti, Hugo Héctor Ortega, Gustavo Juan Hein, Florencia Rey
Laboratorio de Biología Celular y Molecular Aplicada

Several local factors, including components of the insulin pathway, are involved in the ovarian follicular persistence associated with anovulation. In previous studies, we detected alterations of insulin-signaling intermediaries in the ovaries from cows with cystic ovarian disease. Therefore, we aimed to study the protein expression of IR, IRS1, PI3K, total and phosphorylated AKT by immunohistochemistry in ovarian follicular structures from a model of follicular persistence. We also measured the insulin concentrations in serum and follicular fluid by radioimmunoassay. The model was performed with an intravaginal progesterone device to get subluteal concentrations of progesterone, obtaining dominant follicles in the expected day of ovulation (n=5; P0) and follicles that persist for 5 (n=5; P 5), 10 (n=5; P10) or 15 days (n=5; P15) relative to the expected time of ovulation. Controls cows were ovariectomized in proestrus (n=5; C). Serum insulin concentrations were higher in all persistence groups than in control cows and the intrafollicular concentrations were higher in P5, P10 and P15 groups respect to the control group ($p<0.05$). In granulosa cells, the expression of IR was higher in dominant follicles of control group relative to all persistent follicles evaluated ($p<0.05$). In theca cells, the expression of IR was higher in persistent follicles of P0 and P5 groups respect to persistent follicles of P10 and P15 groups ($p<0.05$). While in granulosa, the IRS1 was higher in the dominant follicles than in persistent follicles of P5 and P15 groups ($p<0.05$), the expression in theca cells showed no differences ($p>0.05$). The PI3K and phosphorylated AKT were higher in control group than all persistence groups in both cell populations ($p<0.05$). Total AKT expression showed no differences ($p>0.05$). Alterations of the insulin-signaling in follicular persistence could be involved in a lower insulin response that might be associated with disorders in ovarian functionality.

362. (323) **OBESITY ALTERS THE UTERINE GLUCOSE UPTAKE AND ITS CONTRACTILE ACTIVITY IN RESPONSE TO SALBUTAMOL, A SELECTIVE AGONIST OF ADRENERGIC RECEPTOR B2.**

María Victoria Bazzano, Gisela Sarrible, Nora Martinez, Martín Berón de Astrada, Evelin Elia
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The prevalence of obesity is increasing worldwide. Obesity leads to several reproductive disorders but the molecular mechanisms linking them remain unclear. Previously we described that cafeteria diet (CAF) administration induces obesity and insulin resistance in female rats. An adequate glucose supply and metabolism are essential for the proper endometrial differentiation and myometrial contractile activity, two key processes controlling embryo implantation. The aim of this study was to test whether obesity alters the uterine glucose uptake and contractile activity. For that purpose, 22day old female wistar rats were divided into: Control Group (n=10) that was fed with standard rodent chow diet; and Obese Group (n=10) which was also fed with CAF ad libitum for 60 days. Animals were sacrificed on estrus phase; uteri were removed and used for the myometrial contractile studies, uptake glucose analysis, PCR and WB. We found that basal glucose uptake was similar in uteri from control and obese rats. However, obese rats developed uterine insulin resistance through mechanisms that involved the downregulation of the insulin receptor gene expression ($P<0.05$) without altering glucotransporters 1 and 4 mRNA levels. Moreover, obese rats showed lower uterine adrenergic receptor β2 (β2AR) mRNA ($P<0.05$) and protein levels ($P<0.001$), a key factor regulating relaxation of the myometrium. Obesity did not alter the spontaneous uterine contraction pattern. Salbutamol, a selective agonist of β2AR decreased the uterine contraction pattern in both group. In obese rats, this decrease was detected for higher concentrations of salbutamol than for controls ($P<0.05$). We conclude that obesity alters the uterine environment by inducing uterine insulin resistance and decreasing its sensitivity to a relaxation stimulus. Since the uterus must be relaxed and an adequate glucose supply is needed for embryo nidation, the alterations produced by obesity here described may impair embryo implantation and, consequently, the foregoing pregnancy.

363. (376) **INFLUENCE OF STRESS ON REPRODUCTION: INVOLVEMENT OF THE MELANOCORTIN RECEPTOR TYPE 2 IN OVARIAN RESPONSE TO ADRENOCORTICOTROPIC HORMONE.**

Lucas Etchevers, Eduardo Matías Belotti, Emilia Huber, Natalia Raquel Salvetti, Florencia Rey, Hugo Héctor Ortega, Ayelén Noelia AMWEG
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Cattle undergo numerous environmental, management and nutritional stressors throughout the reproductive cycle. In response to stress, adrenocorticotropic hormone (ACTH) acts on adrenal gland promoting glucocorticoids synthesis and secretion, which are implied in metabolism, inflammatory response and reproduction. The aim of the present study was to evaluate the protein expression of Melanocortin Receptor 2 (ACTH receptor, MC2R) and its accessory protein (MRAP2) in the preovulatory follicles in the ovary of cows treated with ACTH. Holstein cows (n=16) were divided into two groups: Control group (administrated with saline solution) and ACTH group (administrated with 100 UI of ACTH) every 12 hours during 4 days before expected time of ovulation until ovariectomy. MC2R expression was higher in theca interna cells from preovulatory follicles of ACTH group relative to Control group ($p< 0.05$), whereas in granulosa cells it was similar in both groups ($p> 0.05$). MRAP2 expression was similar in both cell types from both groups analysed ($p> 0.05$). In addition, higher concentration of cortisol was detected in follicular fluid and serum of ACTH group relative to Control group ($p< 0.05$). Also, higher concentration of progesterone was detected in serum of ACTH group ($p< 0.05$). These results show that the bovine ovary is able to respond locally to the ACTH released as a consequence of stress. Moreover, these results indicate that ACTH, through its specific receptor, may be involved in regulatory mechanisms related to ovarian functions such as ovulation, steroidogenesis and in the pathophysiology of various reproductive diseases in cattle.

FARMACOLOGÍA Y TOXICOLOGÍA / PHARMACOLOGY AND TOXICOLOGY ORAL SESSION

364. (707) **NADPH OXIDASE CONTRIBUTES TO REDOX IMBALANCE IN RAW 264.7 MURINE MACROPHAGES EXPOSED TO AIR POLLUTION PARTICULATE MATTER**

Lourdes Cáceres, Mariela Laura Paz, Mario Contin, Mariana Garces, Valeria Calabró, Natalia Magnani, Deborah Tasat, Valeria Tripodi, Silvia Álvarez, Daniel González Maglio, Timoteo Marchini, Pablo Evelson
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The exposure to environmental particulate matter (PM) induces pulmonary oxidative stress and inflammation. Alveolar macrophages are suggested to play a central role in this scenario, since they produce inflammatory mediators and reactive oxygen species (ROS) following PM uptake. The aim of our work was to characterize this inflammatory response and to address the main targets of the redox imbalance observed in macrophages after the exposure to Residual Oil Fly Ash (ROFA), a PM surrogate rich in transition metals. The murine cell line RAW 264.7 was exposed to ROFA at 25, 50, or 100 μg/mL for 24 h. Cell viability was not significantly affected under

these experimental conditions. Cell culture supernatants showed increased TNF-α levels after incubation with ROFA 100 µg/mL (control: 480±140 pg/mL TNF-α, p<0.01). Consistently, we observed increased CD80 expression and a 2-fold increase in MHC class II levels (control: 29±3 MHC class II+ cells, p<0.05) when exposed to the highest ROFA dose, assessed as mean fluorescence intensity (MFI) by flow cytometry. Intracellular redox status was evaluated as GSH and GSSG content by HPLC-MS, resulting in increased GSSG levels after incubation with ROFA 50 and 100 µg/mL (control: 0.50±0.06 nmol/mg protein, p<0.01), which led to a decrease in the GSH/GSSG ratio. Given that NADPH oxidase (NOX) is a major source of ROS production, we studied its activity and found a 2-fold increase after incubation with ROFA 100 µg/mL (control: 340±70 AU/min mg protein, p<0.05). These findings suggest that exposure to ROFA leads to inflammatory activation and intracellular redox imbalance in RAW 264.7 cells, which could be attributed to increased NOX activity. Taken together, these results contribute to the understanding of the molecular pathways triggered by air pollution derived PM exposure.

Keywords: AIR POLLUTION - MACROPHAGES – OXIDATIVE STRESS

365. (443) **BIODISTRIBUTION OF A NEAR INFRARED FLUOROPHORE-LABELED ANTI SHIGA TOXIN MOLECULE BY NON-INVASIVE IN VIVO OPTICAL IMAGING IN MICE**

Andrea Lorena Berengeno[1], Facundo José Salinas[1], Belkis Marelli[1], Vanesa Zylberman[2], Yanina Hiriart[2], Santiago Sanguineti[2], Natalia Raquel Salvetti[1], Hugo Héctor Ortega[1]
[1]*ICIVET Litoral - UNL CONICET* , [2]*INMUNOVA* , [3]*CONICET*

Hemolytic uremic syndrome (HUS) is a multisystemic disorder characterized by microangiopathic hemolytic anemia, thrombocytopenia and acute renal damage. Shiga toxin (Stx) producing *Escherichia coli* (STEC) O157:H7 is the most common serotype associated with HUS in children younger than 5 years old. Argentina has the highest incidence of HUS in the world. Recently a new treatment capable of neutralizing the toxic effect of Stx and its variants has been developed. The efficacy and potency against Stx1 and Stx2 of F(ab')2 fragments from an equine antiserum were proved in preclinical models. Although the anti-Stx F(ab')2 pharmacokinetic was shown to be similar to that of immunoglobulin derived molecules, the tissue distribution and bioaccumulation have not been described. Our objective was to evaluate the biodistribution of anti-Stx F(ab')2 labeled with a NIR fluorophore using an *in vivo* optical imaging system in mice. The 800CW Protein Labeling Kit - HMW was utilized to label the anti-Stx and 5 BALB/cCmedc mice (25 days old) were treated with 10 mg/kg. At 0.5, 1, 2, 4, 6, 8, 12 and 24 h after inoculation images were acquired using the Pearl Trilogy Image System (LI-COR Biosciences) with Ex/Em setting at 785/820 nm. After *in vivo* imaging, animals were sacrificed. *Ex vivo* imaging after removal of brain, heart, lungs, liver, spleen, kidneys, stomach, intestines, adrenal glands, eyes, seminal vesicles, prostate and bladder was performed. *In vivo* fluorescence was observed in large vessels, liver and kidneys at 0.5 h after inoculation with a decrease intensity over time. *Ex vivo* localization of anti-Stx in liver and kidneys was confirmed. Fluorescence signal in bladder was detected. This distribution should be associated with a high vascularization of these organs. Our results demonstrate that *in vivo* imaging systems are a valuable technology to understand biodistribution and targeting of new therapeutic biological molecules in experimental models.

366. (122) **CELL LADEN 3D PRINT SCAFFOLD FOR DRUG TESTING.**

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Traditional synthetic substrates and matrices for cell culture have proven to be of only limited utility in efforts to understand and control cell behavior, in large part because they fail to capture biochemical, mechanical, and dynamic characteristics of in vivo environments. However, recent advances in materials chemistry and engineering have provide a toolbox to mimic the complex characteristics of natural extracellular matrices, providing new pathways to explore cell–matrix interactions and direct cell fate under physiologically realistic conditions. Here we perform a morphological and biocompatibility analysis of materials as dynamic substrates and matrices for 3D cell culture, and highlight their use in furthering our understanding of how cells respond to temporal variations in their environment. We compared a commercial bioink and gel combination of an in house matrix (Alginate, gelatin and pluronic F127 as sacrificial material). We used temperature and Calcium chloride as crosslinkers. The cell ladens were HEK293 and HCT116 commercial cell lines and we used the Envision Bioplotter to print a cylinder structures without inner structure on a 6 well plate from bpl file. We performed material degradation studies and biocompatibility. Regarding the stiffness of the structure we employed the range of gut tissue stiffness. The biocompatibility was tested by live/dead cell viability assay (2 µM calcein-acetoxymethyl and 4.5 µM propidium iodide solution). Our results showed that degradability and lasting of the cylinder structure made by in house material was significantly better than the commercial bioink (p=0.04), lasting for 21 days and allowing cell survival and what it seems to be cell proliferation. We consider that the in house 3D cell laden bioinks made of alginate gives sufficient architectural and life support to study cell-cell interactions, and drug testing as it allow to biochemical, mechanical, geometric and dynamic characteristics of in vivo environments to be implemented.

367. (279) **DEVELOPMENT AND VALIDATION OF ANALYTICAL TECHNIQUES UNDER GOOD LABORATORY PRACTICES FOR HIGH COMPLEXITY PRECLINICAL TRIALS**

Gonzalo Santiago, Valeria Gálvez, Enzo Cabaña, Belkis Marelli, Gustavo Juan Hein, Hugo Héctor Ortega
ICIVET Litoral - UNL CONICET

Performing toxicological tests on small laboratory animals (rats and mice) means that there are few quantities of biological samples, which limits the possibility of carrying out commercial methods. Therefore, we aimed to develop and validate analytical micro-methods to determine relevant biomarkers for clinical diagnosis with less than 50 μL of serum sampled from mice, rats and rabbits, in accordance with the principles of Good Laboratory Practices. Serum concentrations of glucose (Glc), triglycerides (Tg), cholesterol (Col), total protein (TP), albumin (Alb) and urea (U) were determined using an ultra-fast UV/Vis spectrometer SPECTROstar Nano (BMG LABTECH GmbH, Germany) and commercial kits designed for human and higher sample volumes. The figures of merit were: limit of detection of 4.71 mg/dL for Glc, 2.72 mg/dL for Tg, 2.72 mg/dL for Col, 0.13 g/dL for TP, 0.05 g/dL for Alb, and 1.25 mg/dL for U ; limit of quantification of 14.29 mg/dL for Glc, 8.25 mg/dL for Tg, 8.25 mg/dL for Col, 0.39 g/dL for TP, 0.16 g/dL for Alb and 3.77 mg/dL for U; quantifiable range of 20-500 mg/dL for Glc, 80-1040 mg/dL for Tg, 50-600 mg/dL for Col, 0.98-9.60 g/dL for TP, 1.55-6.20 g/dL for Alb and 7-140 mg/dL for U. All the techniques were in accordance with international regulations (FDA and EMA) in relation to the intra and inter-precision test, accuracy, inter-laboratories comparison, dilutional linearity and stability at – 20 °C and – 80 °C for 60 days. These validated analytical techniques will allow monitoring pathophysiological changes in different animal models, designed for the study of damages occurred in certain organs (such as liver, pancreas, kidney and heart), and involving very small amounts of samples in agreement to the principle of the 3Rs.

368. (495) **VALIDATION OF AN IN VIVO IMAGING MODEL IN MICE TO STUDY THE COLLAGEN-INDUCED ARTHRITIS USING NEAR INFRARED FLUOROPHORE-LABELED 2-DEOXYGLUCOSE**

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In vivo bioluminescent imaging systems are increasingly being utilized as a reference method in biomedicine due to its advantages of high sensitivity, non-invasiveness, no radioactivity, and low cost. The effectiveness of optical imaging heavily depends the use of validated models. Rheumatoid arthritis (RA) is a systemic autoimmune disease that affects the joint synovium, leading to chronic inflammation, and finally loss of function. The collagen-induced arthritis (CIA) mouse model is induced by immunization with type II collagen (CII) and it is the most commonly studied autoimmune model of rheumatoid arthritis. Previous works from our laboratory has demonstrated that glucose metabolism is increased in stromal and infiltrating cells in this arthritis model. Taking into account these observations, our aim was to evaluate the biodistribution of 2-deoxyglucose (2-DG) labeled with a NIR fluorophore for in vivo optical imaging in mice. 2-DG is a glucose analog that utilizes the GLUT transporters and upon phosphorylation, it is not metabolized further and is effectively trapped within the cell. Male DBA/1 mice (8–12 wk old) (n=3) were immunized with CII emulsified in CFA by intradermal injection at the base of the tail. At day 40 when the symptoms were manifested, 20nmol of IRDye 800CW labeled 2-DG were administrated EV. Non-immunized mice were used as control. The images were acquired at 0, 4, 6, 12 and 24 h after administration with a Pearl Trilogy Image System (LI-COR Biosciences). A specific distribution of 2-DG was observed in arthritic mice joints compared to nonarthritic control mice. The fluorescence was evidenced specifically in distal limb joints and mandibular area, with a significant difference in the fluorescence signal ($p < 0.05$) from 4 hours (3.79+/-1.48), up to 24 hours (3.55+/-0.91) in relation to basal signaling (0.09+/-0.07). These data indicate that targeting metabolic pathways is a novel approach to analyze experimental models of arthritis.

369. (597) **FORMATTING SINGLE-DOMAIN ANTIBODIES FOR THE DETECTION AND INHIBITION OF INFLUENZA VIRUS INFECTION**

Maria Florencia Pavan, Walter Gabriel Sperat, José Manuel Gómez, Nora Mattion, Lorena Itatí Ibáñez
Instituto de Ciencia y Tecnología Dr. César Milstein - CONICET

Influenza is an enveloped RNA virus that causes acute respiratory infections in both humans and animals and is responsible for epidemics all over the world. Even though, vaccines are the most effective way to prevent influenza virus infection, antivirals are also useful tools to control the disease. The emergence of multidrug-resistant viruses has prompted the development of novel antiviral drugs. Single domain antibodies (sdAbs) are small molecules with high stability, solubility and affinity, that can be produced at low cost. For this reason, in this work we propose developing sdAbs capable of binding influenza's hemagglutinin protein (HA), and therefore have the potential to act as antiviral molecules.
In order to develop anti-HA sdAbs, llamas were immunized with recombinant HA, and subsequently a phage library containing genes encoding sdAbs was generated. Molecules obtained after panning the library were modified to obtain bivalent derivates, which bind with higher affinity to the HA protein. sdAbs were expressed in *Escherichia coli* strain BL21 and extracted from the periplasm. Antibodies were subsequently purified by immobilized metal chelate chromatography (IMAC) and protein expression and purification were checked by Western Blot and SDS-PAGE. Binding capacity of mono- and bivalent sdAbs was assessed by ELISA, in plates coated with viral particles of different influenza serotypes. The tested sdAbs displayed a wide heterotypic recognition pattern, since they were capable of recognizing H1, H3, H5, H7 and H9 influenza subtypes. We have also generated chromobodies by fusing mCherry and GFP coding sequences to those of the selected sdAbs, which were expressed in HEK-293 cells at high levels as assessed by confocal microscopy.
The anti-HA modified sdAbs produced in this work are capable of binding strongly to different types of influenza viral strains and therefore will be used to study their ability to inhibit viral infection *in vitro* and *in vivo*.

INMUNOLOGÍA / IMMUNOLOGY ORAL SESSION 4

370. (301) **HLA B51 EXPRESSION AS A RISK FACTOR IN PATIENTS WITH RECURRENT APHTHOUS STOMATITIS AND BEHCET'S DISEASE IN THE ARGENTINE POPULATION**

Andrea Muiño[1], Adriana Lence[1], Laura Harada[1], Mariana Diaz[1], Mario Labbrozzi[1], Pablo Turon[1], Mariana Gandolfo[1], Silvia Aguas[1], Valeria Denninghoff[1], Sara Teper[2], María Jesús Moreno[2], Lidia Isabel Adler[1]
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Recurrent aphthous stomatitis (RAS) is one of the most frequent manifestations of Behcet's disease (BD). Some individuals may have BD at early stages with oral aphthous ulcers as the only clinical manifestation of it. Whereas the BD is associated with HLAB-51expression, the aim of this study was to estimate the association between RAS, HLA-B51 expression and BD in the studied population. The study design was an observational case-control study. A total of 1267 patients were included in this study, 51 with RAS (study group) and 1216 as healthy controls (control group). In both groups, blood samples were taken to perform the HLA B determination. The HLA typing was carried out using the reverse PCR technique (Luminex system).
49% of the patients in the study group (25/51) were female gender and 51% (26/51) were male. The age ranged between 6 and 81 years (mode 22), with an average of 39.46 +/- 1.53 years. 70% (36/51) presented major aphthous. HLA B51 was expressed in 27.4% of the patients with RAS (14/51) and in 15.4% (188/1216) of the control group with an OR 2.06, a CI of 1.10- 3.86 ($p < 0.05$). BD was confirmed in 8 out of 14 (57.14%) patients with RAS and HLA-B51 (these patients fulfilled three of five criteria of the International Study Group for BD).
As a conclusion, our study estimated the association between RAS, HLA B51 expression and Behcet's disease in the studied population. The presence of HLA-B51 may be useful as a determinant for those patients at risk to develop BD

371. (564) **T CELL ACTIVATION IN PATIENTS WITH SEVERE HANTAVIRUS PULMONARY SYNDROME**

Ayelen Iglesias[1], Natalia Periolo[1], Daniel Alonso[1], Rocío Coelho[1], Marina Garcia[2], Pablo Schierloh[2], Carla Bellomo[1], Valeria P Martinez[1]
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Hantaviruses are emerging human pathogens responsible of hantavirus pulmonary syndrome (HPS) in the Americas. Hantaviruses predominantly infect microvascular endothelial cells causing capillary leakage. The hallmark of the disease is the vascular permeability, leading to pulmonary edema in HPS patients.
In order to evaluate the role of the immune response on pathogenesis we performed T-cell phenotypic characterization in acute HPS patients (AP), and when possible, longitudinal analysis during convalescence (CONV). We also studied viral load, IgM/IgG titers and kinetics of neutralizing antibodies in blood samples. We obtained control samples from healthy adult volunteers (HV). Almost all patients presented a severe form of disease.
Analysis of PBMCs showed increased TCD8 and decreased TCD4 cells in HPS patients, resulting in alteration of the CD4/CD8 ratio. The phenotypic analysis of T-cell subpopulations showed an average of 44,94% CD8+/CD38+/HLA-DR+ cells (activated phenotype) in AP (average 10,3 days of illness); 13,06% in CONV (average 84,7 days) and 3,49% in HV. TCD4 cells showed 12,85% CD38+/HLA-DR+ in AP; 3,92% in CONV and 1,77 in HV. Statistic analysis show significant differences between AP and HV (ANOVA "Kruskal-Wallis test"). The average for CD8+/CD38+/CD28- was 13,41% in AP; 3,10% in CONV and 0,80% in HV (differences were not statistically significan).
General analysis of relationship between activated CD8 T-cells and

viral load did not show any correlation; analyzing by sex we found positive correlation in women, in men it was constant. This finding is consistent with higher lethality in women. We also observed differences in activated TCD4 comparing severe and moderate cases. All patients had high IgM/IgG titers in AP. Longitudinal analysis showed decreasing IgM and increasing IgG titers but delay in the development of neutralizing antibodies.
Our analysis revealed an activated state of the immune system. Increased T-cell activation markers in acute patients show tendency to normalize during the convalescence.

372. (586) **HUMAN CATHELICIDINS IMPROVE COLONIC EPITHELIAL DEFENSES AGAINST SALMONELLA TYPHIMURIUM MODULATING CELLULAR PERMEABILITY, TLR4 AND PRO-INFLAMMATORY CYTOKINES**

Maia Solange Marin[1, 2], Mercedes María Burucúa[1, 2], Ravi Holani[3], Graham Blyth[4], Dominique Drouin[5], Anselmo Carlos Odeón[2], Eduardo Cobo[3]
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The intestinal mucosa contributes to frontline gut defenses by forming a barrier and preventing the entry of pathogenic microbes. One remarkable innate role of the colonic epithelium is to secrete cathelicidin, a peptide with broad antimicrobial and immunomodulatory functions. In this study, the effect of cathelicidin in the maintenance of epithelial integrity, Toll-like receptor recognition, and initiation of inflammatory response against Salmonella typhimurium were investigated. Tight junction gene levels and ZO-1 immunolocalization were studied in T84 cells infected with a virulent and drug-resistant S. typhimurium definitive type 104 strain (MOI 5, 4-16h) ± synthetic LL37 (0-40 µg/mL). Normal (ntLL37) and LL37 know-down (shLL37) HT29 colonic cells were pre-stimulated with LL37 (0-20 µg/mL) and infected with S. typhimurium (MOI 1-5, 4-24h). Antimicrobial activity in cell lysates was achieved by bacterial counting and TLR4, TLR9, IL1β and IL18 expression by RT-qPCR. For comparisons a non-paired, two-tailed Student's t-test was used ($P < 0.05$). Exogenous human cathelicidin restored the epithelial integrity in S. typhimurium-infected colonic epithelial by mostly post-translational effects associated with the reorganization of ZO-1 tight junction proteins. No changes were observed in occludin and claudin gene expression. Endogenous cathelicidin showed to contribute to preventing S. typhimurium internalization as studied in intestinal epithelial cells genetically deficient in the only human cathelicidin LL37. Moreover, supplementation of shLL37 cells (i.e., lacking endogenous cathelicidins) with synthetic LL37 (restorative effect) reduced the grade of S. typhimurium internalization in a dose-dependent manner (~50-90% inhibition). Mechanistically, shLL37 cells had significant lower gene expression of TLR4 and IL1β than normal cells in response to S. typhimurium (0.5-1.5 folds). Thus, cathelicidins aided in the early epithelial response against enteric S. typhimurium controlling the invasion of and maintaining the barrier integrity. Endogenous synthesis of cathelicidins occurred key in these functions and principally in the production of sensing TLR4 and pro-inflammatory cytokines.

373. (687) **ANALYSIS OF THE EOSINOPHIL-CHEMOATTRACTANT CYTOKINE PRODUCTION IN INTESTINAL TISSUES OF PATIENTS WITH FOOD ALLERGY**

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Juvenile polyps (JP) are common in pediatric patients with milk allergy and lead to rectal bleeding and diarrhea. We have previously characterized the allergic inflammatory cell infiltrate of the polyp tissue, and here we aimed to investigate the origin of the high frequency of eosinophils, which may be responsible for tissue remodeling.
Biological intestinal samples were obtained by colonoscopy in 7 patients, along with a biopsy of the surrounding tissue (SCT), as a control sample. The production of eotaxin-3 (CCL26) and RANTES, chemokines responsible for eosinophil homing, were assessed by qPCR and confocal microscopy in lamina propria and the epithelial compartment. In addition, Caco-2 and HMEC-1 cell lines were employed to study the induction of chemokine production. Cells were stimulated with IFN-γ (10ng/ml) and IL-13 (10ng/ml), and the activation of STAT-3 and expression of chemokines were analyzed by immunoblotting and qPCR, respectively.
We found a significant increase of eosinophils and eotaxin-3 expression in polyps compared to SCT ($p<.05$), while no difference was observed for RANTES. Eotaxin-3 was mainly produced in the epithelial compartment, which was confirmed by microscopy. Lamina propria and endothelial cells of polyp tissue were also positive for CCL26 staining. The SCT showed a basal expression of this chemokine. Caco-2 and HMEC-1 cells stimulated with IL-13 rendered higher levels of eotaxin-3 transcripts compared to cells incubated with medium or IFN-g ($p<.02$).
In conclusion, we found that the epithelial compartment and endothelium were the main sources of eotaxin-3, being IL-13 responsible for its secretion. These findings may shed light on understanding the etiology of the gut homing of eosinophils in food allergic patients. It may be critical to study the IL-13/STAT-3/CCL26 axis during the novel therapy with biologics (Dupilumab or anti-IL4Ralpha) for patients with eczema and food allergy.

374. (705) **CLASS SWITCH AND IGE PRODUCTION IN GERMINAL CENTERS OF INTESTINAL TISSUES FROM PATIENTS WITH FOOD ALLERGY**

Karina Eva Canziani[1], Melisa Pucci Molineris[2], Luciana Guzmán[3], Viviana Bernedo[3], María Teresa González Villar[3], Eugenia Margarita Altamirano[4], Marcela García[5], Cecilia Muglia[1], Guillermo Docena[1]
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IgE is known as a key mediator of allergic responses; however the production of this antibody has remained largely unknown in patients with food allergy. The intestinal tissue has been proposed as a potential site for production. We have previously reported that patients with juvenile polyps (JP) and high levels of serum IgE, showed an inflammatory cell infiltrate with a Th2-biased and a large number of IgE-producing plasma cells in the gut. The presence of a secondary lymphoid organ-like histology in the stroma of the polyp tissue (PT) prompted us to study the presence of germinal centers (GC) and the local production of IgE antibodies.
PT and surrounding mucosa (SCT) biopsies from patients with rectal bleeding (n=7) were studied by histology, confocal microscopy and RT-qPCR; cytokines were assessed by CBA and ELISA; germinal centers were isolated by laser dissection microscopy (LDM) and analyzed by PCR.
We found germinal center-like cell agglomerates containing CD20+, Ki67+ and AID+ B cells and CD57+ mononuclear cells (follicular helper T cells). We also observed a high frequency of IgE+CD138+ plasma cells (91.25±6.71% of IgE+CD138+ cells of total plasma cells) in the marginal region of germinal centers. SCT showed the absence of these cells. PCR was assessed in RNA isolated from the removed GC with specific primers for different isotype class switch

intermediates (sterile and circle transcripts) and we found bands corresponding to direct (μ to ε) and sequential class switch (μ to γ and γ to ε).
In conclusion, our findings showed that IgE is produced at the mucosa and the local Th2 environment may be strongly linked to the presence of active germinal centers that generated IgE. Likewise, this work provides the first evidence that class switch recombination to IgE occurs in the colonic mucosa of patients with food allergy.

375. (777) **CHARACTERIZATION OF THE A549 CELL LINE AS A TYPE II PULMONARY EPITHELIAL CELL MODEL FOR ANDES VIRUS INFECTION**
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ANLIS.INEI.Instituto Malbrán.Departamento de Virologia

Andes virus (ANDV) cause hantavirus pulmonary syndrome (HPS) in human. Hantaviruses infection is mediated in human cells by integrins β3 and β 1.Multiple cell types contribute to pulmonary barrier including Type I and Type II alveolar epithelium. Most studies were performed on a human tumor cell line, the A549, defined as a model of human AECII. The objective of this research was to establish and characterize an in vitro model of Type II alveolar epithelium using the A549 human lung adenocarcinoma cell line to studied the infection with ANDV.
The viral stocks for ANDV used had titers Vero E6 cell line culture. A549 cell line were infected with multiplicity of infection (MOI=1) for 1 h at 37°C. We analyzed the expression of activation marker (HLA-DR; CD80, CD86), integrin (CD51/CD61) and pro-surfactant protein-C (proSP-C) by flow cytometry. The non-parametric Mann-Whitney test was applied to compare phenotypic markers expression of A549 cells non-infected versus infected.
The presence of virus and viral load was determined by immunofluorescence and RT-PCR (RT-qPCR), respectively.
The expression of CD51/CD61 was detectable on the surface of A549 cells line. This expression was lower than VeroE6 (MFI $p< 0,01$). Surface expression of HLA-DR showed an increased in A549 line infected vs non infected (MFI $p <0,05$). CD86 was activated in A549cell infected vs not infected (MFI $p< 0,05$), while CD80 expressing cells did not change after infection. Conversely, proSP-C was expressed on nearly all the A549 cells no infected (90.0%), while we observed a decrease in infected cells, but this difference was not significant ($p>0.05$).
The results indicated that the A549 cell line is an ideal in vitro model for human study, this will allow to provide knowledge about the immunopathogenesis of ANDV infection.

REPRODUCCIÓN / REPRODUCTION 2

376. (211) **VIP INDUCES GLUCOSE UPTAKE THROUGH PKA/MAPK/PI3K SIGNALING PATHWAYS AND REGULATES AMINO ACID TRANSPORT IN TROPHOBLAST CELLS**
Fátima Isabel Merech, Vanesa Hauk, Elizabeth Victoria Soczewski, Daniel Paparini, Rosanna Ramhorst, Claudia Perez Leiros, Daiana Vota
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Appropriate trophoblast (Tb) metabolism is crucial to allow nutrient transport across the placenta. Alterations of this process may lead to placental insufficiency associated to fetal growth restriction. Glucose and amino acids are essential substrates for the placenta and the fetus and its transfer depends on specific carrier proteins such as GLUT and mainly System A (SNATs) and System L, respectively. mTOR is a serine-threonine kinase activated by PI3K pathway that regulates the expression/activation of amino acid transporters and its role on GLUT transporters in mouse skeletal muscle has been reported. The vasoactive intestinal peptide (VIP) activates cAMP/PKA, PKC and MAPK pathways. VIP expression is regulated by mTOR in central nervous system.We have previously demonstrated that VIP induces mTOR and GLUT1 mRNA expression as well as glucose uptake through mTOR pathways in Tb cells. Here we analyzed the mechanisms involved in VIP-mediated glucose uptake and the role of VIP in the regulation of amino acid uptake in Tb cells. The human Tb cell lines Swan-71 and BeWo were cultured in absence/presence of VIP (50 nM). SNAT1/VIP expression was evaluated by qRT-PCR and flow cytometry. Glucose uptake was measured by flow cytometry using the fluorescent analogue 2-NBDG, and System A activity by incorporation of a specific radiolabelled substrate, ^{14}C-MeAIB, using a scintillation counter. Pharmacological inhibition of PKA and MAPK but not PKC pathways prevented VIP-mediated glucose uptake. Inhibition of PI3K also impaired VIP effect ($n=5$; $p<0.05$). VIP also induced mRNA/protein expression of SNAT1 ($p<0.05$) and modulated System A activation measured as^{14}C-MeAIB uptake. Interestingly, mTOR inhibition with rapamycin reduced VIP expression ($p<0.05$). These results support a role for VIP in the modulation of glucose and amino acid transport in the maternal-fetal interface and propose a bidirectional regulation between VIP and mTOR, thus sustaining its relevance in fetal growth.

377. (213) **NFKB SIGNALLING ON PLACENTAL LEPTIN EXPRESSION INDUCED BY ESTRADIOL**
Malena Schanton[1], Maria Fernanda Camisay[1], Antonio Perez Perez[2], Roberto Casale[3], Bernardo Maskin[3], Victor Sanchez Margalet[2], Alejandra Erlejman[1], Cecilia Varone[1]
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Leptin is a key hormone in placental physiology. It regulates trophoblast proliferation, inhibits apoptosis, stimulates protein synthesis, and regulates fetal growth and development. The mechanisms involved in the regulation of placental leptin expression are not fully understood. Previous results from our lab demonstrated that estradiol (E_2) induces leptin expression involving genomic and non-genomic effects. In this study we aimed to analyze the effect of the transcription factor NFκB on E_2 induction of leptin expression in human placental cells. BeWo cells cultured under standard conditions, as well as human placental explants were used. Western blot, immunoprecipitation and immunocytochemistry were carried out. We found that E_2 treatment did not modify p65 expression. However E_2 increased IκBα phosphorylation suggesting that the transcription factor NFκB, might be affecting estradiol leptin induction. We also evaluated the localization of ERα and p65 NFκB subunit in BeWo cells by immunofluorescence assay. We have found that both proteins are located in the cytoplasm and when they are overexpressed, they migrate to the nucleus. We also analyzed the interaction between ERα and p65 by immunoprecipitation. We observed that they probably interact forming a complex as p65 could be revealed in ERα immunoprecipitation. All these findings suggest that leptin expression is tightly regulated and improve the comprehension of the mechanisms whereby E_2 regulates leptin expression probably involving a cooperative effect between ERα and NFκB.

378. (241) **ALTERATIONS IN THE MTOR PATHWAY IN THE DECIDUA OF DIABETIC RATS DURING EARLY POST-IMPLANTATION**
Sabrina Lorena Roberti, Evangelina Capobianco, Hugo Sato, Romina Higa, Alicia Jawerbaum
Centro de estudios farmacológicos y botánicos

Maternal diabetes increases the risk of anomalies in the embryonic development. During early embryonic development the decidua plays an important role in the histotrophic nutrition. In turn, the mTOR pathway (mechanistic target of rapamycin) is an intracellular signaling pathway involved in nutrient transport and embryonic growth and development. LAT1 (L-amino acid transporter-1) and SNAT2 (Sodium-dependent neutral amino acid transporter-2) are transporters of amino acids regulated by mTOR pathway. Objective: to study the total and phosphorylated protein levels downstream mTOR pathway (4E-BP1 (eIF4E-binding protein 1), AKT y rpS6 (ribosomal protein S6)) and the mRNA levels of mTOR, LAT1 and SNAT2, in the decidua from control and diabetic rats at day 9 of gestation. Methods: Diabetic Wistar rats, obtained by intraperitoneal administration of streptozotocin (50 mg/kg), were mated with healthy males. On day 9 of gestation the decidua was obtained and processed to be used for measurement of total and phosphorylated 4E-BP1, AKT and rpS6 by

Western Blot and for measurement of mRNA levels of mTOR, LAT1 and SNAT2 by qRT-PCR. Results: Reduced levels of phosphorylated 4E-BP1 (27%, p<0,05), AKT (30%, p<0,05) and rpS6 (40%, p<0,05) proteins were found in diabetic decidua, with no changes in total protein levels. mRNA level of mTOR (0,46 fold, p<0,05), LAT1 (0,49 fold, p< 0,01) and SNAT2 (0,54 fold, p<0,01) were also lower in the decidua from diabetic rats than in the decidua from control rats. Conclusion: Maternal diabetes leads to the inhibition of the activity of mTOR pathway in the decidua during early post-implantation, which could compromise the availability of important nutrients for embryo development.

379. (511) **THE MATERNAL TREATMENT WITH CHIA OIL IN DIABETIC RATS (F0) REDUCES THE OVERACTIVATION OF MTOR PATHWAYS IN THE PLACENTA FROM THEIR OFFSPRING (F1) THAT DEVELOPS GESTATIONAL DIABETES MELLITUS.**

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The offspring (F1) from mild diabetic rats (F0) develops gestational diabetes mellitus (GDM), a rat model with increased fetal growth associated with the overactivation of placental mechanistic target of rapamycin (mTOR) signaling. Maternal diets supplemented with polyunsaturated fatty acids (PUFAs) have beneficial metabolic effects in the placenta from pregestational diabetic rats.
AIM: to study the effect of a maternal treatment with chia oil in diabetic rats (F0), on the fetal weight and placental mTOR pathways in their offspring (F1) that develops GDM.
METHODS: control and diabetic rats (F0) were fed with a standard diet or a diet supplemented with 6% chia oil (rich in n-3 PUFAs) (CH group) during gestation. The female offspring (F1) was mated with healthy males. Metabolic parameters and the mTOR signaling pathways (by Western blot) were evaluated in the placenta from control and GDM rats on day 21 of pregnancy.
RESULTS: The development of GDM (fasting glycemia values over 130 mg/dl) was not prevented by the chia oil diet. Fetal weight was increased in the GDM group (26%, p<0.01) but not in the GDM-CH group. The levels of phosphorylated rpS6 were decreased (16% vs GDM group, p<0.05) and the levels of 4EBP1 were increased in the GDM-CH group (41% vs GDM group, p<0.05), indicating a reduction in mTORC1 overactivation. The increase in the phosphorylation of SGK1 (30%, p<0.01) and the reduction of PKCα levels (29%, p<0.05) in the GDM group suggested mTORC2 overactivation that was prevented in the GDM-CH group (p<0.05).
CONCLUSIONS: The maternal dietary treatment with chia oil in diabetic rats does not prevent the development of GDM in their offspring, but prevents fetal overgrowth and the overactivation of the mTOR signaling pathways in the placenta.

380. (530) **PHOSPHORYLATION OF LIMK1 AND COFILIN IS REGULATED BY PAK4 AND OKADAIC ACID- SENSITIVE PROTEIN PHOSPHATASES DURING MOUSE SPERM CAPACITATION**

Liza Jamaica Schiavi-Ehrenhaus, Ana Romarowski, Guillermina Maria Luque, Cintia Stival, Belen Ugo, Paula Balestrini, Martina Jabloñski, Nicolas Gilio, Dario Krapf, Mariano Buffone
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Actin dynamics play a central role in controlling the process of exocytosis in somatic cells as well as in sperm from several mammalian species. In spermatozoa, acrosomal exocytosis, a special type of controlled secretion, is an absolute requisite for fertilization in mammals. This complex exocytic process is controlled by several players including the actin cytoskeleton. We previously have shown the involvement of small GTPases of the Rho family in the signaling pathway that leads to actin polymerization during sperm capacitation. We have also demonstrated the participation of LIMK1 and COFILIN, both downstream effectors of small GTPases that are modulated by phosphorylation. In the present work, we studied the role of phosphatases and PAK kinases in the regulation of LIMK1/COFILIN phosphorylation during mouse sperm capacitation. One of the widely known phosphatases of COFILIN in somatic cells, Slingshot homolog 1 (SSH1), was observed to be expressed in mouse sperm and localized in the acrosome and the principal piece of the flagellum, as well as its characteristic regulator protein, 14-3-3. The time course of SSH1 dephosphorylation is coincident with that observed for COFILIN dephophorylation. Furthermore, okadaic acid- sensitive protein phosphatases were found to dephosphorylate SSH1 and their inhibition resulted in persistent levels of phosphorylated COFILIN. However, flow cytometry analysis revealed that okadaic acid did not significantly affect the percentage of sperm that undergo acrosomal exocytosis. On the other hand, inhibition of PAK4 kinase by a specific inhibitor (PF-3758309) resulted in lower levels of pLIMK1 and a significant decrease in the percentage of sperm that undergo acrosomal exocytosis (p<0.05). In conclusion, our results suggest that PAK4 kinase and okadaic acid- sensitive phosphatases participate in the regulation of LIMK1/COFILIN phosphorylation/dephosphorylation balance, which is essential for the dynamic changes of the actin cytoskeleton during capacitation and acrosomal exocytosis.

381. (50) **GPER MEMBRANE RECEPTOR-MEDIATED ESTROGEN REGULATION OF THE AMH PROMOTER**

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Anti-Müllerian hormone (AMH) is secreted by testicular Sertoli cells from the beginning of fetal life until puberty, when it is inhibited by androgens. In conditions characterized by high estrogen levels, like Peutz-Jeghers and androgen insensitivity syndromes, AMH production is increased. The physiological effects of estrogens are mediated by classical nuclear receptors (ERα and ERβ), and also by a G protein-coupled membrane receptor (GPER). Previously, we demonstrated that estradiol (E_2) mediates the increase of AMH promoter activity in Sertoli cells by ERα. In this study we assessed whether E_2 regulates AMH promoter activity also through GPER in the mouse pre-pubertal Sertoli cell line SMAT1.
Reporter assays were performed in SMAT1 cells co-transfected with a GPER expression plasmid associated with fluorescent protein EGFP, a firefly luciferase-associated AMH promoter plasmid, and a renilla luciferase plasmid as transfection efficiency reporter. Luciferase was measured after incubation with 17β-E_2 (10^{-9} M), GPER-specific agonist G1 (100 nM) or GPER antagonist G15 (100 nM) for 24 hours. Results, expressed as a relative luciferase unit (RLU, mean ± SEM), were compared using a paired-samples Friedman test and Dunn›s multiple comparison post-test.
The expression of GPER-EGFP in SMAT1 cells was confirmed by fluorescence microscopy. Basal AMH promoter activity (0.979 ± 0.081 RLU) increased modestly but significantly after incubation with E_2 (1.062 ± 0.078 RLU, n=10, P=0.048) or G1 (1.109 ± 0.085 RLU, n=10, P=0.022). The co-incubation with antagonist G15 did not produce significant changes in AMH promoter activity compared to E_2 (E_2: 1.062 ± 0.078 RLU, G15 + E_2: 1.027 ± 0.132 RLU, n=10, P=0.227).
Conclusion: Estrogen effect on AMH promoter activity is also partially exerted through GPER in SMAT1 cells. This suggests a mild modulatory action of prepubertal Sertoli cells by estrogens through GPER under physiological conditions, which could be increased in situations of hyperestrogenism.

382. (218) **LEPTIN PROMOTES TROPHOBLASTIC CELL INVASION INVOLVING BETA-CATENIN/WNT PATHWAY**

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Leptin, the adipocyte derived hormone encoded by the Lep gene, is

synthesized by placentaltrophoblasts where it works as a paracrine and autocrine hormone. It has been shown thatduring pregnancy leptin modulates migration and invasion of trophoblastic cells. In recentstudies it has been determined that leptin reduces the level of E-Cadherin and increases theexpression of β1-Integrin in Swan-71 cells.In the present work, we aimed to study the molecular mechanisms that mediate the effect ofleptin regulation of trophoblastic cells invasion in placenta.We use Swan-71 cells, a first trimester trophoblastic human cell line, cultured under standardconditions, as well as human term placenta explants. Swan-71 cells and placental explantswere treated with different concentrations (50, 100 and 250 ng/ml) of recombinant leptin during48 h (Swan-71cells) or 20 h (placenta explants). Metalloproteinases 2 and 9 (MMP-2 andMMP9) were assessed by qRT-PCR and zymography. β-Catenin expression was determinedby Western-Blot and immunofluorescence analysis. Invasion experiments using trans-wellscovered with Matrigel were also performed.We determined that leptin treatment increased invasion of trophoblastic cells. Leptin alsoenhanced MMP-2 and MMP-9 expression and activity. The effect of leptin on the expression ofβ-Catenin as a possible activated pathway mediating invasion was studied. We determined thatleptin treatment increased significantly the level of β-Catenin in Swan-71 cells ($p<0,05$), andpreliminary results in placenta explants confirm our results.Therefore, β-Catenin/Wnt pathway seems to be one of the signalling pathways implicated inincreasing the invasiveness of trophoblastic cells.

383. (634) **EFFECT OF MILD HYPERTHYROIDISM ON HYPOTHALAMIC PROLACTIN SIGNALING PATHWAY IN EARLY LACTATION**

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During late pregnancy the hypothalamic neuroendocrine control of PRL secretion adapts, decreasing dopaminergic tone, to promote adequate lactation. Previously we described that severe hyperthyroidism elevates dopaminergic tone, blunting suckling-induced prolactin (PRL) release that results in early litter death. In this work, we studied the effects of mild hyperthyroidism, which also reduces PRL secretion but allows the maintenance of lactation and litter survival, on hypothalamic regulation of PRL secretion using a dose of T4 (0.1mg/ml/kg/day, s.c.) on late pregnancy (day 19, G19) and early lactation (L2). Hypothalamic mRNA of hyperthyroid (HyperT) and control (Co) rats was obtained and the expression of receptors (R) of thyroid hormone (TRα1, TRβ1, TRβ2), progesterone (PR, PRB), estrogen (ERα, ERβ) and prolactin (PRLR Long isoform) and members of the PRL signaling pathway (STAT5b, SOCS1 and SOCS3) was determined by RT-qPCR. TRβ2 expression decreased on L2 compared to G19 in Co and HyperT ($P<0.001$). PRB showed lower expression on L2 respect to G19 ($P<0.0001$ in HyperT and $P<0.05$ in Co). Also, the HyperT PRB expression was increased with respect to Co on G19 ($P<0.05$). PRLR expression in the HyperT group was increased on L2 compared with G19 ($P<0.01$), while Co remained unchanged. Furthermore, PRLR expression was increased in HyperT compared with Co rats on L2 ($P<0.05$). HyperT also decreased the expression of CIS on L2 with respect to G19 ($P<0.01$), whereas the expression in Co was stable. HyperT increased CIS expression on G19 compared to Co ($P<0.05$) and decreased SOCS1 expression on L2 compared with G19 ($P<0.01$) while there were no differences in Co. Thus, mild HyperT increased PRLR and decreased SOCS1 and CIS expression on L2, maintaining an increased hypothalamic dopaminergic tone that is responsible for the impaired suckling-induced PRL secretion observed in HyperT mothers.

384. (550) **STARD7 DEFICIENCY MODULATES CONNEXIN 43 EXPRESSION AFFECTING GOLGI APPARATUS AND CENTROSOME LOCATION DURING CELL MIGRATION**

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StarD7 belongs to START domain protein family, which is involved in lipid transport, metabolism and signaling. Previous results from exploratory differential gene expression analysis in JEG-3 cells transfected with StarD7 siRNA demonstrated that connexin 43 (Cx43) mRNA is largely downregulated. In the present study we explored the effect of StarD7 siRNA on Cx43 expression and its association with Golgi apparatus and microtubules organization center (MTOC) location during polarized cell movement in the derived-first trimester trophoblast HTR-8/SVneo cells. Additionally, the expression of several extracellular matrix (ECM) proteins was examined. Data from qPCR and western analysis demonstrated a decrease in Cx43 mRNA and protein levels in cells transfected with StarD7 siRNA compared to control siRNA. A significant increase in the mRNA and protein levels of integrin α5, the mature integrin β1, β-catenin and nidogen-1 in silenced cells was determined. Also, StarD7 silencing lead to an increase in the amount of MMP9 secreted to the culture medium. Exogenous StarD7 expression was able to restore Cx43 and nidogen-1 expression in StarD7 silenced cells. Wound healing and transwell assays demonstrated that cell migration was decreased in StarD7 knockdown cells. Finally, StarD7 suppression lead to a disruption in Golgi apparatus organization and also to a lack in the ability of the cell to reorient MTOC/Golgi in a polarized migration.
Collectively, our studies reveal that StarD7 deficiency leads to a diminution in Cx43 expression with a reprograming of genes encoding ECM or ECM associated proteins; and this outcome is linked to Golgi apparatus disruption and a deficiency to appropriately locate MTOC/Golgi during cell migration. All of these findings cause cell polarity defects and a decreased cellular migration.
Keywords: ECM, polarized migration, extravillous trophoblasts, StarD7

385. (574) **HEPARAN SULFATE, PRESENT ON THE EQUATORIAL SEGMENT OF HUMAN SPERMATOZOA, DOES NOT PARTICIPATE IN IN VITRO NUCLEAR SPERM DECONDENSATION.**

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We have previously reported that Heparan Sulfate (HS) is present in the ooplasm and perivitelline space of human oocytes and participates in sperm decondensation *in vivo*. Flow cytometry experiments revealed that HS is also present on the surface of human spermatozoa. The aim of this study was to 1) determine the localization of HS on the surface of human spermatozoa and **2)** evaluate whether this HS could be involved in sperm nuclear decondensation. **1)** Spermatozoa were obtained from normozoospermic donors (WHO), swam up in HTF medium (**W**), incubated in capacitating conditions (overnight, 37 °C, 5 % CO_2, **C**) and exposed to A23187 calcium ionophore (**IAR**). Immunocytochemistry was performed using a monoclonal anti HS antibody and Rhodamine labeled second antibody. Acrosomal status was determined with *Pisum sativum* lectin. **2)** Sperm nuclear decondensation was evaluated *in vitro* in the presence of murine oocytes with or without the addition of 20 mUI/mL heparinase III (Hase). **1)** HS was present on 77±6% **W**, 62±10% **C** and 46±6 % **IAR** spermatozoa (n=3), localizing mainly (67±4% in **W**; 48±8% in **C**, and 38±11% in **IAR**) over the equatorial segment. Acrosomal labeling was observed in 10±4% sperm throughout. Double staining with *Pisum sativum* and anti-HS revealed that HS localization was not related to acrosomal status. **2)** Sperm in vitro decondensation in murine oocytes was not affected by prior incubation of spermatozoa with Hase (62±3 % control vs 55±8 %, NS) treated but decreased significantly when Hase was added to oocytes (25±2 %, $p=0.024$, ANOVA+Tukey, n=3). Results demonstrate that HS localizes primarily over the equatorial segment of human spermatozoa, regardless of physiological status and does not participate in nuclear sperm decondensation *in vitro*. Its possible involvement in sperm interaction with the epithelium of the female tract is currently under investigation.

386. (633) **LYSINE ACETYLATION AS A MODULATOR OF SPERM CAPACITATION**
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Mammalian sperm are unable to fertilize the egg before undergoing a series of biochemical and physiological changes in the female reproductive tract, collectively known as capacitation. Functionally, capacitation is associated with changes in the sperm motility (hyperactivation) and with their ability to undergo the acrosome reaction. At the molecular level, capacitation correlates with activation of the cAMP-PKA pathway, increase in intracellular pH and Ca2+ concentration, hyperpolarization of the plasma membrane potential, lipid modifications and increase in protein tyrosine phosphorylation. How these signaling pathways interact to induce hyperactivation and acrosomal responsiveness is not well understood. Since mature sperm are transcriptionally and translationally silent, they rely on postranslational modifications (PTM) more than any other cell type. Therefore, it is an exceptional model for the study of signaling pathways based on PTM. The importance of phosphorylation, an essential PTM in sperm physiology has been well established. Acetylation as a broad and abundant PTM comparable with phosphorylation, however, has not been well analyzed.
In this context, the general aim of our work was to study the role of protein acetylation in the signaling cascade responsible for the acquisition of fertilizing capacity of mouse sperm. Western blot and immuno-localization analyses with anti-acetyl lysine antibodies showed acetylation of several proteins, with a significant increase detected in capacitated sperm. Therefore, we used deacetylase inhibitors to produce pharmacological hyperacetylation under non-capacitating conditions and evaluated the molecular and functional events associated to capacitation. In this condition, we observed increased PKA activity and [Ca2+]i, CatSper opening and hyperpolarization, all capacitation-associated molecular events. Furthermore, hyperacetylation of non-capacitated sperm promotes hyperactivation and prepares the sperm to undergo acrosome reaction, even in the absence of HCO3- and BSA. Together, these results show that acetylation plays a critical role in acquisition of the fertilization competence of mammalian sperm.

387. (234) **PERINATAL EXPOSURE TO ETHINYLESTRADIOL IMPAIRS PREGNANCY DEVELOPMENT IN MICE**
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Ethinylestradiol (EE) is a potent synthetic estrogen used principally in the female oral contraceptive pill. Due to its widespread use, EE has been detected in river systems around the world. Here we studied how EE exposure around implantation affects pregnancy, particularly concentrating on placentation and uterine remodeling. C57BL/6 pregnant female mice were exposed to 5 µg EE/kg/day, 0.005 µg EE/kg/day or 0.1% ethanol by oral gavage from day 1 to 7 of gestation. The number of implantation sites and the abortion rate in each group was determined at gd 5, 10 and 14 respectively. Weight of fetuses and placentas was recorded and the fetal development was assessed by high frequency ultrasound. Blood velocity in the arteria uterina was analyzed by Doppler measurements. The remodeling of uterine spiral arteries (SA) was studied in HE-stained slides of transversal sections of implantations. We showed that short-term exposure to EE around implantation has negative consequences: the remodeling of SA was impaired in low dose EE-treated mothers as well as a significant higher fetal and placental weight, whereas fetuses of high doses EE-treated mothers die intrauterine. Our work revealed unsuspected short-term effects of EE on pregnancy and urges to more studies dissecting the mechanisms behind the negative actions of EE during early pregnancy.

NEUROCIENCIAS / NEUROSCIENCE 2

388. (561) **INHIBITION OF HISTONE DEACETYLASES AFTER THE ONSET OF BRAIN ISCHEMIA IMPAIRS NORMAL EVOLUTION OF REACTIVE ASTROGLIOSIS AND EXACERBATES THE DAMAGE**
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Brain stroke is still of major concern in medicine and the lack of efficient treatments is because of the incompletely understood molecular mechanisms underlying neuronal death. A key factor promoting neurodegeneration is the reactive astrogliosis (RA) in which astrocytes drastically change morphology, become pro-inflammatory and form the glial scar blocking axonal regeneration. This phenotype follows changes in the programs of gene expression and we showed that activation of transcription factor NFkB is at least one of the pathways responsible for the phenomenon. We aim now to address what happens at the chromatin level in terms of gene regulation by epigenetic mechanisms. We analyzed astrocytic response in vivo using a model of brain ischemia by cortical devascularization and in vitro by exposing primary cultures of astrocytes to ischemic conditions. At 1/2/3 days post lesion (DPL) animals were injected (i.p.) with a single dose of 300mg/kg/day of valproic acid (VPA), an inhibitor of histones deacetylases. At 14DPL the glial scar is formed and astrocyte reactivity is confined only to the region around the ischemic core in control animals. Surprisingly, in VPA injected animals we observed expansion of the damage and immunofluorescence for GFAP and Vimentin showed that astrocytes were highly more reactive. We observed in these animals a higher cell infiltration. Histone 3 acetylation (H3ac) levels in inmunoblot indicated that this mark decreases immediately after ischemia and then increases compared to control. Confocal microscopy revealed a very heterogeneous pattern of H3ac in nuclei from reactive astrocytes. Our results suggest that astrocytes suffer epigenetic modifications in response to brain ischemia and that these changes may be instrumental for the proper evolution of the process of RA. We consider that understanding this mechanism is of high relevance for designing new therapeutic strategies which aim to modulate gliosis and inflammation. PICT2015-1451/UBACYT.

389. (103) **STEROID RECEPTORS AND PHOTORECEPTOR SURVIVAL**
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Photoreceptors survival is affected in various retinal diseases. Steroid hormones and glucocorticoids (GC) can be neuroprotective and it has been suggested that progesterone (PROG) and dexamethasone (DEX) may be beneficial. So the objective is to investigate the mechanisms of neuroprotection involved by PRG and DEX, in two models of mouse photoreceptor degeneration: phototoxic degeneration and degeneration induced by mifepristone (MFP). As well as the location of the GCs and PROG receptors.
We use male balb/c mice (5-7 weeks) reared under cyclic illumination (≤ 60 lux). To generate damage, animals were exposed 48hs to 1500lux or received an injection of MFP (10mg/Kg), with or without steroid treatment (DEX 4mg/Kg/day, PROG 1 or 4 mg/Kg/day) for two days. In both models, the control groups received saline solution. Animals were sacrificed at day 2.
The immunofluorescence revealed the presence of GR and PROG receptors in the retina, data corroborated by WB of the whole retina and also form isolated rod outer segments (ROS). WBs demonstrated the presence of cleaved caspase 3 (CC-3) in both degeneration models, but not in those treated with PROG 4mg/Kg. The apoptotic regulator Bcl-2 showed an increase in injured retinas, while Bcl-XL disappeared in both degeneration models. p-H2AX increases considerably under both damage, but steroids treatment reduced its expression considerably. We found that only the CNE have TUNEL+

nuclei, that was higher in animals exposed to light than in those treated with MFP, in both cases PROG and DEX reduced the number of positive nuclei. The same was seen with CC-3 immunohistochemistry.
We conclude that both PROG and DEX are neuroprotectors because they prevented photoreceptors death in both models of degeneration, demonstrated by the absence of CC-3 and alterations in the levels of Bcl-2, Bcl-XL and p-H2AX, as well as by reducing TUNEL+ nuclei.

390. (314) **REDOX-SENSITIVE TRANSCRIPTION FACTORS AND GLUTAHIONE METABOLISM IN RAT BRAIN AFTER ACUTE FE-DEXTRAN TREATMENT**

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It was previously described that acute Fe-dextran treatment modified redox-sensitive transcription factor related to cell protection against oxidative stress in rat brain. NF-κB DNA binding capacity and nuclear Nrf2 expression levels were affected after 6 h of Fe administration. However, no significant differences in the expression of Keap 1 protein in nuclear or cytosolic fractions of brain extracts were observed. Cellular defense systems involve the expression of antioxidant and detoxification genes, including glutathione S-transferase (GST) and hemeoxygenase 1, among others. The aim of this study was to determine the activation of brain Nrf2/Keap1 signaling pathway, after acute Fe-dextran treatment to rats, assessing the participation of target genes. A single dose of 500 mg Fe-dextran/kg body weight was administered intraperitoneally to male Sprague Dawley rats. Total brain samples or cortex area were obtained from control and treated animals at 6 and 8 h post injection (pi). GST activity and total thiols were determined spectrophotometrically. Glutathione (GSH) content was determined by reverse phase HPLC. Results indicate that GST activity was 2-fold increased at 8 h pi with respect to control values ($p<0.05$). Total thiols content was 1.2-fold significantly decreased in whole brain samples after either 6 or 8 h pi, as compared to control tissue ($p<0.05$). In cortex area, not only the total thiols but also GSH content were significantly decreased after 6 and 8 h of Fe overload. These results suggested that the oxidative stress produced in rat brain by the acute Fe-dextran treatment, produced the translocation of Nrf2 to the cell nucleus and in turn activated genes involved in the antioxidant GSH metabolism response (among other target genes).

391. (316) **GONADOTROPIN-RELEASING HORMONE, GLUTAMATE AND ESTRADIOL INTERACTION IN THE MODULATION OF THE REPRODUCTIVE CYCLE OF THE SOUTH AMERICAN PAINS VIZCACHA (LAGOSTOMUS MAXIMUS).**

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Lagostomus maximus shows peculiar reproductive features such as massive poliovulation and reactivation of the hypothalamic-pituitary-ovarian (HPO) axis during pregnancy with follicular recruitment and ovulation at mid-gestation. Its gonadotropin-releasing hormone (GnRH) neurons at preoptic (POA) and supraoptic (SON) areas express estrogen receptor alpha (ERalpha). Glutamate (GLU) modulates GnRH neurons through its specific receptor NMDA-R in an estradiol (E2) dependent manner. The aim of this work was to study the involvement of E2 on the glutamatergic system and its modulation on GnRH expression. Their involvement on HPO axis activation during gestation was evaluated. Adult vizcachas were used under three experimental conditions: 1) non-pregnant (NP), 2) early-pregnant (EP), mid-pregnant (MP), and term-pregnant (TP) females, and 3) NP ovariectomized (OVX) females injected with E2 (1mg/kg/day) during 5 days (OVX+E2). SHAM animals were used as controls. N=6 per group. E2 serum levels, and hypothalamic GnRH and NMDA-R were studied by ELISA, RIA, Western-blot, and immunofluorescence. MP females showed higher serum E2 levels, and GnRH and NMDA-R expression vs. EP & TP. Hypothalamic explants of NP incubated with GLU +/- GLU receptors antagonist showed significant GnRH delivery increments when NMDA-R was blocked ($p<0.05$). NMDA-R expression was localized both in the POA GnRH neurons and their neighboring neurons. MP females showed abundance of GnRH+/NMDA+ neurons, while TP females showed a prevalence of GnRH-/NMDA+ expression. OVX+E2 showed significantly increased levels of NMDA-R vs. OVX and SHAM ($p<0.05$). Finally, hypothalamic explants of NP incubated with ERalpha agonist + ERbeta antagonist, ERalpha antagonist + ERbeta agonist, or the aromatase inhibitor letrozole, showed significant increase in GnRH delivery related to Controls and E2 supplementation ($p<0.05$). These results suggest that the increase in the expression of NMDA-R at mid-gestation could be induced by increments in E2 levels which would enable GnRH expression during pregnancy (Fundación Científica Felipe Fiorellino, CONICET-PIP110/14, and MICyT-PICT1281/2014).

392. (374) **NEUROANATOMICAL INDEXES IN THE SOUTH AMERICAN PLAINS VIZCACHA: A DIFFERENT STRATEGY OF BRAIN DEVELOPMENT AMONG HYSTRICOMORPHA.**

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The Indexes of Encephalization (EQ) and Gyrification (GI) are used to determine and compare the degree of the brain evolutionary development among species. We studied the degree of brain development in the vizcacha and its comparison with evolutionarily related species. Adult vizcachas (n=25) and embryos between 65 and 133 days (n=18) were used to determine EQ and GI, and fetal time of convolutions development, respectively. EQ and GI indexes from other rodents (myomorphs: rat and mouse, and hystricomorphs: guinea pig, capybara, aguti and chinchilla) were calculated from public data available at Mammalian-Brain-Collections, Universities of Wisconsin and Michigan, (http://neurosciencelibrary.org). EQ was calculated according to Herculano-Houzel, whereas GI was obtained as the quotient between the brain-pial-surface and the external brain-cortical-contour using coronal slices. The vizcacha showed EQ=1.09 and GI=1.14. When both indexes were compared among the hystricomorphs, although the vizcacha´s GI was in the middle, its EQ was one of the lowest of the group. In addition, the average value for both indexes was lower for myomorphs vs hystricomorphs. In the regression analysis for GI in function to the brain-weight including all the studied species, an r^2=0.8375 was determined. However, when suborders were separately analyzed, only the hystricomorph resulted adjusted (r^2=0.8159). Moreover, in the correlation analyzes between EQ and GI, inverse correlation patterns were observed: myomorphs r=0.9943, and hystricomorphs r=-0.8202. Fetal brain analysis showed that transverse sulci and lateral sulci begin to development around embryonic days 80 and 100, respectively. We conclude that the main factor involved in brain development of myomorphs is the encephalic volume increase. However, in the hystricomorphs, an increase in the convolutions is opposed to the increase in the encephalic volume. Finally, unlike the other members of the suborder, vizcacha's brain development would result from the combination of size and convolutions. Fundación Cientíca Felipe-Fiorel-

lino, CONICET-PIP-110/14, MINCyT-PICT-1281/2014.

393. (407) **ENDOGENOUS ADENOSINE MODULATES ELECTRICALLY-EVOKED ACH SECRETION VIA A3 RECEPTORS AT THE MOUSE NEUROMUSCULAR JUNCTION (NMJ)**

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At mammalian NMJ, ATP is co-released with ACh, and metabolized *via* ecto-nucleotidases to adenosine (AD), which modulates ACh release through presynaptic inhibitory A_1 and facilitatory A_{2A} receptors (R). We have demonstrated, by pharmacological and immunohistochemical assays, that A_3AD R are also present at the motor nerve terminals and that they may be activated by AD and its metabolite inosine (INO). Our aim was to elucidate if A_3R are activated by endogenous AD when the nerve is stimulated at 0.5 Hz (50 pulses), 5 Hz (750 pulses) or 50 Hz (750 pulses or 5 bursts of 150 pulses, 20-s interburst interval). In phrenic-diaphragm preparations (CF1 mice), we studied the action of the specific antagonist or agonist for A_3R (MRS-1191 5 μM; INO 100 μM) upon EPP amplitude. At 0.5 Hz (n=4) and 5 Hz (n=4), MRS-1191 did not change the amplitudes of the 1º EPP, mean EPPs, last 20 EPPs and last 20 EPPs/1º EPP ratio, while INO (0.5 Hz n=6; 5 Hz n=4) significantly reduced 1º EPP amplitude, mean EPP and last 20 EPPs. At 0.5 Hz, INO did not alter last 20 EPPs/1º EPP ratio but at 5 Hz this relation was higher than in control ($p<0.05$). During continuous 50 Hz stimulation, MRS-1191 (n=4) did not modify 1º EPP amplitude but increased ($p<0.05$) mean EPP amplitude, last 20 EPP amplitude and last 20 EPPs/1º EPP ratio. In turn, INO (n=5) decreased 1º EPP and mean EPP amplitude ($p<0.05$), did not alter last 20 EPPs and increased last 20 EPPs/1º EPP ratio ($p<0.01$). During intermittent 50-Hz stimulation the results were similar to those observed at 5 Hz for MRS-1191(n=5) and INO (n=5). These findings suggest that during continuous high stimulation frequency enough endogenous AD is generated and accumulated in the synaptic cleft able to modulate ACh secretion via inhibitory A_3R.

394. (466) **MECHANISM OF ACTION OF TESTOSTERONE IN A MOUSE MODEL OF SPONTANEOUS MOTONEURON DEGENERATION.**

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The Wobbler mouse (WR) is an animal model of amyotrophic lateral sclerosis (ALS), a progressive disorder with selective loss of motoneurons, astrocytosis and microgliosis. Although the incidence of ALS is greater in men than in women, it increases after menopause. Therefore, sex steroids may play a key role in ALS. Testosterone, a complex steroid, can bind androgen (AR) or estrogen (ER) receptors, membrane and sigma1 receptors (S1R). It is metabolized on two pathways: 1-aromatase converts androgens into estrogens and 2- 5-alpha reductase metabolizes testosterone into dihydrotestosterone. Here, we studied the effect of exogenous testosterone administered by 10mm silastic tubes s.c. for 2 months to male symptomatic WRs. In the cervical spinal cord (CSC) of testosterone-treated WR´s, we showed a reduction of AR ($p <0.01$), ER ($p<0.05$) and aromatase mRNAs ($p<0.01$) vs. untreated WRs. ER and aromatase mRNAs were higher in WR´s CSC ($p <0.05$ vs control), whereas AR mRNA expression was similar to controls (NS vs control). Five-alpha reductase type 1 mRNA did not show differences between groups. StAR mRNA, an essential protein for neuroesteroidogenesis, showed an increase in WRs vs controls ($p <0.01$), but decreased in testosterone-treated WRs. WR´s gray matter showed higher StAR immunoreactivity ($p<0.001$ vs control; $p<0,01$ vs. WR+testosterone), while white matter regions (ventrolateral funiculus and corticospinal tract) showed similar values in the 3 groups (NS). Strikingly, S1R mRNA expression was significantly increased in testosterone-treated compared to untreated WRs or controls ($p <0.01$ vs WRs or controls). Similarly, testosterone-treated WRs showed higher immunoreactivity for glutamine synthetase, an enzyme necessary for maintaining glutamate concentration, than WR´s in the ventral horn ($p <0.001$), a parameter that was reduced in untreated WRs ($p <0.01$ vs control). The results suggest a multifactorial mechanism of action of testosterone in Wobbler neurodegeneration. Besides classical mechanisms, the role of the S1R deserves further appraisal.

395. (581) **SWEET COMPOUNDS FOR A BITTER PATHOLOGY: STUDY OF THE MECHANISM OF ACTION INVOLVED FOR ISOLATED ANTICONVULSANT COMPOUNDS FROM STEVIA.**

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Despite there are many useful antiepileptic drugs (AEDs), nearly 1/3 patients with epilepsy who have access to AEDs continue to have seizures, and a similar proportion experience unacceptable AED-related adverse effects. Therapy based on the use of medicinal plant extracts has begun to be considered and tested in human refractory epilepsia to classical treatments. Stevia rebaudiana (Bertoni) is native to South America and grown well in Argentina. Steviol glycosides are a group of intensely sweet compounds that have been extracted and purified from Stevia, been stevioside and rebaudioside A the most abundant. In the last 3 years computational and animal in vivo assays, have demonstrated that these compounds have anticonvulsant properties (doi: 10.1089/drrr.2014.0008, doi.org/10.1016/j.kjms.2016.07.002) but the mechanism of action of these compounds is unknown.
Voltage gated sodium channels (VGSCs) are therapeutic targets to treat epilepsy; the known antiepileptic drugs such as phenytoin, carbamazepine and lamotrigine, exert their activity by modifying different electrophysiological properties of VGSCs. Using patch clamp technique we have tested if stevioside and rebaudioside A are able to inhibite two human VGSC isoforms Nav1.1 and Nav1.2, stably expressed in HEK293 cell line. Our results indicate that in hNav1.2 isoform stevioside and rebaudiosideA at 100 µM reversibly inhibit sodium current ($p< 0.02$) and regarding the effect on the steady-state inactivation they shift to the left the Vh1/2 of h curve ($p< 0.03$). In hNav1.1 isoform stevioside block the sodium current ($p<0.04$) and shift to the left the Vh1/2 ($p<0.04$) while rebaudioside A does not exert any effect with respect to the control group.
This work represents a novel research about the mechanism of action of the principal isolated compounds from Stevia, with in vivo anticonvulsant activity in mice and rats. They share the VGSCs as a target like the most classical AEDs but rebaudiosideA present isoform selectivity.

396. (660) **SYNAPTIC CHANGES IN GLUN2A KNOCKDOWN OF MATURE PRIMARY NEURONAL CULTURES**

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NMDA Receptors (NMDAR) are glutamatergic receptors involved in synaptic plasticity, learning and memory processes as well as in several neuropathologies. NMDAR are composed by two GluN1 obligatory subunits and two regulatory subunits: GluN2 (A-D) or GluN3 (A-B). In memory related brain structures, like the hippocampus, GluN2A and GluN2B are the most expressed regulatory subunits. Transcription and translation of both subunits are tightly regulated; while GluN2B expression is characteristic of immature synapses, GluN2A is present in mature and stable synapses. However, changes in GluN2A subunit expression has been shown in some synaptopathologies. In order to better understand the role of GluN2A in synapsis, we built two AAV-eGFP vectors: one codifying

a specific shRNA anti GluN2A (AAV-sh2A), and the other carrying a shRNA scramble as control (AAV-shSc). In mature primary neuronal cultures transduced with AAV-sh2A or AAV-shSc we analyzed dendritic spines and also the expression of two synaptic proteins: Syn-1 and PSD95. As was expected, we observed by qPCR a decrease in GluN2A mRNA only in primary cultures transduced with AAVsh2A, without modifications in the other NMDAR subunits expression. Interestingly in those cultures where GluN2A was knockdown, we saw a significant decrease in GluN1 protein, while GluN2B protein levels did not change. Furthermore, in those cultures we found an increase in dendritic spines number, at expenses of immature spines. In addition, the expression of Syn-1 and PSD95 was raised up in GluN2A knockdown cultures. These results suggest that GluN2A decreased expression seems to induce a rise in the synaptic spines, that would provoke changes in neuronal architecture and the return of the system to an immature state. All these observed changes could help to explain, at least in part, the molecular pathway of some variants of epileptic disease related to GluN2A mutations.

CARDIOVASCULAR Y RESPIRATORIO / CARDIOVASCULAR AND RESPIRATORY 2

397. (85) **CARDIOPROTECTION OF BENZOLAMIDE IN A REGIONAL ISCHEMIA MODEL: ROLE OF ENOS/NO**

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Recent studies from our laboratory show the cardioprotective action of benzolamide (BZ, carbonic anhydrase inhibitor) against ischemia-reperfusion injury. However, the mechanisms involved have not been fully elucidated. To examine the participation of the endothelial nitric oxide synthase (eNOS)/nitric oxide (NO) in the effects of BZ in a model of regional ischemia. Isolated rat hearts perfused by Langendorff technique were submitted to 40 min of coronary artery occlusion followed by 60 min of reperfusion (IC). Other hearts received BZ during the first 10 min of reperfusion in absence or presence of L-NAME, NOS inhibitor. The infarct size (IS) and the post-ischemic recovery of myocardial function were measured. Oxidative/nitrosative damage were assessed by reduced glutathione (GSH) content, thiobarbituric acid reactive substances (TBARS) and 3-nitrotyrosine levels. The expression of phosphorylated forms of Akt, p38MAPK, eNOS and iNOS were also determined. BZ significantly decreased IS (6.2 ± 0.5% vs. 34 ± 4% in IC), improved post-ischemic contractility, preserved GSH levels (3.4 ± 0.4 vs. 2.0 ± 0.31 µg/mg prot) and diminished TBARS (0.49 ± 0.06 vs. 0.74 ± 0.04 nmol/mg prot) and 3-nitrotyrosine (84 ± 5% vs. 127 ± 4%). In IC hearts, P-Akt, P-p38MAPK and P-eNOS decreased (71± 1%, 26 ± 6% and 57 ± 2%, respectively) and iNOS increased (122 ± 1%). After BZ addition the levels of P-kinases and P-eNOS increased (132 ± 1%, 122 ± 1%, 118 ± 5% for P-Akt, P-p38MAPK and P-eNOS, respectively) and iNOS decreased (84 ± 2%). Except for P-Akt, P-p38MAPK and iNOS, the effects of BZ were abolished by L-NAME. Our data demonstrate that the treatment with BZ at the onset of reperfusion was effective to reduce cell death, contractile dysfunction and oxidative/nitrosative damage produced by coronary artery occlusion. These BZ-mediated beneficial actions appear mediated by eNOS/NO-dependent pathways.

398. (486) **MYOCARDIAL ISCHEMIA-REPERFUSION INDUCES INTERSUBUNIT CROSS-LINKING IN CALCIUM RELEASE CHANNEL (RYR2)**

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Introduction: Restoration of coronary flow after myocardial ischemia (I/R) although necessary, can lead to adverse effects such as ventricular arrhythmias attributed to increased RyR2 activity. Experiments from our laboratory showed that complete prevention of oxidative stress decreases the reperfusion arrhythmias. Since the selective inhibition of S-nitrosylation and S-glutathionylation, two oxidative modifications of RyR2, did not attenuate the ventricular arrhythmogenesis other oxidative RyR2 modifications should be involved in the generation of these rhythm alterations. Recently, the increase in the channel activity by redox-mediated formation of disulphide bonds between two RyR2 subunits (cross-linking, XL) was reported. Aim: To identify RyR2-XL of formation during the I/R protocol and its possible correlation with arrhythmic activity Methods: Langendorff perfused rat hearts were subjected to I/R (20/1-3min), in the presence or absence of a non-specific ROS scavenger, MPG (2mM). Epicardial monophasic action potentials, mechanical parameters and RyR2-XL (assessed by Western Blot) were examined. Results: Hearts submitted to I/R showed an increase in ventricular premature beats (VPB) 51 ± 4 with respect to non-ischemic hearts (Ctrl), 3 ± 2 (n = 5-6, p <0.05). At the onset of reperfusion an increase in RyR2-XL was detected (I/R 3.47 ± 0.40 vs. Ctrl 1.64 ± 0.35% of total RyR2, n = 6-8, p <0.05). This post-translational modification was prevented by MPG treatment (0.78 ± 0.49% of total RyR2) and occurred in association with a reduction in VPB: 24 ± 5 (n= 4 p<0.05). Conclusion: We found that RyR2 cross-linking occurred in the intact heart during I/R in coincidence with the outbreak of the arrhythmic activity. The treatment with MPG prevented RyR2-XL in association with a decrease in VPB. Our results suggest the participation of this oxidative change in the increased RyR2 activity, which contributes to the sarcoplasmic reticulum calcium mishandling associated with oxidative stress.

399. (631) **THE CHRONIC ACTIVATION OF THE G-COUPLED-ESTROGEN RECEPTOR (GPER) PREVENTS ISCHEMIA-REPERFUSION INJURY IN THE HEARTS OF OVARIECTOMIZED RATS.**

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During menopause women are exposed to an increase in cardiovascular risk, typically associated with lack of estrogens. However classic hormone replacement therapy (HRT) has not been as successful as expected in preventing such pathologies. It has been proved that many of the beneficial effects that estradiol exert on the cardiovascular system are mediated by GPER (G-coupled-estrogen receptor). Recently our group has described a greater damage in ovariectomized rat's hearts after an ischemia-reperfusion (IR) protocol compared to the control group. The hypothesis of the present work is that specific activation of GPER signaling would be beneficial for cardiovascular health, representing an alternative to HRT.
Bilateral ovariectomy was performed in 3-month-old female Wistar rats, which were randomly assigned to undergo a treatment during one month with either G1 (specific agonist of the GPER) (OVXG1) or vehicle (OVXVH). After the treatment, animals were sacrificed and their hearts used for IR protocols.
Weight gain (40.73± 5.88 g, n=6 vs 55.27±6.66 g; n=9) and the increase in left ventricle mass (55.94±19.08 mg, n=8 vs 124.93±33.33 mg; n=7) were milder in OVXG1. No differences were detected in systolic blood pressure. Chronic G1 administration prevented mechanic damage after the IR protocol in OVXG1 hearts (Left ventricle developed pressure: 57.98±12.75 % vs 10.28±2.65 %; Left ventricle diastolic end pressure: 55.75±3.99 mmHg vs 12.23±1.28 mmHg, n=5, p<0.05). Moreover, G1 reduced the infarct size (23.12±2.32* % vs 51.34±4.38 %; n=6; p<0.05). These results indicate that chronic GPER activation protects the heart from IR injury, and may represent an interesting alternative to prevent cardiac alterations during menopause.

400. (701) **CARDIAC ISCHEMIA/REPERFUSION (I/R) INJURY: RELATIVE ROLES OF SARCOPLASMIC RETICULUM (SR) CA2+ LEAK AND SR CA2+ REUPTAKE.**

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Although the factors contributing to I/R injury are complex, experimental evidence reveals that loss of Ca2+ homeostasis is one of the major contributing mechanisms. Previous experiments indicate that there are two main factors related to Ca2+ handling involved in I/R cardiac injury: SR Ca2+ leak due to phosphorylation of the ryanodine receptors (RyR2) by the Ca-calmodulin dependent protein kinase (CaMKII) at the onset of reperfusion; and the increased Ca2+ sequestration/load that involves CaMKII-dependent phosphorylation of phospholamban (PLN), which, when phosphorylated, increases SR Ca-ATPase activity and Ca2+ sequestration. In an attempt to dissect the relative roles of these factors on mitochondrial dysfunction during I/R, experiments were performed in wild type (WT) mice, double mutant mice (SDKO), with increased SR Ca2+ leak, due to constitutive pseudophosphorylation of RyR2 (aspartic acid replaces serine at RyR2–2814, the CaMKII site) and increased SR Ca2+ reuptake by PLN ablation, and double mutant mice (SAKO) with non-phosphorylatable CaMKII site at the RyR2, by replacement of Ser2814 site by Ala and PLN ablation. Mitochondria were isolated from hearts non-submitted and submitted to I/R (15/10 min). After isolation, mitochondrial membrane potential (ΔΨm) expressed in mV and measured by rhodamine-123 fluorescence quenching) and mitochondrial swelling (light scattering decrease (LSD, in au)), were evaluated. In mitochondria not submitted to I/R there was no differences in ΔΨm among the groups. The short protocol of I/R significantly increased mitochondrial depolarization in SDKO (-114.6±3.1) vs. WT (-137.4±1.4). ΔΨm returned to WT values in SAKO (-143.2±2.28). LSD produced by addition of Ca2+ was also significantly lower in SDKO (0.21±0.04) vs. WT (1.04±0.12) and SAKO (0.95±0.03) after I/R. The results indicated that the enhanced Ca2+ leak due to CaMKII-dependent phosphorylation of RyR2 is more important in determining mitochondrial dysfunction in I/R than the increase in SR Ca2+ reuptake by PLN ablation.

401. (735) **ROLE OF PROTEIN KINASE B (AKT) IN THE CARDIOPROTECTIVE EFFECTS EXERTED BY ORAL ADMINISTRATION OF STEVIOSIDE IN HEARTS SUBJECTED TO ISCHEMIA-REPERFUSION (I-R).**

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Stevia rebaudiana bertoni is an herbaceous plant widely distributed and used for its sweetener character. In previous studies carried out in our laboratory, we demonstrated that oral administration of stevioside (S), a mayor component of stevia, improved the recovery of contractile activity in hearts subjected to I-R and decreased the infarct size. These effects were, at least in part, reverted by the administration of Wortmannin (W), an inhibitor of Akt. The aim of the present study was to investigate the mitochondrial morphology and ATP synthesis capacity after oral administration of S (168 mg/kg for 15 days) and its relation with the activation profile of Akt and GSK3β, in Langendorff perfused rat hearts subjected to I-R. Hearts from female Wistar rats (200-250g) fed ad libitum were used. W (100nM) was added 15 min before I. Mitochondrial ultrastructure was analyzed by electron microscopy, the measurement of mitochondrial ATP synthesis was made by the luciferin-luciferase method and the activation profile of Akt and GSK3β were studied by western blot considering Akt-P/Akt-T and GSK3β-P/GSK3β-T, respectively. ANOVA, n=8/group. Results showed an increase in mitochondrial ATP synthesis rate of hearts treated with S that was partly canceled by the administration of W (C: 66.3±6.5, W: 59.5±6.1, S: 87.3±3.7*, S+W: 64.6±6.9 nmol/min/mg mitochondrial protein; *p<0.05 vs all groups) Likewise, electron micrographs showed better mitochondrial conservation in the group treated with S. Akt presented higher phosphorylation with S treatment (C: 1.26±0.19, W: 1.16±0.07, S: 1.80±0.16*, S+W: 0.86±0.16 AU; *p<0.05 vs all groups). Moreover, GSK3β phosphorylation was higher in the S group with respect to the control group, being partially reverted with W (C: 1.03±0.11, W: 1.04±0.15, S: 1.88±0.05*, S+W: 1.63±0.18 AU; *p<0.05 vs C and W). These results suggest that oral administration of S presents cardioprotective effects that could be partly mediated by Akt activation.

402. (764) **ERYTHROPOIETIN AND ROSUVASTATIN: COMPARATIVE ANALYSIS OF THEIR CARDIOPROTECTIVE ACTIONS IN HEARTS SUBJECT TO ISCHEMIA (IS)-REPERFUSION (RP)**

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In previous work carried out in our laboratory we could show that Akt was involved, at least in part, in the direct cardioprotective effects of rosuvastatin (R,3uM). The aim of the present work was to investigate whether the Erythropoietin (E,2mU/ml), an Akt activator, promoted the same response as that exerted by R in the presence or absence of wortmannin (W,100nM), a PI3K/Akt inhibitor.
Hearts from Wistar rats (200-250g) were subjected to 25 min of IS and 60 min of RP. E and W were added 10 and 15 min before global IS respectively. The contractility was evaluated by left ventricular developed pressure (LVDP), rate-pressure product (RPP), peak rate of contraction and peak rate of relaxation (±dP/dt) and left ventricular end-diastolic pressure (LVEDP). Tissue samples were taken to assess mitochondrial damage by electron microscopies and to evaluate infarct size. Mitochondria were isolated to evaluate the opening of mitochondrial permeability transition pore (MPTP) against different calcium concentrations. Infarct size was also measured by triphenyltetrazolium . ANOVA, (n=6/group).
E improved the postischemic recovery of contractility in the same way as R, although LVDP presented a greater increase than developed by R (5 min RP, RPP(%): C:7±2, W:10±3, R:24±6*, R+W:9±3, E:24±3*, E+W:11±3, *p<0,05 vs C,W, R+W,E+W; LVEDP (%): C:15±2,W:12±2,R: 3±0,2**, R+W:10±2, E:12±2@, E+W:21±3, @ p<0,05 vs C,W,R+W,E+W, **p<0,01 vs C,W, R+W,E,E+W). R and E delayed the opening of the MPTP to 300uM CaCl2, while the use of w caused the opening of the MPTP to 200uM CaCl2. Electron microscopy showed greater mitochondrial conservation in both groups, R and E. The analysis of infarct size in hearts treated with E did not show significant differences with R.
Our results suggest that the cardioprotection exerted by rosuvastatin could be partly mediated by Akt, although it remains to investigate the possible regulation exerted by E on GSK3β.

403. (716) **CAMKII AND OSMOLARITY ARE INVOLVED IN THE ARRHYTHMOGENIC EFFECT OF MOUSE CARDIOMYOCYTES EXPOSED TO EXTRACELLULAR HIGH GLUCOSE**

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The glycaemia is continuously fine-tuned according to our carbohydrate intake and consumption. However this regulation could be disrupted under certain metabolic conditions i.e. impaired glucose tolerance, metabolic syndrome and diabetes mellitus. Given that pre-diabetic and diabetic hearts present calcium (Ca2+) mishandling, we hypothesized that an acute increase in glycaemia causes Ca2+ handling abnormalities capable to trigger arrhythmias. The aim of the present work is to test our hypothesis and elucidate the intracellular pathways.
We used isolated mice cardiomyocytes loaded with Fura-2AM to evaluate intracellular Ca2+ handling by epifluorescence.
The change from normal glucose (NG) buffer (11 mM, 325.61mOsm) to high glucose (HG) buffer (25 mM, 339.26 mOsm) significantly increased Ca2+ transient (CaiT) amplitude (60.4±20.5%) and accelerated CaiT relaxation, although without reaching significant levels. These results were completely normalized with AIP, a highly specific CaMKII inhibitor. On the other hand, HG produced arrhytmogenic events that were significantly reverted by 48.2% with AIP. The same increase in osmolarity by manitol (13.9 mM, 339.26 mOsm), did not

modify CaiT amplitude or Ca2+ dynamics and induced arrhytmogenic events that also decreased with AIP, although in a significantly lower percentage (24.5%) than HG.
We conclude that acute administration of HG induces changes in Ca2+ handling and arrhythmic events that are dependent on CaMKII. We could not discard that the results observed were influenced, in part, by the osmotic effect of HG perfusion. We speculate that HG and hiperosmolarity per se induced arrhythmic effects by different intracellular pathways.

404. (723) **APOPTOSIS IN PRE-DIABETIC HEART IS ASSOCIATED WITH INCREASED SARCOPLASMIC RETICULUM (SR) CA2+ LEAK, SR-MITOCHONDRIA INTERPLAY AND ALTERED MFN-2 AND GRP75 EXPRESSION**
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We have previously reported that pre-diabetic heart presents Ca2+ mishandling, increased reactive oxygen species and CaMKII activity, swelling and depolarized mitochondria, enhanced proximity between sarcoplasmic reticulum (SR)-mitochondria and apoptosis. The communication (interplay) between SR and mitochondrion through Ca2+ ions is pivotal in either physiological or pathological situations. Several proteins are involved in this relationship and Ca2+ trafficking, like mitofusin 2 (Mfn-2), glucose regulated protein (GRP75) and voltage-dependent anion channel (VDAC).
We proposed that in pre-diabetic mice hearts SR Ca2+ leak and the level of expression of these proteins affect the relationship SR-mitochondria, favoring Ca2+ trafficking and apoptosis as part of a CaMKII-dependent pathway.
3H-Ryanodine (3HRy) binding assay and Mfn2, GRP75 and VDAC expression were measured in a pre-diabetic model induced by high fructose diet in WT, AC3I mice (which express a CaMKII inhibitor at heart level), and in S2814D knock-in mice hearts (which have the CaMKII phosphorylation site, Ser2814, mutated to Asp, mimicking a maximal and continuous phosphorylation of this site).
3HRy binding assay revealed that WT pre-diabetic mice hearts had higher Vmax, in ftmol/mg protein, 47.9±5.3, than control, 33.6±4.2 and pre-diabetic AC3I mice, 32.2±5.9. Moreover, the Vmax in pre-diabetic S2814D knock-in mice hearts did not differ (65.8±9.8) from pre-diabetic WT mice. Mfn2 expression was significantly increased in pre-diabetic mice, either WT (53.6±12.4%) or AC3I (91.1±21.9%). GRP75 increased in WT pre-diabetic mice (38.3±9.7%), whereas this effect was prevented in pre-diabetic AC3I mice. In contrast, VDAC expression was similar in all conditions and mice lines.
These results allow us to conclude that the increased SR Ca2+ leak is consequence of CaMKII activation and GRP75 and Mfn2 increased expression would be favoring SR-mitochondria proximity and Ca2+ traffic.

405. (744) **MITOCHONDRIAL PERMEABILITY TRANSITION PORE AND CALCIUM HANDLING IN DIABETIC HEARTS**
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Mitochondrial dysfunction underlies the causes of many cardiac diseases. The mitochondrial death pathway features the sequential loss of mitochondrial membrane potential (ΔΨm), accompanied by irreversible opening of the mitochondrial permeability transition pore (MPTP), release of reactive oxygen species and toxic proteins into the cytoplasm, and activation of caspases. The NHE1 Na+/H+ exchanger has been associated with cardiac disorders and was recently located in cardiomyocytes mitochondria. The specific NHE1 inhibitor, cariporide (HOE642), is cardioprotective through its effects on mitochondria and its beneficial effect in hearts subjected to I/R has been associated with attenuation of MPTP opening and reduction of apoptosis, reducing mitochondrial dysfunction. In addition, inhibition of mitochondrial NHE1 during ischemia delays the progression of ischemic injury by decreasing cell death. Recently, abnormal mitochondrial Ca2+ handling was demonstrated in the hearts from spontaneously hypertensive rats (SHR). SHR heart mitochondria displayed reduced Ca2+ retention capacity and lower ΔΨm compared to control hearts.
We aim to study the role of the MPTP in the cardiac dysfunction of diabetic hearts that leads to diabetic cardiomyopathy. Our results showed decreased swelling after Ca2+ addition in the mitochondria from the obese diabetic heart (ODH) that could be due to reduced Ca2+ uptake activity. In order to define this mechanism we studied the calcium retention capacity (CRC) exposing mitochondria to small Ca2+ pulses to test CRC before MPTP opening. Mitochondria from obese mice showed reduced CRC confirming the increased mitochondrial Ca2+ load. Finally, we studied the role of the NHE1 in the increased Ca2+ load from ODH mitochondria. Inhibiting NHE1 with HOE642 resulted on increased CRC in the ODH mitochondria, confirming the deleterious role of NHE1 on the mitochondrial Ca2+ overload.
Our results show that increased mitochondrial NHE1 expression and NHE1-prompt Ca2+ overload could be important factors for the development of diabetic cardiomyopathy.

406. (763) **NHE1 HYPERACTIVITY IN AN IN VITRO MODEL OF DIABETES**
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Diabetes mellitus (DM) is a disease of growing incidence worldwide and is a significant risk factor for cardiovascular disease. In the heart, intracellular pH (pHi) influences cardiac functionality and its dysregulation is related to various cardiac pathologies. For pHi regulation, several ion transporters intervene, among which is the Na + / H + exchanger (NHE1). Although NHE1 normally plays an important physiological role, aberrant regulation and overactivation of NHE1 contribute to heart disease, including acute ischemia reperfusion damage and cardiac hypertrophy. NHE1 hyperphosphorylation, exaggerated myocyte hypertrophy and apoptotic death was observed in human diabetic ischemic cardiomyopathy.
We aim to dissect the mechanism of NHE1 hyperactivity in diabetic heart using an in vitro model of diabetic cardiomyopathy. Rat myoblast H9C2 cells were treated with a medium that mimics diabetic patient's plasma, that is, increased free fatty acids (FFA) and high glucose (HG). Cells were cultured and treated with a low glucose (LG) medium for 24 hs and then changed to a HG and/or high palmitate (HP) medium. While NHE1 expression levels did not change in any of the conditions tested, NHE1 activity was increased in cells treated with HG, HP and HGHP, showing that both metabolites could be related to the increased NHE1 activity. Considering that NHE1 expression levels remained unchanged, maybe the mechanism involved could be NHE1 phosphorylation. Further studies using kinase inhibitors will help to probe this hyphotesis.

407. (791) **CARDIOTOXIC EFFECTS OF THE ANTINEOPLASIC DOXORUBICIN IN A MODEL OF METABOLIC SYNDROME: OXIDATIVE STRESS AND TRANSPORTERS EXPRESSION IN THE LEFT VENTRICLE**
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Doxorubicin (DOX) clinical use as chemotherapeutic agent is limited due to the development of cardiomyopathies and heart failure.
In the present study we aimed to examine whether Metabolic Syndrome-like conditions in rats, generated by administration of 10% fructose in the drinking water, modify the adverse effects induced by a single dose of DOX and whether the treatment modifies the expression of P-glycoprotein (P-gp) and organic cation/carnitine transporter OCT-N2 in the heart.

Male Sprague-Dawley rats receiving either tap water (C, control) or water with 10% fructose (F) during 8 weeks were treated with DOX (6 mg/kg, ip, md) or vehicle (V) (n=4/group). Three days after injection, echocardiographic monitoring was performed and then rats were sacrificed. Left ventricles were excised to measure oxidative stress markers and protein expression of P-gp and OCTN1/2/3 by western blot.
F increased TBARS tissue levels and decreased the activity of the antioxidant enzyme SOD ($p<0.01$). It is of note that DOX injection produced a further increase in TBARS tissue levels in F rats ($p<0.001$) whereas it had no effect in C animals. DOX decreased ejection fraction and fractional shortening in F rats ($p<0.05$) but had no effects in C animals. P-gp protein levels were significantly lower in F than in C animals ($p<0.05$). DOX did not modify P-gp expression in C animals but caused a decrease in the F group ($p<0.01$). On the other hand, OCT-N1/2/3 protein levels did not change with either F overload or the administration of DOX.
Therefore, it is suggested that a minor efflux of DOX due to a reduced expression of P-gp could contribute to the greater cardiotoxicity of DOX in F rats. Supported by CONICET (PIP 112-201201-00425).

ONCOLOGÍA / ONCOLOGY 6

408. (111) **MOLECULAR MECHANISMS OF ACTION IN TSH-MEDIATED REGULATION OF THYROID CANCER CELLS. ROLE OF PKC**

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The incidence of thyroid cancer has increased significantly within last decades in Argentina as in the rest of the world. Thyroid stimulating hormone (TSH) controls thyroid function by binding to TSH receptor (TSHR) coupled to G protein. TSH hyperactivation could be involved in thyroid diseases and cancer. PKC, a serine/threonine protein kinase, has been widely implicated in malignant transformation, cell survival, motility and invasion. Classical and novel PKC are activated by PLC activation vía tyrosine kinases and G protein-coupled receptors. Numerous studies established that PKC is overexpressed in human cancer and its expression correlates with tumor aggressiveness in prostate, lung and breast cancer. However, the role of PKC in thyroid cancer remains poorly studied. We here addressed the potential role of PKC in TSHR transduction pathways involved in cellular proliferation and tumorigenesis. Analysis of PKC expression in human thyroid cancer cell lines (2 papilar, 1 follicular and 3 anaplastic) revealed high protein and mRNA levels relative to 2 “normal” immortalized cell lines. Treatment of thyroid cancer cells with TSH induced an increase in cellular proliferation compared to untreated cells (Ct) ($p<0.05$ vs Ct) by cell titter blue assay (CTB). Treatment with pan-PKC inhibitors Staurosporine (St) and GF109203X (GF) resulted in a significant inhibition of TSH-mediated proliferation compared to control in every cell line studied by CTB ($p<0.05$ vs Ct). Also, treatment with St and GF diminished AKT and Erk activation in TSH-stimulated cells by western blot. Finally, PKCα-depleted cells by RNAi reduced significantly TSH-induced proliferation by CTB and anchorage-dependent colony formation. Our results show that PKCα is implicated in AKT and Erk phosphorylation by western blot. Our findings establish a potential role for PKC in the control of hormone-induced proliferation that can be explored as treatment to effectively eliminate thyroid cancer cells.

409. (126) **EFFECT OF LEVOGLUCOSENONE AND ITS DERIVATIVES IN THE MALIGNANT PROGRESSION OF HUMAN AND MURINE MAMMARY TUMOR CELLS**

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Breast cancer is a major public health problem being the second cause of cancer related death in developed countries. Conventional therapies (surgery, chemo and radiotherapy) seem to have reached a therapeutic efficacy plateau. In order to propose new therapeutic alternatives, we suggest an unexplored molecule: Levoglucosenone. Levoglucosenone results from pyrolytic treatment of microcrystalline cellulose or cellulose-containing materials, and it has been used as chiral building block for the synthesis of a wide variety of compounds with different biological activitiesNevertheless, its usefulness in oncology remains unexplored till nowadays.
Here, we have evaluated the effect of levoglucosenone (compound 1) and is derivatives (compounds 2, 3 and 4) on *in vitro* and *in vivo* processes related to malignant progression using human (MDA-MB-231) and murine (LM3) mammary tumor cell lines.
All compounds showed a strong antiproliferative effect, with an Inhibitory Concentration 50 (IC_{50}) between 9 and 16 μM, obtained by MTS assay. By flow cytometry we have demonstrated an increase in the Sub G0 cell cycle fraction, compatible with the presence of apoptotic cells. Next, we studied the effect of the compounds on adhesion, migration (wound healing), invasion (transwell), and metalloproteinases (MMPs) activity (zymography). Moreover, we performed an *in vivo* lung colonization assay using the LM3 murine model, syngeneic in BALB/c mice.
In MDA-MB-231 cells, compounds 1 and 2 reduced migratory potential while compounds 3 and 4 also reduced adhesive capacity and decreased MMP-9 secreted activity (*$p<0.05$, ANOVA test). In LM3 cells, compounds 2, 3 and 4 reduced MMP-9 activity but the compound 2 also impaired invasive potential (*$p<0.05$, ANOVA test) and it increased *in vivo* lung colonization (*$p<0.05$ Kruskal-Wallis test).
Based on our results, we believe that these compounds assayed could become in the future in an important alternative for breast cancer management.

410. (133) **IMMUNOSUPPRESSIVE MECHANISMS IN MURINE SPONTANEOUS LMM3 MAMMARY TUMOR**

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Different schedules of immunotherapy against cancer were developed including vaccines combined with antibodies against T reg or anti-myeloid-derived suppressor cells and, more recently, immune checkpoint inhibitors such as antibodies against cytotoxic T lymphocyte-antigen 4 (CTLA-4), programmed death receptor (PD-1) or programmed death-ligand (PDL-1). However, differently from experimental tumors induced by massive doses of chemicals, most tumors originated spontaneously in mice exhibit undetectable immunogenicity. This feature could be shared with most common human cancers and should be taken into account in order to improve immunotherapy strategies. The objective of this work was to study the immunosuppressive mechanisms through the growth of the murine spontaneous LMM3 mammary adenocarcinoma displaying undetectable immunogenicity. We also used the MC-C strongly immunogenic methylcholanthrene-induced fibrosarcoma, as a control. Cytokines production was evaluated by ELISA and cell populations by flow cytometry. LMM3 tumor cells showed a higher expression of PDL-1 compared to MC-C ($p<0$, 05). However, there are no significant differences in the expression of PDL-1 in splenic neutrophils and macrophages in LMM3 and MC-C tumor bearing mice, both in early and advanced stages of tumor growth. On the other hand, incipient and large LMM3 tumor exhibits a higher percentage of CD4+ and CD8+ splenic lymphocytes that express PD-1 ($p<0,05$) and CTLA-4 ($p<0,05$) than MC-C tumor. The presence of IL-10 ($p<0$, 05) and TGF-β ($p<0$, 05) in LMM3 lysates was significantly higher compared to MC-C lysates tumor. Also, the plasma levels of IL-10 ($p<0$, 05), TNF-α ($p<0,01$), and TGF-β ($p<0,05$) increase throughout LMM3 tumor growth. In summary, the undetectable immunogenicity associated with the spontaneous murine LMM3 tumor may be associated with the induction of immunosuppressive factors that would work together to prevent the initiation of an anti-tumor immune response.

411. (269) **SIGNALING NETWORK INVOLVED IN THE GPC3-INDUCED INHIBITION OF BREAST CANCER PROGRESSION**

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We showed that GPC3 overexpression in breast cancer cells prevents metastatic dissemination, as well as it inhibits canonical Wnt and Akt pathways, while non-canonical Wnt and p38MAPK cascades are activated. However, the hierarchical sequence through which GPC3 modulates these pathways has not been determined.
In this study, we aimed to investigate the mechanism involved in the GPC3 effect on breast tumor progression, focusing on Wnt pathway. We employed the murine mammary LM3 cancer cell line (ER -, PR -, GPC3 -), overexpressing GPC3.
We confirmed by cytoplasmic β-Catenin levels and its transcriptional activity, that GPC3 inhibits autocrine and paracrine canonical Wnt signaling. We demonstrated by qPCR microarrays that GPC3 can modulate Wnt pathway in a genomic way. Out of the 84 evaluated genes, only Wnt 5b (non-canonical) was upregulated, 66 genes were downregulated (several Fz), and the expression of 17 genes -including several Wnt factors- was not modified by GPC3 overexpression (3-fold change, $p \leq 0.05$). Our WB from conditioned media indicated that GPC3 is secreted, suggesting that it competes with Wnt factors and thus prevents their binding to Fz.
In the cross-talk studies, we demonstrated that the GPC3-induced inhibition of Akt is necessary for the non-canonical Wnt activation, and for the canonical inhibition, but it has no effect on p38MAPK. The p38MAPK activation was required for the non-canonical Wnt upregulation and for the canonical Wnt and Akt pathways inhibition. The canonical Wnt blocking was crucial for the Akt downregulation as well as for the p38MAPK and non-canonical Wnt pathways activation. Finally, the non-canonical Wnt activity regulated canonical Wnt and p38MAPK pathways, although it had no effect on Akt.
In conclusion, our data indicate that GPC3 is secreted and it operates through an intricate signaling network. From the balance of these interactions, the inhibition of breast metastatic spread induced by GPC3 emerges.

412. (291) **ISOLATION AND CHARACTERIZATION OF NOVEL MURINE MAMMARY CELL LINES WITH DIFFERENTIATED AGGRESSIVE PHENOTYPE**

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Breast cancer is the first cause of death from female cancer. The recurrence of the disease originated at the level of secondary organs, or metastasis, is responsible for 90% of deaths from cancer. The factors that endow these cells with metastatic functions are largely unknown. One of the limitations in the study of tumor cells with metastatic phenotypes is that cell lines maintained in culture lose this ability to invade and colonize tissues. On the other hand, it has been shown that reinjection of cells in animals can lead to their enrichment with aggressive phenotypes. The aim of this work was the isolation and characterization of different cell populations with differentiated metastatic capacities. Following inoculation of the F3II murine mammary carcinoma cell lines, we established cell populations in vitro, one from the primary tumor and another from a metastatic nodule, F3II TP and F3II NM cell lines respectively. To determine their aggressiveness, a series of additional characteristics were compared between these lines and F3II. The three lines showed variations in morphology in culture and a different doubling time, with F3II NM having the highest one. Moreover, F3II NM presented major adhesion capacity and lower clonogenic potential. This could be explained by the differential expression of cell adhesion molecules, such as integrins or cadherins analyzed by flow cytometry. In addition, the migration capacity was analyzed by transwell assay and the results showed differences in this process. Finally, we compared the behavior *in vivo* and we detected variations in tumor progression such as latency, frequency of ulceration, tumor growth and the presence of pulmonary nodules. All things considered, the establishment and characterization of these two new different cell lines with differentiated metastatic capacities will allow us to determine molecular differences involved in the metastatic process.

413. (326) **INHIBITION OF GLUCOSE METABOLISM POTENTIATES METFORMIN CYTOTOXICITY IN CANINE AND FELINE MELANOMA CELLS**

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Área de Investigación-Instituto de Oncología Ángel H Roffo

Cancer cells exacerbate glucose consumption by increasing not only glycolysis but also pentose phosphate pathway (PPP). Feline and canine melanomas are highly aggressive pathologies that have been proposed as an interesting model of human melanoma. The aim of the present work was to investigate the in vitro effects of a combination of metformin (MET, antidiabetic drug, OXPHOS inhibitor) with 2-deoxyglucose (2DG, glycolysis inhibitor) or 6-aminonicotinamide (6AN, PPP inhibitor) on two melanoma cell lines, Sc (canine) and Dc (feline) derived from spontaneous tumors. Sc and Dc were grown as monolayers at subconfluent cell density. After 24 h of culture, Dc and Sc monolayers were treated with 0.5 or 1 mM 2DG, 2.5 or 1 mM MET and 10 or 25 μM 6AN respectively, or a combination of them. Concentration-response curves were also assessed. The antitumor effects of bioenergetic inhibition were evaluated by the acidic phosphatase assay (APH) 5 days after treatments. We found that both cells lines significantly decreased cell viability ($p<0.05$) in a concentration dependent manner after 2DG and MET treatments whereas only Sc was significantly affected by 6AN. In addition, MET cytotoxic effect was significantly potentiated ($p<0.05$) by the combination of both 2DG and 6AN in both cells lines. We further analyzed the mechanism involved in the effectiveness of both combinations by means of specific fluorescent probes (Flow cytometry). We found that MET/2DG and MET/6AN increased intracellular oxidants (DCF, $p<0.05$) and acidic vesicles (NA, $p<0.05$) thus suggesting an autophagic process. The results reported here support further studies to investigate the potential use of this metabolic modulation approach in a clinical setting.

414. (346) **MECHANISM INVOLVED IN THE SYNERGISTIC EFFECT OF METFORMIN AND G6PDH INHIBITION**

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Most cancer cells display a strikingly different metabolism than normal cells. Cancer cells exacerbate not only glycolysis (known as the "Warburg effect") but also TCA cycle and PPP to enhance the malignant phenotype. Previously, we described a synergic cytotoxic effect on eight melanoma cell lines by using metformin (MET, an inhibitor of OXPHOS complex I and an AMPK´s indirect activator) in combination with 6-aminonicotinamide (6AN, an G6PDH inhibitor). The aim of the present work was to determinate the effect of this combination on metabolic parameters and to elucidate the mechanisms involved. We used as study model three melanoma cell lines, hM1 (BRAFV600E) and hM4 (BRAFV600R) established from tumor of IOAHR´s patients and A375 (BRAFV600E) a commercial cell line. As metabolic parameters we evaluate extracellular glucose and lactate. Melanoma cells treated with 6AN and MET/6AN showed a lower glucose consumption and lactate production than cells treated with MET or control cells. Also, we determined the reductive power which decreased in 6AN treated cells. To elucidate the mechanism involved, we measured the intracellular oxidants (DCF-DA probe) and mitochondrial membrane potential (TMRM and Mitotracker CMXRos probe). At short term MET/6AN displayed an increase of mitochondrial depolarization and intracellular oxidants. In contrast, long term survival cells exhibited mitochondrial hyperpolarization. Also, we could determinate a rise on intracellular complexity (SSC) on cells treated with the combination MET/6AN. On the other hand, we evaluated apoptosis and necrosis using two techniques Annexin

V-Propidium Iodide and Orange acridine-Ethidium bromide staining. The combination MET/6AN exhibited a strong rise on late apoptotic or necrotic events. Also, we found an increase on Sub G0 population although we did not detect a change on the cell cycle. Our results reported here support further studies to investigate the potential use of this metabolic modulation approach in a clinical setting.

415. (389) **EFFECT OF THYROID HORMONES ON THE ACTIONS OF ONCOLOGY DRUGS IN BREAST CANCER CELLS.**

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BIOMED-CONICET-UCA

Chemoresistance is a major cause of cancer treatment failure. Many breast cancer cells acquire multidrug resistance (MDR) by upregulating the level or activity of membrane protein such as Pgp, which enable the exclusion of cytotoxic substances from the intracellular environment. Previously we demonstrated that Thyroid Hormones (THs) modulate CYP3A4 expression and Doxorubicin chemosensitivity in T lymphoma cells. However, in breast cancer cells, little is known about these mechanisms that lead to tumor chemotherapy resistance and are crucial to assure the success of treatment. Bexarotene and Lapatinib are recommended for breast cancer treatment but thyroid disfunction, is recognized as an important side effect of such therapies, potentially manageable by TH administration. Being MDR1 the major protein involved in the efflux of cytotoxic agents, we reasoned that Bexarotene- and Lapatinib-induced MDR1 activity may act as an important regulator in breast cancer MDR and that THs could modulate these effects. To this end, we first demonstrated that triple negative breast cancer MDA-MB-231 cells display both TRβ and integrin avb3 dimer (TH membrane receptor). Also, we demonstrate that Bexarotene and Lapatinib inhibits MDA-MB-231 cells proliferation ($p<0,05$) and THs increased cell viability ($p<0.01$) and impaired the action of both drugs. On the other hand, Bexarotene and Lapatinib modulate MDR1 mRNA expression and protein levels. We also evaluate MDR1 activity and found that both bexarotene and lapatinib induce Rho123 exclusion, but TH treatment did not revert this effect. In conclusion, THs affect the action of oncology drugs by mechanisms to be studied, but that would not include the participation of MDRs.

416. (416) **ROLE OF THE PRO-APOPTOTIC FACTOR NOXA IN THE SENSITIVITY OF PATIENT-DERIVED GLIOMA STEM CELLS AGAINST CHEMOTHERAEPUTIC AGENTS**

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Glioblastoma multiforme is one of the most malignant types of central nervous system tumors. Despite advances in treatments it remains largely incurable. This recurrence is attributed to the presence of a highly resistant subpopulation of tumor cells named glioma stem cells. Defective or inefficient apoptosis is an acquired hallmark of cancer cells. Thus, a thorough understanding of apoptosis resistance mechanisms is imperative to unravel novel drug targets for the design of more effective therapies. The BH3-only proteins of the Bcl-2 family can trigger apoptosis by binding to the pro-survival members of this family and neutralizing their activity. This concept has prompted the development of small molecules capable of mimicking BH3-only proteins leading to apoptosis, and thus, sensitizing cancer cells to treatments. Herein, we exposed five patient-derived glioma stem cell lines to routinely used chemotherapeutic drugs (temozolomide, lomustine and vincristine) and BH-3 mimetics (ABT-263 and WEHI-539). Viability assays revealed that the combination of BH3 mimetics that target Bcl-xL with chemotherapeutic drugs led to a marked increase in cell death compared to that triggered by each drug alone. Notably, one cell line resulted particularly sensitive to these combination therapies and this sensitivity correlated with the expression of the BH3-only protein NOXA. ABT-263 not only increased the degree of cell death but also induced NOXA mRNA levels as judged by RT-qPCR analysis. Moreover, we observed that siRNA-mediated downregulation of NOXA protected glioma stem cells from BH3-mimetic-induced cell death. These results indicate that NOXA contributes to glioma stem cell apoptosis and that its expression could represent a predictive biomarker of sensitivity to Bcl-xL inhibitors. Therefore, a proposed strategy to combat brain tumors that express NOXA would consist of combining Bcl-xL inhibitors with agents that potentiate NOXA activity.

417. (544) **HUMAN CLEAR CELL RENAL CELL CARCINOMA: HLA-G EXPRESSION AND MICROVESSEL DENSITY ANALYSE.**

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Renal cell carcinomas (RCCs) are the third genitourinary malignancie, behind prostate and bladder carcinoma. The most aggressive and deadly subtype is the clear cell RCC (ccRCC). The habitual oncological treatments are radio and chemotherapy, but there is a high percentage of failure in this treatment. New strategies are being tested, like immune and angiogenesis therapy, but results are controversial. In this context, the HLA-G molecule appears as a new perspective of anti-tumor therapeutic strategy, since generates suppression and tolerance of the immune system and is expressed abnormally in some types of cancer as a mechanism of immune evasion. On the other hand, microvessel density (MVD), determined by immunohistochemical staining with CD34, is used as indicator of neoformation of sanguineous vessels. Our aim was to create a primary cell culture, using human samples from partial or radical nephrectomies to evaluate MVD and HLA-G expression. Methods: MVD was determined by CD34 staining in the tumor samples and HLA-G expression was determined by real time-PCR from cells grown from primary cell culture. Preliminary results showed that all tumor samples are HLA-G positive, but this expression is not homogeneous. Some samples expressed HLA-G only in the peripheral tumor area, others only in the central area, and others in both. No patient expressed HLA-G in the normal renal parenchyma surrounding the tumor. MVD index was observed to be higher in the peripheral tumoral area than in the central one. We can conclude that the intratumoral heterogeneity observed in the HLA-G expression as much as CD34 expression could be the reason for therapeutic failure. These knowledge, about ccRCC, is important not only for new target therapy but also to improve the diagnosis and prognosis of this important neoplasia.

418. (554) **FUNCTIONAL DIFFERENCES BETWEEN METASTASIC AND NON-METASTASIC OSTEOSARCOMA CELLS AND DIFFERENT POTENCIAL IN THEIR CAPACITY TO INDUCE DIFFERENTIATION**

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Osteosarcoma (OS) is the most common bone malignant tumor, affecting mainly children and young adults. Lung metastasis is a therapeutic challenge during osteosarcoma progression (15–30% survival rate with pulmonary metastasis at diagnosis). Niche establishment is critical for metastasis. Through proteomic analysis we demonstrated differential gene expression related to molecular function between metastasic OS (LM7) and non-metastasic (SAOS2) OS cell lines. Molecular differences were reflected in variations in the differentiation capacity in the two OS cell lines that differ in their

metastatic ability. Differentiation capacity was evaluated by Alizarin Red staining and absorbance (abs) measurement by spectrophotometry. We demonstrated that SAOS2 had higher differentiation capacity towards osteoblastic lineage than LM7 (0.09042±0.0096 abs vs 0.06937±0.0049 abs, respectively) suggesting that LM7 suffer a loss of differentiation potential in the process of gaining metastasic traits. We use conditioned medium (CM) of OS cells lines to evaluate their capacity to induce differentiation and SAOS2 CM increase the differentiation of metastasic and non-metastasic cells towards osteoblastic lineage (LM7: 0.1111±0.02136 abs vs 0.09189±0.01156 abs, SAOS2 CM and LM7 CM respectively), indicating that the paracrine effect of non-metastasic cells may account for calcification observed in lungs. When CM of OS cells were used in tube formation assay, LM7 induced higher morphogenic rearrangements in microendothelial cell (HMEC-1) monolayers, indicating that even though LM7 had a diminished ability to differentiate and to induce differentiation, they can induce microendothelial cell rearrangements, a step associated to the angiogenic cascade. Further proteomic analysis show an increase in calcium ion binding proteins in the CM of SAOS2 . All these points out not only a change of phenotype in metastasic OS cells, but also to a selective ability to induce differentiation in other cells, losing characteristics of the bone microenvironment but gaining traits that could favour the establishment of a suitable metastatic niche.

METABOLISMO Y NUTRICIÓN / METABOLISM AND NUTRITION 3

419. (277) **GLUTAMINE PROTECTS THE INTESTINAL CALCIUM ABSORPTION UNDER OXIDANT CONDITIONS PRESERVING THE MOLECULES OF BOTH TRANSCELLULAR AND PARACELLULAR CALCIUM PATHWAYS**

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Calcium (Ca^{2+}) is absorbed in the intestine through transcellular and paracellular pathways. Menadione (MEN) causes oxidative stress and apotosis in enterocytes, decreasing the intestinal Ca^{2+} absorption. Glutamine (GLN), one important nutrient of these cells, has antioxidant and antiapoptotic properties. Our aim was to study the ability of GLN to preserve the functionality of both pathways of intestinal Ca^{2+} absorption, which are decreased by MEN. We analyzed in chicks the effect of both drugs on intestinal Ca^{2+} absorption, in presence or absence of ruthenium red (RR, inhibitor of TRPV6, molecule of transcellular pathway), and gene/protein expression of components of both pathways by PCR and western blot, respectively. The data were analyzed by ANOVA and Tukey´s test; differences were considered significant at $p<0.05$. In control animals, 60% of Ca^{2+} is absorbed through the paracellular pathway and 40% through the transcellular pathway. MEN alone produced 45% reduction in Ca^{2+} absorption as compared to the control group, being the reduction higher with RR. The previous treatment with GLN prevented the blockage of transcellular pathway caused by MEN. GLN alone did not modify the intestinal Ca^{2+} absorption. MEN decreased gene and protein expression of Calbindin-D28K and PMCA1b, molecules of the transcellular pathway, and Claudin-2, molecule of the paracellular pathway. When GLN was administered prior to MEN, the expression of proteins was similar to that from the controls. In conclusion, both pathways are affected by oxidative stress generated by MEN. GLN preserves the activity and gene and protein expression of molecules involved in both transcellular and paracellular pathways, blocking the oxidative damage and apoptosis triggered by the quinone, thus preventing the decrease in the intestinal Ca^{2+} absorption.

420. (324) **CU OVERLOAD IMPAIRS PROTEOSTASIS IN THE ABSENCE OF ANTIOXIDANT RESPONSE IN BEAS-B AND HCT116 CELLS**

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Copper (Cu) overload is toxic for cells as observed in Wilson's disease. However, the events driving cell death due to Cu ions have not been yet elucidated. Whereas the intracellular accumulation of this metal has been reported to promote oxidative damage to biomolecules, antioxidant therapies have not been reported to be effective in the treatment of Cu imbalance-related disorders. Through gene set enrichment analysis (GSEA) we analyzed the biological processes which are activated or deactivated in the RNA microarray data collected from BEAS-2B and HCT116 cells exposed to 800 μM and 1400 μM Cu(II), respectively. Such concentrations result in nearly 50% cell death after 24 hours. Our gene expression kinetic indicates a strong heat shock response (HSR) and unfolded protein response (UPR) activation along with a slight intrinsic apoptosis signaling in response to the UPR. Notably, no activation of antioxidant response is observed. Cu overload leads to a strong deactivation of all processes related to the progression of the cell cycle, transcription, cytosolic translation, mitochondrial translation and mitochondrial respiration. In conclusion, Cu overload impairs proteostasis but the absence of a consistent antioxidant response suggests that the metal may promote protein misfolding independently of its pro-oxidant features. Interestingly, the strong deactivation of cell cycle progression, protein synthesis and mitochondrial respiration-related processes indicates that Cu-induced cell death may involve signaling pathways which may be exploited to develop new therapeutic approaches for Cu disorders.

421. (794) **METABOLIC AND ANTIOXIDANT PROPERTIES OF ERUCA VESICARIA EXTRACTS IN RATS**

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Oxidative stress generated by xenobiotics, bacteria, bile acids or oxidized food debris contributes to inflammatory diseases and cancer in the gastrointestinal tract. The intake of cruciferous such as Eruca vesicaria (E.v., commercial arugula), source of vitamin C, flavonoids and glucosinolates, has grown extensively in Argentina. The aim of the present study is to evaluate the oxidative balance and the lipid profile after chronic intake of E.v. fresh leaves extracts.
Adult male and female Sprague-Dawley rats (5 months old) were randomly separated for administration of either 2g/kg juice (Juice), 100mg/kg methanolic extract resuspended in water (ME)- richer in polyphenols and glucosinolates than Juice- or saline solution by gavage, 3 times/week, during 4 weeks. After euthanasia, serum triglycerides and cholesterol; epididymal or supraovaric adipocyte area; duodenal, jejunal, ileal, colonic and hepatic lipid peroxidation (TBARS) and antioxidant enzymes activity catalase and superoxide dismutase were measured. Statistical analyses were performed by ANOVA/Bonferroni (n=6/group).
Hypertriglyceridemia ($p<0,05$) and increased adipocyte areas in both sexes ($p<0,05$) were found in ME, not in Juice. Catalase increased in duodenum ($p <0,001$), jejunum ($p<0,05$) and liver ($p<0,01$)in Juice as well as in duodenum ($p<0,05$) and liver ($p<0,001$) of ME. Superoxide dismutase activity increased in duodenum ($p<0,01$), colon ($p<0,01$) and liver ($p<0,005$) in Juice and ME. There were not significant changes in serum cholesterol and TBARS in either group.
The present results suggest that a chronic intake of E.v. leaves could increase antioxidant defenses in gastrointestinal determined by flavonoids or glucosinolates present in the Juice or ME. Moreover, caution should be paid to the possible negative effect in lipidic profile with methanolic extract of E.v. Future studies will be necessary to determine these active compounds and mechanisms involved.

422. (30) **EFFECTS OF YERBA MATE (ILEX PARAGUARIENSIS) ON BONE AND OXIDATIVE STRESS PARAMETERS**

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Yerba mate infusion (YM) intake is very common in South America.

Higher bone mineral density (BMD) were found in rats and postmenopausal women who drank YM vs controls. An association was described between bone loss with age and oxidative stress. The aim of this study was to evaluate the effect of YM with different antioxidant capacity on bone tissue and oxidative stress parameters. 30-day Sprague Dawley rats were divided into 3 groups (n=9/group) which received ad libitum during 90 days: Control (water), YM (25 g/500 ml, 70°C), YM+ (YM 50 g/500 ml, 90°C). The highest antioxidant capacity of YM+ was verified through the percentage of inhibition of the radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) and the content of total polyphenols. After 90 days, the total BMD was performed on tibias by dual-energy X-ray absorptiometry (DXA, Hologic). The morphological analysis were performed on cross sections (500 µm in thickness) using a software (Image J 1.40 - NIH). In addition, oxidative stress parameters: glutathione peroxidase (GPx), catalase and lipoperoxidation were measure in plasma. Results: As expected, no difference in body weight, food and drink intake were observed. In addition, no differences in tibias length and cortical morphological parameters were found. BMD were significantly increased in both YM groups (YM= 10.1% and YM+= 9.1% vs control) without differences between them. The oxidative stress parameters were found decrease in YM groups vs controls, without differences between the YM groups: GPx (μmol.min-1.mg of prot-1): control= 0.014±0.001, YM= 0.011±0.001*, YM+=0.011±0.001* (ANOVA, *p<0.05 vs control); catalase (µmol H2O2 consumidos/min.mg prot): control= 0.30±0.25, YM= 0.15±0.08, YM+=0.12±0.05 (ANOVA, p=0.0592); lipoperoxidation (µM equivalentes 1,2 MDA-TBA): control= 42.70±16.42, YM= 21.07±11.77*, YM+=19.35±17.33* (ANOVA, *p<0.05 vs control). In conclusion, YM infusion showed a positive effect on BMD without differences between its antioxidant capacity. This effect could be due to low oxidative stress in YM groups.

423. (247) **IMPAIRED MITOCHONDRIAL FUNCTION CORRELATES WITH HEART, KIDNEY AND LUNG MORPHOLOGYCAL ALTERATIONS IN ACUTE COPPER OVERLOAD**

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Oxidative damage (OD) and mitochondrial bioenergetic dysfunction are associated to acute overload of copper(II) preceding rat death. The aim of this research is to evaluate if morphological damage in liver, heart, lung and kidney correlates with dose and mitochondrial damage. Sprague Dawley male rats (200 g) received Cu(II) at dose of 5.0; 6.5 and 7.5 mg/kg (ip) and were sacrificed 1h and 6h after treatment. The organs were excised and the samples were processed according to routine methods for obtaining histopathological preparations that were stained with hematoxylin-eosin. The congestion index (CI) was calculated for each organ and dose. Mitochondrial function (oxygen uptake, ΔO2) was evaluated using a Clark type oxygen electrode and mitochondria Complex I-III and II-III activity using spectrophotometric methods. Results indicated that organs presented different indexes of congestion. CI increased with dose, CI minor than 1 corresponds to dose 5 mg/kg; 0.2 to 1.2 (mild-moderate) to dose of 6.5 mg/kg; and CI higher than 2 (moderate to strong) to dose of 7.5 mg/kg (25% survival of rats). Activity of complex I-III and II-III decreased in heart (21%, p<0.001 and 13%, p<0.05) and kidney (23%, p<0.01 and 26%, p<0.01, with CI of 0.6, respectively); in liver only complex I-III activity decreased 16%, p<0.05. With CI greater than 1 (1-2 moderate) ΔO2 decreased with malate-glutamate as substrate mainly in heart (40-46%, CI: 0.6 and 2.2, p<0.01, respectively), and liver (41%, p<0.01) with CI of 2.0. With succinate as substrate, ΔO2 decreased in lung (43%, CI: 1.2 and 20% CI: 2.2, p<0.01) and liver (30-70%, p<0.001) with CI 0.2-2.0. Activity of complexes I-III and II-III decreased with increasing CI, in heart (28-22%, CI:0.6-2.2), kidney (27-36%,CI:0.3-2.6), p<0.001, and lung (32-48%, CI:1.2-2.2, p<0.01) respectively. These results indicate that impaired mitochondrial function correlates with the histological alterations and OD associated to acute Cu(II) overload.

424. (196) **VITAMIN K2 (VK2) SUPPLEMENTATION BLOCKS THE ANTIAPOPTOTIC EFFECT OF INTERFERON ALPHA 2B (IFN-A-2B) ON THE EARLY STAGES OF LIVER CANCER DEVELOPMENT IN RATS**

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VK2 holds anticancer properties. On the other hand, IFN-α-2b inhibits the development of altered (preneoplasic) hepatic foci (AHF) by apoptosis. Objective: to evaluate the effects of IFN-α-2b supplemented with VK2 on the onset of liver cancer development in rats. Methods and Results: Adult male Wistar rats were subjected to a 2-phase model of liver cancer. Rats received 2 ip doses of carcinogenic diethylnitrosamine (150 mg/kg) 2 weeks apart and, one week after, they received 20 mg/kg of the promotor 2-acetylaminofluorene by gavage 3 days/week for 3 weeks (IP group). Animals were divided into 4 groups: IP; IFN: IP rats that received IFN-α2b $6.5x10^5$U/kg ip 3 times/week/3 weeks; VK2: IP rats that received VK2 8 mg/kg ip 3 times/week/3 weeks; and IFN+VK2: IP rats which received both drugs. Animals were euthanized after treatments and livers were obtained. Caspase-3 activity in liver lysates was significantly increased in IFN group respect to IP group. VK2 had no effects on caspase-3 activity. Immunoblot analysis of p53 protein in nuclear fractions showed an increase (+75%*) in IFN group compared to IP group, whereas VK2 treatment reduced the levels of the protein (-54%*). Bcl-2 was evaluated by immunohistochemistry. An astonishing staining outside the AHF was observed in the IP group. By contrast, the IFN group presented less stained cells, distributed both inside and outside the AHF. VK2 IFN+VK2 groups presented a pattern similar to IP group, with more Bcl-2-positive cells within the AHF. Immunoblotting and immunohistochemistry showed no differences in the PCNA and cyclin D1 protein expression between all the studied groups. (*p<0.05vsIP). Conclusion: VK2 administration in the early stages of liver cancer development has no effect on cell proliferation or apoptosis. VK2 interferes in the signaling pathways of IFN-α-2b that leads to undesired final effects. This interaction might be mediated by the antiapoptotic protein Bcl-2.

425. (332) **SOLID PHASE ASSAYS TO DETERMINE THE BINDING TO PROTEINS OF SOME FOOD DYES CONTAINED IN COMERCIAL DRINKS OR IN BAKERY/PATISSERIE COLOURING ADDITIVES**

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Drinks, foods, candies, cosmetics and medicines are coloured with different artificial anionic sulfonated azo dyes. There is a long standing discussion concerning the toxicity of these dyes and their effects on food digestibility as well as if they are really edible and safe. After many worrying research reports, some countries banned certain dyes while they are still allowed in others. Simple methods to compare the protein binding capacity and optimal pH or concentration of different dyes would be a step forward to determine their binding constants/parameters and possible toxicity. Some dyes might bind proteins in the stomach at acid pH by electrostatic interaction of basic residues with the sulfonic groups while others might also bind proteins in the lower tract even at neutral pH through hydrophobic and/or non-ionic interactions. Dyes might also access the bloodstream during gastrointestinal pathologies. Thus, we implemented solid phase assays to quickly assess the effects of incubating milk proteins, BSA and bovine gamma-globulin directly with drinks and beverages including isotonic drinks. 1µl of serially diluted protein samples was spotted on different surfaces in an arrayed manner. Dye binding was imaged and densitometrically quantified. Commercial isotonic drinks coming at a pH of 2.6-2.9 were able to stain quickly, sensitively and linearly the proteins regardless of the many other additives or preservatives present in the drinks (sucrose, citrate, benzoate, salts, etc). Besides, dyes commercialized for cooking, bakery or patisserie as pastes or solutions were also able to stain proteins. Altogether, our solid-phase assays are a simple, promising way to compare the

binding of different food dyes dissolved in drinks or commercialized in pastes or solutions. The effects of pH, solvents, additives, dye concentration and protein types on dye-protein affinities/bonding can be compared as a step previous to the evaluation of dye toxicity by other analytical methods.

426. (250) **INTRACELLULAR REDOX HOMEOSTASIS IN LIVER AND BRAIN ASSOCIATED TO HISTOPATHOLOGICAL DAMAGE IN ACUTE COPPER OVERLOAD**

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Acute copper (Cu(II)) overload generates histopathological lesions (HL), oxidative damage (OD), mitochondrial dysfunction and antioxidant enzymes response to oxidative stress, mediated by the nuclear transcription factor (Nrf2-ARE) signaling pathway. The aim of this study is to evaluate if HL in liver and brain correlates with redox homeostasis, Cu organ content and dose. Sprague Dawley male rats (200 g) were sacrificed at 1h, 6h and 16h after received 5.0; 6.5 and 7.5 mg Cu(II)/kg (ip). The organs were excised and samples were processed for obtaining histopathological preparations that were stained with hematoxylin-eosin. The congestion index (CI) was calculated for each organ and dose. Superoxide dismutase activity (SOD1) was evaluated using spectrophotometric method. Nrf2 expression in cells (cytosol and nucleus) was determined by western blot. Results shows that congestion increased with dose, CI<1:very mild (5mg/kg); CI1-2:mild to moderate (6.5mg/kg), CI>2: moderate-strong (7.5mg/kg). In liver, CI increased from 0 to 2 with Cu content from 1.1 to 8.0 μg Cu/g organ, with no significant changes in SOD1 after 6h (5mg/kg); Nrf2 decreased 48% ($p<0.001$) in cytosol but increased 41%($p<0.001$) in nucleus, indicating nuclear translocation, with stimulated synthesis and activity of SOD1 (1.5 fold ($p<0.01$) at 16 h), without HL. In brain, SOD1 activity decreased 33% ($p<0.01$) to 46% ($p<0.0.001$), when CI and Cu content increase (CI:0-1; Cu:0.25 - 0.40μg/g, 1-6 h, dose 5.0-7.5mg/Kg); but SOD1 increased 3 fold ($p<0.05$) (16h, 5mg/kg). Nrf2 increased 100% ($p<0.01$) only in cytosol, indicating that SOD1 synthesis is not activated at 6h of Cu(II) load, when mild HL were found in brain. Cu(II) overload produces HL in a dose dependent manner, SOD1 protects liver from HL at lower dose, and activation of Nrf2 expression takes place earlier in liver than in brain. SOD1 activity and Nrf2 expression are not involved in the brain protection from HL.

427. (134) **SERUM HEPCIDIN LEVELS AND HFE GEN IN A GROUP OF MALE BLOOD DONORS WITH HIGH IRON INTAKE**

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Excess iron (Fe) intake in subjects carrying *HFE* genotypes C282Y, H63D and S65C may result in Fe overload. Hepcidin plays a major role in Fe homeostasis, although limited information is available on this biomarker. To study the relationship between serum hepcidin levels and *HFE*genotypes, 34 male blood donors (18-62 y) attending the Departamento de Hemoterapia, Hospital de Clínicas (UBA) (2017) were enrolled. Serum hepcidin (sHep) (DRG Hepcidin 25 (bioactive) HS ELISA Kit), serum ferritin (SF) (Advia Centaur Immunoassay) and transferrin saturation (TS%) (IRON2, Tina-quant Transferrin, Cobas) were measured in blood samples negative for infectious diseases and C-reactive protein (PCR-latex, Wiener lab). C282Y, H63D and S65C genotypes were studied in whole blood by DNA extraction (Accupred Genomics DNA Kits) and PCR-RFLP (Bcl-I, Hinf-I and Rsa-I). Daily Fe intake (FeI), nonhem FeI including Fe from flour enrichment (Ley 25630) (nonhem FeI), and hem Fe intake (hem FeI) were estimated by a "Food Consumption Frequency" questionnaire (ARGENFOODS and USDA Database). The mean values+/-SD (range) were: sHep (ng/mL): 34+/-21 (7-80); SF (ng/mL) 208+/-149 (46-701);TS (%):31.6+/-9.1 (20.8-59.1); FeI (mg/d): 24.1+/-9.0 (10.0-47.2); nonhem FeI: 22.0+/-8.3 (9.1-42.2); hem FeI (mg/d): 2.1+/-1.1 (0.5-5.0). No participant presented FeI lower than EAR (6 mg Fe/d), and one donor surpassed 45 mg Fe/d (UL) (NAS, 2001). sHep in WT vs. donors carrying *HFE* mutations was (ng/mL): 32.3+/-20.3 vs. 38+/-22.1 ($p=0.4554$). In 90.9% (10/11) of donors with genotypes associated with type I haemochromatosis, sHep values were between P2.5th and P97th (8.65 and 70.1 ng/ml); even more, one donor C282Y homozygote, showed sHep concentration over P97th. These results suggest no association between serum hepcidin levels and *HFE* mutations in carrier male blood donors in spite of high daily iron intake. Universidad de Buenos Aires, Programación Científica 2016, UBACyT 20720150100004BA

428. (468) **IRON INTAKE IN A GROUP OF MALE BLOOD DONORS FROM CABA. RELEVANCE IN RELATION TO HFE GENOTYPES.**

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Dietary iron (Fe) intake and bioavailability are determinants of Fe status in the general population; however, excess Fe intake can lead to impaired health status, particularly in men carrying mutations associated with the HFE gene. Although in Argentina, feeding habits are characterized by high bovine meat intake (55.5 kg/per capita/y, FAO 2013), wheat flour fortification with Fe is mandatory since 2002. To follow up dietary Fe intake (FeI), 206 male blood donors (18-65 y) attending Hospital de Clínicas, UBA were enrolled in 2012 (n=43), 2013 (n=48), 2014 (n=75) and 2017 (n=40). A "Food Consumption Frequency" questionnaire (ARGENFOODS and USDA Database) was administered. HFE genotypes C282Y, H63D and S65C associated to type I haemochromatosis were analyzed in blood samples (PCR-RFLP). FeI (mg/d) showed a significant increase from 2012 to 2013 (mean+/-SD): 17.9±6.6 vs 26.1±10.3 ($p<0.001$); the FeI values for 2013, 2014 and 2017 samples remained without significant difference: 26.1+/-10.3; 22.2+/-8.8 and 24.2+/-9.0 (ns). FeIf (mg/d) increased like FeI from 2012 to 2013: 7.5±3.6 vs 13.3±8.4 ($p<0.001$); from 2013 onwards, a decrease in FeIf was observed being significant different in 2017: 13.3+/-8.4 vs. 8.4+/-4.8 ($p<0.01$). Conversely, no significant differences were found in hem FeI values (mg/d) from 2012 to 2017: 2.2+/-1.4; 2.1+/-1.1; 2.0+/-1.3 and 2.1+/-1.2. HFE genotypes C282Y, H63D and S65C frequency in this group of male blood donors was 32.4%, being H63D/WT the most prevelant (20.9%). Therefore the decrease in nonhem FeIf, registered in the 2017 male blood donors group, could benefit clinically healthy individuals most likely unaware of any family history of Fe overload. Universidad de Buenos Aires, Programación Científica 2016, UBACyT 20720150100004BA

429. (781) **LOCAL ANAESTHESIA AND PORPHYRIA. IS TOTALCAINE FORTE A SAFE DRUG FOR PORPHYRIC PATIENTS?**

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Acute attacks of Porphyria may be precipitated by exogenous drugs including anaesthetics. There is limited information in the literature about the use of some dental medicines in the acute Porphyrias. The safety of local anaesthetic agents remains a controversial issue because experimental evidence reveals that some of them are porphyrogenic either in animal models (lidocaine) or cell culture (lidocaine,

prilocaine, bupivacaine), however clinical experience has shown that its use in patients with acute Porphyria had no notable adverse effects. Articaine (carticaine, methyl-4-methyl-3-(2-propylaminopropionamide)thiophene-2-carboxylate hydrochloride) is a relatively new local anaesthetic and still untried in acute Porphyria, although some reports established its safety and others classified this drug as unsafe. The aim was to investigate the effect of a commercial preparation, Totalcaina Forte (carticaine chlorhidrate:L-adrenaline, Bernabó Laboratories) on heme metabolism in CF1 mice. Animals received different doses (1, 5, 7 mg/kg, s.c.) and were sacrificed at different times (30 and 60 minutes) after injection. 5-Aminolevulinic acid synthetase (ALA-S), Porphobilinogen deaminase (PBG-D) and Heme oxygenase (HO) were measured in different tissues. No variations of ALA-S specific activity was observed with any of the doses and times assayed. PBG-D activity was unchanged for 1 or 5 mg/kg of Totalcaina, independently of the tissue; although a significant reduction of liver activity (35%, $p<0.05$) was produced for 7 mg/kg at 60 minutes. All the doses augmented liver HO activity (30-70%, $p<0.05$) indicating an alteration in redox status. This conclusion was supported by reduced glutathione (GSH) levels and Catalase activity determination. A 50% ($p<0.05$) reduction in GSH levels and a striking induction of Catalase (7 fold, $p<0,01$) were observed for the dose of 7 mg/kg, 60 minutes. These preliminary results reveals that Totalcaina Forte would be safe but at very low doses, otherwise further studies are needed.

430. (663) **METABOLIC SYNDROME: DEVELOPMENT OF A NEW MURINE MODEL**

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The metabolic syndrome (MetS) is a serious health problem characterized by insulin resistance, dyslipidemia and central obesity. There is a strong correlation between obesity and increased risk for development MetS. Animal models are very useful tools in biomedical investigation. Diet-induced obesity models are used to develop MetS models. CBi is an inbred mouse strain generated by crossing BALB/c, Rockland, NIH and Swiss mice in the Institute of Experimental Genetics, School of Medical Sciences, UNR. Our aim was to emulate a model of MetS in CBi mice. Five weeks old male mice were randomly fed with a high fat diet containing 40% kcal from bovine fat (HFD group)or standard diet (Control group) (n=8/group). At week 16, body weight (BW) gain, showed higher values in HFD (%; 97±32) respect to Controls (54±2)($P<0.005$). Even though the amount of food intake was similar between groups: Control (g/day/animal; 5.50±0.44) and HFD (5.08±0.57), HFD consumed more kcal (kcal/animal; 3271±497.7) than Control (1755±100.3)($P<0.0001$). Insulin resistance was evaluated by an insulin tolerance test, where HFD showed a worse response with a maximum glucose clearance (%; 22.06±13.84) respect to Control (33.09±21.18)($P<0.05$).Then, mice were euthanized and samples of serum, epididimal and retroperitoneal fat pads were taken. HFD showed hyperglycemia: HFD (mean±SD, mg/dl; 138±16.09) vs Control (89±17.59) ($P<0.0001$); hypercholesterolemia: HFD (165±34.51) vs Control (115.3±42.67) ($P<0.05$) and hypertriglyceridemia: HFD (81.89±19.07) vs Control (52.22±11.67) ($P<0.005$). The % of body weight of epididymal fat weight(HFD: 6.39±0.67 vs Control:2.01±0.54) ($P<0.0001$) and retroperitoneal fat weight (HFD: 1.16±0.23 vs Control: 0.39±0.24) ($P<0.05$) were higher in HFD and is taken as an index of central obesity. CBi mice fed with HFD diet developed different characteristics of MetS and constitutes a novel promising model for the study of the syndrome. The importance of this development resides in the fact that it mimics the establishment of the human pathology.

NANOMEDICINA / NANOMEDICINE

431. (44) **LIPOSOMES EFFECT ON SKIN PENETRATION OF ANTILEISHMANIA DRUGS**

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Cutaneous Leishmaniasis is an endemic disease that affects the skin. The adequate formulation of topical vehicles to treat skin diseases is particularly complex. A desirable formulation should enhance the accumulation of the active drugs in the target tissue (the skin), while avoiding that the drugs reach the systemic circulation in toxic amounts.

Liposomes of nanometric size enhance drug´s penetration into the skin, especially if ultraflexible. In order to evaluate the performance of different liposomes we have evaluated the transcutaneous penetration of three drugs chosen for their capacity to act against Leishmania and for their widely variable physicochemical properties: Amphotericin B, Imiquimod and Indole. We incorporated the drugs in fluid or ultra-flexible liposomes and compared the drug´s penetration to the penetration measured when the drugs were applied as solutions/suspensions in buffer.

We applied the formulations onto the surface of human skin ex vivo and incubated for 24 hs in Franz cells. We then quantified the amount of drug found in epidermis vs dermis vs receiving compartment and in skin slices 600 microns thick by UV-HPLC.

We followed the penetration of liposomal lipidic molecules by measuring the fluorescence intensity of a lipidic probe incorporated into the liposomes and applied to mice skin in vivo.

The lipidic component of the liposomes penetrated deeper through canyons than through clusters in the in vivo experiments. The presence of the drugs did not alter this behaviour.

Ultra-flexible liposomes produced enhancement of drug penetration into/through human skin in all cases in comparison with fluid liposomes without detergent, regardless of drug molecular weight. Suspensions of insoluble drugs in buffer provided for better penetration into the skin than drugs incorporated in liposomes, especially for the large MW drug AnfotericinB.

Our results indicate that liposomes can impede the transcutaneous penetration of molecules, in particular the small MW drug Indole.

432. (56) **DIFFERENT KINDS OF POLYMERIC NANOPARTICLES WITH APPLICATION IN ANTIBACTERIAL PHOTOTHERMAL THERAPY**

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Due to the high resistance to antibiotics developed by different bacteria, new methods are currently required for the treatment of infections generated by these pathogens. Among the alternative therapeutic modalities to the conventional ones, photothermal therapy (PTT) stands out. It is based on the absorption of radiation (e.g. NIR light) by molecules or materials and the subsequent generation of heat in the surrounding medium when transforming the incident energy. This phenomenon can be exploited to cause the death of the pathogen. (1) In this sense, with the rise of nanotechnology, different materials have been synthesized and characterized to be applied in PTT. In this work, we demonstrate how is possible to use nanoparticles based on conducting polymers (NP-PANI) generated by different methods (oxidative polymerization from monomer (2) and solvent displacement -nanoprecipitation–(3)) to decrease via PTT the viability of pathogenic bacteria such as *Pseudomonas aeruginosa* and *Staphylococcus aureus*, both responsible for a large number of nosocomial infections. The results show that in both cases more than 80% of the viability is reduced, regardless of the kind of nanoparticle, there being an antibacterial synergistic effect given by the contribution of the nanoparticulate conducting polymer and the action of the NIR light. The PTT phenomenon was corroborated by techniques such as fluorescence microscopy and DNA fragmentation. (1). References:1. S. Bongiovanni Abel *et al.* Biomed. Phys. Eng. Express 4, 2018, 045037.2. S. Bongiovanni Abel *et al.* Nano-

technology 25(49), 2014,3.S.BongiovanniAbel *et al.* Nanotechnology 29, 2018, 125604.

433. (95) **INTRAVENOUS ADMINISTRATION OF ANTI-INFLAMMATORY EXOSOMES FROM HUMAN MESENCHYMAL STEM CELLS SUPPRESS LIPOPOLYSACCHARIDE-INDUCED NEUROINFLAMMATION IN MICE**

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Neuroinflammation is an important component of many diseases of the brain. Recently there has been great interest in the therapeutic potential of the small extracellular vesicles defined as exosomes. Here we investigated the suppression of neuroinflammation by human mesenchymal stem cells (MSCs)-derived exosomes in a classic inflammatory murine model induced by intravenous administration of a low dose of LPS (2.5 mg/Kg). Human MSCs were cultured in a chemically defined protein-free medium for 48 h and $CD63^{+}CD81^{+}$ exosomes were isolated by anion exchange chromatography. Nanoparticle tracking analysis showed a single peak of exosomes (mode size 92.9±2.4 nm) without the presence of other types of vesicles. Exosomes demonstrated anti-inflammatory activity in-vitro by suppressing the up-regulation of IL-1β in LPS-stimulated human brain microvascular endothelial cells in a dose-dependent manner. As expected, pro-inflammatory cytokines were significantly increased in spleen at 2 h and in hippocampus at 6 h after injection of LPS into mice. In hippocampus, IL-1β was mainly expressed by microglia (CD11b+ cells). Intravenously administered exosomes (4-6x10^{9} exosomes/mouse) reduced the expression of IL-1β by 15.8% (p=0.006) and 37.5% (p=0.003) in spleen and hippocampus of LPS-treated mice, respectively. Moreover, exosomes supressed the up-regulation of the chemokine C-C motif ligand 2 (CCL2), an important mediator of inflammation, both in spleen (28.8%, p=0.008) and hippocampus (25.7%, p=0.0006). Exosomes pre-labeled with dye were found in microglia, indicating they had crossed the blood-brain barrier. In contrast, administration of dexamethasone (1.2 mg/Kg) decreased both IL-1β (30.5%, p=0.0002) and CCL2 (24.8%, p=0.007) in spleen but had no significant effect in the hippocampus of LPS-treated mice. The results demonstrated that intravenous administration of exosomes is more effective than dexamethasone in suppressing neuroinflammation induced by systemic administration of LPS. They therefore support previous indications that exosomes may be an effective therapy for any of the multiple causes of neuroinflammation.

434. (261) **ANTIBACTERIAL ACTIVITY OF NANOCOMPOSITE HYDROGELS BASED ON SILVER NANOPARTICLES AGAINST PSEUDOMONAS AERUGINOSA**

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Hydrogels are crosslinked polymers with high capacity to absorb aqueous solution and they are employed as polymeric matrix of nanocomposites. In this sense, these structures are of great interest on biomedical applications.
Antibacterial inactivation of Gram negative (-) bacterium such as the *Pseudomonas aeruginosa* was evaluated when they were treated with nanocomposite hydrogels (PNIPAM-NpAg or PNIPAM-co-6%APTMAC-NpAg). These nanocomposites were developed through a photochemical synthesis of silver nanoparticles (NPsAg) inside of the hydrogels matrixes: PNIPAM or PNIPAM-co-6%APTMAC. This antimicrobial action was studied by means of the diffusion method in agar, which was evidenced by the formation of inhibition halos and was confirmed through the viability determination by counting colony-forming units (CFU/mL) and the live/death assay. The size of the inhibition halos increases with the incubation time (24-120 h) suggesting an important antibacterial effect of both nanocomposite hydrogels. However, the antibacterial activity was in the following order: PNIPAM-co-6%APTMAC-NpAg>PNIPAM-NpAg depending on incubation time against this Gram-negative bacteria. From the results of viability and inhibition halos using as control hydrogels (PNIPAM or PNIPAM-co-6%APTMAC) at different incubation times there were not observed antibacterial action depending on time. The bacterial live/dead assay indicated that the bacterial cell membrane was compromised after treatments with both nanocomposite hydrogels, although PNIPAM-co-6%APTMAC caused a more marked membrane damage effect compared with PNIPAM-NPsAg. Addition, it was in concordance with the observed by the nanocomposite hydrogel PNIPAM-co-6%APTMAC-NpAg that triggers oxidative stress in bacteria. In conclusion, these results reveal that PNIPAM-co-6%APTMAC exhibited high and improved antimicrobial property against *P. aeruginosa* producing a significant decrease in cell viability triggering oxidative stress and cell death by cell lysis.

435. (359) **ALENDRONATE-LOADED NANOARCHAEOSOMES: THE RISE OF AN EFFICIENT FOAM CELLS KILLER.**

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Atherosclerosis is the main cause of most cardiovascular diseases where heart attack and stroke occur, involving a high death rate in the world. In atherosclerosis,plasma level of low-density lipoprotein (LDL) is increased and the lipoproteins infiltrate the artery wall exceeding the capacity for elimination.Activated endothelial cells recruit monocytes, and ultimately plaque macrophages magnify the inflammatory processupon phagocytosis of locally oxidized LDL and transformation into “foam cells”. Foam cells necrosis would be associated with the occurrence of life threatening vulnerable plaque destabilization. Hence, a targeted nano-drug delivery system capable of recognizing and eliminating foam cells in the atheromatous plaquemay constitute a useful anti-atheromatous theranostic tool. As a first approach, in this work we in-vitro screened the impact of scavenger receptor A1(SRA-1) targeted nanoarchaeosomes (NA) prepared from archaeolipids extracted from the archaea Halorubrumtebenquichense and loaded with the bisphosphonate alendronate (A), (ANA) on J774A.1. Upon structural characterization, the uptake and cytotoxicity of NA were determined on J774A.1 and on oxidizedLDL-induced foam cells. Additionally, the effects of NA on J774A.1 mitochondrial membrane potential and lysosomal structure were determined. We observed that NAwere more extensively captured both by J774A.1 and foam cells, than ordinary liposomes made of classical phospholipids. The internalization of NA was accompanied by lysosomal size enlargement and mitochondrial membrane potential interruption.Finally, ANA carrying small amounts (in the order of nanograms/ml) of alendronate efficiently eliminated both macrophages and foam cells. ANA thus, can be presented as the first step towards a foam cells-targeted nano-drug delivery system.

436. (461) **IN VIVO EVALUATION OF 99MTC-POLYMERIC MICELLES PROBES LOADED WITH 2-AMINO-7(8)-FLUOROPHENAZINE N5,N10-DIOXIDE AS A POTENTIAL TARGET & DELIVERY AGENT TOWARD BREAST CANCER TUMORS**

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Breast cancer is a solid tumor characterized by a high level of hypoxic areas which are difficult to treat. In order to these we have developed polymeric micelles (PMs) as a nanocarriers of bioreducible pro-drug 2-amino-7(8)-fluorophenazine N5,N10-dioxide (FNZ) which have the ability to detect and treat hypoxic tumoral tissues. To improve FNZ pharmaceutical properties, the encapsulation was studied using pristine polymeric micelles (PMs) and glycosylated derivatives as a strategy: (a) to increase the solubility, (b) to stabilize the aqueous formulation for intravenous application and (c) to have nanoscale sizes which are suitable for the enhanced permeability and retention (EPR) effect for cancer diagnosis and improved antitumor activity. On the other hand, in order to know the biodistribution

behavior of PMs/FNZ systems we prepared different radio-probes.
This work presents the physicochemical characterization and the *in vivo* evaluation of 99mTc-PMs/FNZ probes. The preliminary results indicate that all the systems under study (Free-FNZ, pristine PMs/FNZ and glycosylated PMs/FNZ) were stable in particle size and Z-potential after its re-suspension in ultrapure water. Finally, the *in vivo* studies using 99mTc-PMs/FNZ, revealed an enhanced circulation time and a good performance in models of mice bearing 4T1 tumor.

437. (499) **NANOSTRUCTURED ARCHAEOLIPID CARRIERS FOR THE TREATMENT OF INFLAMMATORY BOWEL DISEASES**

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Inflammatory bowel diseases (IBD) are chronic relapsing disorders of the gastrointestinal tract, charac¬terized by chronic inflammation and epithelial injury induced by the uncontrolled activation of the mucosal immune system. Dendritic cells and macrophages are key cells in the inflamed mucosa, which produce large amounts of pro-inflammatory cytokines. Overproduction of reactive oxygen species (ROS) is also involved in symptoms of IBD.
Herein, we have prepared nanostructured archaeolipid carriers (NAC) composed by a core of neutral archaeal lipids (NA) as antioxidants and a shell of polar archaeolipids (PA) as hydrolytic, oxidative, and enzymatic attack resistant lipids and carrying dexamethasone (D-NAC) for oral delivery.
The anti-inflammatory and anti-oxidant activities of D-NAC submitted to in vitro digestion were evaluated in a co-culture model of inflamed intestine consisting of human intestinal cell monolayers, Caco-2 cells, and human monocyte-derived macrophages THP-1 cells, stimulated with lipopolysaccharide (LPS) and compared with carriers without archaeal lipids (D-NLC).
D-NAC made of a core of a compritol and NA containing dexamethasone and stabilized by a shell of PA and Tween 80 (2; 2; 1.2; 3% w/w) were prepared by homogenization-ultrasonication. D-NAC showed mean size of 60 ± 9 nm and zeta potential of -32 ± 5 mV and no changes in mean size was observed after in vitro digestion. Lipolysis of NLC was relatively fast, being almost completely digested in 30 minutes. In contrast, the lipolysis was significative lower for NAC, up to 60 min the accumulative lipolytic percentage was only 25%. Digested D-NAC decreased the secretion of TNF-α and IL-8 and showed higher antioxidant activity than digested D-NLC in the co-culture model.
Thus, the ultra-small, highly negatively charge D-NAC resulted highly stable under gastrointestinal conditions, reduced the secretion of the pro-inflammatory cytokines, and showed high antioxidant activity. These D-NAC could be the most promising candidate for IBD treatments.

438. (501) **NANOARC: NANOVESICLES WITH ANTIOXIDANT AND PHOTOPROTECTIVE ACTIVITY AGAINST UVB DAMAGE ON KERATINOCYTES**

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The development of malignant skin tumors is a major problem in the field of dermatology. The mechanisms of photocarcinogenesis are mainly due to UV solar radiation, which alters the DNA structure, the cellular homeostasis and induces reactive oxygen species (ROS). The extreme halophilic archaebacteria produce a unique group of polar (PA) and neutral membrane archaeolipids (NA) that help them to survive in high UV radiation exposure environments where they live. PAs are composed of saturated isoprenoid chains linked by ether bonds to the glycerol carbons in the sn 2,3 position and are highly resistant to hydrolytic, oxidative and enzymatic attack. The NA are carotenoids with high antioxidant activity. The nanoformulation of both types of lipids could generate a product with photoprotective activity of topical application. In this work, we optimize the biomass production of halophilic archaea by a simple batch growth. Then we implemented an extraction process of PA and NA using bio-solvents and we used it to produce nanovesicles (NanoARC). The antioxidant and photoprotective capacity of NanoARC were then evaluated on keratinocyte irradiated with UVB light.
PA-NA extracts obtained with ecological solvents showed high DPPH scavenging ability with a Trolox Equivalent Antioxidant Capacity (TEAC) of 11 ± 5.6, that was superior to that reported for compounds such as a-tocopherol or ascorbic acid. Using PA-NA extract we prepared NanoARC (310 nm and -42 mV Z potential) that showed significant protection against the detrimental effects of cell destruction induced by UVB light (270 mJ/cm2) and ROS release measured by the carboxy-H 2 DCFDA dye. Besides, NanoARC inhibited UVB-induced apoptosis, as indicated by YO-PRO and propidium iodide staining.
The results suggested that the halophilic archaebacteria is a sustainable source of unique biomaterials that can be extracted by ecological solvents and formulated in nanovesicles that can be propose as potential new photoprotective agents

439. (515) **TOPICAL VACCINATION AGAINST INFLUENZA WITH NOVEL IMIQUIMOD ULTRADEFORMABLE NANOVESICLES**

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Development of needle and pain free noninvasive immunization procedures is a top priority for public health agencies. Ultradeformable archaeosomes (UDA) made with total polar archaeolipids, soybean phosphatidylcholine and sodium cholate topically applied can penetrate the SC and induce antigen specific IgG serum titers eliciting a humoral response. Imiquimod (IMQ), on the other hand, is a ligand of TLR 7 that induces the production of several pro-inflammatory cytokines and specific T-cell response. Our hypothesis is that combination of skin penetration and targeting of antigen presenting cells of UDA with immunomodulation activity of IMQ (UDA-IMQ) may offer a topical vaccination option to parenteral vaccination against influenza.
The viability of keratinocytes (HaCaT) and macrophages (J774 cells) upon 24 h incubation with free IMQ and UDA-IMQ (250 ± 94 nm, -26 ± 4 mV Z-potential, IMQ/phospholipid ratio of 62 μg/mg) showed that only free IMQ produces significant cytotoxicity in both cell types.
The release of cytokines, in vitro human skin penetration and the ability to stimulate immune responses when mixed with a seasonal influenza vaccine upon topically application on Balb/c mice of UDA-IMQ and free IMQ were measured. On J774 cells, both free IMQ and UDA-IMQ promoted the release of IL-6, but only UDA-IMQ generated TNF-α. On HaCaT cells, UDA-IMQ promoted more IL-6 release than free IMQ. Penetration studies showed that IMQ accumulation in human skin was 1.5 times higher when applied as UDA-IMQ than as free IMQ. Finally, topically applied on mice UDA-IMQ produced the same serum IgG response and isotype ratio IgG2a/IgG1 (≈1) as the subcutaneous vaccine, with a higher stimulation index and INF-γ levels by splenocytes.
UDA-IMQ was more efficient to achieve a cellular response compared to free IMQ, due in part to its greater accumulation in skin and induction of proinflammatory cytokines. AUD-IMQ can be a safer alternative to injections for vaccination

440. (516) **FIRST STEPS TOWARDS INHALABLE CURCUMIN: CURCUMIN-LOADED NANOARCHAEOSOMES**

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Curcumin (CUR) is a hydrophobic polyphenol with antibacterial properties. However, its low solubility in water hinders its intravenous administration, decreases its oral absorption and systemic bioavailability. In aqueous medium, archaeolipids auto-assemble into

nanovesicles called nanoarchaeosomes that, unlike liposomes, remain structurally stable when submitted to nebulization stress. The incorporation of CUR into ARC would allow the formation of a stable aqueous suspension of CUR, protecting its chemical structure and potentiating its antibacterial activity, thus allowing, for example, its sustained release onto bacterial biofilms located in the lungs. Formulations of CUR loaded in nanoarchaeosomes -AC (archaeolipids:tween80:CUR 1:0.4:0.04 w:w)- and in ordinary liposomes -LC (HSPC:cholesterol:CUR 1:0.33:0.04 w:w)- were prepared by the lipid film hydration method and sizes were homogenized by sonication, extrusion and filtration. The AC formulation had a final lipids:CUR ratio of 1:0.023 w:w whereas LC displayed a lower loading of CUR, of 1:0.003 w:w. The average diameter of AC nanovesicles, measured by dynamic light scattering, was 130 nm, with a polydispersity index (pdi) of 0.22 and a Zeta potential of -18 mV. For the LC formulation the size and pdi value were higher: 305 nm and 0.84, respectively; and lower Zeta potential of - 4 mV. By evaluating the changes in the absorption spectrum of free CUR vs CUR in nanovesicles, it was observed that *CUR was nearly eight folds more extensively partitioned within the lipid bilayer of nanoarchaeosomes than in liposomes*. Furthermore, cytotoxicity of both formulations was evaluated on the alveolar epithelium cell line A549 to determine ranges of safe concentrations for eukaryotic cells. Overall, the *AC formulation provided a stable colloidal aqueous suspension of CUR*, superior to the one obtained with liposomes in terms of size homogeneity and incorporation efficiency, key factors to achieve a successful nebulization of nanovesicles.

441. (531) **CHARACTERIZATION AND ANTIBIOFILM ACTIVITY OF LIPID NANOVESICLES CONTAINING ESSENTIAL OIL OF THYMUS VULGARIS**

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The clinical relevance of biofilms (matrix-enclosed communities of bacteria that are adherent to surfaces) is related to their wide occurrence together with their ability to cause relapsing infections characterized by chronic inflammation, tissue damage and the significant difficulties in their eradication. The antibiotic tolerance shown by bacteria in the biofilm has been attributed mainly to the matrix, that restricts the access of antibiotics by binding them to their components or inactivating them by enzymes. In that way, lipid nanovesicles containing antimicrobial agents (AMA) could penetrate the biofilm matrix and remain there releasing AMA in high doses.
We have recently shown that the presence of total polar archaeolipids of Halorubrum tebequichense (TPA) in lipid nanovesicles increased their chemical and colloidal stability. We hypothesized that the encapsulation of essential oil of Thymus vulgaris (AETv - a potent AMA that has low solubility in water and rapid decomposition) in archaeolipid nanovesicles would allow to obtain a stable aqueous suspension of AETv with efficient antibacterial and antibiofilm activity.
Archaeolipid nanovesicles (soy phosphatidylcholine: TPA: Tween 80 1:1:1.6 w:w) of 129 ± 23 nm, Z potential -7 ± 1.5 mV and nanospherical structure, containing 41.6 ± 14.6 mg/ml of AETv (Arc-AETv) were obtained by the lipid film hydration method. The encapsulation of AETv in Arc-AETv increased the solubility of AETv 20.8 times with regards to an aqueous suspension. After 150 days of storage, Arc-AETv maintained the concentration of AETv and was stable colloidally. Finally, Arc-AETv presented antibacterial activity on both planktonic S. aureus and biofilm at lower concentrations than Tween 80 1% v/v suspension of AETv and AETv in lipid nanovesicles lacking TPA (Lipo-AETv).
In conclusion, the incorporation of AETv in archeolipid nanovesicles favored obtaining a stable formulation with effective antibiofilm activity.

442. (532) **NOVEL NANOVESICLES INCREASED STABILITY AND ANTIOXIDANT ACTIVITY OF SUPEROXIDE DISMUTASE**

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Inflammatory bowel diseases (IBD), such as Crohn's disease or colitis are associated with an imbalance, comprising increased reactive oxygen species (ROS) and decreased antioxidant activity. Antioxidant enzymes, such as superoxide dismutase (SOD), play a key role in diminishing oxidative stress and have attracted considerable attention on treatment of ROS-related disorders. However, clinical applications of SOD are very limited due to its instability. On the other hand, we have previously demonstrated that nanovesicles prepared with archaeolipids (ARC) have greater resistance to hydrolytic attacks, oxidation and lipolysis, unlike vesicles prepared with phospholipids named liposomes (LIPO). Therefore, we propose ARC as a useful oral delivery system for SOD that can provide protection to proteolysis during the gastrointestinal transit. ARC loaded with SOD (ARC-SOD) were prepared by thin-film hydration followed by extrusion method. Nano-sized (240 ± 8.9 nm), monodisperse (polydispersity index, PdI: 0.237 ± 0.02) and negative Z-potential (-34.34 ± 1.39 mV) were obtained. Colloidal stability of ARC-SOD was measured during storage and after simulated digestion process. Antioxidant and anti-inflammatory activity on macrophages stimulated with H2O2 or lipopolysaccharides (LPS) for both digested and undigested vesicles, was determined by MTT assay and measuring the levels of proinflammatory cytokines, respectively. Intracellular ROS levels were identified using flow cytometry. Effectiveness of the ARC-SOD was compared with LIPO-SOD. ARC-SOD suppressed the levels of IL-6 and TNF-α and ROS production on macrophages stimulated LPS as compared with free SOD or loaded in ordinary LIPO. After in vitro digestion, size, PdI and the antioxidant activity of ARC-SOD was retained, while that of LIPO-SOD was lost. In conclusion, ARC-SOD conferred SOD with better stability and enhanced therapeutic potential, offering a promising oral delivery option in treatment of IBD.

443. (571) **DEVELOPMENT OF SIRNA IMMUNONANOVEHICLES FOR CANCER TREATMENT**

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Colorectal cancer (CRC) is a high incidence tumor. Patients with CRC are treated mainly with surgery, chemotherapy, radiotherapy, leading to radioquimioresistance of tumors. Consequently, multimodal treatment strategies have a greater probability of success because these therapies affect diverse targets simultaneously. Gene therapy, which involves the use of vectors to transfer genetic material, combined to radio or chemotherapy, is an example of this. Small interfering RNA (siRNA) is an efficient tool for gene silencing used to design gene therapies. Preliminary studies from our laboratory showed that Peroxirredoxin II (PrxII) enzyme, which is overexpressed in CRC, is involved in radioquimioresistance. Silencing the expression of this enzyme with siRNA produces a radioquimiosensitization and a decrease in cell growth of tumors in vivo. Also, it has been shown that CRC tumor cells overexpress, mostly, the epidermal growth factor receptor (EGFR), which has been associated with radio and chemoresistance, as well as being an indicator of poor prognosis; making it attractive for active targeting of these nanovehicles. The aim of this work is to generate nanoimmunovehicles, in particular immunoliposomes containing a siRNA against PrxII and directed by a VHH against the expression of EGFR, combining this strategy with oxaliplatin. siRNA-PrxII/PEI complexes were obtained, characterized and encapsulated in liposomes in a N/P 3 ratio. VHH against EGFR was synthesized and conjugated to maleimide residues contained in the lipid formulation. Immunoliposomes containing siRNAPrxII/PEI with VHH coupled to its surface generated greater inhibition of proliferation in a CRC line (LoVo) respect to immunoliposomes containing a control siRNA. Likewise, the co-administration of siRNAPrxII/PEI immunonanovehicles combined with oxaliplatin produced even greater inhibition of proliferation respect to the treatments without the addition of the antineoplastic drug; a fact that shows that combined therapies tend to be more efficient

than monotherapies.

444. (600) **OXIDATIVE DAMAGE AS A TOXICITY MECHANISM OF SILVER BIONANOPARTICLES IN HUMAN TROPHOBLASTS**

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Within the nanomaterials, the metal nanoparticles have gained a great popularity due to their potential antimicrobial activity. Silver nanoparticles (AgNPs) biosynthesized by metal-reducing culture supernatant of *Pseudomonas aeruginosa*, have demonstrated an important antibacterial activity. Recently we showed that AgNPs are cytotoxic for human trophoblasts. The aim of this study was to deepen the mechanism of toxicity of these nanoparticles in the human trophoblast.

HTR8/SVneo cell line was exposed for 6 and 24 h at different concentrations of AgNPs (0.3-1.5 pM). Cell viability, ROS production and endogenous defenses (glutathione content (GSH), catalase (CAT), superoxide dismutase (SOD) and glutathione s-transferase (GST) activities) were determined. Biomarkers of macromolecules oxidative damage were evaluated, for protein oxidation (AOPP method) and genotoxic damage (comet alkaline assay).

The exposure of HTR8/SVneo cells to AgNPs produced a decrease in cell viability (CI_{50} for 6h and 24 h were 1.21 pM and 0.81 pM respectively). All the AgNPs concentrations evaluated induced an increase in ROS production and GSH content. The antioxidant enzymes SOD and CAT increased the activity at the highest concentrations assayed (1.5 pM at 6 h and 0.75-1.5 pM at 24 h). While GST activity, a detoxifying enzyme, decreased after AgNPs treatment. Regarding oxidative damage to biomolecules an increase in protein oxidation and genotoxic damage were observed in cells exposed to AgNPs at the highest concentrations assayed. To elucidate whether oxidative stress is a toxicity mechanism triggered by AgNPs toxicity trophoblast cells were preincubated with the antioxidant NAC (N-acetylcysteine, 2 mM). The treatment with NAC reverted cell death, protein oxidation and genotoxic damage.

Therefore, the AgNPs biosynthesized by *P. aeruginosa* are cytotoxic to human trophoblast cells and oxidative imbalance would be the toxicity mechanism involved in cell death and macromolecule damage triggered by these nanoparticles.

445. (618) **APTAMER VEHICULIZATION FOR THERAPY AND MOLECULAR IMAGING**

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Aptamers are oligonucleotides that have the characteristic of recognizing a target with high affinity and specificity. They have significant advantages in terms of size, production and modification and they are a versatile and low-cost tool for the development of new biotechnological platforms, as well as the development of therapy and imaging agents. The biggest challenges are to increase blood circulation and to prevent the degradation by nucleases. Sgc8c is an aptamer that recognizes the PTK7 receptor, described as a tumor target that has been previously studied, as a molecular imaging probe. Therefore, the goal of this work was the vehiculization of Sgc8c using different nanosystems to improve their delivery, as well as their stability.

For this purpose, Sgc8c-(CH2)6-NH2 was firstly conjugated to NHS-Alexa647 fluorophore (Sgc8c-ALEXA) and its vehiculization was studied, using: (i) preformed pegylated liposomes (LPS), (ii) polymeric micelles (PMs) and (iii) hydroxypropyl-beta-cyclodextrin (HPβ-CD). The fluorescent probe was efficiently included in all the systems. The average particle size of loaded-systems was similar to that of the empty-systems. The Zeta-potential of the charged systems showed evidence that the probe would be included within the particles. After 24 hours, the 96% of LPS/probe was found in the release medium, so its effective release being manifested. The images obtained by TEM showed that the data fit in good agreement with DLS.

Finally, the pharmacokinetic studies included the administration of the free-probe and the nanosystem/probe. Secondary pharmacokinetic parameters quantifying aptamer exposure and residence time were obtained through non-compartmental analysis. Although observations showed high intra- and interindividual variability, aptamer exposure was significantly greater for LPS/probe. Data obtained hitherto, would suggest that the preformed pegylated liposomes would increase the residence time of Sgc8c-ALEXA.

446. (648) **POLYMERIC MICELLE ENCAPSULATING 2,9(10),16(17),23(24)-TETRAKIS[(6-METHYLPYRIDIN-2-YL)OXY]PHTHALOCYANINATOZINC(II) FOR ENHANCED PHOTODYNAMIC THERAPY**

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A major difficulty in photodynamic therapy is the poor solubility of the photosensitizer (PS) under physiological conditions which correlates with low bioavailability. PS aggregation leads to a decrease in the photodynamic efficiency and a more limited activity in vitro and in vivo. To improve the aqueous solubility and reduce the aggregation of 2,9(10),16(17),23(24)-tetrakis[(6-methylpyridin-2-yl)oxy]phthalocyaninatozinc(II) (PcZn), the encapsulation into the biocompatible T1107 poloxamine polymeric micelles was investigated. T1107 (10% wt/vol) were prepared by dissolving the required amount of copolymer in milli-Q water (pH 7–8) at 4°C followed by the equilibration of the system at 25° C. For the incorporation of PcZn into micelles, a 0.5 mM solution of PcZn in acetone was added dropwise to the different micellar systems (5 mL) with magnetic stirring and the samples were shaken for 48 h at 25°C. Morphological evaluation showed the formation of PcZn-loaded spherical micelles in the nanosize range. The UV–visible absorption spectra of PcZn was recorded in DMSO, water-DMSO 2% and polymeric micelle T1107 in the 500-800 nm range. The Q-band of PcZn-loaded micelles displayed a slight broadening as well as a bathochromic shift from 674 to 683 nm in comparison with that obtained in THF. Besides, the two small vibrational bands of PcZn in THF at 609 and 644 nm merged into one broad peak with a maximum at 644 nm. These results suggest that PcZn is present in an aggregated form in the micelle core. However, PcZn is less aggregated upon encapsulation in comparison with PcZn in water–DMSO 2%. The photostability of PcZn-loaded micelles was analyzed in water by measuring the decrease in the intensity of the Q-band under irradiation with a red light. PcZn-loaded micelles was photostable and it was more stable than that in water–DMSO 2%. The aqueous solubility of PcZn-loaded micelle was increased up to 35 times.

447. (717) **TRASTUZUMAB-CONJUGATED HOLLOW POROUS SILICA NANOPARTICLES FOR HER2+ TARGETED THERAPY IN BREAST CANCER**

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Conjugation of nanoparticles (NPs) with ligands of cancer specific tumor biomarkers is a potent therapeutic approach to treat cancer diseases with high efficacy. In order to optimize HER2+ targeted therapies in breast cancer; we developed and characterized Tras-

tuzumab-NPs conjugates. First we synthesized from a mold, hollow and porous silica nanoparticles (HPNPs) by sol-gel technique using tetraethylorthosilicate (TEOS) as a silicon precursor and as a guide for the porous structure Tris Amine and CTAC, which are inexpensive and have plenty commercial availability. HPNPs were analyzed by DLS and TEM obtaining spherical NPs of similar sizes (110±7.4 nm) with an internal diameter hollow of 97±5.2nm and pores of 2-5.4nm with narrow distribution. In addition, Z potential has a negative value and the FTIR showed a characteristic band spectrum for silica without interferences. Afterwards, the monoclonal antibody Trastuzumab (Tz) used in therapy for breast tumors HER2+ was adsorbed nonspecifically on the surface of the HPNPs, thereby obtaining a therapeutic nanotool. The binding was characterized by TEM and FTIR and remained stable for at least 30-40 days, even in the lyophilized formulation. The maximum adsorption condition to avoid aggregates was 0.2 mg Tz/mg HPNPs. Tz-HPNPs were generated in several mass ratios and analized their cytotoxic effect on BT-474 (HER2+) and MDA-MB231 (HER2-) breast cancer cell lines for 72 hours. We found that Tz-HPNPs produced a significant decrease on BT-474 viability, compared to the treatment with Tz as a single agent ($p < 0.001$), using a higher concentration than IC50 (0.19+0.09). The cytotoxic effect could be targeted towards HER2 since IgG-HPNPs (control) was not able to decrease the BT-474 viability. However, part of the concentrations evaluated showed some toxicity in the MDA-MB231 cell line. In conclusion, Tz-HPNPs are a promising strategy for the development of novel HER2-targeted therapeutic agents to improve Tz treatment response.

448. (757) **TPGS BASED 99MTC NANO RADIOPHARMACEUTICALS: POTENTIAL APPLICATION IN BREAST CANCER DIAGNOSIS**

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There is only one useful SPECT radiopharmaceutical (^{99m}Tc-sestamibi) for breast cancer diagnosis and follow up and its utility carries with some limitations. Our aim was to evaluate the performance of three micellar ^{99m}Tc nano radiopharmaceuticals (i-TPGS; ii- TPGS and Soluplus®; iii- TPGS and Soluplus® with glucose) as radioisotopic imaging probes. Radiolabeling was performed by direct method and a syngeneic orthotopic model of BALB/c mice injected with 4T1 cells, was used for scintigraphic diagnosis evaluation with a gamma camera. The uptakes visualized in the images were validated by biological distribution measuring radioactivity accumulation in organs of interesr by Solid Scintillation Counter. Nano radiopharmaceuticals sizes were measured by Dynamic Light Scattering at 25° and 37°C. TPGS and Soluplus® radiopharmaceutical was the only one that allowed scintigraphic visualization of the tumor. Nano radiopharmaceuticals sizes increased at 37°C for system ii and iii (more drastically for the latter). Biological distribution studies showed uptake values that backed up scintigraphic results for each radiopharmaceutical assayed. In conclusion, mixed system of TPGS and Soluplus® stands as a potential candidate to be used in breast cancer imaging diagnosis.

449. (766) **CONTROLLED RELEASE OF CIPROFLOXACIN FROM CHITOSAN-BIOGLASS COMPOSITE COATINGS OBTAINED BY ELECTROPHORETIC DEPOSITION (EPD)**

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Nowadays,great efforts are being made in regenerative medicine or bone implants area in order to develop materials that directly interact with microorganisms. Biopolymers, such as chitosan (Ch) are interesting to be use in the controlled release of drugs. Usually, polymeric coatings are associated with bioglass (BG) and other biomaterials which provide the coatings with specific properties. In adition Ciprofloxacine (CF) is an antibiotic commonly used in bone infections. BG-Ch-CF coatings can be considered as a drug delivery system where it is necessary to graduate the release rate of Ciprofloxacine The proposed aims are to optimize the manufacturing of composite coatings of Ch-BG-CF in one stage by Electrphoretic depsition (EPD) and to evaluate the antibacterial effects of ciprofloxacine.
The fabrication of composite coatings was performed on stainless steel AISI 316L using an aqueous suspension of components. Before EPD, the substrates were cleaned with acetone and the Ch-BG-CF suspension was stabilized ultrasonically, where the chitosan stabilizes the complex system electrostericaly. The quantification of ciprofloxacine incorporated in the coatings was performed by HPLC-UV. Antimicrobial standard tests were performed on Staphylococcus aureus.
The EPD process produced homogeneous composite coatings by applying 1.5 V for 2 min. An increased in the weight of the coating is observed with the deposition time, thus regulating the thickness of the coatings. However, a reduction in the EPD rate was observed afterward 300 sec. SEM images of coatings Ch-BG and Ch-BG-CF shown surface homogeneity. The release of CF was initially high, being sustained in a smaller amount for 4 weeks. The antimicrobial test after 10 days of immersion in PBS of the composite coatings, developed a zone of inhibition of 18.9 mm, confirming its bacteriostatic effect on *Staphylococcus aureus*

450. (165) **EXOSOMES AS NANOCARRIERS OF APOTRANSFERRIN FOR THERAPEUTIC USE**

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Exosomes are biological extracelullar vesicles with a diameter ranging between 20 and 100 nm which are fundamental for intercellular communication, RNA, DNA and protein transport. Exosome capacity for biologic information transfer makes them an attractive tool for therapeutic agent nano-delivery to specific target cells. In addition to their small diameter, the exosome bilipidic structure protects the cargo from degradation and immunological detection; allows them to overpass the blood brain barrier and increases cargo molecule stability, solubility and bioavailability.
Previous studies in our laboratory demonstrated the pro-myelinating and differentiating effect of apoTransferrin (aTf) in the central nervous system (CNS). The present work focuses on aTf nano-encapsulation for targeting to oligodendroglial cells in the CNS through intranasally administered endosomes in cuprizone- demyelinated mice.
We isolated exosomes from different sources; human plasma, mouse plasma, neuroblastoma cell line (N2a), oligodendroglioma cell line (OLN-93) and primary culture of astrocytes. We characterized exosomes by Western blot using typical exosome markers CD63, alix and Tsg101 to verify their purity and identity. To verify their structural integrity and average size, we performed scanning electron microscopy (SEM) and dynamic light scattering (DLS). We conclude that the nanoparticles isolated are exosomes and that their structure is not compromised, allowing us to proceed to the loading, tracking and targeting phase of this study.

GENÉTICA / GENETICS 1

451. (465) **CLINICAL, BIOCHEMICAL AND GENETIC CHARACTERIZATION OF ACUTE HEPATIC PORPHYRIAS IN A COHORT OF ARGENTINE PATIENTS**

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The Acute Hepatic Porphyrias (AHPs) are characterized by an acute neuroabdominal syndrome including both neuropsychiatric symp-

toms and neurodegenerative changes.
Acute Intermittent Porphyria (AIP) is an autosomal dominant disorder caused by a deficient activity of Porphobilinogen deaminase, producing a markedly increase in the urinary excretion of 5-aminolevulinic acid and porphobilinogen.
Variegate Porphyria (VP) is an autosomal dominant disorder associated to a deficiency of the penultimate enzyme of the heme biosynthetic pathway the Protoporphyrinogen oxidase. Patients with VP may present a broad spectrum of clinical manifestations characterized by cutaneous photosensitivity and neurological symptoms which can occur separately or together in affected individuals. Cutaneous photosensitivity is characterized by skin fragility, erosions, blisters, millia and pigmentary changes in sun exposed areas. Neurological symptoms are similar to those of AIP.
Two main hypotheses explain the pathogenesis of nervous system dysfunction: the ROS generation by autooxidation of 5-aminolevulinic acid accumulated in liver and in brain; and heme deficiency in liver and in neural tissues generating an oxidative status, a component of the neurodegenerative process.
We review results obtained from AIP and VP families studied at clinical, biochemical and molecular level at the CIPYP. The relationship between the porphyric attack and oxidative stress was also evaluated in AHP patients, to identify a marker of neurological dysfunction.
We studied 116 AIP families and 30 VP families (609 and 132 individuals respectively). Genotype/phenotype relation was studied. Oxidative stress parameters and plasma homocysteine levels were measured in 20 healthy volunteers, 22 AIP and 12 VP individuals.
Of the 354 carriers of an AIP mutation, 74.9% were women, 64.8% of them were symptomatic. In VP, 68.9% of the individuals carry a mutation from which 54% were
symptomatic; most of them had skin symptoms. No significant difference in oxidative stress parameters and homocysteine levels between the groups analyzed was found.

452. (473) **MDR1 POLYMORPHIMS AND ACUTE INTERMITTENT PORPHYRIA**

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Acute Intermittent Porphyria (AIP), a metabolic disease caused by an inherited deficiency of Porphobilinogen deaminase (PBG-D), is characterized by a neuroabdominal syndrome. The reduction of enzyme activity is not enough for the triggering of these symptoms and crisis may be precipitated by several factors, including therapeutic drugs. More than 50 polymorphisms in the multidrug resistance (MDR1) gene, that codes for drug transport P-gp protein, are of clinical importance, among them: exon 12 (c.1236C>T), 21 (c.2677G>T/A) and 26 (c.3435C>T) with high incidence in Caucasians. Several P-gp substrates are unsafe drugs for AIP patients. The aim was to evaluate the role of MDR1 in AIP triggering. Studied population: Control (N=60, no AIP) and AIP (n=34) that had clinical symptoms, biochemical alterations characteristic of this disease and the mutation in PBG-D gene was detected. Exons 26, 12 and 21 were genotyped by PCR-RFLP; haplotypes were performed with SNPStats program. The polymorphic T allele frequency was significantly elevated ($p<0.05$) in AIP group respect to control for exon 26 (0.57 vs 0.36) and exon 12 (0.54 vs 0.33) while in exon 21 a high frequency of A allele was observed (0,14 vs 0.03). Genotypic frequencies of polymorphic allele was also found in homozygosis in a high frequency in AIP group for exon 26: 32% (Control: 5%) and exon 12: 29% (Control: 8%). Haplotype study revealed a higher frequency of TTT in AIP respect to control ($p<0.05$). The elevated frequency of T allele observed in AIP patients could be indicating the involvement of MDR1 polymorphisms in AIP onset. Due to similar results previously observed in Porphyria Cutanea Tarda population, this conclusion could be extend to other Porphyrias in which manifestations are associated with pharmacological triggering factors. To further support this conclusion, it is necessary to study individuals with latent AIP.

453. (476) **ROLE OF GLUTATHIONE S-TRANSFERASE GENE POLYMORPHISMS IN THE ONSET OF PORPHYRIA CUTANEA TARDA IN HIV INFECTED INDIVIDUALS**

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A high association of Porphyria Cutanea Tarda (PCT) with HIV infection (15%) is found in Argentina. To date slight evidence exists about if triggering factors of PCT, a rare toxicogenetic disease, in HIV patients are related to the infection and/or the therapy. Glutathione S-transferase (GST) is a member of Phase II drug metabolizing system and also involved in the drug detoxification system. Genetic polymorphisms that alter the activity of GST may affect the level of hormones and xenobiotics. Some polymorphisms in this gene are of clinical importance, among them: GSTT1 null, GSTM1 null and GSTP1 (c.313A>G). The aim was to analyze if these polymorphisms are involved in the triggering of PCT in HIV patients. With this purpose a population of Control (no PCT, no HIV, n=30), HIV (no PCT, n=30), PCT (n=30) and PCT-HIV (n=30) were studied. Analysis of GSTT1 null and GSTM1 null polymorphisms was performed by multiplex PCR; this assay discriminate the null genotype (homozygous deletion, absence of band), of the heterozygous and homozygous presence of genotypes. The analysis of haplotypes was also done. The homozygous deletion frequencies for GSTT1 were: 8.3% (Control), 6.7% (HIV), 0% (PCT) and 14.3% (PCT-HIV). The null genotype frequencies for GSTM1 were: 41.7% (Control), 53.3% (HIV), 33.3% (PCT) and 32.1% (PCT-HIV). The frequency of the deletion in homozygosis of GSTM1 null showed an opposite result than that observed for GSTT1 null. The fact that GSTT1 null was in a higher frequency in PCT-HIV group ($p<0.05$) could indicate a possible involvement of this polymorphism in the onset of PCT in infected individuals. On the contrary, GSTM1 null would not be related with the onset of PCT by antiretroviral and/or other triggering factors.

454. (154) **NEW RESULTS ABOUT THE RELATIONSHIP BETWEEN CYP2C9 SNPS AND ACUTE INTERMITTENT PORPHYRIA MANIFESTATION**

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Alterations produced by polymorphisms would contribute to cause illness. So, the identification of variants in specific sequences of some gene is of very clinic importance. Porphyrias are a group of metabolic hereditary pathologies in which only the presence of the mutation in the gene codifying the deficient enzyme is not sufficient for porphyria manifestation. Stress, low calories intake and many porphyrinogenic drugs are known as triggering factors. So, genetic variants in xenobiotic metabolization enzymes have an essential role in symptomatology manifestation. It was suggested that SNPs in CYP2C9, that affect its activity, would play a role in Acute Intermittent Porphyria (AIP), the most frequent acute porphyria in our population. With the aim of verify this hypothesis we continued with the analysis of some reported SNPs in different exons: CYP2C9*3 (exon 7), CYP2C9*7 (exon 1) CYP2C9*6, CYP2C9*9 and CYP2C9*10 (exon 5) in a control population and in a group of AIP patients biochemically and genetically diagnosed at CIPYP. We started this study with the analysis of CYP2C9*3 in 30 healthy volunteers and 40 AIP patients, 11 symptomatic and 29 asymptomatic, and also the other SNPs mentioned above, in 10 samples selected from the same group of AIP. Molecular typing was performed by PCR and automatic sequencing. Considering the total population (70) the frequencies found for A/A and A/C genotypes were 0.95 and 0.05 respectively while C/C genotype was not present in Argentinean population. Surprisingly CYP2C9*3 was not present in AIP patient and neither SNPs in both exons 1 and 5, but we found others reported SNPs in introns 1, 6 and 7 which have no effect on the enzyme activ-

ity. According to our results, these genomic variants are not present in our population. We will continue analysing other regions of this CYP as well as SNPs described for CYP2C19.

455. (460) **SPECTRUM OF GENETIC VARIANTS ASSOCIATED WITH FAMILIAL HYPERCHOLESTEROLEMIA AND PHENOTYPE / GENOTYPE RELATIONSHIP IN CASES OF OUR POPULATION**

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Familial hypercholesterolemia (FH) in Argentina has a frequency of 1/217, is identified with a score (DLCN) 6-8¨probable¨, >8¨definitive¨. Is caused by mutations in genes: LDLR, APOB and PCSK9, or others like APOE. We present an update of the spectrum mutations in our population and a family with a non-conventional phenotype/genotype relationship.
We evaluated 100 index cases (IC) with NGS: 87 adults and 13 children; 8 were severe forms. Identifies variants were traced in 36 relatives. 47% of IC were carriers of variants; 83% in LDLR, 11% in APOB and 6% in APOE. When relatives were included the detection rate reaches 54%. The most common DLCN value was 7(17%) with 42% of variants; for DLCN values ≥8, 60% or more presented variants; while DLCN of 5-6 15%. We identified 40 different variants, 1 in PCSK9. 29% of variants were null allele type, 25% VUS, 9% indels and 4% large rearrangements. 8 variants were observed in more than one IC; the most frequent LDLR c.2043C>A 11.2%(6). Exons with most mutations; LDLR 14, 7 and 4, which represents 23% of the IC. 52.9% of cases were phenotype +/genotype + agreement; 39.7% phenotype +/genotype - and 1.5% phenotype -/genotype +, observed in two cases detected in the family cascade study, one of them carries two rare variants, the LDLR c.1895A>T VUS (observed in two other IC) and another in PCSK9 that could moderate the HF phenotype.
FH presents great heterogeneity in our population, the most common variant, c.2043C>A, was found in 11% of the IC, 25% of the variants lack functional studies and have an uncertain significance. The family cascade study favors the identification of carriers that increase the cost-benefit ratio of the genetic study, it is necessary to identify variants that can modulate the phenotypic expression and still apply little in our population.

456. (733) **NEW TECHNIQUES OF MOLECULAR BIOLOGY FOR THE DIAGNOSIS OF IODIDE ORGANIFICATION DEFECTS.**

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Iodide Organification defects (IOD) represent 10% of cases of congenital hypothyroidism (CH) being the main genes affected that of TPO and DUOX2. From eight patients with clinical and biochemical criteria suggestive with CH associated with IOD, TPO and DUOX2 genes were analyzed (Patients 1-8). In principle sequencing by the Sanger technique was carried out. In those cases in which a single mutation was identified in the TPO gene, NGS technique was used. A custom panel targeting 8 genes associated with dishormonogenesis (TPO, IYD, SLC26A4, TG, DUOX2, DUOXA2, TSHR, SLC5A5) has been designed in order to amplify all exons and exon-intron of the respective genes by multiplex PCR. Sequencing of these amplicon libraries was carried out by using the Miseq Ilumina platform. 4 novel mutations have been identied (two en TPO, one in TG and 1 in DUOX2). The heterozygous compound to the novel mutation c.2695delC, p.Q899Qfs*21 and the c.2895_2898delGTTC, p.S965Sfs*30 was identified in the DUOX2 gene in Patient 1. Sequencing analysis of TPO gene revealed the following inactivating mutations: c.1993C>T, p.R665W and c.2395G>A, p.E799K in Patient 2; c.1186_1187insGGCC, p.R396Rfs*77 and c.1682C>T, p.T561M in Patient 3; c.1496C>T, p.P499L and c.1682C>T, p.T561M in Patient 4, c.920A>C, p.N307T (novel) and c.1727C>A, p.A576E in Patient 5; c.1186_1187insGGCC, p.R396Rfs*77 and c.1727C>A, p.A576E in Patient 6. In addition to mutations identified in the TPO gene, the NGS revealed mutations in other thyroid genes. So, Patient 7 showed the novel TPO mutation: g.IVS16-2A>C and the IYD mutation: c.874C>T; p.R292C . Patient 8 carries the TPO mutations: c.920A>C, p.N307T (novel) and the c.1682C>T, p.T561M and a novel TG mutation: c.1804G>A, p.V602I. The use of new molecular biology techniques is a valuable tool for understanding the molecular pathophysiology and for the diagnosis and treatment of this type of thyroid defects.

457. (120) **CYTOCHROME P450 ISOENZYMES SINGLE NUCLEOTIDE POLYMORPHISMS (SNPS) ARE REALLY INVOLVED IN PORPHYRIA CUTANEA TARDA DEVELOPMENT ?**

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It was suggested a role for some CYP1A1 and CYP1A2 isoformes in PCT development but the results from different populations are controversial. We analised three polymorphisims, one in CYP1A2 and two in CYP1A1 in a group of Argentinean PCT patients and in a control group. One hundred and twelve PCT patients, 36 H-PCT and 76 A-PCT were analysed employing PCR-RFLP and each variant was compared with 89 controls. The Fisher exact test was used to detect differences in alelles and genotype frequencies, odds ratio and 95% confidential interval.For CYP1A2*1F polymorphism was the only case in which the wild type C allele was more frequent than the mutant A allele which has the highest transcriptional activity been the risk allele for PCT development according to Wickliffe et al (2011).For CYP1A1 the m4 polymorphism wich there are not report about the effect of the nucleotide or aminoacid change for enzyme activity our result show that's the A allele is a risk factor for porphyria triggering although the program Predict snp did not show any deleterious effect for these aminoacid change. For the m2 polymorphism were in accord with those obtained with Cascorbi et al (1996), for the G variant more active than wt variant being the risk factor when we compared H-PCT vs A-PCT. For m1 polymorphism did not show significative difference although previous report showed that C variant has the highest catalytic activity (Wei Liu et 2014).According snpSTATs program the risk haplotype for m4-m2-m1-1A2 polymorphism was C-G-C-C.

INMUNOLOGÍA / IMMUNOLOGY 4

458. (41) **MODULATION OF SPECIFIC TH22 RESPONSE BY MDR M. TUBERCULOSIS ISOLATED IN ARGENTINA**

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Tuberculosis (TB) is one of the main infectious disease affecting hu-

mans. Innate and adaptive immune responses are needed to control M. tuberculosis (Mtb) infection. Although TH1 T lymphocytes (LT) are essential to control Mtb growth, other LT, such as TH22 would participate in this response. Previously we demonstrated that the local multidrug-resistant (MDR) Mtb strains M and Ra differentially modulated TH1 and TH17 responses. So, the aim of this study was to explore the TH22 response induced by these strains.
Peripheral blood from 25 MDR-TB, 24 fully drug-susceptible (S-TB) patients and from healthy donors (HD) were evaluated. Epidemiological and bacteriological data of patients were collected. PBMCs were cultured alone or with M, Ra or H37Rv γ irradiated strains (2:1 Mtb to PBMC ratio) for 6 days. Then, cells were tested for CD4, CD8, IL-17 and IL-22 expression by FACS and IL-22 amounts were determined in cultured-supernatants by ELISA. IL-22 secretion from Mtb-stimulated PBMC was higher in MDR-TB and S-TB patients than HD ($p<0.05$) in coincidence with higher proportion of Mtb-expanded Th22 cells. S-TB patients showed the highest percentage of LTCD4+ and LTCD8+ expressing IL-22 ($p<0.05$). Similar trend was observed for LT cells co-expressing IL-22 and IL-17 ($p<0.05$) while the proportion of LT expressing IL-22 but not IL-17 was similar among groups. No differences were found in the modulation of TH22 response induced by the strains. Interestingly, in MDR-TB the lower percentage of LT expressing IL-22 the greater bacillary load and severity of lesions ($p<0.05$). As IL-22 is involved in the control of Mtb growth and tissue repair, the low proportion of ex vivo Mtb-expanded TH22 cells in MDR-TB could reflect a deficient host ability to reduce mycobacterial burden and the low effectiveness of the anti-TB treatment leading in severe tissue destruction with a prolonged bacillary load.

459. (53) **BRUCELLA INFECTION OF HUMAN ENDOMETRIAL STROMAL CELLS INDUCES A PROINFLAMMATORY RESPONSE**

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Brucellosis can induce abortion in humans and animals. Therefore, the interaction between uterine cells and Brucella is important for understanding the pathogenesis of this disease. Inflammatory phenomena have been shown to promote abortion by other pathogens. In this work, we evaluated the inflammatory response related to Brucella infection of a human endometrial stromal cell line (T-HESC). For this, T-HESC cells were decidualised in-vitro, infected with B. abortus 2308 or B. melitensis H38, or stimulated with conditioned medium (CM) from infected human macrophages, and changes in cytokines and chemokines were measured by ELISA. We demonstrate for the first time that B. abortus and B. melitensis can infect and proliferate in T-HESC. Expression levels of the decidualisation marker prolactin did not change in Brucella-infected T-HESC as compared to non-infected T-HESC (182±13 pg/ml for B. abortus, 176±14 pg/ml for B. melitensis vs. 192± 8 for non-infected cells). The infection induced an increase in interleukin8 (IL-8: 3233± 112 pg/ml for B. abortus, 3907±140 pg/ for ml B. melitensis vs. 1154± 188 pg/ml for non- infected), IL-6 (18 ±2 pg/ml, 17 ±2.3 pg/ml vs 0± 0 respectively) and monocyte chemoattractant protein 1 (MCP-1: 4022±298 pg/ml, 2253±42 pg/ml vs. 1375 ±106 pg/ml respectively) secretion at 48 h post- infection. Further, the stimulation of T-HESC for 48 h with CM from B. abortus-infected macrophages induced a significant increase of IL-8 and MCP-1 as compared to stimulation with CM from non-infected macrophages ($p<0.01$). These results suggest that human endometrial stromal cells may provide a local inflammatory environment during Brucella infection both directly and through interactions with infected phagocytes, potentially contributing to the pregnancy complications of brucellosis.

460. (304) **CD4+ T CELLS INDUCE PLATELET ACTIVATION THROUGH THE RELEASE OF EXTRACELLULAR VESICLES**

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Atherosclerosis is an inflammatory and thrombotic disease. Both platelets and lymphocytes play important roles in atheroma plaque development. However, knowledge on the interaction between these cells and its consequences is limited. Extracellular Vesicles (EVs) have emerged as a novel intercellular communication system. By carrying bioactive lipids, miRNAs and proteins they can modulate target cell functions. EV secretion is increased in diverse pathologies such as cancer, infections and atherosclerosis. Also, previous results from our laboratory have shown that activated CD4+ T cells (Th1) produce more vesicles compared to resting T cells. Therefore, in this work, we studied platelet-lymphocyte interaction through EVs. First, we characterized EVs secreted by activated CD4+ T cells by detection of canonical EV markers CD63 and CD81 by immunoblot. The EV-excluded endoplasmic reticulum (ER) marker calnexin was, as expected, present in cell lysates but not in EVs. EV visualization by electron microscopy revealed that EVs secreted by CD4+ T cells had a spherical shape and a diameter that ranged from 50 to 200 nm. Incubation of purified EVs (ultracentrifugation) with human washed platelets induced platelet hemostatic responses such as αIIbβ3 integrin activation, platelet aggregation and degranulation (CD63 exposure) in an extracellular calcium-dependent manner. Furthermore, CD4+ T cell derived EVs also induced P-selectin exposure, suggesting a pro-inflammatory role for this interaction. We also tested EVs purified from plasma and from the human mammary carcinoma cell line MCF-7 and did not observe platelet activation, suggesting a specific effect for CD4+ T cell derived EVs. In summary, we describe an alternative mechanism of leukocyte-platelet cross-talk mediated by EVs that is of potential relevance for the earliest aspects of inflammation and hemostatic cell responses. Alternatively, this process may also contribute to the exacerbation of leukocyte activation, and intercellular adhesion and migration during the initial phases of vascular injury and the atherosclerotic disease.

461. (377) **POTENTIAL OF BACULOVIRUS AS EXPRESSION AND DELIVERY SYSTEM OF HETEROLOGOUS ANTIGENS OF TRYPANOSOMA CRUZI IN IMMUNIZATION ASSAYS**

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Trypanosoma cruzi, the etiological agent of Chagas disease, has intracellular (amastigote) and extracellular (trypomastigote) stages in the vertebrate host. TcTASV is a multigenic family unique to *T. cruzi* present in all strains of the parasite analyzed so far and expressed in parasite stages infecting the mammalian host. Subfamilies TcTASV-A and TcTASV-C are the most numerous and have differential expression: TcTASV-A is expressed intracellularly in amastigotes/ trypomastigotes while TcTASV-C is expressed at trypomastigote surface and secreted (Garcia et al, 2010; Bernabó et al, 2013; Caeiro et al, 2018). A previous prime-boost vaccination assay with TcTASV-C (DNA/protein) delayed the time of appearance of bloodstream trypomastigotes and partially improved the infection outcome in challenged mice, which was mediated by a humoral response directed to the TcTASV protein motif (Caeiro et al, 2018). We hypothesize that the efficacy of the vaccination protocol could be improved summing a cellular immune response against an intracellular antigen, like TcTASV-A. The baculovirus (BV) system is a promising platform for vaccine delivery that elicit potent cytotoxic immune response when antigens are displayed at the capsid. Hence, we engineered a recombinant baculovirus expressing TcTASV-A fused to the major nucleocapsid protein VP39 (BV-TcTASV-A). Expression of VP39-TcTASV-A was confirmed by Western blot and immunofluorescence. C3H/He mice were immunized by a first dose of rTcTASV-C (25μg) adjuvanted with aluminium hydroxide, followed by a boost with BV-TcTASV-A (1.10^7 PFU) plus rTcTASV-C (25μg) 21 days lat-

er. Mice immunized with TcTASVs elicited a strong anti-TcTASV-C humoral response (title>1/204800). Intracytoplasmic cytokines measured by flow cytometry evidenced differential CD8+/IFNγ+ and CD4+/IFNγ+ populations after stimulation with TcTASV-A (5,2%) and TcTASV-C (0.7%), respectively (p<0.001 vs control group). We conclude that this immunization scheme elicited an accurate cellular response to the intracellular TcTASV-A while maintaining a robust humoral response to the surface/secreted TcTASV-C that could hopefully improve protection against *T. cruzi*.

462. (391) **CONDITIONING OF MYELOID REGULATORY CELLS BY THE DECIDUALIZATION PROCESS**

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The decidualization process involves phenotypic and functional changes on endometrial cells and modulation of mediators with immunoregulatory properties that may condition maternal leukocytes, as dendritic cells (DC) and macrophages, to a regulatory fate. In fact, Myeloid regulatory cells (Mregs) have recently been focus of attention since their presence at the materno-fetal interface and their potential role as immune-suppressors. Here, we focus on the impact of decidualization process in conditioning CD14+ cells to Mregs subsets. For this, we collected conditioned media (CM) of Human endometrial stromal cell line (HESC) decidualized with MPA+dbcAMP for 8 days (Dec-CM) or not (Non-dec CM). Then, isolated monocytes from peripheral blood mononuclear cells from healthy donors were cultured with rhGM-CSF+rhIL-4 for 5 days in the absence/presence of CM. We observed that Dec-CM was able to inhibit CD14+ cells differentiation to immature DC, in a concentration-dependent manner, evidenced by the persistence of high-proportions of CD14+CD1a- cells after 2 and 5 days of culture (p<0.05, Friedman test with Dunn's post-test). Moreover, in the presence of Non-Dec and Dec-CM, monocyte-derived cells displayed a higher HLA-DR expression and a lower expression of CD86 (p<0.05, Friedman test) compared to unconditioned cells. Interestingly, we found that the presence of Dec-CM induced a higher IL-10 production and also promoted HLA-G expression on monocyte-derived cells (p<0.01, Friedman test); both markers of tolerogenic DC-10 subset. In fact, when Dec-CM conditioned cells were challenged with LPS (0,2µg/ml, 16h), it was prevented the increase of CD83 expression, the production of IL-12p70 and TNF-α while induced an even higher IL-10 production (p<0.05 *vs* LPS-treated unconditioned cells, Friedman test). These results show that Dec-CM promotes a tolerogenic semi-mature profile on monocyte-derived cells and suggest the induction of different subsets of Mregs during the decidualization process, whose may play a key role in embryo implantation and pregnancy maintenance.

463. (418) **CD32 LIGATION DECREASED THE THRESHOLD FOR CD4+ T CELL ACTIVATION**

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Low affinity receptors for the Fc portion of IgG (FcγRs) represent a critical link between innate and adaptive immunity. The expression and function of FcγRs in myeloid cells, NK and B-cells have been well characterized. However, whether T-cells express FcγRs and if so, what function might exert, is still unknown.We demonstrated that 2.4%±0.4 of resting CD4+ T-cells express CD32 (FcγRII) on the surface (n=18) and 8.5%±1.9 was present also in the intracellular compartment (n=9), revealing the existence of a cytoplasmic pool.
Activation of CD4+ T-cells induced a higher expression of CD32 either in the cell surface (0.87%±0.2 vs 10.6%±1.8 unstimulated vs activated cells, respectively; p<0.0001, n=7) or intracellularly (63.7%±11.7, n=7).
We also observed that CD32+CD4+ T-cells were capable of bind aggregated IgG (aIgG) in a dose dependent manner, mimicking the immune complexes, the natural ligands of FcγRs. Importantly, this effect was abrogated when cells were preincubated with a blocking CD32 antibody IV.3 (14.1%±2.2 vs 3.4%±0.6, for aIgG and IV.3 plus aIgG, respectively, p<0.01, n=8).Purified CD4+ T-cells cultured onto IgG-coated (cIgG) plates or stimulated by specific CD32 cross-linking increased the proliferative response of PHA-stimulated CD4+ T-cells (11.8%±2.4, 15.8%±4.7 and 3.7%±0.9, respectively, p<0.01 and p<0.05; n=10) compared with non-pre-treated PHA-stimulated CD4+ T-cells (xxx).These stimuli also promoted the release of a wide pattern of cytokines including IL-2 (p<0.001), IL-5 (p<0.001), IL-10 (p<0.0001), IFN-γ (p<0.05) and TNF-α (p<0.001) compared with control cells (n=10).Blocking CD4+ T-cells with CD32 neutralizing antibody previous to the cIgG stimulation diminished both, the proliferation rate as wells as the cytokine production at levels similar to the control cells, showing that the effect observed is, at least in part, due to CD32 specific activation.Collectively, our results suggest that CD32 exerts a stimulatory action on CD4+ T-cells function by decreasing the threshold needed for cell activation.

464. (475) **INCREASED LPS-INDUCED PRODUCTION OF TNF BY MONOCYTES AFTER LONG-TERM EXPOSURE TO PROSTAGLANDIN E2 IS MEDIATED BY HOMOLOGOUS RECEPTOR DESENSITIZATION**

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Prostaglandin E2 (PGE2) is a pleiotropic agent produced during acute and chronic inflammation. Presence of PGE2 during LPS stimulation of monocytes results in the inhibition of LPS-induced production of TNF. Previously, we have shown that long-term exposure of monocytes to PGE2 resulted in an increased ability to produce LPS-induced TNF. Here, we analyzed the mechanism of this paradoxical effect.
Monocytes were purified from peripheral blood using CD14+ magnetic microbeads. Monocytes were cultured for 15 hs in the presence of PGE2 (10^{-8}M), or not (control), and then stimulated with LPS (25 ng/ml). Production of TNF was evaluated by ELISA or intracytoplasmic staining and flow cytometry.
Monocytes pre-treated for 15 hs with PGE2 showed an increase in the production of TNF compared to control cultures: 75 +/- 8 vs 56 +/- 7% TNF+ cells (n=9, p<0.05). Addition of a second pulse of PGE2 (10^{-7}M, 10 min before LPS stimulation) led to inhibition of TNF production in control cultures but not in PGE2-pre-treated cultures: 15 +/- 11 vs 66 +/- 9% TNF+ cells (n=7, p<0.01). Dose-response curves of TNF production vs PGE2 (serial dilutions 10^{-11} to 10^{-7}M) indicated that, in control monocytes, PGE2 inhibited TNF production with a mean log IC50 of -9.0 +/- 0.2, whereas PGE2-pre-treated cultures showed a mean log IC50 of 7.2 +/- 0.6 (n=5, p<0.001). Incubation of control cultures with antagonists of EP2 and EP4 receptors before the second pulse with PGE2 mimicked the curves obtained with PGE2-pretreated monocytes (mean log IC50 of -7.5 +/- 0.6, n=3, p<0.001 compared to control and p>0.2 compared to PGE2-pre-treated cultures). Blocking of endogenous PGE2 production in control cultures using COX2 inhibitor Celecoxib also led to increased LPS-induced TNF.
According to these results, increased TNF production in PGE2-pretreated monocytes could be explained by PGE2-receptor desensitization disrupting the negative feedback elicited by endogenous PGE2.

465. (493) **DIFFERENT REGULATION OF CIRCULATING AND RENAL CYTOKINES IN SHIGA TOXIN TREATED IL10 DEFICIENT MICE**

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Hemolytic Uremic Syndrome is a disease triggered by Shiga toxin (Stx) characterized by hemolytic anemia, thrombocytopenia and renal failure. Although the role of inflammatory factors in this disease is well documented, the role of anti-inflammatory cytokines, such as IL-10, is less clear.Previously we demonstrated that IL-10 deficient mice (IL-10-/-) showed a higher survival after Stx2 inoculation, associated with lower renal damage reflected by reduced plasma creatinine levels. In this work, we studied the profile of pro- and anti-inflammatory factors in blood and kidney.
Before or 3h after administration of 1LD100 of Stx2 e.v., IL-10-/- and Wt mice were euthanized and plasmas were collected. The kidneys were excised and the tissues homogenized in HEPES buffer. The plasma (pg/ml) and renal levels (pg/mg total protein) of IL-10, Corticosterone, IL-6 and TNF-α were quantified by ELISA.
Even though IL-10 was not detected in Wt plasma, before and after Stx2, renal level of this cytokine was higher before than after Stx2 inoculation (Wt0h:57.31±8.16/WT3h:26.72±4.47*,*<p0.05 vs 0h, n=4 per group). The circulating corticosterone levels were only increased in IL-10-/- mice 3h after Stx2 (IL-10-/-0h:24100±10000/ IL-10-/-3h:337900±56000*, *<p0.05 vs 0h, n=5 per group).
In contrast, IL-6 and TNF-α were only increased in IL-10-/- mice after Stx, in plasma and kidney (IL-6 plasma: Wt0h:ND/Wt3h:2.9±2.9; IL-10-/-0h:2.5±3.5/IL-10-/-3h:13.1±3.4; IL-6 kidney: Wt0h:0.22±0.13/ Wt3h:0.17±0.01; IL-10-/-0h:0.18±0.02/IL-10-/-3h:0.30±0.11, n=2 per group; TNF-α plasma: Wt0h:0.75±0.71/Wt3h:0.66±0.56; IL-10-/-0h:2.5±2.9/IL-10-/-3h65.7±13.1*, *<p0.05 vs 0h, n=5 per group; TNF-α kidney: Wt0h:69.6±9.1/Wt3h:96.9±19.6; IL-10-/-0h:72,5±2,9/ IL-10-/-3h;149.0±18.44, n=2per group).These results show that IL-10 is basally expressed in renal tissue, and could be modulated by Stx2. On the other side, in IL-10-/- mice, the inflammatory cytokines evaluated as well as glucocorticoids are upregulated by Stx2 inoculation. To finally conclude whether renal cytokines or the balance of pro- and anti-inflammatory of them is related to kidney protection, other cytokines (i.e. TGF-β) are being assayed.

466. (526) **S-LAYER GLYCOPROTEIN FROM LACTOBACILLUS KEFIRI ENHANCED TLR-INDUCED ACTIVATION ON HUMAN MACROPHAGES THROUGH C-TYPE LECTIN RECEPTORS ENGAGEMENT**

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Surface layers are nanostructured (glyco)-proteinaceous cell envelopes ubiquitously found in different species of *Archaea* and *Bacteria*. We have previously demonstrated that the S-layer glycoprotein from probiotic *Lactobacillus kefiri* CIDCA 8348 (SLP-8348) favored the LPS-induced response on murine macrophages (Raw 264.7 cells), and SLP´s glycan moieties are involved in that effect. In this work, we aim to study the ability of SLP-8348 to enhance TLR-induced response on human macrophages using different agonists as well as to investigate the involvement of the recognition by C-type lectin receptors (CLR) in that synergistic effect. SLP-8348 was removed from bacterial cells with 5 M LiCl and exhaustively dialyzed against PBS. Human macrophages (THP-1 cells) cultured in RPMI-10% FBS were incubated with SLP-8348 (10 µg/ml) or TLR-agonists such as E. coli LPS, Salmonella Typhimurium flagellin (FliC) and poly-IC, or combination of SLP-8348+agonist at different concentrations. Cell activation was assessed by quantification of secreted IL-6 by ELISA after 24h of incubation. In a similar way to the previous report for murine macrophages, although SLP-8348 did not induce cell activation by itself, it favored significantly the LPS-induced activation on THP-1 cells (P<0.005). Noteworthy, the same results were obtained using FliC or poly-IC as stimuli (P<0.05). For all TLR-agonists tested the effect was completely abrogated by the presence of the Ca^{+2}-quelating agent EGTA. Moreover, the inhibition of CLR pathways using Syk inhibitors resulted in a significative reduction of secreted IL-6 by SLP-8348+LPS-activated macrophages (50% decrease, P<0.05). Taken together, all these results strongly suggest that the CLR-TLR cross-talk triggered by simultaneous cell stimulation with SLP-8348 and TLR-3, -4 or -5 agonists is responsible for the enhanced macrophage´s activation. These findings, along with its known ability of self-assembly, do make SLP-8348 a unique structure with high potential in vaccinal applications as well as interesting mediators of microbe-host immune modulation.

467. (528) **STRATEGIES TO ENHANCE THE ADJUVANT EFFECT OF MINTHOSTACHYS VERTICILLATA ESSENTIAL OIL**

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In a previous study we demonstrated that *Minthostachys verticillata* essential oil (2.5 and 5 mg/ml) has adjuvant effect of humoral immune response. However, the antibody levels were lower than those observed with $Al(OH)_3$. Microencapsulation technique is used in the pharmaceutical industry for increase the biological effectiveness of drugs. The aim of this study was to determine the effect of higher doses of essential oil (EO) as adjuvant of humoral immune response. In addition, microencapsulation of EO was assayed and its cytotoxic effect was evaluated. Balb/c mice (n=4 per group) were immunized by subcutaneous injection (day 0) and revaccinated (day 14 and 28) as follows: Group 1:100 µl saline solution; Group 2:100 µl OVA 0.2 mg/ml; Group 3: OVA+$Al(OH)_3$ 0.5 mg/ml; Group 4: OVA+incomplete Freund adjuvant 50%; Group 5-7: OVA+EO (10, 20 and 40 mg/ml). Seven days after last revaccination, serum samples were collected and analyzed for antigen-specific total antibodies (IgG, IgM, IgA) by indirect ELISA. The incorporation of EO into microcapsules of hydroxypropyl methylcellulose and maltodextrin was determined by spray drying. The cytotoxic effect of microencapsulated EO was evaluated on bovine mammary gland epithelial cells (MAC-T). Any mouse died, and no signs in the inoculation zone neither generalized reactions were observed in the immunized groups. Groups 5, 6 and 7 did not showed significant differences in antibody levels respect to group 2. This result suggests that the adjuvant effect of EO is not dose-dependent. The yield of the EO microencapsulation by spray drying was 24%. The microencapsulated EO decreased cell viability in a dose-dependent manner. The average viability percentage of microencapsulated EO 2.5 mg/ml was 78.6 % (p<0.01 respect to control). This result is encouraging considering that EO at 2.5 mg/ml act as an adjuvant; therefore the microencapsulated EO will be evaluated as a potential adjuvant of the immune response.

468. (540) **SURVIVAL OF MATURE DENDRITIC CELLS DEPENDS ON CLUSTERIN EXPRESSION**

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Clusterin is a multifunctional glycoprotein present in almost all tissues and body fluids. It is involved in a number of physiological and pathological processes including apoptosis, protein homeostasis, Alzheimer´s disease and cancer. Although clusterin expression by myeloid cells has been reported, its influence on macrophage and dendritic cell (DC) function has not been analyzed. Here, we showed that clusterin plays a critical and non redundant role in the promotion of mature DC survival. First, we analyzed whether activation of human monocyte-derived DCs resulted in clusterin production. Cells (2x105/ml) were cultured for 48 h with or without LPS (10 ng/ml) and clusterin production was evaluated by ELISA in cell supernatants. No detection of clusterin was observed in unstimulated DCs. Activation of DCs by LPS effectively induced clusterin production: 15.4 ± 4.2 ng/ml (mean ± ES, n=5, p<0.01 vs unstimulated cells). Similar results were observed using DCs stimulated by Pam3Cys, CpG and ManLam (not shown). The expression of clusterin in LPS-activat-

ed DCs was confirmed by confocal microscopy (n=3). To analyze a possible role of clusterin in DC function we performed a knockdown (KD) strategy using clusterin shRNA carrying lentiviruses and a scramble (SCR) construction as a control. We selected two lentiviruses able to knockdown >85% of clusterin expression, as evaluated by western blot and real time PCR. Unexpectedly, we found that silencing clusterin expression (CLU-KD) resulted in a massive cell death of DCs upon LPS stimulation, evaluated by anexin-V/propidium staining: 63.7 ± 11.8, 19.7 ± 4.9, and 25.2 ± 6.5% of death cells, for LPS-stimulated CLU-KD DCs, LPS-stimulated scramble DCs, and unstimulated DCs, respectively (mean ± ES, n=4, p<0.01 CLU-KD vs scramble or unstimulated DCs). These observations uncover a novel function of clusterin that might play an important role in the control of the adaptive immune response.

469. (549) **HYPOXIC CD4+ T CELL PRODUCE PRO-INFLAMMATORY EXTRACELLUAR VESICLES**

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An increasing body of evidences show that hypoxia induce inflammation. This is clinically relevant in different conditions. For instance, ischemia in organ grafts increases the risk of inflammation and graft failure or rejection. The adaptation of cells to hypoxia rely on the transcription factor HIF-1α, which is inactive in normoxia but is activated in hypoxic conditions. Extracellular Vesicles (EVs) have emerged as a novel intercellular communication system. By carrying bioactive lipids, miRNAs and proteins they can modulate target cell functions. However, the inflammatory function of EVs secreted by hypoxic CD4+ T cells has not been explored.
Herein we analyzed the effect of hypoxia in the pro-inflammatory activity of EVs produced by CD4+ T cells. Briefly, CD4+ T cells purified from the blood of healthy donors were activated via CD3/CD28 stimulation and cultured in vitro in either normoxia (21% O2) or hypoxia (2% O2) for 48 h. Hypoxia induction was confirmed using a stable CD4+ T cell line transfected with a hypoxia response element fused to GFP (Jurkat-HRE). EVs were purified from cell culture supernatants by ultracentrifugation and the purity confirmed by western blot using classical EV-markers (CD81, CD63, syntenin-1 and exclusion of gp96). EVs were then added to human monocyte-derived macrophages and cytokine production was determined 24 h later by ELISA. We observed that secretion of IL-6 and IL-1β are induced in macrophages treated with hypoxic CD4+ T cell-derived EVs but not by EVs produced by normoxic macrophages (p<0.05).
Together, our results demonstrate that CD4+ T cells activated in hypoxia release EVs that, in turn, induce a pro-inflammatory phenotype in macrophages. The molecules present in the EVs responsible for inducing inflammation are currently being investigated.

470. (557) **SUPERANTIGENS OF THE EGC OPERON: SEG, SEI, SEO AND SEM DIFFERENTIAL EFFECT ON IL-6 SIGNAL TRANSDUCER (GP130), IL17 RELEASE AND γδt CELLS.**

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Superantigens (SAgs) are enterotoxins that promote massive cytokine release and immunosuppression. Most of them are produced by extracellular bacteria, such as *S. aureus and S. pyogenes*. IL-17 is a crucial cytokine in the eradication of these pathogens. Here, we investigated the effect of four egc operon natural SAgs (SEG, SEI, SEO and SEM) on the innate immunity and its IL-17 arm.
SAgs induce production of higher concentrations of IL-6 (ELISA) on PBMCs compared to control (p<0.0001). IL-6 acts directly to promote the development of Th17 by activating gp130-STAT3 pathway. Despite all four SAgs induced IL-6 production, only SEI, SEG and SEM significantly increased IL-17A(p<0.01).
We evaluated the ability of these SAgs to bind gp130 by Surface Plasmon Resonance. SEO and SEG showed higher affinity (KD=~1.10-6) compared to SEI and SEM (KD=~1.10-5), but all of them showed distinct kinetic behavior. Significantly, mutant SAg in TCR binding site did not display interaction
We also evaluated these SAgs' effect on human γδ T cells by FC and ELISA. While SEI did not activated γδ T cells, SEG, SEO and SEM activated γδ T cells from 0.1μM (p<0.05). At 10μM they induced death by necrosis and apoptosis. In addition, we found a significant production of IFN-γ and TNF-α starting at 0.1μM (p<0.05), and no detectable IL-17A. Notably, mutant SAgs lacking the ability to bind αβ TCR, activated γδT cells as much as wild type SAg.
We concluded that direct interaction of SAgs with gp130, a key participant in inflammation, immune regulation and activation of the Th17 arm, may explain part of the wide effects of these toxins, and would have straight influence over γδ T lymphocytes. Furthermore, SAgs promote an early necrosis and apoptosis in this cell type, with release of type 1 cytokines, which would conspire against the eradication of these extracellular bacteria.

471. (559) **REPOLARIZATION OF HUMAN M2 MACROPHAGES RESTORES NK CELL-MEDIATED FUNCTIONS: IMPLICATIONS IN HUMAN RENAL CELL CARCINOMAS.**

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Macrophages represent the most abundant population within the tumor microenvironment and they have been associated with poor prognosis in many cancer patients due to local and systemic effects that facilitate tumor growth, aberrant angiogenesis, metastases and immunosuppression. We previously demonstrated that anti-inflammatory macrophages (M2) inhibit NK cell-mediated cytotoxicity against tumor cells and IFN-γ production upon stimulation with cytokines. Also, we demonstrated that M2-driven up-regulated expression of the inhibitory receptor CD85j (ILT-2) on NK cells restrains NK cell degranulation through interaction with HLA-G expressed by M2. Therefore, in this work we characterized the macrophage polarization status and the expression of CD85j in NK cells from human renal cell carcinoma (RCC) biopsies. Flow cytometry analysis revealed a higher percentage of $CD68^+$ (macrophages) infiltrating cells within tumors compared with renal healthy parenchyma (HP) (p<0,05). These tumor infiltrating macrophages displayed lower CD274 and up-regulated CD206 expression when compared to HP macrophages, suggesting that they are skewed towards a M2 phenotype. Moreover, compared with HP infiltrating NK cells, tumor infiltrating NK cells of RCC patients displayed overexpression of CD85j (p<0,05). CD85j was also overexpressed on peripheral blood NK cells from RCC patients compared to healthy donors (p<0,01), suggesting that tumor-driven immunosuppressive effects affect the phenotype of peripheral blood NK cells. Finally, as repolarization of M2 may negatively impact tumor growth, we explored the consequences of repolarization of M2 on NK cell effector functions. Exposure of in vitro differentiated M2 to poly I:C promoted the acquisition of an M1 phenotype that resulted in a restoration of NK cell degranulation and IFN-γ production. Overall, intratumoral macrophages in RCC phenotypically resemble M2 and repolarization of M2 with poly I:C reverted M2-mediated NK cell suppression. Therefore, repolarization of intratumoral macrophages may promote the reacquisition of NK cell-mediated tumoricidal functions, appearing as an interesting therapeutic option in RCC patients.

472. (567) **MODIFICATION OF IMMUNE RESPONSE TO DIPHTHERIA TOXOID BY CYTOKINE NEUTRALIZATION**

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Introduction: In previous works, we demonstrated that infection of mice with Lactate dehydrogenase elevating virus (LDV) -a RNA arterivirus that induces NK, macrophages and B-cell activation- modified Ab specificity to different antigens models such as hGH and OVA. This

effect was correlated with secretion of specific cytokines. Recently, we found that an interleukin -IL-17A- is involved in this mechanism. *Objective:* Expand the studies to an antigen of clinical relevance, such as Diphtheria Toxoid (DTx). *Methods:* C57BL/6J mice (n=18) were inoculated subcutaneously with 25µg of DTx in Alum Adjuvant. At day 15, the mice were boosted with the Ag. At days 4, 7 and 11, half of the animals (n=9) were inoculated intraperitoneally with 150*µ*g of anti-IL-17A MAb in PBS. Another mice group (n=18) were treated as before but infected with 2×107 50% infectious doses of LDV in saline at day 0. Bleeding was performed at days 8, 21, and 32. Serum lactate dehydrogenase (LDH) levels were determinated enzymatically and the titer of Ab anti-DTx was evaluated by indirect ELISA, whereas IL-17A concentration was measured by ELISA sandwich. *Results:* MAb to IL-17A increased LDH levels in infected-animals treated with DTx (2802±57 and 5020±208 U/L, 21 and 32 dpi, respectively, P<0.001). In infected-mice treated only with DTx the levels none changed. Titers of anti-DTx Ab increased by MAb treatment (1/65000±7100 to 1/131100±28900, control and anti-IL17A treated, respectively, P<0.01).However, in infected-animals anti-DTx Ab decreased by MAb treatment (1/130000±9900 to 1/69000±2800, control and anti-IL-17A treated, respectively, P<0.001). Furthermore, MAb treatment augmented plasmatic IL-17A concentration in animals treated with DTx in comparison with control (1203.5±161.9 pg/ml and 10.6±0.3 pg/ml, P<0.01, respectively) this effect was also detected in LDV-infected animals inoculated with DTx (680.7±218.3 pg/ml and 10.1±1.2 pg/ml, respectively, P<0.05). *Conclusions:* This work indicated that IL-17A is capable of modulating the immune response towards DTx.

473. (569) **OBTENTION OF SPECIFIC CAMELID SINGLE DOMAIN ANTIBODIES, VHH OR NANOBODIES, AGAINST MURINE IGG1 FOR DEVELOPMENT OF A NEW METHOD FOR PURIFICATION OF MONOCLONAL ANTIBODIES**

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CEVAN - ICT Milstein - CONICET

Last 30 years, monoclonal antibodies (mAbs) industry has grown exponentially, due to their wide use in research, diagnosis and therapy. Currently, most used method for mAb purification is affinity chromatography with protein A (ProtA). However, it presents some disadvantages as acidic elution and high cost. On the other hand, biotechnological application of camelid single domain antibodies (VHH or nanobodies) has notably increased in the last years. These nanobodies present several advantages as small size, high solubility and physicochemical stability. For this reason, our goal was to obtain VHH against murine IgG1 (mIgG1) to be used as ligand in a new resin for mAb purification.
Firstly, two llamas were immunized with a mix of murine IgG (total and subtype 1). After immunization, both llamas presented a high humoral response against mIgG1 measured by ELISA. Two immune VHH libraries were constructed starting from llama peripheral blood and library quality controls were performed. Both libraries presented high size (more than $5x10^8$ CFU/ml) and 100% of full-length insert clones. Phages expressing specific VHH against mIgG1 were obtained after two rounds of selection. Reactivity of total phage libraries and eluted phages was tested by polyclonal Phage-ELISA. For 88 selected clones, specific reactivity was confirmed in VHH-ELISA. The 24 most reactive nanobodies were analyzed by fingerprinting, in order to evaluate VHH diversity. As a result, 14 different digestion patterns were obtained and 9 of them were confirmed as unique by sequencing.
In conclusion, two immune VHH libraries were constructed and specific nanobodies against mIgG1 were obtained by Phage Display methodology. Even more, 14 out of the 24 most reactive nanobodies presented a unique fingerprinting pattern confirming VHH diversity. Further characterization of these nanobodies will allow us to develop a new method based in VHH as ligand, with potential wider use and lower cost for mAb purification.

474. (578) **B CELLS EXPRESSING IL-33 RECEPTOR ARE EXPANDED IN THE ACUTE PHASE OF PRETERM BIRTH**

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Preterm birth (PTB) is one of the most recurrent complications during pregnancy affecting 8-18 % of all pregnancies worldwide. Up to 40% of all PTB are caused by clinical or subclinical infections. Recently, an immune-protective mechanism, involving IL-33 signaling in decidual B-cells, has been demonstrated to be sufficient to prevent LPS-induced PTB in pregnant mouse. Interestingly, we have simultaneously demonstrated that IL-33 receptor (ST2) is significantly up-regulated in B cells during pregnancy.
Hence, we aimed to characterize here the expression of ST2 in B cells in the early phases of PTB.
PTB model: Pregnant C57BL/6 females on gestational day (gd) 16 were challenged with different doses of LPS (E.coli 055:B5; 5 to 50 µgr/mouse, i.p.) or PBS as control. PTB was defined as birth occurring before gd 18. We choose 10 µg/mouse, as it was the minimum dose capable of triggering 100% of PTB without affecting maternal health.
Using this model, we evaluated by flow cytometry, the expression of ST2 in different B cell subsets in the spleen, peritoneal cavity and uterine-draining lymph nodes (para-aortic lymph nodes (PLN)), 5h post LPS challenge.
Although relative distribution of main splenic B cell populations was not significantly altered, percentages of ST2-B1 cells ($B220^{low}CD23^{-}CD21^{-}ST2^{+}$) were significantly increased in PTB mice compared to controls (C) (%Mean±SEM; C=16.7±1.7, PTB=35±5.6*; *p<0,01vsC , Unpaired t-test, n=7). Similarly, percentages of total ST2-B cells ($CD19^{+}B220^{+}ST2^{+}$) were significantly increased in PLN of PTB mice as compared to controls. (%Mean±SEM; C=7±0.6, PTB=14.4±3.2*; *p<0,01vsC, Mann Whitney test, n=7). No significant differences were observed concerning ST2 expression in peritoneal B cells.
We showed here that ST2-expressig B cells rapidly expand upon LPS induction of PTB. This result reinforces the idea of IL-33/ST2 axis in B cells as a pivotal mechanism launched to suppress infection-induced inflammation during pregnancy that can lead to PTB.

475. (619) **AUTOGRAPHA CALIFORNICA MULTIPLE NUCLEAR POLYHEDROSIS VIRUS INDUCES ANTIVIRAL ACTIVITY AGAINST FOOT-AND-MOUTH DISEASE VIRUS IN PIGS**

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Foot-and-mouth disease (FMD) is a highly contagious viral illness that affects cloven-hoofed animals causing serious economic losses. The inactivated vaccine against foot-and-mouth disease virus (FMDV) requires approximately 7 days to induce an effective protective response. This is not fast enough to control FMD outbreaks and rapid antiviral strategies are needed. The baculovirus Autographa californica multiple nuclear polyhedrosis virus (AcMNPV) are DNA virus that infect insects. They cannot replicate in mice but induce a strong innate immune response and establishes a nonspecific antiviral status able to protect them at short term against challenges with FMDV among other viruses. AcMNPV has never been tested in mammals of veterinary interest. In this study we evaluated the immunostimulary effect of AcMNPV on swine to induce antiviral activity against an FMDV infection.
Peripheral blood mononuclear cells (PBMCs) purified from pigs were treated with budded virions of AcMNPV at different moi or mock infected for 16 h and supernatants were assessed for antiviral activity. Values of 344.9±107.4 U/ml (moi 0.1) - 1742.7±183.1 U/ml (moi 10) for anti-VSV activity and 634.7±172.7 U/ml (moi 0.1) - 1742.4±435.6 U/ml (moi 10) for anti-FMDV activity were detected.

Levels of 80.4±21.4 pg/ml (moi 0.1) - 708.4±108.4 pg/ml (moi 10) for IFN-α were measured by ELISA, and its neutralization with Mab reduced the antiviral activity (P<0.01), demonstrating its involvement. Five nine weeks old-pigs were inoculated intravenously with AcMNPV at 1x10^{9}PFU and sera were taken at different times. The sera of the immunized animals were able to protect porcine non immune cells against in vitro challenges with FMDV (5827.8±2493.1 U/ml at 3 hpi). The antiviral activity correlated temporally with the production of IFN-α (652.7±139.4 pg/ml at 3 hpi), with peaks of induction within the first 6 hpi. We conclude that AcMNPV is a promising biotechnological tool for antiviral strategies in pigs.

476. (637) **EFFECT OF LACTOBACILLUS RHAMNOSUS CRL1505 ON THE IL-17A/GM-CSF AXIS IN THE INTESTINAL MUCOSA AND NEUTROPHIL HOMEOSTASIS OF BONE MARROW IN A MODEL OF IMMUNOSUPPRESSION BY CYCLOPHOSPHAMIDE**

Andrés Daniel Gramajo Lopez[1], Lucila Saavedra[1], Elvira María Hebert[1], Julio Cesar Villena[1], Susana Alvarez[2], Susana Salva[1]

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Gut microbiota is able to control immunity in distant tissues through regulation of granulopoiesis at primary immune sites as bone marrow (BM). The dietary supplementation with probiotic *Lactobacillus rhamnosus* CRL1505 (Lr05) improves steady-state and emergency granulopoiesis in immunosupressed hosts. While the Lr05 viability is an important factor to achieve optimal effects, it is possible to stimulate immunity using non-viable Lr05. The aim of this work was to study the effect of oral administration of viable or non-viable Lr05 (Lr05V or Lr05NV, respectively) on the IL-17A/GM-CSF axis in the intestinal mucosa and neutrophil homeostasis of bone marrow, in a model of immunosuppression by cyclophosphamide (Cy). Adult Swiss-mice were orally treated with Lr05V or Lr05NV during five consecutive days. On day 6, lactobacilli-treated and untreated control mice received one intraperitoneally dose of Cy (150 mg/kg). The Cy did not change the frequency of IL17A-producing innate lymphoid cells on the small intestinal lamina propria (IL-17A$^+$RORγt$^+$CD4$^-$ NKp46$^+$). However, both Lr05V and Lr05NV are able to increase these cells after the administration of the Cy. These results were correlated with an increase in the levels of serum IL-17A ($p<0.05$), without detecting differences in the serum levels of GM-CSF between the different experimental groups. On the other hand, Cy decreased HSCs (Lin$^-$Sca-1$^+$c-Kit$^+$) and MMP (Gr-1$^+$Ly6G$^+$Ly6C$^-$) in BM. Lactobacilli treatments were able to significantly promote early recovery of HSCs and MMP ($p<0.05$). In addition, at 3 days post-Cy, Lr05V and LR05NV induced a high expression of GM-CSF and a lower expression of CXCL12 in BM, compared with the Cy control group ($p<0.05$). In conclusion, both Lr05V and Lr05NV are able to activate beneficially the IL-17A/GM-CSF axis and accelerate the recovery of Cy-induced immunosuppression by increasing BM myeloid progenitors. Non-viable *L. rhamnosus* have the potential to be a good and safe resource for reducing chemotherapy-induced immunosuppression in cancer patients.

477. (647) **CD39 EXPRESSION ON CD8+ TUMOR-INFILTRATING T CELLS IS ASSOCIATED TO EFFECTOR MEMORY PHENOTYPE AND METABOLIC STRESS.**

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Previously we have demonstrated that exhausted tumor-infiltrating CD8+ T cells (TILs) exhibit high expression of the ecto-nucleotidase CD39. In this work we aimed to characterize the phenotype of the CD39+ TILs. Using the B16-F10-OVA cancer model we determined by FACS that CD8+CD39high TILS exhibited an effector memory phenotype (CD62L-CD44+), while CD8+CD39-TIls represent a heterogeneous population (central memory, effector memory and naïve). CD8+CD39high TILS showed higher frequency of KLRG-1+CD127- cells (short-live effector phenotype) than CD39low CD8+TILS ($p\leq0,05$). In addition, we observed that CD8+CD39high TILs showed higher expression of CD8α than CD39- or CD39int CD-8TILs ($p\leq0,0001$ in both cases). In spleen and lymph node, CD39int CD8+T cells showed higher CD8α expression than CD39- cells. Similar results were observed in other cancer models like 4T1, MCA-OVA and CT26. We also evaluated markers of metabolic stress and we detected that CD8+CD39high TILS (obtained ex vivo) exhibited higher expression of p-mTOR, pS6 and p-AMPK than CD8+CD39-TILS ($p\leq0,005$, $p\leq0,0001$ and $p\leq0,001$ respectively). After αCD3/αCD28 stimulation CD8+CD39highTILs showed lower expression of pmTOR and PS6 than CD8+CD39-TILs ($p\leq0,05$). Studying breast cancer patients we observed that high frequency of CD8+CD39+ T cells from tumor or metastatic lymph node (MLN) showed a memory effector phenotype. Most of CD8+CD39- TILs cells exhibited phenotype of central memory or effector memory while in MLN most of this population correspond to naïve phenotype. CD8+CD39+ TILs showed higher expression of CD8α than CD39- TILs ($p\leq0,05$). All together or results demonstrated that expression of CD39 on CD8+ T cells is associated to a short-live effector phenotype and metabolic stress. CD39 emerges as a target for treatments aimed to restore CD8 T cells anti-tumor immunity.

478. (649) **LY9 AGONIST REDUCES GERMINAL CENTER AND PLASMABLAST RESPONSE IN TRYPANOSOMA CRUZI INFECTION: ROLE OF INKT AND MZB CELLS.**

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Ly9 is an inhibitory receptor, a SLAM family member, present on all lymphocytes. Its highest expression is found on innate-like lymphocytes such as iNKT and MZ B cells. Treatment of mice with a Ly9 agonist selectively depletes splenic MZ and B1 B cells, reduces iNKT cells number and impairs IL-4 and IFN-γ production by iNKT. It was reported that iNKT cells promote the early seeding of germinal center (GC) reaction to infections and that MZ B cells could be also involved in GC reaction due to their location and high expression of CD1d.

The aim of our work is to evaluate the effect of a Ly9 agonist on the GC and extrafollicular response in T cruzi infection. For this, 1 day before intraperitoneal infection with 5000 trypomastigotes of T cruzi (Tulahuén) or PBS (control), C57BL/6 mice were intraperitoneally injected with anti-Ly9 (Ly9-mice) or with control isotype. Splenic lymphocytes were evaluated by FACS. Five days post-treatment, Ly9-mice exhibited a strong reduction in the frequency of MZB cells (B220+ CD19+ CD21hi CD23lo) and iNKT cells (CD3int α-GalCer-CD1d-Tetramer+ B220-) were diminished in numbers and activation (quantified by CD69 expression) compared with controls ($p<0.05$). At 15 days post-infection (dpi), Ly9-mice had a lower frequency and number of GC B cells (B220+ CD19+ FAS+ GL7+) and Plasmablasts (B220lo CD138int) than infected controls ($p<0.05$).

Finally, we analyzed the phenotype and functions of iNKT cells at early stages of infection. T cruzi infection increased the frequency of IL-4- and IFN-γ-producing iNKT cells and triggered CXCR5 and FAS-L expression in a percentage of iNKT, at 4 dpi. In this context, iNKT and MZB cells could be involved in the GC and extrafollicular responses in T cruzi infection. Further studies are needed to test our hypothesis.

479. (674) **FUNCTIONAL AND IN SILICO CHARACTERIZATION OF NEUTRALIZING INTERACTIONS BETWEEN ANTIBODIES AND THE FOOT AND MOUTH DISEASE VIRUS IMMUNODOMINANT ANTIGENIC SITE**

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Foot-and-mouth disease (FMD) is one of the major animal diseases of agronomic relevance and it is caused by the FMD virus (FMDV). The FMDV control is mainly exerted by neutralizing antibodies that recognize functional-relevant viral regions (antigens; Ag). Few FMDV-Ab complexes had been studied by crystal diffraction methods and characterized at a quasi-atomic level, but there are no investigations that extrapolate this information to analyze unknown structures from different FMDV strains antibodies.
Currently, we have evaluated the effectiveness of eight computational programs to model the interaction between serotype C FMDV site A Ag and two different monoclonal antibodies (mAbs). Specifically, we model all possible point mutations through the amino acid residues of a viral antigenic peptide and generate a computational profile of the Ag/mAb interaction energy. These results were compared with the interaction data, obtained previously by ELISA. The best correlation was obtained with the program FoldX showing a Pearson correlation (r) of 0.7, with a high significant level of confidence (simple Mantel test).
Secondly, we model multiple amino acid mutations representative of FMDV outbreaks interacting with the referred mAbs. The emerging data from this experiments showed a coefficient R2 of 0,53 for the FoldX program which also yields the better AUC value (0.91) in a ROC analysis.
Finally, we cloned the coding sequence of further two mAbs that also recognize and neutralize site A epitopes from a serotype A virus. Then, we implemented a protocol for modeling their molecular structure as well as their interaction with viral Ag, assisted by the information obtained with peptide ELISA test mutational profiles. By means of those results, we developed a differential and functional epitope fine map for those mAbs.

480. (684) **TIM-3 INCREASE DURING ANTI PD-1 TREATMENT IS ASSOCIATED WITH DISEASE PROGRESSION IN PATIENTS WITH NON-SMALL CELL LUNG CANCER AND RENAL CARCINOMA**

Estefanía Paula Juliá, Pablo Mando, Manglio Miguel Rizzo, Florencia Tsou, Romina Luca, Alicia Inés Bravo, Walter Astorino, Jose Mordoh, Claudio Martín, Estrella Levy
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Cancer immunotherapies targeting PD-1/PD-L1 axis have shown efficacy in a wide range of cancers. However, not all patients benefit from treatment. In a cohort of 18 non-small cell lung cancer (NSCLC) and 7 renal carcinoma (RC) patients, we assessed immune cell populations and soluble mediators in peripheral blood before (PRE) and after (POST) 8-12 weeks of therapy with anti-PD-1 mAbs Pembrolizumab or Nivolumab. The aim was to identify potential biomarkers of response.
We used an automated hematology analyzer to study white blood cell counts and flow cytometry to analyze lymphocyte subpopulations (CD4, CD8 and regulatory T cells and NK cells) and markers of activation/differentiation on T cells. Plasmatic C-Reactive Protein (CRP) and cytokine concentration were measured using CRP assay and Cytometric Bead Array, respectively.
12 patients presented stable disease or response (SD-R) while 9 patients progressed (PD). Response was not evaluable in 4 patients. No differences were observed in any of the markers analyzed in pre-treatment samples between PD and SD-R patients. So we compared the variation (POST minus PRE median values) of each marker between both response groups. Patients with PD, in contrast to SD-R, presented an increase in the percentage of TIM-3+ within CD4 (median variation [IQR]: +2.8% [+1.7–+4.1] vs -1.6% [-2.9– -1.2], p=0.0018) and CD8 (+5.5% [+4.0–+6.5] vs -1.9% [-2.3– -0.38], p=0.0009) T cells. PFS analysis showed that increase of TIM-3 expressing cells was deleterious for survival (CD4 p=0.001; CD8 p=0.002). Evaluation of soluble mediators' variation showed significant differences between PD and SD-R patients in CPR (+9.9mg/l vs -5.9mg/l, p=0.03) and IL-8 (+8.8pg/ml vs -3.1pg/ml, p=0.015) plasma levels.
TIM-3 expression in TILs has been previously described as a resistance mechanism to anti-PD-1 therapy. The evaluation of TIM-3 in peripheral blood lymphocytes may be a more accessible and useful tool to monitor progression to this therapy.

481. (699) **AUTOLOGOUS T CELL ACTIVATION FOSTERS ABT-199 RESISTANCE IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) AND SELECTS MALIGNANT CELLS WITH AN AGGRESSIVE PHENOTYPE.**

Esteban Enrique Elías, Ana Colado, Maricef Vergara Rubio, Gregorio Cordini, Denise Risnik, Horacio Fernandez Grecco, Fernando Raimundo Bezares, María del Rosario Custidiano, Julio César Sánchez Avalos, Ángeles Vicente, Gonzalo Martín Garate, Mercedes Borge, Mirta Giordano, Romina Gamberale
IMEX-CONICET, Academia Nacional de Medicina

BCR signaling and activated T cells from the microenvironment favor malignant cell activation, proliferation and survival in CLL. ABT-199, a specific BCL-2 inhibitor, is highly cytotoxic against unstimulated CLL cells. We reported that T cell activation induces ABT-199 resistance in CLL cells (Elías-Haematologica-2018). To further characterize resistant CLL cells, PBMC from CLL patients were cultured for 48hs without (control) or with anti-CD3 (aCD3) to activate T cells, and then ABT-199 was added to the cultures. Leukemic cell survival, activation and proliferation capacity were evaluated by flow cytometry. While control CLL cells treated with ABT-199 showed more than 93% of cell death after 48hs of ABT-199 treatment, CLL cells from aCD3 cultures are still alive with ABT-199 at 120hs (%CD19+ viable cells: 63±8 vs 34±7 for aCD3 vs aCD3+ABT-199) and show similar levels of the activation marker CD86 (n=9). Interestingly, CLL cells in aCD3+ABT-199 cultures showed increased size (n=9, $p<0.01$) and higher Ki67 expression (n=6, $p<0.05$) compared to aCD3 cultures. To overcome ABT-199 resistance we previously used the BCR kinase inhibitor (BCR-KI) GS-9973 (Elias-Haematologica-2018). Here we showed that GS-9973 did not directly affect leukemic cell survival or modify ABT-199-induced cell death (n=10), corroborating that its effects were through impairment of T cell activation (Colado-Cancer. Immunol.Immunother-2017). Finally, we reported that GS-9973 reduces phagocytosis of rituximab-coated CLL cells (Colado-Cancer. Immunol.Immunother-2017), while ABT-199 enhances it (Elias-Haematologica-2018). We here found that obinutuzumab-coated CLL cells phagocytosis was reduced by GS-9973 (n=5, $p<0.05$) and ABT-199 seemed to enhance it (%phagocytosis: 40±8 vs 68±9 for Obinutuzumab+GS-9973 vs Obinutuzumab+GS-9973+ABT-199; n=5), although this difference is not statistically significant yet. Moreover, ABT-199 did not affect CD107a degranulation by NK cells induced by anti-CD20-coated CLL cells (n=5). Our results confirm that activated leukemic cells from the microenvironment might not be properly targeted by ABT-199 monotherapy and encourage its combination with BCR-KI and anti-CD20 antibodies.

ONCOLOGÍA / ONCOLOGY ORAL SESSION 3

482. (149) **ANTITUMORAL AND IMMUNOMODULATORY ROLE OF HISTAMINE IN BREAST CANCER**

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Investigaciones Biomédicas (BIOMED) UCA-CONICET

It is well known that histamine is a key regulator of immune cell functions and it also modulates cancer cell proliferation. The aim of this work was to investigate the effect of histamine and its H4 receptor (H4R) agonist (JNJ28610244) on tumour growth and in the immune tumour microenvironment as a whole, in a triple negative breast cancer (TNBC) syngeneic model developed in immunocompetent mice. Tumours of the TNBC cell line 4T1 were established in Balb/c mice. Treatments employed: histamine (1 or 5 mg/kg) and JNJ28610244 (1 or 5 mg kg). Results show that histamine treatment (5 mg/kg) reduces tumour growth more effectively than JNJ28610244. Histamine but not the agonist increases tumour apoptosis and it reduces

the number of intratumoural vessels. Histamine also reduces immunosuppression through the modulation of the tumour microenvironment, as it increases the tumour secretion of IFN gamma and reduces the number of T regulatory (Treg) lymphocytes in lymph nodes and spleen.
A lower concentration (1 mg/kg) of JNJ28610244 reduces tumour size while no immunomodulatory effects are observed in the immune cell subsets studied. In contrast, a higher concentration (5 mg/kg) is not able to decrease tumour growth probably because of the immunosuppressive effect produced in the tumour microenvironment, showing increased levels of interleukin (IL)-10 and decreased levels of IFNγ in tumours and increased infiltrating Treg cells in tumour draining lymph nodes. These results highlight the critical interplay between tumour cells and host immune response that determine the clinical therapeutic outcomes and suggest that histamine is a key pleiotropic mediator with therapeutic benefits in TNBC.

483. (312) **MULTIVARIATE ANALYSIS OF IMMUNE CELLS POPULATIONS INVOLVED IN TUMOR GROWTH**
Antonela Del Giúdice, María Celeste Capitani, Matías Fusini, Leandro Mainetti, O. Graciela Scharovsky, Ricardo Di Masso, María José Rico, Viviana R. Rozados
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M-406 mammary adenocarcinoma appeared in an inbred CBi mouse. CBi- mice were artificially selected by body conformation from CBi. When CBi- mice are s.c. challenged with M-406, 100% of the tumors are rejected; conversely, tumor grows exponentially in CBi. To explain the participation of the immune system in this behavior, CBi and CBi- females (N=9), were inoculated with M-406, blood samples were taken on days 0, 7 and 14, and CD4+, CD8+, Treg and Th17 cells were quantified (flow cytometry). Day 0- CD4+: CBi<CBi- (P=0.0133), CD8+: CBi>CBi- (P=0.0041), Treg: CBi>CBi- (P=0.0003) and Th17: CBi<CBi- (P=0.0111) (Student's t). The analysis with the multivariate technique of principal components, generated two components (PC). CBi: PC1- day 0 (mean ± SEM,0.4 ± 3.18), day 7 (-2.8 ± 5.14), day 14 (2.4 ± 2.14) explained 79.27% of the variance and was negatively correlated with CD4+ (P<0.0001); PC2- day 0 (-0.6 ± 1.57), day 7 (-3.4 ± 1.09), day 14 (3.9 ± 1.85), explained 20.23% of the variance and was negatively correlated with CD8+ (P<0.0001). No associations were observed for Treg and Th17. CBi-: PC1- day 0 (15.3 ± 4.10), day 7 (-21.5± 5.30), day 14 (9.5 ± 3.5) explained 98.25% of the variance and was positive and negatively correlated with CD4+ (P<0.001) and Th17 (P<0.0001), respectively; PC2- day 0 (0.4 ± 0.95), day 7 (-0.5 ± 0.62), day 14 (0.8 ± 0.97) explained 1.57% of the variance and was negatively correlated with CD8+ (P<0.0001). No association was observed for Treg. 1) Treg cells were not associated with tumor growth/rejection. 2) The antagonistic behavior observed in both lines of mice challenged with M-406 could be mainly explained by the different evolution of CD4+ and Th17 cells during tumor growth (PC1) 3) PC2 (CD8) explains the variance found in CBi.

484. (363) **TNFA BLOCKADE IMPROVES ANTITUMOR INNATE IMMUNE RESPONSE AND OVERCOMES TRASTUZUMAB-RESISTANCE IN HER2+ BREAST CANCER**
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HER2 positive (HER2+) is a subtype that affects 13-20% of breast cancer (BC) patients. They receive trastuzumab (T), an anti-HER2 monoclonal antibody, but 40-60% of them relapse. Therefore, new strategies to overcome trastuzumab resistance are needed. We recently demonstrated a novel tumor immune evasion strategy where TNFα induces upregulation of the expression of the transmembrane glycoprotein mucin 4 (MUC4) to impair trastuzumab binding, preventing antibody mediated killing of BC cells. Etanercept (E), an inhibitor of TNFα, downregulated MUC4 expression and sensitized de novo trastuzumab-resistant BC xenografts to trastuzumab. The aim of this work was to study whether etanercept improved antitumor innate immune response (IIR) mediated by trastuzumab.
We used the de novo trastuzumab-resistant and TNFα-producing cell line JIMT-1 to establish s.c. tumors in female nude mice. Animals were treated with IgG, T, E or T+E (5 mg/kg each) i.p. twice a week. Treatment with T+E significantly reduced tumor growth in 72,4% ($p<0.01$) vs. the control group, IgG. Spleen NK cells from T+E group showed an increase in the degranulation marker CD107a by flow cytometry ($p<0.05$) vs. IgG. Moreover, spleen NK cells from T and T+E groups showed an enhanced trastuzumab-dependent degranulation in an ex vivo assay ($p<0.01$) vs. IgG. In addition, T+E treatment also reduced total myeloid cells (CD11b+) infiltration in tumor microenvironment (TME) ($p<0.01$) vs IgG, but granulocytic and monocytic subtypes distribution remained unchanged. MUC4 and cyclinD1 expression determined by Western blot were downregulated in tumors treated with T+E ($p<0.01$ and $p<0.05$, respectively) and AKT phosphorylation was inhibited ($p<0.01$) with respect to IgG, T and E.
These results suggest that TNFα blockade downregulates MUC4 expression reduces tumor burden and improves the IIR, increasing NK degranulation and generating a less suppressive TME. Patients with HER2+ MUC4+ BC could be eligible for the combined therapy T+E to overcome/avoid resistance.

485. (616) **TUMOR-SUPPRESSIVE FUNCTIONS OF 4-METHYLUMBELLIFERONE ON HUMAN AML CELLS: MODULATION OF SENESCENCE, CD44 EXPRESSION AND MITOCHONDRIAL STATUS.**
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Beside the improvement in acute myeloid leukemia (AML) therapy, half of patients die due to complications related to treatment. CD44, the main hyaluronan receptor, has been proposed as a therapeutic target in AML. Down-regulation of this receptor has been associated to inhibition of cell proliferation and metabolic reprogramming including mitochondrial changes which sensitize to chemotherapy in different tumors. 4-methylumbelliferone (4MU) has shown promising effects as a potential new drug in cancer. Recently, it has been demonstrated that 4MU down-regulates CD44 expression in breast cancer cells. However, little is known about 4MU effects on hematological malignancies. Previous results of our lab showed that 4MU inhibited cell proliferation in a dose dependent manner in AML cell lines without inducing apoptosis at lower doses but showing senescense-associated heterochromatin foci (SAHF+) cells by DAPI stain. Considering this, we hypothesize that 4MU would be a potential new drug for the treatment of acute leukemia. The aim of this work was to evaluate the effect of low doses of 4MU on CD44 expression, senescence modulation and mitochondrial status in human AML cell line (U937). Results showed that treatment with 4MU reduced significantlyl CD44 expression in a dose dependent manner as assessed by FC ($p<0.05$) and WB. Also, 4MU increased the mean of fluorescence of TMRE ($p<0.05$) and NAO ($p<0.01$) by FC suggesting an increment of mitochondrial mass. Fluorescence microscopy using MitotrackerRed showed mitochondrial elongation, accumulation of these organelles and changes on their distribution. In order to evaluate senescence we performed a SA-β-galactosidase colorimetric assay. 4MU increased SA-β-Gal+ cells percentage ($p<0.05$) in U937 cell lines. In view of these results, we conclude that 4MU down-regulates CD44, with an increment in mitochondrial mass and the induction of senescence in U937 cell line. These findings broaden the knowledge of the potential use of 4MU in acute leukemia treatment.

486. (90) **MAGEB2 ENHANCES RDNA TRANSCRIPTION AND GLOBAL PROTEIN SYNTHESIS**
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MageB2 is a pro-proliferative and tumor specific protein belonging to MAGE family. Previously, we observed a reduced expression of MageB2 caused a reduction in the level of ribosomal RNA precursor (pre-rRNA). Here, we study the mechanisms involved in the regulation of ribosomal biogenesis by MageB2 as well as the effect on protein synthesis.
The regulation of rDNA transcription is achieved via growth factor-dependent signaling pathways (mainly PI3K/AKT/mTOR and RAS/MEK/ERK) modulating the activities of Pol I-specific transcription factors, such as upstream binding factor (UBF). UBF phosphorylation enhances its association with Pol I and pre-rRNA synthesis.
By using specific inhibitors of PI3K/AKT/mTOR and RAS/MEK/ERK pathways we observed the negative effect on UBF phosphorylation is contrarested when MageB2 is expressed. According to this, by performing Chromatin Immunoprecipitation assays we observed a reduced amount of pUBF is recruited to rDNA promoter in the HCT116 MageB2 KO in comparison with parental cell line.
Next, we performed a proteomic study to identify proteins differentially expressed in HCT116 and HCT116 MageB2 KO cells. Notably, more than 35 ribosomal proteins were identified with reduced levels in MageB2 KO cells, supporting the idea that MageB2 is involved in ribosome biogenesis. In this way, abolition of MageB2 expression could lead to less ribosome production and therefore less content in ribosomal proteins.
Finally, we used the non-isotopic SUnSET technique to measure protein synthesis. According to a reduced number of ribosomes, we observed a 30% reduction of global protein synthesis in the HCT116 MageB2 KO, which is reverted by ectopically expressing MageB2 in this cell line.
Together, these results suggest a mechanism whereby MageB2 through its ability to upregulate phosphorylation of UBF, Pol I transcription and consequently, ribosomal biogenesis is able to affect global protein synthesis, collaborating in this way to the elevated proliferation rates required by cancer cells.

487. (108) **THE RESPONSE OF CANCER STEM CELLS (CSC) TO CHEMOTHERAPY USING A MURINE INVASIVE BLADDER CANCER MODEL**

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Introduction: Bladder Cancer (BCa) is in one of the most common tumors of the urogenital male tract and an important worldwide cause of death, being the muscle Invasive, is one of the worst prognosis. The standard treatment is the radical cystectomy, although, alternative treatments are being tried in order to provide better standard of living to the patients, such as tumor transurethral resection followed by radiotherapy and chemotherapy. Cancer Stem Cells (CSC) are a minority tumor cell population, associated to the lack of treatment response. The development of an in vitro assay that can predict treatment response, would be useful for patients with this pathology. Objective: Evaluate CSC modulation under treatment with chemotherapy drugs like Doxorubicin (Doxo) and Cisplatin (CisPt) using a murine BCa cell line (MB49-I). Results: Through a cell viability assay in monolayer, measured by MTS, half minimal inhibitory concentrations (IC50) were obtained, being 0,1μM for doxorubicin and 9 μM for cisplatin. Cancer sphere forming assay in low adherence conditions, an accepted method for CSC quantification, was carried out and treating or not the cells seeded with IC50 of Doxo or CisPt. Sphere formation efficiency of control MB49-I was 13%. Both treatments reduced this efficiency by 95% ($p<0,0001$) and the sizes of spheres also was reduced (Doxo: 36%; CisPt: 57%) ($p<0,0001$). The quantitative analysis by qPCR for Oct-4, a pluripotency-associated gene, showed that Doxo increased that expression (185%), while CisPt treatment decreased it (25%) regard to control. Both drugs inhibited the number of CSC, however, the modulation of one pluripotency marker, was different. An in vivo assay analysing the growth of spheres under these treatments, will give us relevant information about their tumorigenic potential

488. (397) **WNT/B-CATENIN SIGNALING INHIBITION AS PART OF TEMOZOLOMIDE-ANTITUMOR MECHANISM IN PROLACTINOMAS**

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Pituitary tumors are one of the most frequent intracranial tumors, some of them may become locally invasive/aggressive with resistance to conventional therapy. For these clinical complex entities, new therapies are still needed. Temozolomide (TMZ), a chemotherapeutic agent used in glioma treatment, in now being implemented for resistant/aggressive pituitary adenomas treatment. However, molecular mechanisms that mediate sensibility or resistance to TMZ in pituitary tumors are still unclear. Dysregulation of Wnt/β-Catenin signaling has been associated to different types of cancer. Moreover, its inhibition has been proposed as adjuvant in TMZ treatment in a vast number of tumors. Our objectives were to evaluate in prolactin secreting experimental models *in vivo* and *in vitro*, the effect of TMZ on critical processes for tumor growth and in particular on Wnt/β-Catenin signaling activation. Rat prolactinoma MMQ cells were treated *in vitro* with 200 μM TMZ (or DMSO as control) or were subcutaneously injected in Nu/Nu mice for *in vivo* experiments. After tumor development, 15mg/kg TMZ (or control) were orally administered to animals. TMZ treatment markedly reduced prolactinoma hormone production at mRNA ($p=0,0001$) and protein levels ($p=0,0066$), and slightly restrained tumor growth *in vivo*. Among Wnt pathway components, TMZ reduced *β-Catenin* ($p=0,0025$) and *CyclinD1* ($p=0,0001$) mRNA expression *in vivo*. β-Catenin strongly correlated with PRL ($p=0,0086$) and *CyclinD1* ($p=0,0001$) in this model. *In vitro*, TMZ inhibited tumor lactotroph cells proliferation ($p=0,005$), increased cell apoptosis rate ($p=0,042$) and promoted G2/M cell cycle arrest ($p=0,013$). PRL showed a trend of reduction and β-CATENIN activation was inhibited by TMZ ($p=0,048$). With regard to angiogenesis, VEGF secretion was inhibited *in vitro* while no effects were observed *in vivo*. We conclude that TMZ reduces prolactin production and restrains tumors growth in experimental prolactinoma models, and acts in part by inhibiting β-Catenin activation. These findings open the opportunity of new therapies for resistant prolactinomas.

INMUNOLOGÍA / IMMUNOLOGY ORAL SESSION 5

489. (498) **INDUCTION OF HIF-1A BY HIV-1 INFECTION IN CD4 T CELLS PROMOTES VIRAL REPLICATION AND DRIVES EXTRACELLULAR VESICLE MEDIATED INFLAMMATION**

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Background: Human Immunodeficiency Virus type 1 (HIV-1) is a very important global pathogen that preferentially targets CD4+ T cells and causes Acquired Immunodeficiency Syndrome (AIDS) if left untreated. Although antiretroviral treatment efficiently suppresses viremia, markers of immune activation and inflammation remain elevated in HIV-1 infected patients. Our aim was to analyze whether HIF-1α (a transcription factor that coordinates cellular metabolism and immune functions) and Extracellular Vesicles (EVs), play a role in HIV-1 associated inflammation.
Methods: CD4+T cells isolated from the blood of healthy donors were activated with anti-CD3/CD28 antibodies and infected in vitro with HIV-1. Then, EVs purified by differential centrifugation were added to non-infected CD4+T cells and macrophages. Supernatants were harvested 24 h later and cytokine production was evaluated by CBA kit. To evaluate the role of mitochondrial ROS (mROS) and

HIF-1α signaling in the production of proinflammatory EVs, we used the pharmacological inhibitors MitoTEMPO(500μM) and Equinomycin(1nM) respectively. HIV-1 replication was evaluated measuring p24 expression by FACS and ELISA.
Results: We show that cytosolic dsDNA generated in infected CD4+ T cells during the HIV-1 replication cycle promotes the mitochondrial ROS-dependent stabilization of the transcription factor Hypoxia Inducible Factor-1α (HIF-1α), which in turn, enhances viral replication ($p<0.001$). Furthermore, we show that induction of HIF-1α promotes the release of pro-inflammatory Extracellular Vesicles (EVs). These EVs foster inflammation by inducing the secretion of interferon-γ by bystander CD4+ T cells ($p<0.05$) and of IL-6 and IL-1β by bystander macrophages through an mROS/HIF-1α-dependent pathway ($p<0.05$).
Conclusion: This study demonstrates that HIF-1α plays a crucial role in HIV-1 pathogenesis by promoting viral replication and the release of EVs that orchestrate lymphocyte and macrophage-mediated inflammatory responses. These results pave the way to explore the possibility of targeting HIF-1α and/or EVs pathway to restore immune condition in HIV-1 infected individuals.

490. (595) **SEMEN EXOSOMES INHIBIT THE ZIKA VIRUS INFECTION ON DENDRITIC CELLS**

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Surprisingly, it has been reported sexual transmission of Zika virus (ZIKV), changing our view of arbovirus-host interaction. Infectious ZIKV persist in semen after symptoms onset at higher viral loads. Seminal plasma (SP) contains trillions of exosomes, small extracellular vesicles that mediate intercellular signaling. Knowing that semen is not merely a carrier for sexually transmitted diseases; our aim was to evaluate the role of semen exosomes (SE) on ZIKV infection of dendritic cells (DCs).
SP and monocytes were obtained from healthy donors. SE were purified using size exclusion chromatography. DCs were obtained from monocytes (GM-CSF/IL-4, 5days). ZIKV binding and infection was evaluated by qPCR, UFP/ml or by flow cytometry. Phenotype was analyzed by flow cytometry and cytokines production by ELISA. We infected DCs (2X10^5) with ZIKV (5x10^5 UFP) in the presence or absence of SP(1/1000-dil), SP-SE-depleted (1/1000-dil) or SE (200ug/ml) for 48hs. We observed the presence of SP abrogated the infection, while SP-SE-depleted not ($p<0.05$-n=3). SE also abrogate ZIKV infection ($p<0.05$-n=4). Exosomes purified from blood didn't exert virus inhibition (n=2). DCs (1X10^6) were treated with SE (200ug/ml) 60min and then incubated with ZIKV (5x10^5 UFP) 2hs, cells were washed twice to remove free virus. We observed that ZIKV binding to DCs (n=5) was not altered in SE treated DCs ($p<0.05$-n=7), but when we measured the expression of IFN-β mRNA, we found that SE induce an increase IFN-β expression in DCs incubated with ZIKV ($p<0.05$-n=6), suggesting that IFN-β is involve in the abrogation of ZIKV infection on DCs.
We analyzed the functionality exerted by SE to infected DCs. DCs exposed to SE showed an up-regulation of CD86 and CD83 ($p<0.05$-n=4), a decrease of IL-12 ($p<0.05$-n=2) and IL1-β ($p<0.05$-n=2) production and an increase of IL-10 ($p<0.05$-n=2). Our observations suggest that SE abrogate ZIKV infection on DCs by inducing an increase expression of IFN-B.

491. (602) **THE GOOD SIDE OF INFLAMMATION: STAPHYLOCOCCUS AUREUS PROTEINS SPA AND SBI CONTRIBUTE TO ABSCESS FORMATION AND RESOLUTION OF SSTI**

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Staphylococcus aureus is the major cause of skin and soft tissue infections (SSTI). During SSTI the inflammatory response is critical to contain the bacteria. This study was aimed at elucidating the contribution of staphylococcal proteins SpA and Sbi, two important inducers of inflammation, to the development of abscess and resolution of skin infection. Mice were inoculated by the subcutaneous route with $1x10^8$ CFU of *S. aureus* strain Newman or the isogenic mutants that do not express Sbi (Sbi-) or SpA (SpA-). At day 3 after inoculation, skin lesions from mice inoculated with the wild type strain had the structure of organized abscess with defined fibrous walls and focalized polymorphonuclear infiltrated surrounding the bacterial community. On the contrary, skin lesions from mice challenged with the SpA- or Sbi- mutants showed a disorganized structure with pour fibrous capsule formation, low neutrophil focalization, higher number of bacterial colonies and extended necrosis in dermis and epidermis. By day 7 post-inoculation, only mice challenged with the wild type strain exhibited evidence of wound healing, including epidermal regeneration and evidence of hair follicle development. Conversely, mice inoculated with the SpA- or Sbi- mutants presented extended epidermal necrosis and ulceration. By day 7 decreased numbers of bacteria were only found in the abscess of wild type inoculated mice ($p<0.01$). Proper abscess formation and resolution of the skin injury found in mice challenged with the wild type strain correlated with increased levels of IL-1β, IL-6, TNF-α and CXCL10 ($p<0.05$) as well as CXCL1 ($p<0.01$) in the abscess compared to those induced in response to the SpA- mutant. Taken together these results indicate that the expression of SpA or Sbi is critical for the induction of the inflammatory response that leads to proper abscess formation and resolution of the infection during staphylococcal SSTI.

492. (681) **THE ARYL HYDROCARBON RECEPTOR AS A POTENTIAL THERAPEUTIC TARGET AGAINST DENGUE VIRUS INFECTION**

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The aryl hydrocarbon receptor (AHR) is a ligand-activated transcription factor that has been classically associated with the mechanism of clearance of xenobiotics. Recently, numerous studies have indicated that AHR has the ability of modulate the immune system. It has been shown that AHR is involved in a negative feedback mechanism with NFκB. Regarding to viral infections, it was shown that the stimulation of AHR reduces the survival of mice infected with influenza A virus. Dengue virus (DENV) belongs to the Flaviviridae family and has four different serotypes (DENV-1,2,3,4) that are capable of causing illness in humans after the bite of infected mosquitoes of the genus Aedes. DENV is considered the most important arthropod-borne virus all over the world. The only vaccine that is been developed had shown low effectivity and no specific treatments exists, thus the development of new antiviral strategies is crucial. In the present study we have evaluated the possibility of modulating the activity of AHR to control DENV infection in vitro. In order to achieve this, we used AHR ligand agonists and antagonists to modulate the activity of the receptor to treat A549 cell cultures before the infection with DENV1-4. From the supernatants of the cultures, titrations were carried out to evaluate the viral yields. Also, real-time RT-PCR and indirect immunofluorescence to quantify the viral genome and protein, respectively, were carried out. The treatment with 20μM of the AHR antagonist CH223191, not only decreased the viral yield in a 95±4%, but also diminished the viral protein expression in a 84,5±0,5%. The preliminary data obtained, allowed us to demonstrate that the AHR signaling pathway is involved in the DENV replication process in vitro, and it is a potential therapeutic target against flavivirus infection.

493. (689) **EXPRESSION OF GAL-1, GAL-3 AND GAL-9 IN MACROPHAGES INFECTED WITH MTB AND ITS MODULATION BY CORTISOL AND DEHIDROEPIANDROSTERONE.**

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Tuberculosis (TB) is an ancient disease caused by the intracellular pathogen, *Mycobacterium tuberculosis* (Mtb). We have previously demonstrated that patients with pulmonary TB presented increased plasma levels of pro- and anti-inflammatory cytokines, cortisol, Galectin-1 (Gal-1) and Gal-3, along with a marked decrease in dehydroepiandrosterone (DHEA). Macrophages, which play a central role in the response against Mtb, were also found modulated by cortisol (Gc) and DHEA. At physiological conditions, DHEA (in presence of Gc) increased phagocytosis, autophagy induction, while decreasing the number of colony-forming units. Expanding this issue and given the central immunomodulatory role of galectins, we have studied the expression of Gal-1, Gal-3 and Gal-9 mRNA in cultured macrophages (derived from THP-1 cell line: Mf -THP-1) infected with Mtb in presence or absence of Gc (1 µM) and/or DHEA (0.1 µM), during 24 h (n= 5/treatment). Mtb-infection increased the expression of Gal-3 mRNA in Mf-THP-1 compared with uninfected ones ($p<0.03$), with Gal-1 and Gal-9 showing no differences. Treatments with hormones separately did not modify the Mtb-induced effect. However, the combination of both hormones significantly increased Gal-9 ($p<0.01$) and decreased Gal-1 ($p<0.05$) respect to only stimulated cultures; implying an important role in Mf-THP-1 microbicidal functions.

494. (750) **SPECIFIC TREG CELL DEPLETION ALLOWS THE EMERGENCE OF PARASITE-SPECIFIC CD8+ T CELL IMMUNITY DURING TRYPANOSOMA CRUZI INFECTION**

Cintia Araujo Furlan, Santiago Boccardo, Constanza Rodriguez, Facundo Fiocca Vernengo, Jimena Tosello Boari, Adriana Gruppi, Carolina Lucia Montes, Eva Virginia Acosta Rodriguez

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Regulatory T cells CD4+Foxp3+ (Tregs) present dual roles during infections as they limit immunopathology but also restrain effector immune responses. During *Trypanosoma cruzi* (Tc) infection, Tregs role remain controversial. We previously demonstrated that after Tc infection, Tregs undergo a marked reduction in frequency at peripheral organs that was sustained over time. In the acute phase, Tregs became activated and acquired a phenotypic and transcriptional profile consistent with suppression of type 1 inflammatory responses. Additionally, the increase in Treg numbers by adoptive transfer experiments resulted in an impaired CD8 response accompanied by increased parasite levels. In order to further assess the biological relevance of the relative reduction in Tregs frequency during Tc infection, we evaluated here whether specific depletion of Tregs at different time points modulates the magnitude of effector responses, parasite burden and tissue damage. For this purpose, DEREG mice were infected with 5000 trypomastigotes and at days (d) 5 or 11 post-infection (pi) were injected with diphtheria toxin (DT) or PBS as control. A kinetic analysis revealed that while no differences were observed by DT-treatment at d11pi, mice injected at d5pi showed reduced parasite burden ($p\leq0.0006$) and increased Tc-specific CD8+ T cells frequency and absolute numbers ($p\leq0.0098$) in spleen and liver at d19pi, in comparison to PBS-injected counterparts. Furthermore, DT-treatment at d5pi also improved CD8 effector function, as shown by the increase in the frequency of splenic Tc-specific CD8+ cells able to degranulate (CD107a+) and produce IFN-gamma and/or TNF upon *in vitro* parasite stimulation in comparison to the control group ($p\leq0.0166$). Finally, Tregs depletion at 5 or 11 dpi did not alter the activity of biochemical markers of tissue damage in comparison to PBS-treated mice. Our results suggest that limited response of activated Tregs during Tc infection is necessary for the emergence of protective anti-parasite CD8+ T cell immunity.

CARDIOVASCULAR RESPIRATORIO I

CARDIOVASCULAR RESPIRATORY 3

495. (99) **ROLE OF AMP ACTIVATED PROTEIN KINASE (AMPK) IN THE CARDIOPROTECTIVE EFFECTS EXERTED BY TRIIODOTHYRONINE (T3) IN THE MYOCARDIUM SUBJECT TO ISCHEMIA-REPERFUSION**

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Experimental evidence supports the hypothesis that thyroid hormones play an effective role in the cardioprotection against ischemia-reperfusion injury. Recent studies have suggested that T3 could activate AMPK, a key enzyme that regulates the cellular energy metabolism, which in previous studies carried out in our laboratory exerted beneficial effects for the recovery of ischemic-reperfused myocardium.

The aim of the present study was to investigate the effects produced by the acute treatment with T3 (60 nM) and the pharmacological inhibition of AMPK by Compound C (CC; 10 µM), in isolated rat left atria subjected to 75 min simulated ischemia (Is)-75 min reperfusion (Rs). Atria were incubated in Krebs-Ringer containing 10 mM glucose, 95% O_2 - 5% CO_2, pH 7,4. For Is, the incubation medium contained 10 mM 2-deoxy-D-glucose, 95% N_2 - 5% CO_2, pH 6,8. ANOVA, followed by Tukey test was used, n=8/group.

Results showed that T3 increased AMPK activation during Is, which was prevented by CC (End stabilization period (ESP): 1.50±0.09, Is-Rs: 2.34±0.02*, Is-Rs+T3: 2.70±0.07*#, Is-Rs+CC: 1.30±0.08, Is-Rs+T3+CC: 1.33±0.07 AU; *p<0.05 vs ESP, Is-Rs+CC, Is-Rs+T3+CC; #p<0,05 vs Is-Rs). During reperfusion T3 increased contractile function recovery, which was prevented by CC (Is-Rs: 37.1±3.6, Is-Rs+T3: 51.2±1.6*, Is-Rs+CC: 37.8±2.1, Is-Rs+T3+CC: 35.6±3.8; *p<0.05 vs all groups). Mitochondrial ATP synthesis rate and tissue ATP content was enhanced by T3, effect that was reverted by CC (Is-Rs: 32.0±6.2, Is-Rs+T3: 51.1±6.6*, Is-Rs+CC: 28.3±3.8, Is-Rs+T3+CC: 39.8±2.4 nmol/min/mg mitochondrial protein; Is-Rs: 434±54, Is-Rs+T3: 608±94*, Is-Rs+CC: 304±50, Is-Rs+T3+CC: 286±48 pmol/mg tissue protein; *p<0.05 vs all groups). Cellular viability was enhanced by T3, effect prevented by CC (Is-Rs: 66±4, Is-Rs+T3: 79±3*, Is-Rs+CC: 57±2, Is-Rs+T3+CC: 58±3; *p<0.05 vs all groups).

The results suggest that AMPK is involved, at least in part, in the protective effects exerted by T3 in the myocardium subjected to ischemia-reperfusion, increasing contractile recovery, mitochondrial ATP production and cellular viability.

496. (150) **THE ROLE OF THIOREDOXIN 1 IN THE ISCHEMIC POSTCONDITIONING DURING EARLY STAGES OF ATHEROSCLEROSIS**

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Thioredoxin-1 (Trx1) has cardioprotective effects against ischemia/reperfusion (I/R) injury, and participates in ischemic postconditioning (PostC). However, whether Trx1 expression is altered and the PostC protective effect is abolished during early stages of atherosclerotic disease is unknown. The objective were evaluate whether a murine high-fat diet (HFD)-fed model that consistent with early stages of atherosclerosis increased oxidative stress and consequently abolished the cardioprotection conferred by PostC. We used C57/BL6 mice fed with control diet (CD) or HFD for 12 weeks. Isolated mice hearts were subjected to 30min of ischemia and 120min of reperfusion (I/R group). For PostC group, after ischemia, six cycles of R/I were performed (10sec per cycle) at the onset of reperfusion. We assessed infarct size and Trx1 expression. Also, we measured oxidative stress by hydrogen peroxide (H2O2) production and levels of reduced (GSH) and oxidized (GSSG) glutathione. We evalueted mitochondrial function on state 3 and 4. In CD-PostC group reduced infarct size (CD-I/R: 52.14 ± 2.8 vs. CD-PostC: 36.58 ± 1.8, P< 0.05), and this cardioprotection was abolished in HFD-exposed mice. HFD increased H2O2 levels (CD: 0.91±0.09nmol/mg protein vs. HFD: 1.27±0.15nmol/mg protein, P<0.05), produced a shift towards an oxidized intracellular environment (GSSG/GSH2), and increased Trx1 expression with higher fractions of oxidized protein (reduced Trx1/oxidized Trx1: HFD-Baseline 23% lower than in CD–Baseline). HFD fed mice had higher oxidized Trx1 levels and therefore, when carrying out an I/R or PostC protocol, no significant changes were observed among the groups (decreased reduced Trx1/oxidized Trx1: HFD-I/R: 29%; HFD-PostC: 31%). State 3 mitochondrial oxygen consumption in basal conditions decreased 24% in HFD-exposed mice and PostC improved state 3 values only in CD mice. We demonstrated that alterations in redox state at early stages of atherosclerosis abolished cardioprotective induced by PostC, even with increased Trx1 levels.

497. (587) **THIOREDOXIN-1 IS REQUIRED FOR THE CARDIOPROTECIVE EFFECT OF SILDENAFIL AGAINST ISCHEMIA-REPERFUSION INJURY AND MITOCHONDRIAL DYSFUNCTION IN MICE.**

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Sildenafil is a phosphodiesterase type 5 inhibitor indicated in erectile dysfunction and pulmonary hypertension, which confers cardioprotection against myocardial ischemia/reperfusion (I/R) injury. Thioredoxin-1 (Trx1) is a protein which contains redox-sensitive cysteine residues and acts as an antioxidant in cells. The aim of this study was to determine if Trx1 system participates in cardioprotection exerted by sildenafil in an acute model of I/R, and to evaluate mitochondrial bioenergetics. Langendorff-perfused hearts from wild type mice (WT) and a dominant negative (DN-Trx1) mutant (C32S/C35S) of Trx1 were assigned to placebo or sildenafil (0.7 mg/kg i.p.) and subjected to 30 min of ischemia followed by 120 min of reperfusion. WT mice treated with sildenafil showed a significantly smaller (41%) infarct size. This protective effect was not observed when sildenafil treatment was administered to DN-Trx1 mice. After I/R, treatment with sildenafil preserved state 3 oxygen consumption from WT mice (137.9±7.6 vs. 140.9±11.0, P<0.05) but had a milder effect in DN-Trx1 mice only partially protecting state 3 values (113.7±3.0 vs. 135.8±7.2, P<0.05). Treatment of WT mice restored respiratory control (RC) after I/R, which resulted only 8% lower than in basal conditions. The same treatment in DN-Trx1 mice was not as effective as in WT mice and RC values after I/R was 24% lower than in basal conditions. We show for first time that active Trx1 is required for the onset of the cardioprotective effects of sildenafil on I/R injury and the preservation of mitochondrial function.

498. (688) **SYMPATHETIC HYPERACTIVITY ABOLISHES THE MYOCARDIAL PROTECTIVE EFFECT OF VAGAL STIMULATION IN MICE WITH CARDIAC GSA OVEREXPRESSION**

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Although important myocardial protective effects of vagal stimulation (VS) have been demonstrated experimentally, the clinical results are not entirely conclusive. We hypothesized that the dysautonomia that accompanies heart disease may interfere with myocardial protection. The objective was to study whether sympathetic cardiac hyperactivity abolishes the protective effects of classical preconditioning and preconditioning mimicked by VS in transgenic mice with cardiac overexpression of GSα protein.

Transgenic FVB mice with specific cardiac overexpression of the GSα protein (TG) and their respective non-transgenic controls (WT) were subjected to 30 min of regional ischemia and 2 hours of reperfusion (I/R). Both TG and WT mice received VS for 10 min before ischemia or 3 preconditioning cycles for 5 min of ischemia and 5 min of myocardial reperfusion (PC) before prolonged ischemia (n= 5-7 per group). All animals were catheterized to measure ventricular function. Hearts were dyed with Evans Blue and incubated in TTC to measure the infarct size (IS).

The heart rate (HR) of the WT and TG mice was 438±11 and 664±14 (p<0.05), respectively. VS reduced HR by 10% in WT animals (p<0.05 vs basal), and only by 6% in TG mice (p=NS vs basal). The VS and the PC significantly reduced the IS with respect to the control group in the WT groups (I/R-WT: 56±2%, VS-WT: 44±3%, PC-WT: 35±2%) (p<0.05). The IS of the I/R-TG group was similar to the non-transgenic group (I/R-TG: 57±5%, p=NS vs I/R-WT). However, in the TG animals the protection of VS (55±5.0%) and PC (52±2.0%) was lost (p=NS vs I/R-TG).

In conclusion, preischemic VS reduced infarct size to a similar degree to ischemic PC. This protective effect of VS and PC was not observed in TG mice with cardiac sympathetic overactivity due to overexpression of GSα protein.

499. (35) **PERICARDIAL TISSUE REGENERATION BY URINARY BLADDER MATRIX SCAFFOLDS IN A PRECLINICAL PORCINE MODEL**

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Closing the pericardium after cardiac surgery is recommended to prevent postoperative adhesions to the sternum. However, it is frequently not possible. Synthetic materials have been used as substitutes, with limited results due to impaired remodeling and fibrotic tissue formation. Extracellular matrix scaffolds, specifically urinary bladder matrix scaffolds (UBM-ECM), recruit and facilitate the incorporation of native cells to promote constructive remodeling that resemble more the native tissue.

Aims: Evaluate the host response to UBM-ECM scaffolds in pericardium defects closure after cardiac surgery; with focus in magnitude, strength and quality of adhesions and epicardial retraction, in a preclinical porcine model.

12 Landrace pigs were subjected to median sternotomy and a 5x7cm pericardial defect was created. The test group (n=6) was subjected to UBM-ECM repair with a device of 5x7cm. Animals in the control group (n = 6) were left with the pericardium open defect.

Animals were euthanized at 8 weeks. Endpoints included cardiac function (Echocardiography), gross morphology with adhesion assessment, mechanical testing and histology.
Softer adhesions were found in ECM group, but strong adhesions and injury of the coronary bed were found in control group. The load at failure showed no differences between the ECM and native pericardium (199.9±59,2g vs. 405.3± 99.89g P=0.0536), but tissue was weaker than native pericardium (44.23±15.01g/mm vs. 146.5±24.38g/mm P<0.01). In ECM pigs, histology resembled that of native pericardium, with neovascularization vessels and few signs of inflammation. Control pigs showed fibrotic tissue with mononuclear infiltrate and no organized collagen fibers. Both groups had normal results without cardiac motility disorders.
Conclusions: In this experimental setting, ECM scaffolds contributes to enhance pericardial repair with tissue characteristics that protect against the formation of postoperative retrosternal adhesions, whereas cardiac function is not affected by the implant.
Keywords: Pericardic tissue repair, Biologic scaffold, Pericardioplasty, Extracellular matrix.

500. (92) **CARDIOPROTECTION BY A CANNABIS SATIVA SP EXTRACT AGAINST ISCHEMIA-REPERFUSION INJURY: INVOLVED MECHANISMS**

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The aim was to determine the effects of acute treatment of a cannabinoid extract (CBE), obtained from Cannabis Sativa sp against ischemia-reperfusion injury. The extract was characterized and quantified by gas chromatography coupled to mass spectrometry (GC-MS). The profile was: Δ9-tetrahydrocannabinol (Δ9-THC) : 1,35mg/ml; cannabidiol (CBD): 0,51mg/ml and cannabinol (CBN): 0,34mg/ml. Isolated rat hearts perfused by Langendorff system were assigned to the following experimental groups: Non-ischemic control (NIC): 110 min of perfusion; Ischemic control (IC): 30 min of global ischemia (GI) and 60 min of reperfusion (R) and CBE: 0,1ug/ml of the extract was administered during the first 10 min of R. Infarct size (IS) was determined by TTC staining. Systolic and diastolic function was assessed by left ventricular developed pressure (LVDP) and the left ventricular end diastolic pressure (LVEDP), respectively. To assess the oxidative stress, the glutathione reduced (GSH) content and the concentration of thiobarbituric acid reactive substances (TBARS) were measured. The expression of phosphorylated forms of eNOS, PKCε and Akt were also determined by western blot. CBE significantly decreased IS (2.3 ± 0.5 % vs. 31 ± 2 % in IC) and improved the post-ischemic recovery of myocardial function (LVDP: 64 ± 9 % vs. 17 ± 3 %; LVEDP: 15 ± 7 vs. 35 ± 9 mmHg) compared to IC group. The GSH content significantly increased (0.86 ± 0.10 vs. 0.78 ± 0.11 µg/mg protein) and TBARS decreased (0.55 ± 0.05 vs. 1.10 ± 0.45 nmol/mg protein) in CBE treated hearts. The expression of P-eNOS, P-PKCε and P-Akt decreased approximately 60 % in IC and increased approximately 150 % in CBE, both compared to NIC. These data demonstrate that CBE reduced the cell death and myocardial contractile post-ischemic dysfunction and attenuated the oxidative damage produced by ischemia-reperfusion. These beneficial actions appear mediated by Akt/PKCε/eNOS-dependent pathways.

501. (430) **MORPHOFUNCTIONAL BEHAVIOR OF CENTRAL BLOOD PRESSURE PULSE WAVES AND ITS POTENTIAL ASSOCIATION WITH HEMODYNAMIC PARAMETERS IN HYPERTENSIVE PATIENTS**

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Evaluation of central blood pressure pulse wave (CBPPW) could be useful as prognostic markers of arterial hypertension , its morfology is determinated by ventricular ejection and arterial elasticity. The application of fractal algorithms in CBPPW provides a mathematical model which could predict their conduct. The conservation of fractal features could estimate the adaptative capacity of the system. It is proposed to analyze the morphofunctional behavior of CBPPW by fractal algorythm and its potential correlation with systolic blood pressure (SBP), diastolic blood pressure (DBP), stroke volume (SV), and hart rate (HR). 260 CBPPW from adults, non-trated, hypertensive patients, average age 57,8±14,2 years, have been considered. Higuchi's algorithm (HA) was applied in systolic peaks (sp) and diastolic valleys (dv) from digitalized images of CBPPW obteined by Mobil-O-Graph. The CBPPW registered: SBP [136,4±18,35 mmHg], DBP [86,7 ±15,4 mmHg], SV [73,97±12,44 ml] and HR [71,81 ±12,97 bpm]. Fractal dimension (FD) and the Coefficient of correlation (R2) (below 0.8 would indicate the loss of fractal features) were determinated. Results: mean (M) and standard deviation (±) of DF and R2 were calculated: M=0,042±0,02; R2(sp): M=0,52±0,31; FD(dv): M=0,091±0,017; R2(dv): M=0,56±0,33; Pearson's coefficient of correlation (r) was obtained between FD stablished with SBP, DBP, SV and HR: FD(ps) vs SBP: r=-0,001 (p=0,96); DF(ps) vs DBP: r=0,09 (p=0,46); FD(ps) vs SV: r=0,06(p=0,59); FD(sp) vs HR: r=0,15(p=0,16); FD(vd) vs SBD: r=-0,08(p=0,5); FD(dv) vs DBP: r=0,21(p=0,082); FD(dv) vs SV: r=-0,37(p=0,0016); FD(dv) vs HR: r=0,23(p=0,039). HA could reveal loss of fractal features of the CBPPW. The correlation of variables suggests that FD(ps) does not manage to associate with hemodynamic variables as FD(vd) does, therefore, SBP, DBP, SV and HR associates its behavior to dv in advance of sp of CBPPW, in hypertensive patients.

502. (555) **MYOCARDIAL CELL DAMAGE IN A MODEL OF MINIMAL HEPATIC ENCEPHALOPATHY**

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Sarcopenia and heart failure are common features of chronic liver disease (CLD). The loss of skeletal muscle (SM) and its function contributes to morbidity and mortality. Its prevalence in patients with CLD is estimated at 40-70%. Hepatic encephalopathy (HE) has a similar prevalence and, in addition, there is a correlation between the two. The CLD decreases the detoxification capacity of ammonia, so SM and astrocytes become the main route of ammonia, mainly through glutamine synthetase (GS). We hypothesized that a model of subclinical hepatic encephalopathy (MHE) showing moderate hyperammonemia and almost normal liver could show a starting point for early events in myocardial (HM) damage. The Wistar rats were divided into 2 groups, (i) simulated surgery and (ii) group with stenosed portal vein, MHE. GS, oxygen consumption, nitric oxide production, high resolution light microscopy (HRLM) and electron transmission microscopy (ETM) were evaluated, after 14 days of surgery, in left HM. The results showed a significant increase in GS ($p < 0.01$) in the MHE group with reduced oxygen consumption and a significant decrease in nitric oxide. HRLM showed that the HM triad (sarcolemma, T-tubes and sarcoplasmic reticulum) was widely dilated with a convergent structure. This membrane traffic system was undulated with an increased surface, to the detriment of the fibrillar structure. ETM, confirmed these, showing subcellular edema, with detachment of fibrillar structures, swollen mitochondria with loss of ridges and matrix density, disruption of the nuclear membrane and an increase in the number and size of subsarcolemal vacuoles. The triad was also altered showing the dilatation of this system and the focal interruption. The most relevant ETM data were focal myofibrillolysis. These results suggest that under these conditions, in early

stages of HE, hyperammonemia could induce myocardial cell damage, with or without CLD.

503. (580) **EFFECT OF GALECTIN 3 ON VENTRICULAR REMODELING AFTER MYOCARDIAL INFARCTION IN MICE**

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We have previously showed that the lack of Galectin 3 (Gal3KO) reduced the macrophages (MØ) infiltration, alters MØ polarization and fibrosis during the early wound healing after myocardial infarction (MI). Here, we aimed to study the effect of genetic mutation of Gal3 on early and chronic ventricular remodeling (VR) in mice. Adult male C57 and Gal3KO mice were subjected to permanent coronary artery ligature or SHAM. After 1 and 4 weeks (w) post-MI echocardiography was performed and ejection fraction (EF %) was calculated. Then, animals were euthanized and the hearts were harvested, snap frozen or fixed in formaldehyde. Infarct size and fibrosis were quantified in cardiac slices stained with Masson's Trichrome and Picrosirius Red from animals of 1w and 4w post-MI. Myocytes cross sectional area (MCSA) was also measured in slices stained with Rhodamine in 4w animal group. Results: X±SEM; * p<0.05 C57 vs Gal3KO. At 1w post-MI EF (%) was reduced from 47.3±2.3 to 37.5±3.3* and fibrosis in infarct zone from 30±1.2 to 18±0.4* while MMP2 activity (RU) increased from 0.8±0.1 to 2.0±0.4* in C57+MI and Gal3KO+MI respectively. Fibrosis at remote zone was 1.7±0.4 (C57+MI) and 1.3±0.1 (Gal3KO+MI, P=NS). At 4 w post–MI EF% was 40.25±3.8 (C57+MI) and 35.5±3.7 (Gal3KO+MI, P=NS). Infarct size (%) was similar between groups 33±2.9 and 30±4.9 while fibrosis at the MI zone (%) was reduced from 86±0.7 to 70±2.1* in C57+MI and Gal3+MI respectively. At remote zone, collagen concentration (%) was reduced from 3.5± 0.5 and 1.9±0.6*, while MCSA (mm2) was 559±17 and 520±10 in C57+MI and Gal3KO+MI respectively. In summary, the lack of Gal3 affected the evolution of the early wound healing and unfavorable modified the early and chronic ventricular remodeling after MI in mice.

504. (785) **EFFECTS OF PRE-ISCHEMIC VAGAL STIMULATION ON THE INFARCT SIZE AND LEFT VENTRICULAR REMODELING IN A MYOCARDIAL ISCHEMIA AND REPERFUSION MICE MODEL**

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The objective was to study the effects of brief vagal stimulation (VS) applied before ischemia on acute myocardial infarction and its long-term evolution, analysing the left ventricular remodeling in an experimental ischemia/reperfusion model. Mice were randomly assigned to different protocol groups (n=5-6). Animals underwent regional myocardial ischemia during 45 min, followed by either 2 h or 28 days of reperfusion, with or without 10 min of pre-ischemic vagal stimulation (VS) and every protocol had their respective Sham group. The following parameters were measured: left ventricular function (LVF), by catheterization of the carotid artery; infarct size (IS) by TTC; Ejection Fraction (EF), Shortening Fraction (SF), Isovolumetric Relaxation Time (IVRT) by echocardiography and morphometric analysis was determined by comparing left ventricle weight with the length of the tibia (LVW/LTi) and the tail (LVW/LT). VS+IR-2h had smaller IS compared to IR-2h (45±2% and 67±3%, respectively, p<0.001). IR-28d group showed a LVEDP of 6.91±1 mmHg which is significantly higher than Sham-28d (3.81±0.2 mmHg; p<0.01). Likewise, there is a decrease in the EF (IR-28d: 58.8±3.1 vs Sham-28d: 74±1.6, p<0.01), SF (IR-28d: 32.7±2.6 vs Sham-28d: 35.5±1.4, p<0.01), and a difference in the IVRT (IR-28d: 30.3±1.2 vs Sham-28d: 21.3±1.1, p<0.001). Pre-ischemic VS improves this data: EF (VS+IR-28d: 68.6±3.7 vs IR-28d, p<0.05), SF (VS+IR-28d: 32.7±2.6 vs IR-28d. p=n.s.) and IVRT (VS+IR-28d: 23.7±1.2 vs IR-28d. p<0.01). Finally, data on LVW/LTi in Sham-28d was 4.81±0.06 while in IR-28d raised to 5.64±0.4 (p<0.05) and didn't changed in VS+IR-28d (5.71±0.3, p=n.s.) In conclusion, VS applied before ischemia confers cardioprotection, reducing the acute infarct size and improving long-term LVF.

505. (706) **EFFECTS OF INTENSE EXERCISE IN A MODEL OF SIMPATIC HYPERACTIVITY IN MICE**

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The aim was to study the effects of intense exercise (IE) training on myocardial hypertrophy, ventricular function (VF) and structural histological parameters in mice with and without specific cardiac sympathetic hyperactivity by overexpression of the protein Gsα. Male FVB (noTG) and Gsα protein overexpression (TG) mice were randomized in sedentary (SED) and IE group. Mice were subjected to IE performed by swimming during 90 minutes twice a day during 6 days/week. After 4 weeks, VF and myocardial response to Isoproterenol (ISO) was studied in catheterized animals. Myocardial collagen concentration, myocytes cross sectional area (MCSA) and capillary density was also quantified. Results: X±SEM (*p<0.05). At baseline, systolic function as compared SEDnoTG and SEDTG with noTG+IE and TG+IE respectively was similar. IE increased the relaxation time (t_{63}, msec) in noTG+IE (10±0,3) as compared with SEDnoTG (8±0,7*). Also reduced the response to ISO as evaluated by +dP/dt (mmHg/sec) from 78±14%, in SEDnoTG to 34±9%* in noTG+EI; heart rate from t_{63}73±7% in SEDnoTG to 27±6%* in noTG+EI and prolonged the t63 from -6±4% in noTG+EI to -29±6%* in SEDnoTG. In contrast, no differences was observed in ISO response in TG+IE. IE tended to increased the collagen concentration and capillary density either in noTG and TG group (P=NS). Finally, IE significantly increased MCSA (μm^2) in both control and TG group from 254±14 in SEDnoTG to 471±20* in noTG+EI and from 354±19 in SEDTG to 527±23* in TG+EI. Our results suggest that IE does not modify structural parameters, however, in nonTG mice reduce myocardial reserve in response to ISO. The presence of sympathetic hyperactivity in mice that overexpress Gsα subjected to IE, showed similar behavior although did not show significant differences, suggesting that overexpression of Gsα can be a useful tool to understand the effects produced by sympathetic hyperactivity in the VF after perform IE.

REPRODUCCIÓN / REPRODUCTION 3

506. (223) **LARGE-CONDUCTANCE CALCIUM-ACTIVATED POTASSIUM CHANNELS (BKCA) MODULATE TESTICULAR MACROPHAGES OXIDATIVE STATE: POSSIBLE IMPLICATIONS IN MALE INFERTILITY.**

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Ion channels play a major role controlling membrane potential. One particular member, the large-conductance Ca^{2+}-activated K^+ channel (BKCa), has been localized to non-excitable cells, i.e. peripheral macrophages (MACs) and Leydig cells.
This study examined the expression of BKCa channels in testicular MACs (tMACs) and its potential role in the regulation of the oxidative state in these immune cells.
We previously described an increase in MAC cell number in testes of men suffering from hypospermatogenesis (H) or Sertoli cell only (SCO) syndrome. Now, we detected, by immunohistochemistry, the expression of BKCa in testes of infertile men. Co-immunolocalization studies revealed BKCa channels expression in CD68-immunopositive MACs, suggesting a possible role of BKCa in tMAC physiology. Moreover, an increase in lipid peroxidation (tiobarbituric reactive species assay, N:7,24±3,42[a]; H+SCO:19,13±2,73[b] pmol MDA/mg tissue; $p<0.05$) and protein expression of catalase and superoxide dismutase 1 (SOD1) was found in infertile testes compared to testes without abnormalities (N) (immunoblot; Catalase, N:1,00±0,11[a]; H+SCO:6,53±1,26[b]; SOD1, N:1,00±0,17[a]; H+SCO:5,60±1,47[b]; $p<0.05$).
Since no functional assays can be performed in testicular biopsy tissues, two alternative experimental models were used: tMACs purified from adult Syrian hamsters and non-testicular human MACs (THP1 cell line). Both MAC models expressed BKCa channel, as assessed by immunocytochemistry and RT-PCR. We used a highly selective BKCa channel blocker, Iberiotoxin (IbTx), to address the impact of BKCa activity on MAC oxidative state. Following 1h incubations in the presence of IbTx (100 nM), lipid peroxidation (B:24,28±3,64[a]; IbTx:42,93±3,86[b] fmol MDA/μg protein) and catalase expression were significantly increased (B:1,00±0,01[a]; IbTx:1,61±0,11[b]), while SOD1 expression was down-regulated (B:1,00±0,02[a]; IbTx:0,39±0,09[b]) in hamster tMACs ($p<0.05$). Similar results were obtained in THP1 MACs.
Thus, our studies describe a modulatory role of BKCa channels in hamster tMACs and human THP1 MACs oxidative state. Whether our results can be extrapolated to human tMAC remains to be elucidated.

507. (317) **IS HUMANIN A CYTOPROTECTIVE FACTOR AGAINST OXIDATIVE STRESS IN OVARIAN CELLS?**
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Humanin (HN) exerts cytoprotection against oxidative stress in cardiac, retinal and pancreatic cells. Previously, we reported that inhibition of endogenous HN in a human granulosa-like tumor cell line (KGN) increases apoptosis and that exogenous HN increases viability of KGN cells. In this study, we aimed to evaluate the action of HN against oxidative stress in ovarian cells. We analyzed the effect of HN on ROS production in cultured KGN cells using a fluorescence indicator (DCF). We incubated KGN cells with DCF (10 μM) for 30 min and HN (1μM) for further 30 min. Afterward, KGN cells were exposed to H2O2 (150 μM) and we analyzed DCF fluorescence. HN per se did not modify ROS production (C: 1689.6 ± 17.5; HN: 1677.6 ± 10.3, ns) but decreased ROS in cultured KGN incubated with H2O2 (H2O2: 2157.3 ± 49.1; H2O2+HN: 1929.3 ± 47.5, $p < 0.05$, Student's t test). Also, we explored the expression and function of HN in Chinese hamster ovary (CHO) cells. We observed that CHO cells express HN by immunofluorescence. Considering that serum withdrawal may induce oxidative stress, we evaluated the action of HN in CHO cells in this condition. HN did not modify CHO cell viability in the presence of serum (C: 1.07 ± 0.04, HN 0.25 μM: 1.09 ± 0.05, HN 0.5 μM: 1.12 ± 0.05, HN 1 μM: 1.12 ± 0.02, ns). However, HN increased CHO cell viability in the absence of serum (C: 0.81 ± 0.04, HN 0.25 μM: 1.00 ± 0.06, HN 0.5 μM: 1.03 ± 0.02, HN 1μM: 0.95 ± 0.06, $p < 0.05$ vs control. ANOVA). Since in a pro-oxidative environment HN increases CHO cell viability and decreases ROS in KGN cells, our results suggest that HN could play a cytoprotective role against oxidative stress in ovarian cells.

508. (547) **THE LIPID TRANSPORT PROTEIN STARD7 LEVELS MODIFIES MITOCHONDRIAL DYNAMICS**
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Mitochondria are dynamics organelles crucial for cell function and survival implicated in oxidative energy production. Mitochondrial lipids affect several important functions such as respiratory metabolism, membrane architecture, protein import, mitophagy and mitochondrial dynamics. StarD7 is a lipid transport protein that carries phosphatidylcholine (PC) to the mitochondria. Previous studies have shown that StarD7 knockdown induces alterations in mitochondria and endoplasmic reticulum morphology with a reduction in mitochondrial PC content, however how different StarD7 levels affects the mitochondrial dynamics was unexplored. Here, we generated a HTR-8/SVneo stable cell line expressing the StarD7-I isoform (StarD7-I) that has a mitochondrial targeting signal. We demonstrated that StarD7 overexpression promotes altered mitochondrial morphology with greater mitochondria motility. Mitochondrial targeting photoactivable (PA-GFP) protein assays indicated that mitochondria from StarD7-I-overexpressing cells were able to produce transient fusions. StarD7-I cells maintain the mitochondrial membrane potential and generate lower ROS levels than control cells. Additionally, an increase in the expression of Drp-1 fission protein was detected in StarD7-I cells. Based on these data, we concluded that StarD7-I overexpression leads to transient mitochondria fusion events without affecting the mitochondrial membrane potential.

509. (548) **PRODUCTION OF REACTIVE OXYGEN SPECIES (ROS) IN EXTRAVILLOUS TROPHOBLAST CELLS: ROLE OF KRÜPPLE-LIKE FACTOR 6**
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ROS are involved in cellular signaling pathways including cell proliferation, apoptosis, and migration. ROS generation and antioxidants action dynamic balance is critical for avoiding cell injury and even cell death by oxidative stress (OS), mainly in high-energy demanding tissues such as the placenta. Indeed, OS is associated with several placental dysfunctions. Krüppel like transcription factor 6 (KLF6) is known to regulate differentiation, proliferation, angiogenesis and apoptosis in different cell contexts. KLF6 immunoreactivity is higher in the placental bed of preeclamptic pregnancies than in those of uncomplicated pregnancies. This correlates with an increase in the migratory capacity of KLF6-silenced HTR8/SVneo extravillous trophoblast cells. Previous results showed an early and transient response of KLF6 to several stressors such as: hypoxia, chlorpyrifos, H2O2 and a decrease of fetal bovine serum in the culture media. Herein, we addressed whether KLF6 modulates redox balance and the possible pathways involved. Downregulation of KLF6 expression in HTR8/SVneo cells transfected with a KLF6-specific siRNA (siK) led to an increase in ROS levels compared to scramble siRNA (siC) transfected cells as measured by flow cytometry using H2DCFDA dye. After 48 h of transfection, ROS levels in siK-cells were higher than those induced in siC-cells treated with 100 μM H2O2 for 3h, but cell viability was not affected. Cell cycle analysis revealed a slightly but significant increase of the population in the sub-G1 and G2/M phases in siK vs siC-cells. KLF6 downregulation also reduced cell proliferation measured by BrdU labeling. Immunofluorescence, western blot, and qRT-PCR assays revealed that neither the canonical antioxidant Nrf2 pathway nor the unfolded protein response were activated. Instead, components of the unfolded protein response, involved in stress survival mechanisms, were downregulated in KLF6-silenced cells. Altogether, these results strongly suggest a new role for KLF6 in redox homeostasis of human trophoblast cells.

510. (575) **OSMOTIC STRESS AND HUMAN AMNION AQUAPORINS**

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Amniotic fluid (AF) is essential for normal fetal growth and development and aquaporins (AQPs) may be crucial in its regulation. To date, four AQPs (AQP1, AQP3, AQP8, AQP9) were described in human amnion and emerging evidence showed that an altered expression of these proteins might be associated with abnormal AF volume such as oligohydramnios or polyhydramnios. However, the etiologies of these syndromes are unknown.
Recently, we demonstrated that AQPs facilitate water transport across the human amnion, being the AQP1 the most important contributor. However, the effect of osmotic stress on the regulation of these proteins was not studied yet.
Our aim was to study the effect of hypo and hyperosmolar stress on the expression and function of the human amnion AQPs.
This study was approved by the ethics committee of the Hospital Nacional Dr. Prof. A. Posadas. Human amnion explants were cultured in hypo (150 mOsm) and hyperosmolar (400 mOsm) conditions generated by dilution of the culture medium or addition of a sucrose solution, respectively. Net transepithelial water movements across the human amnion were measured using a modified Ussing chamber. AQP1, AQP3, AQP8 and AQP9 expressions were assessed by Western Blot and semiquantitative RT-PCR.
Hyperosmolar condition compared to isoosmolar condition, showed a significantly decreased in AQP1 and AQP9 expressions (n=7; $p<0.01$), while AQP8 significantly increased (n=7; $p<0.05$), and AQP3 did not change. Osmotic permeability (pOsm) decreased 44% (n=5; $p<0.001$).
However, in hypoosmolar condition, AQP1, AQP8 and AQP9 expressions significantly increased (n=6; $p<0.01$) while AQP3 significantly decreased (n=6; $p<0.001$). pOsm increased 48% (n=5; $p<0.001$).
Our findings showed for the first time, that the expression and function of human amnion AQPs are regulated by changes in the osmolarity. Our work provides new evidence that changes in AQP expressions may represent adaptive responses or cause AF volume aberrations such as oligohydramnios and polyhydramnios.

511. (335) **EFFECT OF NITRATIVE STRESS ON PLACENTAL AQP9 AND ITS ROLE IN THE PATHOGENESIS OF PREECLAMPSIA**

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Preeclampsia is a gestational hypertensive syndrome of unknown etiology unique to human pregnancy. Increased levels of reactive nitrogen species (RNS) were described in preeclamptic placentas. RNS promotes the formation of peroxynitrite (ONOO-), that covalently modify proteins by nitration of tyrosine residues [3-nitrotyrosine (3NTp)] and lead to a non-functional protein. Nitration of a wide range of placental proteins were found increased in preeclampsia. Previously, in these placentas we reported an overexpression of AQP9 with a lack of functionality.
Our aim was to evaluate the effect of nitrative stress mediated by ONOO- on placental AQP9 expression and functionality.
This study was approved by the ethics committee of the Hospital Nacional Dr. Prof. A. Posadas. Explants from normal term placentas were cultured under conditions of nitrative stress induced by 100 μM de ONOO-. The cell viability, cytotoxicity, and cell damage indexes were evaluated. AQP9 expression and water uptake were also studied. The amount of 3NTp-AQP9 in trophoblast from preeclamptic and normal patients was determined using an immunoprecipitation assay.
In the presence of 100 μM of ONOO-, AQP9 protein expression significantly increased (n=7; $p<0.05$), however mRNA expression did not change, suggesting that the degradation of the protein was reduced. In this condition, water uptake was significant decreased (n=5; $p<0.02$) and not sensitive to HgCl2. On the other hand, we found that 3NTp-AQP9 increased 1.5-fold in preeclamptic placentas compared to normal ones (n=4; $p<0.05$).
Our results proposed that in preeclamptic placentas, the nitrative stress promotes the formation of 3NTp-AQP9. Consequently, the accumulation of this non-functional nitrated protein in the cytosol may adversely affect the survival of the trophoblast cells increasing the shedding of aponecrotic trophoblast fragments into maternal circulation which may induce the maternal syndrome.

512. (118) **THE DECIDUALIZATION INDUCED BY VIP ACTIVATES RETICULAR STRESS AND UNFOLDED PROTEIN RESPONSE AND CONDITIONS ENDOMETRIAL RECEPTIVITY**

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The vasoactive intestinal peptide (VIP) induces the decidualization program and conditions the immunoregulation of the implantation process. Endometrial stromal cells undergo reticular stress (RS) and unfolded protein response (UPR), which will allow them to induce a physiological sterile inflammatory response through inflammasome activation and IL-1beta production. Here, we focus on VIP effect on the RS and UPR pathways induced by the decidualization process and whether modulates inflammation.
We used an in vitro model of human decidualization represented by human endometrial stromal cell line (HESC) differentiated with VIP (10-7M) or with medroxyprogesterone (MPA 10^{-6}M) and dibutyryl-cAMP (2,5 10^{-3}M) during 8 days.
VIP significantly increased the expression of ATF6, a RS-sensor, and CHOP, an UPR marker ($p<0.05$, Student T-test). Then we found increased NLRP3 expression in decidualized cells by VIP or MPA-dbcAMP able to activate the inflammasome increasing IL-1β expression and production. IL-1beta production was tested by FACS ($p<0.05$, Student T-test). In fact, AEBSF (an inhibitor of ATF6-pathway) prevented this increase. Moreover, when we performed an in vitro model of embryo implantation using Blastocyst-like spheroids (BLS) transfered to monolayers of VIP-decidualized cells, AEBSF-treatment decreased the invasion index highlighting the relevance of ATF6-UPR pathway ($p<0.05$, Student T-test).Finally, the present results were confirmed in endometrial biopsies from patients with recurrent implantation failures (RIF) and fertile women. In endometrial biopsies from RIF patients we found not only a reduced expression of VIP and VPAC2 (VIP inducible receptor) also in ATF6 in comparison with fertile women ($p<0.0001$, Mann Whitney).
The present results suggest that VIP contribute to the decidualization process inducing RS/UPR associated with the production of IL-1beta and alterations in these pathways, as observed in RIF patients might precondition endometrial receptivity.

513. (351) **PRENATAL HYPERANDROGENZATION ALTERS HEPATIC LIPID METABOLISM IN A MURINE PCOS MODEL**

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Prenatal androgen excess is considered as one of the main factors contributing to the development of Polycystic Ovary Syndrome (PCOS). Most of PCOS patients present different metabolic disorders such as nonalcoholic fatty liver disease (NAFLD) and metabolic syndrome.
This study aimed to evaluate the effect of prenatal hyperandrogenism on the hepatic lipid pathways at adult life (90 days of age).
Pregnant rats were injected with testosterone and a control group was obtained by the injection of vehicle. The prenatally hyperandrogenized (PH) female offspring (N=150) and control offspring (C, N=96) were characterized according to the estrous cycle as irregular ovulatory (PHiov) and anovulatory (PHanov) phenotypes. We quantified the gene expression of enzymes involved in lipogenesis (Acaca, Acacb, Fas, Scd1) and of modulators of b-oxidation (Ppara

and Cpt1) by qPCR. We also quantified the hepatic triglyceride content by an enzymatic kit.
We found that Acaca and Acacb mRNA levels were not affected in the PH groups ($p>0.05$). Fas and Scd1 mRNA levels were higher in the PHiov phenotype than in the control group ($p<0.01$). Ppara mRNA levels were not affected and Cpt1 mRNA levels were increased in the PHiov group ($p<0.01$). No differences were found in hepatic triglyceride content in the PH groups as compared to controls ($p>0.05$).
We conclude that, although mRNA levels of Fas and Scd1 are increased in the PHiov group, their effect may be compensated by an increase of mRNA levels of Cpt1 in the same phenotype. In this way, the accumulation of triglyceride is avoided. These effects are not observed in the PHanov phenotype, in which there were no differences as compared to the controls in any of the genes studied.

514. (451) **METABOLIC SYNDROME IN FEMALE MOUSE: EFFECT ON FERTILITY AND OVARIAN ANGIOGENESIS**
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Metabolic syndrome (MS) is a cluster of conditions that comprises at least three of these features: overweight, hypertension, hypertriglyceridemia, hyperglycemia and low HDL. Metformin (MET) is a hypoglycemic drug used for type 2 diabetes. MET is capable of improving fertility in these women.
Objective: To evaluate oocyte quality, in vitro fertilization, follicular development, and ovarian Platelet-Derived Growth Factor B (PDGFB) in a MS female mouse model. To study ovarian effects of MET.
Methods: C57BL/6 female mice, four weeks old, were fed with a high fat diet (HFD) during 17 weeks. Control animals received standard diet (SD). A group of HFD also received MET. The estrous cycle and glycemia was evaluated. The animals were sacrificed and serum was collected to measure cholesterol and triglycerides. One ovary was used to obtain proteins and the other for histological studies.
Another set of animals was superovulated with eCG and hCG. Animals were sacrificed and the number of ovulated oocytes counted. By an in vitro fertilization we measured the percentage of fertilized oocytes and calculated the percentage of these oocytes that developed to blastocyst stage.
Results: HFD group showed higher weight, cholesterol and triglyceridemia. Not differences were found in the number of ovulated or fertilized oocytes. The percentage of embryos that developed to blastocyst was lower in the HFD group. MET reversed this effect. The HFD group spent more time in diestrous and less time in estrous and the MET group spent more time in metaestrous and less time in estrous compared to HFD group. Also, MET raised ovarian PDGFB. Finally, follicular dynamics was altered in HFD animals and MET improved it, raising the percentage of corpora lutea and reducing the percentage of atretic follicles.
Conclusions: MS alters follicular dynamics and oocyte quality. MET administration would have a beneficial effect on fertility in MS.

515. (472) **EFFECT OF A HYALURONAN SYNTHESIS INHIBITOR, 4-METHYLUMBELLIFERONE (4MU), ON A STROMAL ENDOMETRIAL CELL LINE**
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Endometriosis is a benign gynecological disease affecting 10% of women of reproductive age, characterized by the presence of endometriotic foci outside the uterine cavity. We have already demonstrated that 4MU has anti-angiogenic properties in two angiogenesis models, both in vitro and in vivo, of endometriosis and it has been reported that binding of hyaluronan to its CD44 receptor is involved in proliferation, migration and invasion in cancer cells. We evaluated the effect of 0, 0.1, 0.5, 1, 2 and 4 mM 4MU in an in vitro endometriosis model. A human stromal endometrial cell line, T-HESC, was stimulated with different concentrations of 4MU. Cell proliferation was evaluated after 24 h with the cell proliferation reagent WST-1; photomicrographs were taken at time 0 h and time 20 h, and the area of a scratch closed by the cells was calculated in a wound healing assay; and the gelatinase activity in conditioned media was evaluated by zimography. Only $p<0.05$ was considered as statistically significant. Cell proliferation of T-HESC was significantly inhibited by 1, 2 and 4 mM 4MU. Even at 0.5 mM 4MU, the migration of the cells was significantly inhibited and the scratch was closed in a low percentage. Preliminary results of MMP-2 and MMP-9 activities revealed that these gelatinases would not be modulated after 4MU treatment. More studies are needed to understand the implication of hyaluronan synthesis inhibition on the migratory capacity of the cells and which molecules are involved in this matter. Nevertheless, given our background on targeting hyaluronan on ENDOMETRIOSIS models we are encouraged to continue investigating on this path.

TRANSDUCCIÓN DE SEÑALES Y MECANISMOS MOLECULARES DE ENFERMEDAD / SIGNAL TRANSDUCTION 2

516. (386) **EFFECT OF YERBA MATE (ILEX PARAGUARIENSIS) AND ITS POLYPHENOLS IN RETINAL DEGENERATIVE DISEASES**
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Age related macular degeneration (AMD) is a progressive disease which leads to irreversible loss of vision. Premature cellular senescence of the retinal pigmented epithelium (RPE) is suggested to play a central role in the etiology of AMD. Polyphenols are postulated to protect against degenerative diseases development. Yerba mate is an important source of polyphenols in our country and two of its most abundant polyphenols are caffeic acid (CAF) and chlorogenic acid (CHL). The aim of this work is to study the role of yerba mate, CAF and CHL in the protection of oxidative stress-induced retinal degeneration. Methods: RPE cells (ARPE-19 cell line), were incubated with CAF (70μM) or CHL (100μM) for 2 hours and then exposed to H_2O_2 (150μM) for 90 minutes. Cells were collected at different time points following damage. BCL-2 expression and γH2AX phosphorylation were analyzed by Western blot. RNA abundance of ROS detoxifying genes NRF2 and SOD2, was evaluated by qPCR. Male C57BL6/J mice were treated with 40 mg/day of yerba mate by oral administration for 3 weeks and then injected with NaIO3 50 mg/kg. Two hours post injection mice were euthanatized and the RPE was isolated for western blot analysis. Results: CAF and CHL increased BCL-2 expression ($p < 0,05$) at 4 and 24 h following treatment. Both polyphenols enhanced NRF2 and SOD2 RNAm levels at 2 and 4 h after exposure and decreased histone H2AX phosphorylation. Yerba mate consumption up-regulated BCL-2 expression in mice RPE cells ($p<0,05$) compare to control animals but also following $NAIO_3$ damage. Conclusions: Yerba mate and its polyphenols protect the RPE cells from oxidative damage by enhancing antioxidant defense and pro-survival genes. These findings suggest that yerba mate could be an important nutraceutic in the prevention of AMD.

517. (395) **EFFECT OF ARSENIC IN EPITHELIAL CELLS WITH IMPAIRED ACTIVITY OF THE CFTR**
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Cystic Fibrosis, an inherited disease affecting 1:2500 newborn, is characterized by mutations in the CFTR, gene encoding a cAMP-regulated chloride channel. Little is known about the effects of envi-

ronmentally relevant levels of arsenic on ion channels including the CFTR. The aim of this study was to determine how arsenic affects mammalian cells with a diminished activity of the CFTR; it is known that the metalloid elicits its effect through mitochondrial pathways which, in cystic fibrosis is deeply compromise. IB3-1 (a bronchial cell line derived from a cystic fibrosis patient with a DF508/W1282X CFTR genotype) and S9 (IB3-1 cells transduced with an adeno-associated viral vector to stably express wt-CFTR) were exposed to As III (NaAsO2) (0-200 uM) for 2-24 h. Crystal violet assay results in S9 and IB3-1 epithelial cells showed increase susceptibility in S9 (compare to IB3-1) to As 10-200 uM when exposed for 2-24 h. Similar response were found in apoptosis profile measured by Annexin-V and Propidium iodide apotosis/necrosis assay and flow cytometry (significant differences were assessed by ANOVA and Fisher's LSD at p =0.01). This might be due to mitochondrial activity impairment and its involvement in apoptosis. Using synchrotron technology at the Synchrotron facility in Brazil, we determined speciation in cells exposed for 2-24 h to As (III) (50-100 uM). This result showed us that As was mainly found as As^{+3} form and that might be binding sulfur, such as As-Glutathione, a well-known detoxifying pathway for As. Taken together, this results partially explains the importance of mitochondrial functionality and GSH detoxifying pathway in cells exposed to As III, even at concentrations of As below permitted levels in drinking water, and for as little as 2 h of exposure. Our next step is to study the effects of the metalloid on the expression and the activity of CFTR.

518. (435) **WNT5A ACTIVATES NF-KB AND PROMOTES CYTOKINE RELEASE IN MELANOMA CELLS**

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Melanoma is the most deadly type of skin cancer and has a poor prognosis when not diagnosed at early stages. Wnt5a is a secretory glycoprotein involved in the non-canonical Wnt signaling pathway that plays an important role in melanoma by increasing motility, invasion, proliferation and resistance to apoptosis. Previous results from our lab have demonstrated the ability of Wnt5a to activate the canonical NF-kB pathway. The aim of this work was to further study the molecular mechanism and functional consequences of this crosstalk.

Our first goal was to identify proteins from both pathways that are required for the crosstalk. By using RNA interference and dominant negative mutants we determined that ROR1 and Dvl2 (from the Wnt5a pathway) and TRAF2 and NIK (from the NF-κB pathway) are essential for the phosphorylation of p65 dependent of Wnt5a. In contrast, ROR2, Dvl1, Dvl3 and RIP are dispensable. Next, we determined that Akt activity is required for Wnt5a-dependent phosphorylation of p65. Both, the PI3K inhibitor LY-294002 and a shRNA against Rictor prevented the phosphorylation of p65 upon Wnt5a treatment. In contrast pharmacological inhibitors of IKK, PKCδ and GSK3β did not affect Wnt5a-dependent p65 phosphorylation.

To determine whether Wnt5a promotes the production of cytokines, culture media from Mewo melanoma cells stimulated for 32h with Wnt5a were evaluated using a Human Inflammatory Antibody Protein Array. This analysis revealed that Wnt5a increased the production of several cytokines including GM-SCF, IL-6, IL-8, IL-11, MCP-1 and TNF sRI. We validated these results by ELISA and found 2-fold increase in IL-8 levels at 4h ($p<0.05$) up to 14-fold increase at 32h ($p<0.001$) upon treatment with Wnt5a. Similar results were obtained in Skmel-28 and 1205Lu.

Taken together these results indicate that Wnt5a might have and immunomodulatory effect on melanoma promoting pro-inflammatory environment that enhances tumor survival.

519. (527) **PIAS4 SUMO-E3 LIGASE REGULATES TAU AND PHOSPHO-TAU LEVELS AND STABILITY**

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Over the past few years, SUMO conjugation has been increasingly related with neurological diseases associated with abnormal protein accumulations. In particular, some of the PIAS SUMO-E3 ligases are directly linked to these processes. These E3 ligases can regulate protein-protein interactions, intracellular trafficking as well as aggregation and degradation of key neuronal substrates, therefore the dysregulation of their activity is linked to neurodegeneration. Among the different neurodegenerative diseases, tauopathies are characterized by the formation of intracellular tau deposits. These aggregates are composed mainly of hyperphosphorylated tau, but also some other modified tau species such as ubiquitinated and SUMOylated tau. SUMOylation of tau proteins may contribute to changes in protein solubility and proteolytic processing. However, the intrinsic molecular mechanism and its physiological relevance is still under investigation. In this work we analyzed the ability of PIAS family (PIAS1, PIAS2a, PIAS2b, PIAS3 and PIAS4) to modulate total and phospho-tau intracellular levels. We found that PIAS4 promotes tau and phospho-tau accumulation, increasing tau stability probably by inhibiting it´s degradation by the ubiquitin-proteasome system. PIAS4 effect over tau protein is dependent on PIAS4 E3 ligase activity.

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520. (535) **A HIGHER LEVEL OF O-GLCNACYLATION OF SP1 DOWN REGULATES GENE EXPRESSION OF PI CLASS GLUTATHIONE S-TRANSFERASE IN DIABETIC MICE**

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Diabetes mellitus is characterized by chronic hyperglycemia caused by defects in secretion and/or action of insulin. Hyperglycemia creates free radicals producing oxidative stress, which debilitates the endogenous antioxidant defense system. Glutathione S-transferases (GSTs) are a multigene superfamily of enzymes that catalyze the conjugation of glutathione with electrophilic compounds including those produced during oxidative stress. GSTP, one of the GST isoenzymes, catalyzes conjugation of glutathione with different electrophilic substrates with a particularly high affinity for small unsaturated aldehydes. Little is known about the regulation and expression of GSTP in diabetes. The aim of this study was to evaluate how GSTP is regulated in diabetes. Animals were diabetized with a single dose of streptozotocin. Diabetic animals were treated with vanadate (STZ+V), or insulin (STZ+I) or no treatment (STZ). Total GST activity and expression of GSTP mRNA decrease right after the onset of diabetes and remained low throughout the whole period of the experiment (32 days). Insulin abolished hyperglicemia and restored both GST activity and GSTP mRNA levels. However, vanadate, a well-known insulin mimetic agent, restored the GST activity but only showed a partial effect on hyperglycemia. Since the 5'-regulatory region of mouse GSTP gene contains activator protein-1 (AP1) and SP1 sites involved in transcription activation, we measured both AP1 and Sp1 glycosylation levels in all groups. Both Sp1 and Sp1 O-glycosilated increased in liver of diabetic animals while insulin treatment impaired overexpression and hyperglycosilation maintaining both parameters at the levels of control group. On the other hand, vanadate treatment yielded expression and glycosilation of Sp1 lower than those of control group. JUN protein levels were lower than control in all groups, whereas cFOS protein expression did not differ between treated and control groups. These results suggest that Sp1 hyperglicosilation might be involved in the changes of GSTP expression caused by diabetes.

521. (553) **CHANGES IN GENE EXPRESSION IN TISSUES OF A MOUSE LACKING EXPRESSION OF ALL 7 GENES ENCODING TRPC CHANNELS (HEPTAKO). A PRELIMINARY ANALYSIS.**

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rreguy, Sarasola Maria de la Paz, Cerchietti Leandro, Birnbaumer Lutz
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TRPC genes encode non-selective Ca2+-permeable cation channels implicated in the mechanism of store operated Ca2+ entry. TRPC channels have been increasingly linked to a diverse number of pathologies. The mutation, down or upregulation of any of these channels may lead to diseases that include cardiopathies, neuronal disorders and immune deficiencies among others. This highlights the potential of TRPC channels to become novel therapeutic targets and also provides evidence of their physiological function. The absence of specific inhibitors that limits the study of these channels lead to the development of KO mice. Knock out (KO) of each and every one of the 7 TRPC genes, separately, has yielded mice with distinctive phenotypes, some favorable, others detrimental to the health of the mouse. It is important to note that TRPCs 1-7 have overlapping functions that could mask the effect of removing just one of these channels. Combining, by breeding, the seven KO alleles has yielded a mouse strain we refer to as HeptaKO. Surprisingly, the mice are alive and breed spawning heptaKO descendants. To date no comprehensive analysis of this mouse has been done that could explain the effects observed in the simple KOs.
Our laboratory has obtained RNAseq data from eight tissues (Liver, Heart, Spleen, Testis, Lung, Kidney, Midbrain and Forebrain) of the heptaKO mice and their WT counterparts. Here we will describe the preliminary results obtained from the analysis of the samples that could explain the differential response to different diseases involving TRPC channels and also why the sevenfold mutant mice are alive and breed normally.
KF is a CONICET Postdoctoral Research Fellow; MVR is a Postdoctoral Research Fellow, SS is a CONICET Assistant Career Investigator, MdPS, is a CONICET Technical Assistant (CPA), LC is an Assistant Professor, LB is an UCA Professor of Biomedical Sciences.

522. (588) **INTERACTION BETWEEN THE ORAI1-BASED CA2+ ENTRY MECHANISM AND THE RAS GTPASE SYSTEM AS SEEN BY FRET BETWEEN ORAI1 AND H-RAS AND MODULATION OF STORE-OPERATED CA2+ ENTRY (SOCE)**

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In test experiments, performed as part of training course in analysis of fluorescence resonance energy transfer (FRET), we co expressed Orai1-YFP and human CFP-HRas) in HEK293 cells hoping to get no FRET signal between them. Unexpectedly we discovered direct interaction between these two proteins at the level of the cellular plasma membrane. This finding suggested that the Ras signaling cascade(s) and SOCE interacted, as Orai1 I the canonical SOCE channel. We decide to test for this interaction at the functional level and we quantified Ca2+ entry using the Fura2 method, which reports changes in cytosolic Ca2+ in response to release of Ca2+ from the endoplasmic reticulum (ER), activating STIM1 at RE and finally the assembly of the Orai1-based Ca entry channel (CRAC) allowing Ca to enter from the extracellular space. We performed two types of experiments: In one we tested the effect of HRas expression on thapsigargin (Tg)-evoked store depletion and Ca2+ entry and on EGF-EGFR evoked SOCE and in the other we suppressed Tg-evoked SOCE by overexpression of Orai1 and asked whether expression of HRas would relieve this inhibition). HRas expression tested positive in both types of test: It inhibited Tg- and EGF-EGFR-evoked SOCE, and it suppressed inhibition of Tg-evoked SOCE by Orai1, the degree of suppression being dependent on relative expression levels of the transfected proteins, never reaching more than 60-70%. We conclude that the Ras signaling and the Orai1-mediated Ca2+ entry mechanisms interact functionally, and propose that one or more steps of Ras activated signaling cascades are modulated by cytosolic Ca2+ changes. We are currently testing for possible differences among H-Ras, K-Ras and N-Ras, and also for the impact that the activation state of Ras using dominant positive (G12V, Q61L) and dominant negative (S17N) mutants) may have in the above described assays.

523. (613) **HSP90 CO-CHAPERONE FKBP51, IS UP-REGULATED UPON SERUM DEPRIVATION AND MAY PLAY AN IMPORTANT ROLE IN REGULATING METABOLIC STRESS.**

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The hypothalamic-pituitary-adrenal (HPA) axis plays a fundamental role in the response to external and internal stimuli. FK506-binding protein 51 (FKBP51) is an Hsp90 co-chaperone that plays a fundamental role in regulating the glucocorticoid receptor (GR) activity and therefore keeping the HPA axis functioning. FKBP51 inhibits GR activity by decreasing GR hormone-binding affinity and nuclear translocation. In order to act as a GR regulator, FKBP51 needs to be SUMOylated, a process enhanced by the SUMO E3 ligase, PIAS4. Interestingly FKBP51 has also been described as an important regulator of energy balance playing an important role in metabolic homeostasis. Both stress and metabolic regulation share common regulatory pathways centered in the hypothalamus. FKBP51 shows a high expression in the hypothalamus and may act as an interplayer between both pathways. Exposure to nutrient overload or to nutrient inhibition is considered to be a metabolic stressor. In this work we show that FKBP51 expression is upregulated upon serum deprivation in HEK 293 cultured cells. Also, we demonstrate by co-immunoprecipitation assays that FKBP51 interaction with AKT1 and Beclin1, which are tightly involved in the metabolic status of the cell, is differentially affected when FKBP51 is SUMOylated, and that this interaction seems to be altered when cells are exposed to a lack of nutrients.

524. (632) **ROR2 INHIBITS PROLIFERATION OF MELANOMA CELLS BY DELAYING CELL CYCLE PROGRESSION**

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Wnt5a and its receptor ROR2 play an important role in cancer. Our aim is to determine the role of ROR2 in melanoma. We have shown that ROR2 overexpression decreased proliferation by negative regulating Cyclin D1 and Cdk4 levels and by increasing p21 levels. Here we studied how ROR2 regulates cell cycle progression.
ROR2 overexpression decreased Retinoblastoma phosphorylation and its cytoplasmic localization, while ROR2 silencing increased it. ROR2 overexpression decreased G2/M population (8,9% vs 24,5% in control cells, ($p<0,01$)) at the expense of cells in both G1 and S. Silencing of ROR2 also altered the cell cycle, most noticeably by decreasing the number of S phase cells from 40,6% to 21,1% ($p<0,01$). We performed time course experiments on G1-phase synchronized cells to evaluate the transition to G2/M phase. Seventeen (17,2% ± 4,8%) percent of A375-empty cells transitioned to G2/M by 18h vs. 0,6% ± 0,4% of A375-ROR2 ($p<0,01$). These cells required an additional 6h to reach similar values (18,91% ± 0,72% at 24h).
In the case of M2 cells, none (M2-scramble) and 9,6% ± 2,7% cells (M2-shROR2) transitioned to G2/M phase after 16h. It took 24h for the M2-scramble cells to reach similar values (10,32% ± 1,02%). In the same experimental setting we found that Cyclin A levels rapidly peaks at 12h after G1 release and then down curved until almost disappear by 24h. In contrast, in A375-ROR2 Cyclin A slowly increased until reaching its peak at 24h. Meanwhile, in M2 cells Cyclin A peaks at 28h and 16h in scramble and ROR2 shRNA cells, respectively. Similar conclusions were obtained from the analysis of Cyclin B levels.
These experiments revealed that ROR2 slows-down G1-G2/M transition by regulating the abundance of cell cycle proteins. Thus, ROR2 has an anti-tumorigenic role in melanoma which has import-

ant implicancies for the understanding of Wnt5a signaling.

525. (653) **SIGNAL TRANSDUCTION PATHWAYS INVOLVED IN OXER1-DEPENDENT CELL MIGRATION**

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Among lipoxygenase products of arachidonic acid metabolism, 5-HETE, 5-HPETE and 5-oxo-ETE, act through a membrane receptor named OXER1. We found that human adrenocortical H295R cells express OXER1 and that, in this cell type, this receptor is involved in PKA- and PKC-dependent stimulation of steroidogenesis. Other authors have postulated that 5-oxo-ETE is a potent activator of human neutrophil migration and prostate cancer cell proliferation. Both effects are mediated by the activation of its receptor. Using a H295R cell line stably overexpressing OXER1, we found that 5-oxo-ETE, agonist of OXER1, produced an increase in cell migration and proliferation. We also detected increased migration in the wild-type cells. In this work we studied the signal transduction pathways involved in the action of 5-oxo-ETE on H295R cells.
H295R cells were cultured under different conditions, cell migration was evaluated by measuring wound healing 24 h after scratch and Western blot assays were performed using specific antibodies that recognize intermediates in different signal transduction pathways.
Cell migration was differently affected when inhibitors of different signal transduction pathways were used: while no effect was observed in 5-oxo-ETE- induced migration using an inhibitor of PI3K/AKT pathway, an increase was found using a PKA inhibitor and a reduction was produced by inhibition of ERK1/2 and P38. In accordance with this, Western blot analyses revealed a time-dependent elevation in ERK1/2 and P38 phosphorylation after 5-oxo-ETE stimulation, while pAKT levels remained constant.
In conclusion, OXER1 activation in H295R cells is involved not only in PKA- and PKC-dependent activation of steroidogenesis, but also in other cell functions such as migration through other signal transduction pathways.

526. (669) **MAP KINASE PHOSPHATASE-3 AND ITS ALTERNATIVE SPLICE VARIANT ARE REGULATED BY ANGIOTENSIN II IN ADRENOCORTICAL CELL LINE H295R**

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The concerted action of kinases and phosphatases regulates key biological events. MAP kinase phosphatases (MKP) dephosphorylate MAPK and thus regulate MAPK-dependent processes such as proliferation, differentiation and apoptosis. MKP-3, a member of MKP family, is induced by different proliferative stimuli and dephosphorylates ERK1/2. As ERK participates in several cellular functions regulated by angiotensin II (AII), we aimed to analyze MKP-3 expression and regulation by AII in human adrenocortical cell line H295R. In addition, we evaluated AII effect on P-FOXO1 dephosphorylation (a novel MKP-3 substrate) and also on the induction of p21, target for this transcription factor. The expression of MKP-3 and its splicing variants L and S were detected and up-regulated by AII. RT-PCR analysis showed a significant 2-fold increase in MKP-3L mRNA levels after 30 min stimulation. MKP-3S mRNA levels displayed a similar temporal profile to MKP-3L. P-ERK1/2 and P-FOXO1 levels were rapidly increased after AII-stimulation and decreased concomitantly with MKP-3 protein induction, as revealed by Western blot analysis. Furthermore, we show that AII upregulated p21, which is in agreement with the dephosphorylation and activation of P-FOXO1.
In summary, our data suggest that in H295Rcells, AII regulates P-ERK levels, MKP-3 and MKP-3-related events. MKP-3 regulation involves the expression of both its transcripts. Since MKP-3S protein lacks important regulatory sites, it is expected to display different properties and function from MKP-3L. Thus, L and S variants of MKP-3 could differentially regulate ERK-dependent events in adrenocortical cells upon AII stimulation.

527. (695) **ESTROGEN-RELATED RECEPTOR ALPHA IS INVOLVED IN THE REGULATION OF MITOFUSIN 2 EXPRESSION IN ADRENOCORTICAL HUMAN CELLS**

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Mitofusin 2 (Mfn2) is a mitochondrial protein that participates in mitochondrial fusion, protects against apoptosis and activates mitochondrial metabolism in mammalian cells. Mfn2 expression is downregulated in obesity and in type 2 diabetic patients and mutations in this protein cause neurodegenerative diseases, like Charcot-Marie-Tooth type 2A neuropathy. It has been described that Mfn2 gene expression regulation involves several transcription factors. Mfn2 can be induced directly by the cooperative action of PPAR-γ coactivator-1α (PGC-1α) and estrogen-related receptor-α (ERRα). We have previously shown in adrenocortical human cells that Angiotensin II (Ang II) regulates Mfn2 expression by increasing its mRNA and concomitantly its mitochondrial localization. The aim of this work was to study the role of ERRα on the regulation of Mfn2 expression in adrenal cells. We observed in H295R adrenocortical human cells that stimulation with Ang II modulates ERRα protein in a time-dependent manner. Then, we demonstrated by qPCR that ERRα overexpression increases Mfn2 mRNA in control and in Ang II-stimulated cells (Mock vs. ERRα: 1 vs. 4.51, Mock + Ang II vs ERRα + Ang II: 5.46 vs. 8.77 ***$p<0.001$, relativized to Mock). In H295R cells overexpressing ERRα, Ang II treatment promotes a one-fold increase in Mfn2 protein content compared to non-stimulated cells. These results indicate an ERRα-dependent modulation of Mfn2 expression, suggesting a possible role of this transcription factor in Ang II pathway, in H295R cells.

GENÉTICA / GENETICS 2

528. (129) **MOLECULAR FEATURES IN CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS WITH MUTATED IGHV4-34 B CELL RECEPTORS.**

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IGHV4-34 is the most frequently used gene in chronic lymphocytic leukemia (CLL) patients expressing B-cell receptor (BCR) immunoglobulin with somatically hypermutated immunoglobulin heavy chain variable region (IGHV) genes. A significant proportion of IGHV4-34 mutated (M)-CLL is assigned to distinctive quasi-identical BCRs, named stereotyped. We have analyzed the mutational profiles of IGHV4-34 CLL patients in order to refine the molecular characterization of the disease. Results were correlated with cytogenetics and FISH data. A total of 290 patients (173 males; mean age: 64.8 years) were evaluated. The study was approved by the Institutional Ethics Committee. All individuals provided their informed consent. Twenty seven (9.2%) patients expressed the IGHV4-34 gene, 5 (18.5%) exhibited unmutated IGHV status (>98% germline identity; GI), while 22 (81.5%) corresponded to the M subgroup (<98 GI). Seven IGHV4-34 M gene rearrangements belonged to subset #4 and 2 to subset #16. JH6, DH2 and DH5 were predominantly used in stereotyped CLL while JH4, DH2 and DH3 in non-stereotyped cases. A longer mean heavy chain complementarity-determining region 3 (VHCDR3) length in stereotyped rearrangements (20.9pb) compared to non-stereotyped ones (16.2pb) was observed. The distribution of Replacement/Silent (R/S) mutations showed higher R/S ratios within VHCDR2, VHFR2 and VHCDR3 in stereotyped, while VHFR3 was the most involved in non-stereotyped rearrangements. Trisomy 12 was more frequent in non-stereotyped cases (57%) compared to stereotyped ones (20%) while abnormal karyotypes were only found in non-stereotyped patients (33%), suggesting a better outcome for IGHV4-34 stereotyped patients. Both subsets

expressed similar mutational pattern and aminoacid changes at codons P45-S, E55Q and S64-I while codon 40 S40T mutation was not found in our series. Our data showed substantial differences in the mutational profile of stereotyped and non-stereotyped IGHV4-34 CLL patients, supporting that cells react in a specific way in face to the antigen selection.

529. (135) **NOTCH1 AND TP53 MUTATIONS IN CHRONIC LYMPHOCYTIC LEUKEMIA. THEIR RELATIONSHIP WITH CYTOGENETIC, FISH AND IGHV STATUS OF PATIENTS.**

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Molecular studies have revealed a number of recurrently mutated genes in chronic lymphocytic leukemia (CLL). Among them, the study of NOTCH1 and TP53 gene mutations showed significant importance in CLL prognosis. The aim of this study was to evaluate NOTCH1 and TP53 mutations in our CLL patients, in order to analyze the type and frequency of these alterations. Results were correlated with cytogenetics, FISH and IGHV mutational status studies. A total of 60 patients were evaluated. Mutational status was analyzed by PCR followed by bidirectional sequencing, and compared with public databases. The study was approved by the Institutional Ethics Committee. All individuals provided their informed consent. Three (5%) cases showed the NOTCH1 c.7541_7542delCT mutation. These patients had unmutated (UM) IGHV (100% germinal identity) and two of them showed very complex karyotypes. For TP53 mutation analysis, a selected group of 24 patients with TP53 deletion by FISH analysis was evaluated. Nine (37.5%) cases showed mutated TP53 (TP53-M), all of them with more than 20% of cells with TP53 deletion; exons 4-8 were involved. Two insertions and 2 deletion with change of reading frame, and 5 replacement point mutations (4 transitions and 1 transversion), were observed. The polymorphism analysis of codon 72 (rs1042522) that encodes arginine (Arg) or proline (Prol) showed association of the Pro/Pro genotype with TP53-M (p=0.047). The presence of TP53-M was not significantly related to IGVH mutational status. Cytogenetic analysis showed that total cases with TP53-M had abnormal karyotypes, compared to 25% of patients with TP53-UM. One case had both NOTCH1 and TP53 mutations. Our data constitute the first evaluation of NOTCH1 and TP53 mutations in patients with CLL in our country and provide information about the molecular heterogeneity of this pathology.

530. (438) **ANALYSIS OF BIRC3 ALTERATIONS IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA.**

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BIRC3 (Baculoviral IAP repeat containing 3) gene (11q22.2), located ~6Mb centromeric to the ATM (Ataxia telangiectasia mutated) locus (11q22.3), is a negative regulator of non-canonical NF-kB signaling pathway. We evaluated BIRC3 mutations and deletions in chronic lymphocytic leukemia (CLL) patients in order to have a better biologic characterization of the disease. Results were correlated with cytogenetics, FISH and IGVH (immunoglobulin heavy chain variable region) mutational status. A total of 80 patients were evaluated. Mutational status of exons 7, 8 and 10 was analyzed by PCR followed by bidirectional sequencing and compared with public databases. BIRC3/MALT1 Dual Color Dual Fusion Probe (Zytovision, Bioars) was used. The study was approved by the Institutional Ethics Committee. All individuals provided their informed consent. No BIRC3 mutations were observed. Three patients showed variants: rs17881197, rs7124969 and rs1055088, within exons 7, 8 and 10, respectively, all of them without clinical significance. The latter variant was associated to abnormal karyotype, TP53 deletion and unmutated (UM) IGHV. BIRC3 deletion was evaluated by FISH analysis in a selected group of 17 patients with ATM deletion. BIRC3 deletion was observed in 16/17 cases with the following distribution: 58% showed similar values in both genes, 25% had higher percentage of ATM deletion and 17% of cases showed increased frequency of BIRC3 deletion, suggesting clonal evolution. In addition, 77.8% of patients expressed UM-IGHV and 76.5% had abnormal karyotypes, 53.8% of them complex karyotypes. Interestingly, 64.3% of cases also showed TP53 deletion, associated to bad prognosis. To our knowledge, this is the first evaluation of BIRC3 alterations in CLL patients in our country. These results suggest very low frequency of BIRC3 mutations and highly variable size of deletions at 11q22 chromosomal region in our series. We identified a high-risk molecular group whose heterogeneity warrants further studied in the search for clinical correlations.

531. (459) **DELETIONS OF THE LONG ARM OF CHROMOSOME 6 IN MULTIPLE MYELOMA PATIENTS. ASSOCIATION WITH PROGNOSTIC FACTORS.**

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Multiple myeloma (MM) is a malignancy of mature plasma B cells. In this pathology, classical parameters are not accurate enough to predict the outcome of patients at early-stage disease. Nowadays, the genetic profile of tumor cells is one of the most relevant prognostic factors that could contribute to the identification of different risk groups. Chromosome unbalances, are common events, being the most frequent gains on 1q, 19p and 9q and losses on 1p, X, 13q, 14q, and 6q. We investigated the impact of deletionsof the long arm of chromosome 6 (del6q) in MM patients. Results were correlated with clinical parameters and overall survival (OS). A total of 150 bone marrow samples of newly diagnosed patients were studied: 89 (59.3%) cases with structural abnormalities(SA) and 61(40.6%) with normal karyotype (NK). Among the SA group, 35 samples showed del6q (39.3%) (17 with complex karyotype; CK), and 54 (60.7%) with other alterations than del6q (19 with CK). Clinical parameter comparisons showed that all patients with del6q (with simple or CK) showed increased levels of creatinine (p=0.0378) and beta2 microglobulin (B2M) (p=0.0039) with respect to NK group. A higher percent of cases with kappa light chain, bone marrow infiltration and lytic bone lesions was detected in cases with del6q with respect to those with NK (p=0.0022, p=0.0004 and p=0.0364, respectively). No significant differences in OS were found between cases with del6q as the only abnormality (87.7 months) respect to those with NK (123.7 months).However, a significant short OS (28.4 months) for patients with CK and del6q, was found (p=0.0021).Our data showed the association of del6q with adverse prognostic factors, but without clinical impact by itself, supporting the importance of genomic complexity in MM clinical evolution.

532. (715) **F8 GENOTYPE CHARACTERISATION OF THE FIRST ARGENTINE SERIES OF PATIENTS WITH MILD HAEMOPHILIA A: NOTABLE PREVALENCE OF RECURRENT MUTATIONS.**

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Haemophilia A (HA) is the commonest X-linked coagulopathy caused by deleterious mutations in *F8*. Mild-HA associates with minor reduction in the clotting activity of factor VIII (FVIII:C) to 5-40 UI/dL. Perhaps due to their mild phenotype expression, mild-HA patients are rarely genotyped although they represent 35-40% of HA cases worldwide. The scarce published data indicate that mild-HA shows notable differences with severe-HA (FVIII:C<1 IU/dL) in its population genetics and mutational pool turnover.
Objective: to characterise the *F8*-genotype in a large series of Argentine patients with mild-HA and to discuss its mutational dynam-

ics.
Population: 64 apparently unrelated families affected with mild-HA countrywide, 97 individuals including index-cases and relatives. Our *F8* analysis algorithm includes: -genomic DNA extraction from peripheral blood leukocytes, -a mutational screening by PCR-amplification of all coding and regulatory regions of *F8* over all 26 exons (38 amplimers) and conformation sensitive gel electrophoresis (CSGE), -mutational characterisation by Sanger sequencing of CSGE anomalous amplimers. Duplication of exon 13 (Dup13) was detected by tail-to-head PCR-analysis.
The mild-HA-causative mutation (established by genotype/phenotype assignment criteria) was identified in 61 families (detection efficiency 95%). Thirty-four families (56%) showed 14 recurrent mutations (repeated 2-5 times), whereas the remnant 27 families, non-recurrent *F8*-defects. Among the recurrent mutations, we found 11 missense in 26 families highlighting p.Arg612Cys* (n=5) and p.Arg550Cys (n=3) among others (n=2), a splicing defect on c.601+5G>A (n=3), the Dup13* (n=3) and a synonym change (n=2) (*reported with Italian origin). Non-recurrent mutations included 24 missense, an *ins-del* and two splicing defects.
Our findings demonstrate that our practical approach is adequate to characterise the mild-HA-causative *F8*-genotype in patients and relatives, highlight the prevalence of missense defects in mild-HA (50/61, 82%) and indicate that the higher frequency assessed for recurrent mutations in mild-HA respect to severe-HA may reflect the higher mutational turnover of the severe-HA pool.

533. (542) **ALTERNATIVE END-JOINING REPAIR PATHWAY INDUCES CHROMOSOMAL REARRANGEMENTS IN HUMAN CELLS TREATED WITH ETOPOSIDE**

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Chromosomal rearrangements (CR) involving the MLL (mixed-lineage leukemia) gene cause secondary malignancies associated with the treatment of human tumors with etoposide (ETO). The incorrect repair of DNA double-strand breaks (DSB) by alternative end-joining (alt-EJ) pathway generates CR, genomic instability and tumorigenesis. The role of alt-EJ in the generation of CR induced by ETO in human cells deficient in the cohesion subunit Rad21 (homologous recombination defective) and in DNA-PKcs (one of the main factors of classical nonhomologous end-joining) was evaluated. HeLa Rad21kd cells and their non-silencing NS control cells were treated with ETO 2μg/ml for 1-2h in the presence or absence of the chemical inhibitor of DNA-PKcs, NU7026 10μM. After 2h of treatment, an increase in the percentage of G2 cells with DSB (88.2%-93.4%), analyzed by flow cytometry, was observed. At 10h post-treatment (PT), the immunofluorescence analysis of nuclei with more than 20 γH2AX foci (DSB biomarker) in G1 post-mitotic binucleated cells showed a significant increment in HeLa Rad21kd and HeLa NS exposed to NU7026-ETO (82.8±2.1% vs. 21.7±3.2%, p=0.0001) compared to cells treated with ETO alone (Rad21kd =36.4±5.9% vs. NS=7.7±1.8%). Abnormal repair of ETO-induced DSB led to inter-chromosomal exchanges and mainly, dicentric chromosomes at second metaphases (28h PT), being this frequency 3.1-times higher in NU7026-ETO-treated HeLa Rad21kd cells than in NS (2.42±0.33 vs. 0.78±0.16, p=0.0001). Moreover, MLL gene rearrangements at band 11q23 using fluorescence in situ hybridization procedure were found in 7.2% and 4.9% interphase nuclei of Rad21kd and NS cells exposure to the combination of NU7026-ETO, respectively. Meanwhile, the percentage of MLL gene rearrangements after ETO treatment was similar in both cell lines (NS= 6.3% and Rad21kd= 5.9%). These results indicate that ETO-induced DSB go through the successive cell division and that alt-EJ plays an important role in the CR formation involving MLL gene.

534. (236) **MITOCHONDRIAL HAPLOGROUPS IN ONCOHEMATOLOGICAL, COLORECTAL AND BREAST CANCER SAMPLES FROM CABA AND BUENOS AIRES PROVINCE (ARGENTINA)**

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Mitochondrial DNA (mtDNA) variants -a unique SNPs combination- define specific haplotypes, which are gathered in haplogroups showing ethnic differences and a continental-specific distribution. mtDNA haplogroups are suggested to be associated with certain pathologies, including cancer. Aims: 1-Identify mtDNA haplogroups in patients with oncohematological (OncoHemCa), colorectal (CRC) or breast cancer (BrCa) and in control samples, 2-Compare frequencies with those published for the Argentine population, and 3-Analyze the possible role of mtDNA haplogroups as risk factors. Sample set (blood): 96 cases with OncoHemCa and 272 controls, from a public hospital in La Plata (BsAs, Argentina), plus 68 CRC and 54 BrCa cases, recruited at private hospitals from CABA (Argentina) and La Plata, respectively. A sequencing approach and multiplex PCR-APLP assays were used to determine mitochondrial haplogroups. Assignments were performed using bioinformatic tools, phylogeographical criteria and local databases. Regarding OncoHemCa cases, 57.3% were assigned to Amerindian, 36.5% to European/Middle Eastern and 6.2% to African lineages. Controls showed 63.5% of Amerindian, 33.9% of European/Middle Eastern and 2.6% of African ancestry. About CRC cases, 25% were assigned to Amerindian, 69.1% to European/Middle Eastern and 5.9% to African lineages. mtDNA ancestry of BrCa cases was 29.6% Amerindian, 64.8% European/Middle Eastern and 5.6% African. Maternal lineage distribution showed significant differences among cases from the public versus private healthcare system (p<0.001). Adjusted multivariate logistic regression models estimated an OR=2.96 (CI95%,0.9-9.65, p=0.07) for individuals carrying African mtDNA when analyzing OncoHemCa. No differences in haplogroups distribution were seeing among CRC and BrCa samples. Observed frequencies in cases and controls from public and private institutions are in line with those reported for this region. More samples will be added in future studies, particularly controls from private hospitals, which will allow us to better analyze the role of mtDNA haplogroups as risk factors, a research area that was not yet deeply studied.

535. (239) **EVALUATION OF GERMLINE AND SOMATIC VARIANTS OF 141 CANCER PREDISPOSITION GENES IN PATIENTS OF HBOC**

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Hereditary Breast and ovarian cancer syndrome (HBOC) is a genetic condition. The majority of HBOC risk is due to germline variants in BRCA1 and BRCA2 genes, however, around 60% of high-risk breast and/or ovarian cancer families are BRCA negative. The detection of somatic variants in cancer samples is challenging due to the presence of inherited germline variants, sample heterogeneity, and genomic instability. Somatic alterations can be identified from massively parallel sequencing data by directly comparing the DNA sequence from tumor samples with their matched normal samples.

The aim of this study was to determine the somatic and germline mutation spectrum in high-risk BRCA1/2 mutation-negative HBOC patients from Argentina.
Tumor and matched normal samples were collected from eleven patients. The DNA was extracted from peripheral blood and tumoral tissue. Library preparation was performed using the GeneRead (Qiagen) workflow for cancer predisposition panel (141 genes) .Clonal amplification and sequencing was performed on the Ion PGM platform (Thermofisher).
We could identify somatic variants in 5 of 11 samples analyzed due to low DNA quality of 6 of the samples. After quality and functional filtering we found 278 somatic variants on Tumor suppressor genes (16.8%) and Oncogenes (5.72%). The most frequent somatic altered genes were: POLE, SMARCA4, ATR. Regarding sequence ontology: 60,5% were nonsense, 33% missense, 3% stopgain and 1,8% splice site. Mutational signature have been also analyzed showing the presence of signatures 19 and 30 mainly.
The developed paired tumor/normal workflow allowed us to identified tumor exclusive mutations. This approach helps to understand the genomic biology of HBOC tumors. Our data shows that NGS based gene panel sequencing is an tool for identify germline and somatic variants. However, we still have to improve the methodology to increase the efficiency of the workflow that is dependent of preanalytic parameters.

536. (572) **ANALYSIS OF TELOMERE LENGTH IN WOMEN WITH POLYCYSTIC OVARY SYNDROME**

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Polycystic ovary syndrome (PCOS) is the most common endocrine disorder affecting women in their reproductive age. We aimed to determine the absolute LT (LTa) in women with PCOS in comparison with healthy controls and their association with metabolic variables and the presence of hyperandrogenism.
We analyzed 86 control women and 130 PCOS patients (16-46 years of age). Measurements of biochemical, clinical, anthropometric and hormonal variables were made. Biochemical hyperandrogenism (HA) presence was determined (total testosterone levels higher than 0.9ng/mL). LTa determination was performed on genomic DNA from peripheral blood leukocytes by Real Time PCR absolute quantitative method. The relationship kpb of telomeric sequences and copies of the single copy gene RPLPO (radio T/S) was determined. Statistical analysis were carried out by one-way ANOVA and linear regression. PCOS patients have a higher weight, body mass index (BMI), greater waist circumference (WC), higher levels of triglycerides (TG), and fasting plasma glucose as compared to controls. An inverse relationship was observed between LTa and age (p=0.004). PCOS patients presented increased LTa as compared to controls (p=0.001, adjusted for age: p=0.005). Moreover, we found higher levels of LTa in PCOS-HA women as compared to PCOS-NHA and Controls (p=0.004). In PCOS patients, we found an association between higher LTa and lower BMI (p=0.040), lower WC (p = 0.004), lower TG levels (p = 0.049), lower DPB (p=0.001) and higher c-HDL (p=0.004).
In conclusion, LTa presents an inverse relationship with age in the studied population. The significantly increased LTa in PCOS patients (as compared to controls) could be a consequence of the presence of different metabolic, but mainly, hormonal components. A lower LTa was associated with presence of metabolic syndrome components, while biochemical HA was associated with higher LTa. Our results contribute to knowledge about the role of LT in the pathophysiology of PCOS.

537. (112) **MOLECULAR ANALYSIS OF AN ARGENTINE DYSTROPHINOPATHY COHORT: DIAGNOSTIC ALGORITHM, GENETIC ASSESSMENT AND DMD GENE CHARACTERIZATION**

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Introduction: Dystrophinopathies are X-linked recessive diseases caused by mutations in DMD gene. Hitherto there is no effective treatment for these pathologies, which enhances the importance of performing genetic assessment in order to detect mutation carriers and prevent diseased newborns. However, two mutation-specific gene therapies were recently approved: Exon 51 Skipping (Eteplirsen) and Premature Stop Codon Read-through (Ataluren). Therefore, accurate detection and characterization of the causing mutation is essential to allow genetic counseling, patient follow-up and determine the suitable gene therapy.
Materials and Methods: We have analyzed 200 boys with clinical diagnosis of Dystrophinopathy, 12 symptomatic women, 240 females at-risk of being carriers and 15 prenatal diagnoses. A diagnostic algorithm was designed for each case, implementing MLPA, PCR, Whole Exome Sequencing, Sanger Sequencing, STRs segregation analysis and HUMARA assay.
Results: The selected strategy allowed disease confirmation in 71.7% (152/212) of the affected boys and symptomatic females. 12 were candidates for Eteplirsen, while 22 were suitable for Ataluren. On the other hand, we were able to establish as carriers 72/255 women/fetuses, while could exclude from being carriers/affected 143/255. As for gene characterization, we could establish an association between the most frequent deletion/duplication intron breakpoints and the abundance of STR loci and, we have detected 3 haplotypes blocks within the SNPs identified by the Exome technique.
Conclusions: In the present work, we have characterized a Dystrophinopathy argentine population and contributed to the understanding of the genetic/molecular basis of these pathologies. This study was supported by PTC Therapeutics and University of Buenos Aires, Argentina.

ONCOLOGÍA / ONCOLOGY 7

538. (599) **hTERT EXPRESSION IS REGULATED BY THE ACTIVATION OF HSF1**

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Cancer cells achieve proliferative immortality by upregulating telomerase. hTERT is the catalytic subunit with reverse-transcriptase activity, which forms complexes with a template functional RNA, Hsp90, p23, and other accessory proteins. Recently, we demonstrated that two Hsp90-binding immunophilins, FKBP51 and FKBP52, are overexpressed in cancer cells and associated to hTERT. FKBP51 is also an antiapoptotic factor that undergoes nuclear-mitochondrial trafficking and binds to the hTERT•Hsp90 nuclear heterocomplex in a peptidylprolyl-isomerase (PPIase)-independent manner enhancing telomerase enzymatic. This effect is PPIase-dependent. hTERT nuclear localization is favored by FKBP52 via the cytoplasmic Hsp90•FKBP52•dynein retrotransport machinery, and because FKBP52 anchors hTERT to nucleoskeleton structures. In this study we analyzed the regulation of hTERT expression and subcellular relocalization. The disruption of hTERT heterocomplex with radicicol (Hsp90 inhibitor) or by overexpression of Hsp90-interacting TPR peptide, delocalizes nuclear hTERT to the cytoplasm. This Hsp90-free hTERT is degraded via proteasome unless it is targeted to mitochondria, where it seems to complement the antiapoptotic effects of FKBP51. Oxidative stimuli (H2O2, arsenite, BSO, tert-butyl-hydroperoxide, etc.) also disengage hTERT from nuclear structures favoring its nuclear export. Importantly, oxidative stress increases hTERT expression. Because high ionic strength, high glucose, heat-shock, etc. also show similar effect, we postulat-

ed that the HSF1 activation could be involved. This was confirmed due to the lack of hTERT induction in HSF1-KO cells compared to wild-type cells, and by the high basal expression of hTERT due to the mere overexpression of HSF1, even in the absence of stimuli. It is concluded that overall expression level of hTERT depends on HSF1 activation, whereas its subcellular localization is commanded by Hsp90.

539. (624) **PREDICTIVE BIOMARKERS OF RESPONSE TO TREATMENT WITH BACILLUS CALMETTE-GUERIN (BCG) IN PATIENTS WITH SUPERFICIAL BLADDER CARCINOMA**

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Intravesical administration of live attenuated Bacillus Calmette-Guerin (BCG) is the main therapy for intermediate/high grade non-muscle invasive bladder cancer (NMIBC). However, the response rate is only 60%, with a 5-year recurrence rate of 30-40%. In addition, for those patients (pts) with tumors staged as T1 or Cis (carcinoma in situ) that do not respond to BCG, the risk of progression to muscle-invasive disease could reach 50%. Intravesical BCG acts as a local immunomodulator, inducing a massive response of inflammatory cells (Th1 polarization) and ultimately the generation of a cytotoxic response that eliminates the tumor. Our hypothesis is that pts with a pre-existing tumor microenvironment of Th2-polarized lymphocytes and eosinophils would be susceptible to polarization towards Th1 after administration of BCG and respond to therapy. Instead, pts that already have a Th1-polarized tumor microenvironment, would not respond to BCG, probably because the tumor has already developed escape mechanisms to the Th1 response. In the search for a biomarker score to predict BCG response, pre-treatment biopsies of NMIBC pts (n=26), we evaluated by immunohistochemistry the polarization of the tumor microenvironment, quantifying the density and degranulation of eosinophils and T-bet+ (Th1), GATA-3+ (Th2) lymphocytes, all at maximal specific immune population focus. All pts received a 6-week induction plus a 3-week maintenance intravesical instillations of 120mg BCG, Danish strain SSI. Non-responders were defined as any recurrence after BCG treatment. A Th2 score was defined combining lymphocyte polarization GATA3+/Tbet+ (G/T) plus eosinophils density plus eosinophils degranulation. We observed a modest tendency towards the higher Th2 score, with response to BCG (no-recurrence) (Fisher´s exact test, p=0.23).G/T top quartile (>38) is clearly associated to BCG response, although near statistical significance (p=0.063). Given these preliminary results, a prospective study will be initiated to evaluate the score as a predictive biomarker of clinical response to BCG for NMIBC pts.

540. (709) **ROLE OF CAMP EFFLUX MEDIATED BY MRP4 IN PANCREATIC CANCER CHEMORESISTANCE**

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Pancreatic ductal adenocarcinoma (PDAC) ranks among the most lethal of human malignancies. This is due to several factors: lack of early diagnosis, extensive local tumor invasion, early systemic dissemination, and extremely poor response to chemotherapy. Thus, there is an urgent need to improve the therapeutic of PDAC. Previous results from our laboratory indicate that cAMP efflux mediated by MRP4 is critical in PDAC cell proliferation, migration, tumorigenicity, and tumor growth rate. Therefore, the inhibition of MRP4 should be considered an alternative strategy for pancreatic cancer treatment, either alone or combined with chemotherapeutic agents. In this study, we hypothesized that the efflux of cAMP by MRP4 could be responsible of an adaptive advantage, critical in the development of chemoresistance. We treated BxPC-3 human pancreatic cancer cells with clinically used chemotherapeutic drugs which are not substrates of MRP4 (10μM gemcitabine, 50μM 5-fluorouracil, or 5μM paclitaxel; 24 h). Western blot analysis demonstrated a significant increase in MRP4 protein levels in all treated cells ($p<0.01$). Chronic (8 months) treatment with crescent doses of gemcitabine reduced sensitivity to this agent, with a significant shift in IC50 ($P<0.01$) and a concomitant increment of MRP4 levels. Moreover, the addition of cAMP to BxPC-3 cells (100μM) activated proliferative (pERK/ERK) and survival (pAKT/AKT) pathways, which are key in the adaptation to chemotherapy. Also, incubation with cAMP and not its metabolites, adenosine or 5'AMP (50μM), was able to induce a transient increase in Ca+2 intracellular levels, suggesting a direct effect of extracellular cAMP on tumor cells. Collectively, our results indicate that exposure to chemotherapeutic agents induces MRP4 expression, augmenting cAMP efflux, which in turn may act as an autocrine factor on neoplasic cells and as a paracrine factor in the tumor microenvironment. Inhibiting MRP4-cAMP transport may represent a novel therapeutic strategy to prevent or delay PDAC chemoresistance.

541. (610) **CEEFOURIN-1: THERAPEUTIC POTENTIAL OF MULTIDRUG RESISTANCE PROTEIN 4 (MRP4) PHARMACOLOGICAL INHIBITION IN ACUTE MYELOID LEUKEMIA AND PANCREATIC DUCTAL ADENOCARCINOMA**

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Multidrug resistance-associated protein 4 (MRP4) transports anionic compounds and the dysregulation of its expression has been historically associated with drug resistance in several pathological conditions, including cancer. Thus, this protein is a potential therapeutic target in some types of neoplasias. Ceefourin-1, a specific inhibitor of MRP4, has recently been developed. Taking in consideration that MRP4 is the principal transporter of cAMP and that the balance between intra- and extracellular levels of this cyclic nucleotide is crucial in acute myeloid leukemia (AML) and in pancreatic ductal adenocarcinoma (PDAC), ceefourin-1 seems to be a promising compound for cancer therapy. The aim of this study was to assess the efficacy and mechanism of action of ceefourin-1 as an anticancer drug in AML and PDAC models. We evaluated the effect of ceefourin-1 on cAMP extrusion in AML (U937; HL-60) and PDAC (Panc1; BxPC3) cell lines through concentration response curves in a radio-binding assay. Both systems revealed a significant decrease in cAMP efflux in basal and stimulated (25μM forskolin) conditions. Ceefourin-1 inhibition of MRP4 activity was confirmed by measuring intracellular cAMP levels by FRET using Epac-SH187 as a cAMP molecular sensor in HEK293T cells. Treatment of leukemic and pancreatic cancer cells with different concentrations of ceefourin-1 showed that viability is affected only at the highest concentration (100μM; 200μM). MRP4 inhibition with ceefourin-1 and a non-specific MRP4 inhibitor (MK-571) has a concentration-dependent anti-proliferative effect ($p<0.01$) in these cell lines. Finally, acute toxicity was evaluated in Balb/c mice treated with ceefourin-1 for two weeks (sc; 2 and 10 mg/kg /3 times a week). No significant toxic effects were observed, except for mild leukocytosis only with the highest dose. These results show that ceefourin-1 represents a promising selective MRP4 inhibitor for AML and PDAC and leads us to propose future experiments to test its efficacy in vivo.

542. (617) **DEREGULATION OF NON-CODING RNAS IS ASSOCIATED WITH CLINICAL OUTCOME OF CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA**

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Patients with Acute Lymphoblastic Leukemia (ALL) are stratified into risk groups according to biochemical parameters, cytogenetic and molecular signatures, and early response to therapy. Despite differential treatment, there are some patients that recur in all risk groups. One strategy to better understand the biology of childhood ALL is to study the transcriptome of leukemic cells in order to identify gene expression profiles driving the outcome. In this study, we sought to identify gene expression profiles that could predict childhood ALL outcome and acute therapy-related toxicity. We collected samples by bone marrow aspiration at time of diagnosis and isolated RNA. Then, we performed paired-end transcriptome analysis (RNAseq) from 29 pediatric patients with de-novo ALL. Clinico-pathological characteristics were evaluated and recorded. We performed differential gene expression analysis between risk groups, presence of relapse and acute grade-3/4 toxicity, considering that genes were differentially expressed if the FDR adjusted p-value≤0.05. We performed multivariate analyses including the risk group as a covariate for relapse and toxicity. In all comparisons, we found that several of the Differentially Expressed Genes (DEG) corresponded to non-coding RNAs (ncRNA). The most deregulated gene between high risk and intermediate risk patients was a long intergenic ncRNA (Log_2FC=-20, adj.p=0.02). When we compared patients with and without acute grade-3/4 toxicity, 12% of the DEG were ncRNA. Finally, when compared patient with and without relapse we detected 42.5% of the DEG as ncRNA. Among these, we identified two micro-RNAs: miR-6727 (log_2FC=5, adj.p=0.02) and miR-4317 (log_2FC=-17, adj. p=0.0003). Particularly, miR-4317 has been reported to be also down regulated in biopsies of cutaneous malignant melanoma and gastric cancer. There is now robust emerging evidence that alterations of ncRNAs are highly associated with tumor development and progression. The detection of differential expressed ncRNAs might help to improve childhood ALL prognosis and identify new potential therapeutic targets.

543. (746) **CHLORIDE CHANNEL CFTR IS ASSOCIATED TO CANCER STEM CELLS**

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The cancer stem cells (CSC), responsible of tumorigenesis, secondary focus formation in metastasis and chemoresistance. We have previously demonstrated that while the NF-κB coactivator RAC3 is required to maintain the CSC and increase the chemoresistance in colorectal cancer, the NF-κB target gene, cystic fibrosis transmembrane conductance regulator CFTR exerts an anti-apoptotic role.
The aim of this work was to determine if CFTR is expressed in human colorectal cancer cell lines and if this is mainly associated to CSC.
We found that it is expressed in the human colorectal HCT116 cells, as determined by RT-PCR and western blot.
When we performed the analysis of CFTR expression from public repository microarrays data of the human colon cancer cell lines HT29 and CACO-2, we found that the CFTR expression was higher in colonospheres (CSC enriched) from primary HT29 than in cells growing as monolayers and significantly higher in CD133+ (CSC enriched) than in CD133- CACO-2 cells (6 arrays, including 3 CD133+ replicates and 3 CD133- replicates; expression values: 2470,30 ± 396,13 vs. 582,05 ± 25,73 respectively; $p < 0,01$).
The functional associations among CFTR and RAC3 were analyzed using Cytoscape and we found that one of the genes in this network was Vimentin, a typical mesenchymal marker that we previously demonstrated as upregulated by RAC3 overexpression. The analysis of this network by gProfiler software, showed the association to cell differentiation and maturation processes for CFTR and some of these genes.
We conclude that while the CFTR/RAC3 relation type remains to be determined, the high CFTR expression could be mainly associated to CSC in human colorectal cancer.

544. (749) **MUTATIONAL LANDSCAPE OF THE HEAT SHOCK PROTEINS IN CANCER.**

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The Heat Shock Proteins (HSP) are important in cancer development, progression and some are targets for anticancer therapy. The HSP genes are evolutionary conserved and the expression of the different members is variable. A comprehensive study of HSP mutations in different cancers is lacking. The purpose of this work is the mutational profile analysis of HSP in breast, prostate and ovarian cancers. The data were retrieved from the TCGA. We examined the mutations in the complete HSP family genes in 3 different tumor types and compared the HSP mutations with the top 10 mutations of the genome. The Mutation Annotation Format files, which contained somatic or germline mutations generated from whole exome sequencing were downloaded. We observed that mutations in the whole genome appeared in 84% of the breast cancer samples: TP53 (34%), PI3K (33%), TTN (19%), and CDH1 (14%) while the HSP mutations were observed in 8% of the samples: SACS (2%), DNAJC13 (2%) and BBS10 (1%). In prostate cancer mutations appeared in 43% of the samples: TP53 (11%), SPOP (10%) and TTN (9%), while the HSP mutations were infrequent appearing in 5% of the samples: SACS (5%), HSPA8 (1%), DNAJC13 (1%) and HSPA4 (1%). A high level of gene mutations appeared in ovarian carcinomas, in 94% of the samples: TP53 (88%), TTN (34%) and MUC16 (12%), while the HSP mutations were relatively infrequent appearing in 13% of the samples: SACS (4%), DNAJC11 (2%), and DNAJC14 (2%).
Genomic Analysis of Important Aberrations (GAIA), was used to figure out the most significant recurrent CNV in the HSPs. No significant amplification or deletions were observed in HSPs.
Our study revealed that mutations in HSP genes occurred at low frequencies and abnormalities in CNV cannot explain the HSP expression variability observed.
Keywords: Heat Shock Proteins, cáncer, mutations, CNV.

545. (205) **INDUCED PLURIPOTENT STEM CELL-DERIVED MESENCHYMAL STEM CELL DECREASE ENDOTHELIAL DAMAGE CAUSED BY SHIGA TOXIN TYPE 2**

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Shiga toxin 2 (Stx2) is key in microangiopathic events that occur in Hemolytic Uremic Syndrome (HUS). Exposure to lipopolysaccharide (LPS) and Stx2, cause endothelial damage in the renal glomerulus inducing one of the most relevant issues that come to kidney failure in this disease. Mesenchymal stem cells (MSC) are multipotent cells that possess known tissue regenerative properties. Induced Pluripotent Stem Cell-Derived Mesenchymal Stem Cell (iPSC-MSC) has similar characteristics to Mesenchymal Stem Cell (MSC). Our objective was to study if iPSC-MSC could reduce the endothelial damage/dysfunction caused by LPS and Stx2 exposure. For this purpose, we used an in vitro model of endothelial damage using HUVEC cells incubated with LPS and/or Stx for 24 h. We found that iPSC-MSC exposed to LPS decreased their migratory capacity measured as the migrated area after a wound in the cell monolayer compared to Control cells, but the combination of LPS+Stx reversed this effect (area cm2(x102), Control: 5,1±0,4; LPS: 3,6±0,2*; Stx: 3,8±0,3*; LPS+Stx: 6,5±0,4*,*vs. Control, p<0,05). Also, the angiogenic response was increased when HUVEC cells were plated in matrigel with conditioned media (CM) from iPSC-MSC exposed to LPS and/or Stx (Number of branches points from HUVEC, Control:6±0,5; LPS:8±0,2; LPS+Stx:20±0,8*; Stx:18±0,1*,*vs. Control, p<0,05). In this sense iPSC-MSC exposed to CM from HUVEC with LPS and/or Stx improved their adhesion properties to fibronec-

tin and released pro-angiogenic factors such as bFGF and VEGF measured by ELISA (% of adhesion, Control: 100; LPS: 120±11; LPS+Stx: 253±16*; Stx: 198±17*, pg/ml of bFGF, Control:5,1±0,1, LPS: 7,2±0,4, LPS+Stx:19,9±0,7*, Stx:15,3±0,1*and pg/ml of VEGF, Control:9±0,1, LPS: 12±0,3, LPS+Stx:21±0,9*, Stx:18±0,3*, *vs. Control, p<0,05). In conclusion LPS and/or Stx induce in iPSC-MSC pro-angiogenic properties that contribute to decrease the endothelial damage caused by LPS+Stx, promoting endothelial tissue repair.

546. (805) **FXYD5/DYS IS ASSOCIATED WITH HIGH RISK OF RECURRENCE IN ENDOMETRIAL CANCER (EC) AND MODULATES CELL MIGRATION AND NF-KB ACTIVATION IN EC CELLS**

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Endometrial cancer (EC) is the sixth most common cancer in women worldwide; its incidence isincreasing. Current EC diagnosis involves subjective evaluation of tissue biopsy. Uterine aspirates(UA) are a reliable biomarkers source for EC diagnosis. In a cell invasive model (HGE), we reportedincreased expression of FXYD5/Dysadherin (FXYD5/Dys), a cell-surface glycoprotein associated toinvasion/metastasis. In other tumors, FXYD5/Dys regulates cell migration, in part through NF-KBpathway activation, also associated with EC progression. In this study we aimed 1) to modulateFXYD5/Dys expression in EC cells and determine its impact in cell behavior, 2) to evaluateFXYD5/Dys and NF-KB pathway activation interplay, 3) to assess FXYD5/Dys expression in ECpatient endometrial tissues and UA biopsies. Materials/Methods: 1) HGE and Hec1a EC cell linestransfected with pcDNA3-Dys or FXYD5/Dys-siRNA to overexpress or knockdown FXYD5/Dys.qRT-PCR/protein expression analysis/functional assays were done 2) NF-KB pathway activationwas evaluated by assessing target genes 3) FXYD5/Dys expression was evaluated byimmunohistochemistry (IHC) of a Tissue Microarray (TMA) and by UA qRT-PCR. EC RNAseqdataset (Survexpress database) was used for survival analysis. RESULTS: FYXD5/Dys-siRNAtransfected HGE cells showed decreased cell migration and increased cell adhesion, whilepcDNA3-Dys transfected Hec1a cells depicted increased cell migration. FXYD5/Dysoverexpression activated NF-KB pathway in Hec1a cells, triggering CCL2 and TNF-α. IHC revealedassociation between high FXYD5/Dys expression and high-risk recurrence in EC TMA. FXYD5/Dysexpression levels showed correlation (r=0.53) between EC tissues and UA matched-samples.Higher FXYD5/Dys levels were found in UA from high-risk compared to low-risk recurrence.Survival analysis revealed association between higher FXYD5/Dys, CCL2 and TNF-α mRNA levelswith lower survival rates. CONCLUSION: FXDY5/Dys modulates migration and NF-KBactivation In EC cells. FXDY5/Dys expressed in EC tissues and UA are associated with high-riskrecurrence. FXYD5/Dys expression and NF-KB activation target genes are novel markersof EC survival outcome.

547. (780) **UPREGULATION OF LACTATE DEHYDROGENASE B EXPRESSION AND CHANGES IN CELL METABOLISM ARE ASSOCIATED TO A NOVEL EPITHELIAL CADHERIN SPLICE VARIANT. STUDIES IN BREAST CANCER CELL MODELS AND TUMOR SAMPLES**

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Breast cancer (BC) is the most common female cancer and the leading cause of cancer death in women worldwide. Alterations in Epithelial cadherin (E-cadherin) expression/function have been associated with BC, but molecular mechanisms involved in E-cadherin deregulation have not been fully elucidated. We have reported a novel human E-cadherin alternative splice variant (E-cadvar) mRNA. Stable transfection of E-cadvar in MCF-7 BC cells (MCF7Ecadvar) led to fibroblast-like cell morphology, E-cadherin wild type downregulation, changes in epithelial-to-mesenchymal transition markers, reduced cell-cell adhesion and increased cell migration/invasion. A Two-Dimensional Differential Gel Electrophoresis/Mass Spectrometry protein analysis identified lactate dehydrogenase B (LDHB) enzyme as the highest overexpressed protein (Fold=+9.34). LDHB catalyzes interconversion of pyruvate and lactate and was proposed to provide cancer cells with metabolic flexibility. One dimension SDS-PAGE/Western immunoblotting analysis identified a 35 kDa LDHB protein form in MCF7Ecadvar cell extracts. Immunofluorescence microscopy analysis revealed a LDHB signal localized to the cell cytoplasm in both cell lines, being 3.45 times higher in MCF7Ecadvar cells than in control cells. MCF7Ecadvar conditioned media had lower ($P<0.05$) glucose levels and higher ($P<0.05$) lactate concentration than control cells. MCF7Ecadvar cells depicted increased ($P<0.05$) MCT1 and MCT4 lactate transporters mRNA levels. MCF7Ecadvar cells treatment with 2 deoxy-glucose led to a decrease ($P<0.05$) in MCF7Ecadvar cell viability, but not in control cells. Modulation of LDHB expression in MCF7Ecadvar cells with a specific siRNA resulted in a reduction ($P<0.05$) of cell viability. A positive association between E-cadvar and LDHB mRNA expression levels (Odds Ratio 0.06, CI 0.01-0.40; $P<0.01$) was found in human breast tumors. While 80% of tumors depicted low E-cadvar and LDHB mRNA levels, 82% had high E-cadvar and LDHB transcript levels. These studies identified changes associated to E-cadvar expression in BC cells, revealing a relationship with the expression of LDHB, an enzyme linked to BC aggressiveness and poor prognosis.

548. (440) **DICER1 GERMLINE AND SOMATIC MUTATIONS ASSOCIATED WITH DIFFERENT HEREDITARY TUMOURS IN PAEDIATRIC PATIENTS.**

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Carriers of germline DICER1 mutations are predisposed to a rare cancer syndrome, the DICER1 syndrome, associated with tumours such as pleuropulmonary blastoma (PPB), ovarian Sertoli-Leydig cell tumours (SLCT), multinodular goiter (MNG), cystic nephroma (CN), embryonal rhabdomyosarcoma (ERMS) and others. Germline mutations in DICER1 cause an alteration in miRNAs processing, deregulating target oncogenes and leading to elevated risk of tumorigenesis. In most reported cases, there is a heterozygous germline mutation and a somatic second hit mutation.
Aim: Analyse the presence of germline and somatic alterations in the DICER1 gene in 6 patients with paediatric tumours associated with DICER1 spectrum.
Methods: Automated sequencing of DICER1 gene from gDNA extracted from blood and formalin-fixed, paraffin-embedded tumour tissues of affected subjects and relatives.
Cases: 5 girls (P1-P5) and 1 boy (P6), (P1: bilateral SLCT, P2 and P3: SLCT and MNG, P4: MNG and uterine myosarcoma, P5: vaginal rhabdomyosarcoma, and P6: CN). Chronological age at diagnostic was 5, 12, 15, 16, 13 y 2, respectively.
Results: We detected 5 novel heterozygous mutations: p.Trp1098* in P1, p.Phe351fs*1 in P2, p.Arg1596Glyfs*24 in P4, c.3269+1G>A in P5, p.Asp244Glyfs*27 in P6, and one previously described heterozygous deletion p.Asp1437Metfs*16 in P3. These mutations (P1-P4 and P6) predict the presence of a truncated protein losing its essential domains for the enzymatic activity and, in one case (P5) an alteration in the splicing process. The tissue analysis revealed p.Asp1709Glu in MNG and p.Glu1705Lys in SLCT in P2, and p. Glu1705Lys in MNG in P3, previously described as hotspot somatic mutations.
Conclusions: We report five novel germinal mutations in DICER1 gene and two somatic hotspot mutations in MNG and SLCT tissue samples. These findings confirm that a second hit event is involved in the mechanism of MNG and SLCT development. Molecular analysis of DICER1 gene allows to perform genetic counselling about familial recurrence risk.

549. (381) **IS WHITE ADIPOCYTES BROWNING INDUCED BY**

BREAST CANCER CELLS?

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Stromal-epithelial interactions mediate the development and progression of breast cancer. Adipocytes are the predominant stromal cell type in breast tissue. We showed that explants from human breast cancer adipose tissue (hATT) secrete a different set of factors compared to that from normal tissue explants (hATN), which may induce browning of white adipocytes. However, adipocyte characteristics involved in this process remain poorly understood. Here, we evaluated the effects of conditioned media (CMs) of hATN and hATT on expression and localization of different brown and white fat-specific markers on 3T3-L1 adipocytes by indirect immunofluorescence. Interestingly, adipocytes exposed to hATT CMs displayed characteristics that morphologically resembled brown adipocytes. These brown-like adipocytes showed increased UCP1 and Glut4, and number of micro-lipid droplets (LDs, stained for perilipin). Contrarily, adipocytes exposed to hATN CMs increased LDs size, characteristic of white adipocytes. Importantly, immunostaining showed that perilipin was homogeneously distributed on the surface of large-LDs, while a lower intensity signal for UCP1 and Glut4 was observed. Therefore, these findings led us to hypothesize that the changes observed in hATT attached to the tumor could result from a browning process of white adipocytes influenced by the adjacent cancer cells. To demonstrate this, we used indirect co-culture of 3T3-L1 adipocytes and various breast cancer cells. Surprisingly, transwell co-culture with NMuMG or LM3 decreased UCP1 expression ($p<0.001$ One-way Anova), decreased GLUT4 expression ($p<0.01$ One-way Anova) and increased HSL expression ($p<0.001$ One-way Anova), compared to control 3T3-L1 adipocytes. Also, compared to adipocytes cultured alone, the adipocytes indirectly co-cultured with breast cancer cells often showed smaller cell size and smaller LDs. Therefore, we may conclude that breast cancer cells could induce white adipocyte remodeling, probably being the first stage of adipocyte browning. We think soluble factors, as well as cell-cell contact might be involved.

ENDOCRINOLOGÍA / ENDOCRINOLOGY 3

550. (198) **THREE FAMILIES WITH HYPOTHYROIDISM AND MONOALLELIC THYROGLOBULIN MUTATION ANALYZED BY NEXT GENERATION SEQUENCING (NGS).**

Sofia Siffo, Mauricio Gomes Pio, Ezequiela Adrover, Maricel Molina, Karen Scheps, Cintia E. Citterio, Elena Bueno Martínez, Rogelio Gonzalez Sarmiento, Carina Rivolta, Héctor M. Targovnik
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Thyroid dyshormonogenesis due to thyroglobulin (TG) gene mutations have an estimated incidence of approximately 1 in 100,000 newborns. Up to now, one hundred and thirty-seven mutations in the human TG gene have been identified and characterized associated to thyroid diseases.
In three families, with two siblings affected each, only one mutated allele was detected even after sequencing by Sanger all exonic coding sequence, the promoter región and the exon/intron boundaries of the TG gene. This can be considered as straightforward cases of haploid insufficiency in a context of recessive inheritance. In all three families the monoallelic mutation was a nonsense mutation, in one case it was the p.R296* (family A), in another case it was p.R787* (family B) and in the remaining one it was p.E1854* (family C).
The objective of the present work is to analyze by next generation sequencing (NGS) in order to identify possible oligogenicity in our monoallelic cases harboring additional mutations in other thyroid genes that could contribute to the CH phenotype. A custom panel targeting 8 genes associated with dishormonogenesis (TPO, IYD, SLC26A4, TG, DUOX2, DOUXA2, TSHR, SLC5A5) was used for NGS. All exons and exon–intron boundaries of these genes were amplified by multiplex PCR and sequenced using the Miseq Ilumina platform. The NGS confirmed the monoallelic TG mutations in the three families and showed no potentially pathogenic mutations asociates in the other 7 genes analized. Interesting discrepancies in the number of allele variants of some polymorphism were observed between sequencing by NGS and by Sanger and consequently the nucleotide variants in the primers of both sequence methods were analyzed.
In conclusión, It is likely that the apparent absence of a second mutation could be explained by technical limitations of the sequencing analysis, that would indicate the possible amplification of only one allele.

551. (206) **SYSTEMATIC ANALYSIS OF THYROGLOBULIN MUTATIONS FOUND IN PATIENTS WITH HYPOTHYROIDISM AND IN THE GENOME AGGREGATION DATABASE.**

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TG is a large glycosylated protein secreted by the thyrocytes into the follicular lumen by exocytosis and it plays an essential role in the process of thyroid hormone synthesis. The human TG gene is a single copy gene of 270 kb long that maps on chromosome 8q24.2-8q24.3 (chr8: 133,879,203-134,147,147; GRCh37/hg19 assembly) and contains an 8,453 nucleotides in the coding sequence divided into 48 exons. The human TG mRNA encodes a polypeptide chain of 2,767 amino acids.
In the present work, we include the analysis of 51 patients from 33 unrelated families with TG mutations identified in our present (p.R296*/c.3001+5G>A, p.C1281Y/c.5686+1G>T) and previous studies. All patients underwent clinical and biochemical evaluation. Sanger sequencing as well as bioinformatics analysis were performed. Our observation shows that mutations in both TG alleles were found in 29 families (9 as homozygote and 20 as heterozygote compound), whereas in the remaining four families only one mutated allele was detected. 29 different mutations were identified, 34 of the 102 TG alleles encoded the change p.R296*. Additionally, we describe the TG mutation analysis in the Genome Aggregation Database (gnomAD). This website to the present spans 123,136 exome sequences and 15,496 whole-genome sequences from unrelated individuals sequenced as part of various disease-specific and population genetic studies. In total, 346 clearly pathogenic TG variants were described, 61 nonsense mutations, 226 splice site mutations (acceptor site [AG], 14; donor site [GT], 31; exonic splice region, 28; intronic splice region, 153) and 59 frameshifts (insertion, 14; deletion 45). The most frequent mutation that causes a premature stop is p.R296* (96 of 277,236 alleles, all heterozygous).
In conclusion, the identification and characterization of TG mutations is undoubtedly a valuable approach to study the TG structure/function relations and also provides an important tool for clinical diagnosis and genetic counseling.

552. (253) **IODINE ACTION ON THE METAMORPHOSIS OF XENOPUS LAEVIS AND ITS UTILITY AS AN ANTAGONIST OF THYROID DISRUPTION GENERATED BY CONTAMINANTS SUBSTANCES PRESENT IN GROUNDWATER USED FOR HUMAN CONSUMPTION.**

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Intake of groundwater with endocrine disruptors is a risk for exposed populations. Amphibian larvae are very sensitive to compounds with thyroid disruptor action. Objectives: Evaluate antagonistic effect of iodine on the action of groundwater contaminated with thyroid-disrupting substances, in the metamorphosis of amphibians by means of a bioassay of chronic toxicity. Xenopus Laevis larvae were used immersed in water: a) filtered network as control (C) (n=5), b) groundwater of 30-meter depth of the Glew city with aggregate of iodine (6.7gr/l) (ANI) (n=8) and c) groundwater without iodine (AN) (n=8). After 69 days of exposure, the morphological assessment was performed by determining weight, height and stages of metamorphosis (premetamorphosis, prometamorphosis and climax) according to the Nieuwkoop and Faber criteria. The statistical analysis was performed with the Fisher and Tukey tests. The weight and final size did not differ between the groups. The metamorphosis was completed in 100% of the C, 60% of the ANI ($p<0.0001$) and 42.8% of the AN ($p<0.0001$), observing difference between the experimental groups ($p<0.02$). The total time of metamorphosis was higher in AN (67 ± 1.5) vs. C (55 ± 1) and ANI (51 ± 0.6) ($p<0.02$). The premetamorphosis time was higher in AN vs. ANI ($p<0.05$), being similar in C and AN. The duration of prometamorphosis it was: C: 24 ± 2 days, ANI: 25 ± 2.1 days, AN: 36 ± 0.5 ($p<0.0001$). The morphological changes, dependent on thyroid hormones, caused by the groundwater are reversed with iodine; suggesting a possible antagonistic effect on the iodine transporter of the substances present in the water of the study zone.

553. (384) **CHARACTERIZATION OF EXTRACELLULAR VESICLES RELEASED BY TRIIODOTHYRONINE-STIMULATED MICE DENDRITIC CELLS (DCS): ROL IN PARACRINE DC ACTIVATION**

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We provided evidence of triiodothyronine (T3) stimulation of mice dendritic cells (DCs), driving pro-inflammatory and cytotoxic responses, exploited in an antitumor DC-based vaccination protocol. Extracellular Vesicles (EVs) exhibit a crucial role in cellular communication and EVs secreted by DCs (EVs-DCs) may be involved in the amplification of the immune response. Our aim was to characterize the populations of EVs-DCs and assess their role in paracrine DC communication after T3 exposition. Immature bone marrow DCs (iDC) were obtained from WT C57BL/6 mice and stimulated (or not) with T3 (5nM-18h, DCs-T3). Secreted EVs-DCs from iDCs and DCs-T3 (EVs-DCs-T3) were isolated by differential ultracentrifugation at 2,000g (2K, large EVs); 10,000g (10k, Microvesicles); and 100,000g (100K, small EVs: sEVs). Morphological analysis of EVs was conducted by Transmission Electron Microscopy (TEM) and dynamic light scattering (DLS), and molecular characterization of EVs by western blot analysis (CD63 and CD81). A functional assay evaluated the syngeneic DC profile induced by treatment with EVs-DCs-T3 (markers of DC maturation: MHCII, CD86, and CD40, Flow Cytometry-FACS; and IL-12, FACS and ELISA). Statistical analysis: ANOVA-SNK. Data obtained from TEM analysis of the different fractions from both, iDCs and DCs-T3, showed the presence of secreted EVs. Size profile analysis revealed a significant higher frequency of 150-600 nm for 2K and 10K, whereas 100k exhibited more than 90% ($p<0.01$) of EVs sized 30-150 nm (sEVs). DLS analysis showed a similar pattern. Besides, sEVs secreted by DCs-T3 significantly increased the expression of DC phenotypic maturation markers, and the production and secretion of IL-12. This study allowed the morphological characterization of EVs populations secreted by mice DCs that in turn induced the activation of syngeneic iDCs, endowing these cells with a Th1-type driving phenotype that may be involved in the adaptive response induced by T3 exposition to DCs.

554. (318) **ACTIVINS AND PROLACTINOMA DEVELOPMENT I. GENDER DIFFERENCES**

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Activins are members of the transforming growth factor beta (TGFβ) family of proteins. They are homodimers composed of βA subunits (activin A) or βB subunits (activin B). Besides their known function on pituitary gonadotrophs, activins also modulate lactotroph population, being inhibitors of cell proliferation and prolactin secretion. Its inhibitory function is modulated by inhibins and follistatin (FST). Up today, the role of activins in prolactinoma development remains unknown. We propose that alterations in the pituitary activin-inhibin system (Ac-In) are involved in prolactinoma development. In this work, we studied the pituitary expression (mRNA by RTqPCR) of Ac-In components in two experimental models of prolactinoma: mice deficient in dopamine receptor type 2 (Drd2 -/-) and mice over-expressing the β subunit of human chorionic gonadotropin (hCGβ+). These animal models present sex differences: an increase in the pituitary size and hyperprolactinemia are observed only in adult female transgenic mice, but not in males. We found that pituitaries from Drd2-/- and hCGβ+ females (prolactinomas) present decreased expression of several components of Ac-In system: βA and βB subunits, and activin receptors (ActRIB, ActRII), concomitant with an increase in FST expression (activin antagonist). On the other hand, male pituitaries (both mice models) present higher expression of Ac-In components compared to females, without genotype differences. According to these results, we postulate that: 1- As activins are inhibitors of lactotroph function, a decreased expression of Ac-In system (βA, βB, receptors), concomitant with an increase in FST expression, is involved in prolactinoma development in transgenic female mice (Drd2-/- and hCGβ+); 2- The higher expression of Ac-In system found in male pituitaries (absence of adenoma), could be protecting this sex from prolactinoma development.

555. (320) **ACTIVINS AND PROLACTINOMA DEVELOPMENT II. EFFECTS OF AN OVARIECTOMY**

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It was previously shown that an ovariectomy (OVX) prevents pituitary hyperplasia in Drd2-/- and hCGβ+ female mice, indicating the participation of ovarian factors in the development of prolactinoma. However, a hormone replacement with estradiol after OVX does not restore tumor development. These results suggest the participation of other ovarian factors. In this work, we propose that gonadal inhibins are involved in the development of these pituitary tumors. Inhibins and activins are members of the TGFβ family of proteins. Activins are homodimers composed of βA subunits (activin A) or βB subunits (activin B). Inhibins are heterodimeric proteins composed of a α subunit and a β subunit (βA or βB), giving rise to inhibin A (α–βA) or inhibin B (α–βB). Gonadal secreted inhibins antagonize, in the pituitary, the activin inhibitory function on lactotroph population. We postulate that an OVX, induces alterations in the pituitary activin-inhibin system (Ac-In), preventing prolactinoma development. Results: Pituitaries from adult Drd2-/- and hCGβ+ female mice (prolactinomas) show a reduced mRNA expression (analyzed by RTqPCR) of the Ac-In system components (βA and βB subunits, ActRI and ActRII receptors) when compared with WT counterpart. On the contrary, when Drd2-/- and hCGβ+ female mice are ovariectomized at 2-month-old, they do not develop prolactinoma in the adulthood, and the pituitary expression of Ac-In system remains similar to those expressed in WT counterpart. According to these results we postulate that, after OVX, the decline in circulating inhibins restores the pituitary activin inhibitory-function on lactotroph population, preventing the development of prolactinoma.

556. (629) **PRL-1 EXPRESSION IN A PROLACTINOMA EXPERIMENTAL MODEL: A POSSIBLE ROLE IN THE TUMORAL DEVELOPMENT**

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PRL-1 (phosphatase of regenerating liver 1) is a subclass of protein tyrosine phosphatases involved in proliferation, migration, invasion and growth tumor, and have been proposed as potential biomarkers of tumor progression. Our aim was to investigate whether PRL-1 is expressed in a lactotroph tumoral experimental model and analyze its correlation with the cell cycle regulatory protein p21, during the pituitary tumorigenesis. To accomplish this objectives Fisher male rats were treated with estradiol benzoate (EB) during 10, 20 and 30 days (d) to induce an experimental pituitary tumor (n: 9). As control group (C) normal pituitary glands (n: 3) were used. The subcellular localization was visualized using immunocytochemical techniques (confocal and electron microscopy). A semiquantitave study by western blot (WB) was used. Statistic analyzes: Anova-Fisher and correlation analyze ($p<0,05$). At electron microscopy and confocal level, tumors exhibited PRL-1 immunolocalyzation predominantly in cytoplasmic level. By WB PRL-1 showed a significant increase during prolactinoma development, reaching the highest level at 30d and demonstrating a lineal correlation with p21 ($r=0,86$ $p<0,05$). At this time of the experimental model with also visualized an increase of the proliferative index (ki 67). Our investigation is the first to show PRL-1 expression in a lactotroph tumoral experimental model correlated with p21 protein. This finding would imply the existence of others possibles counterregulatory mechanisms of proliferation during the prolacinoma development.

557. (783) **ANGIOGENIC MEDIATORS AND NOTCH PATHWAY COMPONENTS ARE ASSOCIATED WITH DOPAMINERGIC D2 RECEPTORS IN HUMAN PITUITARY ADENOMAS**

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Pituitary adenomas (PA) account for the 25% of intracranial tumors. Dopaminergic agonists, as Cabergoline (CG), are the first line therapies for these tumors. CG through D2 receptor (D2R) reduces tumor volume and hormone secretion in prolactinomas and in other types of PA. However, some patients do not respond to CG and need radiation and/or surgery.
Angiogenesis is essential for tumor growth and metastasis, but its relevance in pituitary tumors development is still controversial. Notch signaling is involved in angiogenesis regulation as in other cellular processes such as proliferation and cell renewal. In previous work, we demonstrated Notch pathway components expression in human pituitary adenomas and experimental models. In this work, we aimed to determine the association between angiogenesis, Notch pathway components and D2R in human PA. The expression of D2R, NOTCH2-3 receptors, the target gene HES1, the ligand JAGGED1 and the angiogenic markers VEGF, FGF2 and ENDOCAN were determined by RT-qPCR. Ki-67 proliferation index and α-SMA-based microvessel density were evaluated with immunohistochemistry.
A strong positive correlation between D2R and VEGF was observed ($R2=0,96$; $p=0,02$; $n=14$). Furthermore, we found that VEGF also correlated with Notch target gene HES1 ($p=0,03$; $n=11$). The microvessel density and the vascular area in Non-functioning tumors were higher than in Functioning tumors; however, the vessel size was lower in Non-functioning. No correlation was found between the expression of the previously stated genes or α-SMA values with tumor proliferation index.
Our results demonstrate an interaction between D2 receptor, angiogenesis and Notch signaling in pituitary adenomas and suggest that CG treatment response could be associated to the activation state of these pathways. Therefore, a combined approach consisting of CG and VEGF or Notch inhibitors could be more effective for resistant pituitary adenoma treatment.

NEUROCIENCIAS / NEUROSCIENCE 3

558. (61) **COGNITIVE ASSESSMENT IN PATIENTS WITH GENERAL ANXIETY DISORDERS AND ALLOSTATIC LOAD TREATED WITH ALPRAZOLAM: SEX AND AGE INFLUENCE**

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Introduction: General anxiety disorders (GAD) are frequently associated to chronic stress with possible negative cognitive consequences. As well, anxiolytic drugs may induce cognitive adverse events. The aim of this study was to determined sex and age related differences in the cognitive performance of patients with GAD treated with alprazolam. Methods: Patients with GAD (DSM IV), with >6 in the Hamilton anxiety scale (HAM-A), > 18 de neuroticism (NEO-FFI inventory) and normal Mini-Mental state examination. Cognitive assessment included, the Five Point Test (FPT), the Fluency Verbal Test (VFT), the Continuous Performance Test (CPT) and the Digit Spam Test (DST). Cognitive variables and allostatic load parameters were determined before and after 12 weeks of treatment. Two way ANOVA and the Student t test were determined to analyze age and sex related factors. Results: 35 women (48.6 ±12 years) and 19 men (44.2 ±12.8 years), 28 patients with <50 years (60.7%) and 26 with ≥ 50 years (69.2%) were included. Women showed a higher level of anxiety with a lower cognitive performance before treatment in the FPT and in the DST (Tow way ANOVA $p<0.05$). After treatment, anxiety symptoms were reduced in all patients, but allostatic load was significantly reduced in the younger patients ($p<0.05$). Cognitive performance did not get worse except for the number of repetitions in the FPT in female sex ($p<0.05$). The DST performance was lower after treatment in female sex the same as anxiety ($p<0.05$). Conclusion: In this preliminary study clinical and cognitive differences were found regarding sex and age, in congruence with other studies that show sex and age related differences in stress and therapeutic response.

559. (63) **EXPRESSION PATTERN OF CALBINDIN INMUNOREACTIVITY IN NON-DYSPLASTIC TEMPORAL POLAR CORTEX FROM PATIENTS WITH TEMPORAL LOBE EPILEPSY AND HIPPOCAMPAL SCLEROSIS**

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Introduction : Alterations in the expression of calcium-binding proteins have been reported in focal cortical dysplasias, but only a few studies were performed in the non-dysplastic cortex in patients with resistant epilepsy. The aim of this study was to determine the expression of calbindin inmunoreactivity and the degree of astrocytic reaction in the non-dysplasic temporal polar cortex resected durign surgical procedure in patients with temporal lobe epilepsy (TLE) and hippocampal sclerosis (HD) without dysplastic alterations. Methods: In all patients, clinical, neurological, EEG, video-EEG, MRI, psychiatric and neuropsychological evaluation have been performed prior to surgery. Patients with cortical dysplastic alterations detected by MRI and / or by histopathology were excluded. Microtome histological sections form the polar temporal cortex were processed by immunohistochemistry (immunoperoxidase) with anti-calbindin antibodies (CB) and anti-acid gliofibrillary protein (GFAP). A semiquantitative analysis of the immunoreactivity was carried out using the program (Image J) and the Student's Test was applied. Results: Twenty seven patients (14 women and 13 men, age 32.04 ± 9.53 years) and 5 post-mortem controls were included. Compared to the controls a

significant increase in the percentage of CB + cells in layer II of temporal pole cortex was found in tissue from patients with epilepsy ($p < 0.05$), as well as an increase in the reactive area for CB in layer II and layer III ($p < 0.05$). No differences were observed reagarding optical density ($p > 0.05$). In addition an increase in the expression of GFAP ($p < 0.05$) was found. Conclusions: In this exploratory study an increase in the expression of CB in layer II (marker of GABAergic interneurons) was found, associated with a greater astrocyte reaction, both alterations would be triggered by the epileptic activity in the polar temporal cortex. It is unknown yet if these changes have prognostic implications, it will be investigated in future studies.

560. (173) **PARABENS REPOSITION AS ANTICONVULSANT DRUGS.**

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Parabens are a homologous series of esters of p-hydroxybenzoic acid, used as antimicrobial preservatives in foods, drugs and cosmetics for over 50 years. It has been shown that propylparaben blocks sodium channels in rat brain slices, reducing the excitability of hippocampal neurons (doi:10.1016/j.neuro.2016.09.019). In addition, it has anticonvulsant activity against Maximal Electroshock Seizure test (doi:10.1007/s10822-007-9136-9) and pilocarpine-induced seizures, and reduces the neuronal damage and excitability induced in this last model(doi:10.1016/j.neuro.2017.01.009).
The aim of this study was to fully characterize the mechanism of action of propylparaben, other esters of the series (methylparaben, ethylparaben, buthylparaben and benzylparaben) and p-hydroxybenzoic acid, the main metabolite of parabens, on human NaV1.2 sodium channels. We tested the effect of these compounds on NaV1.2 channel heterologously expressed in HEK293 cells by applying the patch-clamp technique in the whole cell configuration.
All parabens, with the exception of methylparaben, inhibited Nav1.2 currents in a state- and use-dependent manner. Both effects increased as the side of the hydrocarbon chain of the ester group augmented. The state-dependency was observed as a left-shift in V1/2 for steady-state inactivation curve, which was twice and three times greater, respectively, for buthylparaben and benzylparaben compared with propylparaben ($p<0.001$). The use-dependent inhibitory effect was observed as a faster decay in currents evoked in a train of voltage-steps at 50 Hz ($p<0.05$). Propylparaben and benzylparaben were also tested at lower frequencies, where, consistently, they showed a minor and slower inhibitory effect (25 Hz vs. 10 Hz; $p<0.01$). Finally, p-hydroxybenzoic acid also inhibited Nav1.2 currents, with a relatively lower potency in comparison with parabens and thus, it may be responsible for a residual blocking effect after the metabolism of parabens, extending its action.
Parabens profile showed that their effect could be grater in the epileptic focus with respect to normal neurons which would be beneficial in antiepileptic treatment.

561. (243) **ONE-DIMENSIONAL VALUES IN THE SAME MAGNETIC RESONANCE IMAGES OF THE PREFRONTAL LOBE AND OTHER REGIONS OF THE BRAIN IN TWO AGE RANGES**

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Considering the importance of the functions that involve the prefrontal lobe (PFL), the objective of this study is to quantify in parasagittal magnetic resonance images (MRI) by age ranges, one-dimensional values of the PFL in normal aging and simultaneously those of other regions of the brain that share the same images.MATERIALS AND METHODIn MRI equidistant 4 mm from the parasagittal plane of 64 female subjects (41-60 and 61-84 years) without diagnosis of mental or neurological disease neurological, in the prefrontal area, between reliable anatomical references with the Scion Image for Windows program were measured the 2 segments and other 2 in the rest of the brainThe segments that we denominate DA and AV corresponding to the prefrontal region and DP and VP . to the rest of the brainRESULTSLength in cm (mean ± SE) right of 41-60 and 61-84 years: DA 5.46 ± 0.08 and 5.02 ± 0.07 and AV 4.48 ± 0.07 and 4.11 ± 0.06; DP 12.91 ± 0.11 and 12.88 ± 0.10; VP 12.53 ± 0.12 and 12.55 ± 0.10. The DA and VA lengths are greater ($p < 0.01$ ANOVA) at the age of 41-60 years compared to those of 61-84 years, as well as the left segments. Not the DP and VP.The Pearson correlation coefficients are between age and right and left female values of DA -0.64 and -0.55; of AV -0.63 and -0.40; of DP -0.11 and -0.14; of VP -0.04 and -0.05. For AD and VA, the correlations with age are significant ($p < 0.01$). CONCLUSION: the significant decrease with age of measured lengths over the prefrontal region in relation to other regions of the brain is compatible with greater deterioration of this with aging.

562. (245) **MORPHOLOGICAL STUDY OF PRECUNEUS IN MAGNETIC RESONANCE IMAGING**

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The precuneus is an important center of functional and structural integration of the human brain of complex and heterogeneous anatomy (Bruner, 2016) where the first symptoms of Alzheimer's disease are located (Calvino, 2015).The objective is to obtain absolute and relative longitudinal values of the precuneus by age ranges in parasagittal magnetic resonance imaging (MRI) of female control subjects.MATERIALS AND METHODIn an MRI equidistant 4 mm from the parasagittal plane of 49 women (21-84 years) without mental or neurological in the precuneus area, between 4 reliable anatomical references with the Scion Image for Windows program were measured the segments that we denominate anterior, upper, posterior and lower that form the perimeter.The perimeter and the percentage of each segment (mean ± ES) of the groups of 21-40, 41-60 and 61-84 years were calculated.RESULTSThe right perimeter (mm) mean ± ES of 21-40; 41-60; 61-84 years is 136.34 ± 4.63, 138.06 ± 2.57; 136.66 ± 3.44 does not differ with age.The percentage contributed by the segments to the right perimeter of 21-40; 41-60 and 61-84 years: anterior 17.49 ± 0.97; 17.37 ± 0.65; 16.78 ± 0.82, upper 29.31 ± 1.23; 30.30 ± 0.84; 31.13 ± 0.50, posterior 24.26 ± 1.0; 24.45 ± 0.81; 24.55 ± 0.67 and lower 28.94 ± 0.83, 27.88 ± 0.56; 27.54 ± 0.67. The percentages differ among themselves ($p < 0.01$ ANOVA) in all age ranges except between upper and lower of 21-40 years.The left values are not significantly different from the right.Conclusion: In IPRM of female control subjects: 1-the precuneus perimeter does not vary significantly with age, 2- its shape increases its heterogeneity after 40 years.

563. (469) **ELEVATED PLASMA LEVELS OF IL-12 STRONGLY CORRELATE WITH A HIGHER PERCENTAGE OF NON-CLASSICAL MONOCYTES AND MORE ACTIVATED CLASSICAL MONOCYTES IN THE BLOOD OF PATIENTS WITH MAJOR DEPRESSIVE DISORDER.**

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Major Depressive Disorder (MDD) is a prevalent and disabling condition that comprises a set of pleomorphic syndromes with emotional, cognitive, visceral and behavioral symptoms domains. Despite the public impact of depression its etiology remains unknown. However, in the last years, evidences suggesting the role of the innate and the adaptive immune system in MDD have emerged. Monocyte-macrophage lineage orchestrates the initiation and resolution phases of innate and adaptive immunity, with significant impact on protective immunity and immune-mediated pathological damage. Monocytes can be defined in three populations as classical (CD16negCD14bright), intermediate (CD16+CD14+) and non-classical (CD16brightCD14neg) subsets. Several studies have shown that

non-classical monocytes are increased in patients with inflammation processes and infectious diseases. We aimed to characterize the plasma levels of IL-12 and IL-6 together with the monocyte subsets distribution and their activation status in patients with MDD. Blood samples were obtained from patients enrolled in a multicenter prospective cohort study approved by the IRB. Written informed consent was obtained for all participants. In a sample of patients with MDD and no other medical illness or substance abuse (N=33), 36% of patients showed higher levels of IL-12 and 54% of patients higher levels of IL-6 compared with a matched distribution of age and gender healthy control group (N=20). Furthermore, 27% of patients exhibited increased levels of both cytokines. Interestingly, a significant increase of non-classical monocytes fraction in patients with MDD positively correlated with higher levels of IL-12 ($r=0.51$, $p<0.001$). Noteworthy, the percentage of activation markers CD40+CD86+ in classical monocytes was increased in patients with MDD and higher levels of IL-12 ($p<0.05$) or IL-6 ($p<0.01$) compared to patients with low cytokine levels or healthy controls. We have identified a clear pro-inflammatory profile in a fraction of patients with MDD suggesting that this condition should be contemplated for diagnosis and therapeutic approach.

564. (661) **SERA FROM PREDIABETIC PATIENTS INDUCE CHANGES IN NEURONS AND GLIA IN MIX PRIMARY CULTURES**

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Type 2 diabetes (DBT2) is a metabolic disorder clinically characterized by an increase in blood glucose levels. It is well known that DBT2 causes neurodegeneration, however, molecular mechanisms involved in this process are not clear yet. Several hypotheses were proposed in order to elucidate molecular changes and cascades involved in neurodegeneration caused by DBT2. Amongst them, the ones related to RAGE products, stress signaling and chronic inflammation are the best investigated. Furthermore, high glucose levels were also proposed as a mechanism for neurodegeneration. Pre-diabetes 2 (preDBT) is considered to be a step before DBT2, with glucose levels intermediate between normal and DBT levels. PreDBT is thought to be reversible. However, it is not well understood if pre-DBT syndrome per se, or the progression to DBT2, could cause neurodegeneration or synaptic plasticity impairment. In order to find out which mechanisms are involved in these processes, and to identify whether they start during pre-DBT, we decided to investigate if neurodegeneration could take place in these early stages. For this reason, we incubated primary mixed cultures (astrocytes and neurons) with sera from patients with altered fasting glucose (AG) for seven days. As controls we used sera from persons with normal fasting glucose values (NG). Sera from both groups differed only in glucose values, and had normal values for insulin, cholesterol and triglycerides. Our results indicate that sera from AG patients induced changes in astrocytes number and shape. These cultures also showed altered neuron morphology, together with a decrease in the number of neurons counted. Both the increase in astrocyte and the reduction in neurons percentage correlate with glucose levels. These preliminary results would lead us to hypothesize that the increase in glucose values could start changes in neurons and glia that are compatible with neurodegeneration.

565. (711) **BENEFICIAL EFFECTS OF COMBINED TREATMENT WITH MORPHINE OR METHADONE AND OMEGA-3 FATTY ACIDS FOR THE TREATMENT OF PAIN**

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In spite of their undesirable side-effects, such as sedation, respiratory depression, constipation and development of tolerance and dependence, opioids such as morphine (Morp) and methadone (Meth) still remain the most effective drugs for the treatment of severe pain. Efforts have been made to optimize opioid treatment strategies, in search of the most effective drug with minimal side-effects, as well as the possibility of drug combinations. Recent evidence has shown that some omega-3 fatty acids (ω-3 FA) can relieve pain. The objective of this study was to evaluate and compare the analgesic activity using the hot plate test (HPT) and some side effects of morphine and methadone either alone or in combination with treatments with ω-3 FA after 16 days in rats. We found that compared to control, acute and chronic ω-3 FA (720 mg/kg/day) administration increased the response latencies indicating that ω-3 FA has antinociceptive effects. Co-administration of chronic ω-3 FA and Morp (5 mg/kg) or Meth (3 mg/kg) administration revealed a higher antinociceptive efficacy than the individual treatments in rats in HPT. Also, we observed that the combined treatments reduce the tolerance to analgesic effects, sedation and body weight loss. These data might contribute to new therapeutic approaches for the treatment of severe pain. and may mean higher response rates and lower side-effects associated with Morp or Meth treatment. More studies are required to understand the action mechanism underlying the use of the combination of Morph or Meth treatment.

566. (726) **SINGLE CHAIN FV FRAGMENTS AS A TOOL FOR AB OLIGOMERS DETECTION**

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Alzheimer's disease (AD) is a neurodegenerative disorder of the Central Nervous System that affects millions of people in the world. AD involves progressive lost in cognitive functions due to neuronal death in hippocampus and other related areas. From the Aβ theory point of view, AD was first characterized by the presence of amyloid plaques composed by Aβ peptides aggregates. Years after, it was discovered that neither Aβ peptides, nor amyloid plaques were responsible for synaptic failures and neuronal death. In parallel, it was shown that soluble Aβ aggregates (from 3 to 50 monomers) called Aβ oligomers (AβOs) were the main toxins in this pathology. Currently, although palliative therapy for AD exists, there are no treatments that inhibit the progression of this pathology. The development of new biotechnology tools that help to investigate changes in AβOs levels at AD earlier stages would allow delaying the onset or progression of EA symptoms through secondary prevention therapies, leading to an improvement in AD patient's quality of life. In this context we built an Adeno associated Vector (AAV) for transiently expressing a single chain variable fragment antibody (scFv) that binds specifically AβOs (AAV-scFv-NUSC1Glu). Thus, we sought to develop an assay that allows us to differentially detect AβOs levels in AD models samples. We expressed the AAV-scFv-NUSC1Glu in cell lines and collected the scFv from the supernatant. Then we tested two different ELISA assay models for AβOs detection: A Direct ELISA and a Competitive ELISA. Preliminary results showed that a competition essay was more effective for ABOs detection. However, more essays could be performed in order to improve ABOs detection.

INMUNOLOGÍA / IMMUNOLOGY 5

567. (307) **KIDNEY TRANSPLANT PATIENTS CONVERTED TO BELATACEPT SHOW DIFFERENT IMMUNOPHENOTYPING COMPARED WITH DE NOVO BELATACEPT-TREATED PATIENTS**

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Belatacept is a CTLA4-Ig fusion protein that contain the extracellular domain of CTLA-4 coupled to the Fc fragment of a human immunoglobulin G1 antibody. It blocks the costimulatory signals mediated by B7-CD28 and it has been used in kidney transplant patients. However, the incidence of adverse clinical events mainly in early, but not late post-transplantation such as an increased rate of acute rejection precludes the use of Belatacept in all transplant patients. The aim of this study was to compare the cell immunophenotyping in kidney transplant patients treated with Belatacept from the begining (de novo) with those converted to Belatacept from calcineurin inhibitors. For both groups of patients, the immunophenotyping were performed after at least 9 months of the introduction of Belatacept to the immunosuppresive regimen. Peripheral blood mononuclear cells were isolated from 30 transplant patients (16 de novo). There was not statistically significant difference in patients age, BMI, transplantation time, leukocytes numbers between both groups. Several costimulatory molecules and their ligands were measured on monocytes and lymphocytes by flow cytometry. There was not statistically differences on monocytes expression of CD80, CD86, B7H2, CD40 and PD-L1 between both groups of patients. However, there was a slightly higher expression of SLAM (p=0.05) on monocytes derived from converted patients. On T cells, de novo patients had higher levels of CD27 (p=0.01) and CD28 (p=0.05) but lower of ICOS (p=0.03) compared with converted patients. Then, we assessed the lymphoproliferation capacity of PBMC from de novo and converted group of patients. Regardless of the group of patients, proliferation was the same in response to PHA but de novo showed higher allostimulatory capacity than converted patients (p=0.02). Overall these results show that Belatacept converted-patients has a different leukocytes immunophenotype than de novo and this could affect the allostimulatory capacity.

568. (311) **LACTOBACILLI INHIBITS THE FORMATION OF NEUTROPHIL EXTRACELULLAR TRAPS IN A PMA INDUCTION MODEL.**

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Neutrophils are part of the first line of immune defense. Neutrophils utilize different mechanisms to protect hosts such us phagocytosis, degranulation and production of neutrophil extracellular trap (NET). NETs are DNA web-like extracellular structures that immobilize and kill bacteria due to the adhesion of antimicrobial proteins. Uncontrolled NETosis leads to liberation of cell-free DNA and proteases that damage the surrounding cells and exacerbate inflammation. In the gut environment, the presence of beneficial microbes could reduce intestinal inflammation and protect the host. We investigated the capacity of two lactobacilli strains L. delbrueckii subsp lactis (CIDCA 133) and L. delbrueckii subsp bulgaricus (CIDCA 331) to inhibit NET formation.
Human neutrophils were isolated from heparinized venous blood using gradient centrifugation. Cells were incubated with or without lactobacilli at different multiplicity of infection (moi). PMA was used as NET inducer. NETs were visualized by immunofluorescence microscopy. ROS was determined by luminometry.
Results show that in the absence of stimuli lactobacilli lack the ability to induce NETs. Moreover, lactobacilli (MOI 10) have the ability to significantly reduce NETs formation in response to PMA challenge. Strain CIDCA 133 showed a stronger capacity to decrease the number of netotic cells than strain CIDCA 331 (66,09 % ± 3,09 vs 27,69 % ± 0,17, respectively) after PMA treatment. Lactobacilli showed different capacity to stimulate ROS generation, with strain CIDCA 133 as a strong inducer and strain CIDCA 331 as a non-inducer. Neither of these strains were able to inhibit ROS generation in PMA treated cells.
Our results showed that lactobacilli strains had the ability to reduce NET formation but not to inhibit ROS generation in PMA treated cells. NET formation is tightly related to ROS generation and autophagy in PMA induced NETosis. It is possible that lactobacilli are affecting the autophagy process, therefore, reducing NET formation.

569. (315) **LACTOBACILLI STRAINS ENHANCED HUMAN B DEFENSIN 2 (HBD-2) EXPRESSION IN FLAGELLIN-INDUCED CULTURED INTESTINAL CELLS.**

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Human defensins are small cationic peptides which contribute to the mucosal host defences. Gut epithelia produce β-defensins (HBD-1 and HBD-2). HBD-1 has a constitutive expression whereas HBD-2 is inducible by endogenous stimuli such as inflammatory cytokines or by exogenous microbial factors (PAMPs) in a toll-dependent manner. HBD-2 expression is promoted by NF-kB. Certain probiotic strains could exhibit immunomodulatory effects at intestinal level including the induction of HDB-2 expression. The aim of this study is to investigate the capacity of two lactobacilli strains (L. delbrueckii subsp lactis CIDCA 133 and L. delbrueckii subsp bulgaricus, CIDCA 331) to induce HBD-2 expression in human enterocyte-like cells (Caco-2 and HT-29).
Monolayers were incubated with lactobacilli (20 bacteria per cell) for 24 h at 37°C. HBD-2 expression was measured by qPCR. Positive controls were stimulated with flagellin from Salmonella sp. (FliC). NF-KB expression was assessed by using a reporter HT-29 cell line. Lactobacilli strains alone were unable to induce HBD-2 expression. However, the co-incubation of cells with lactobacilli and flagellin significantly increased HBD-2 expression. UV or heat-killed strains had also the capacity of inducing HBD-2 expression. Lactobacilli strains per se were unable to increase the expression of NF-kB. Previous incubation of the intestinal cells with lactobacilli followed by FliC stimulation reduced by 50% the percentage of NF-kB positive cells. Results showed that the lactobacilli strains enhanced the HBD-2 expression induced by FliC. The FliC pathway involves TLRs and expression of NF-kB. Interestingly, lactobacilli down-regulated the NF-kB expression. Several proinflammatory and antimicrobial factors are up-regulated by NF-kB. Lactobacilli strains under study had the capacity to stimulate the expression of HBD-2 without exacerbate NF-kB response thus indicating a non-inflammatory pathway of modulation.

570. (328) **HUMAN NEUTROPHIL RESPONSE TO SHIGA TOXIN**

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Shiga-toxin-producing E coli (STEC) infections can cause hemolytic uremic syndrome (HUS), a life-threatening condition. These non-invasive bacteria colonize the intestine where they release the Shiga toxin (Stx) which, after reaching the blood stream and binding to Gb3Cer receptor, causes the characteristic events of the HUS: hemolytic anemia, thrombocytopenia and renal failure. Recently, it has been proposed that Stx is transported in circulation in extracellular vesicles (EV) released by leukocytes and platelets. Neutrophilia is a typical finding in patients with HUS, and elevated neutrophil (PMN) counts are considered a poor prognostic factor. The aim of this research is to determine if PMNs produce EV in response to Stx type 2 (Stx2) and to analyze its cytotoxic capacity. 10^7 PMN isolated from human peripheral blood from healthy donors were incubated with Stx2 (0.1 μg/ml) or with vehicle for 4hs. Afterwards, supernatants were centrifugated at 16000 x g for 30 min to obtain the EV (EV-Stx or EV-Veh). Later, we studied their cytotoxic effect on VERO cells, as a parameter to determine the presence of Stx2 in the EV. The viability percentage of VERO cells cultured for 48hs with EV-Veh or EV-Stx was 86±3% and 68±2%, respectively (n=6; $p<0.05$). Analysis by transmission electron microscopy indicated that a fraction of

PMN vacuolizes in response to Stx2 during 3hs, and showed vesicles released by a PMN. Using FLICA, a fluorescent probe for active Caspase-1, we detected in real time imaging by confocal microscopy that Stx2 induces the activation of Caspase-1, which appears to be released from the cell in structures that might be EV. These preliminary findings suggest that Stx2 stimulate PMN to produce EV that could contain Stx2 together with other cellular components.

571. (336) **HUMAN NEUTROPHILS SECRETE IL-1B UPON INFECTION WITH SHIGA TOXIN-PRODUCING ESCHERICHIA COLI**

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Shiga toxin-producing E coli (STEC) infections can cause disease with diverse severity, from bloody diarrhea (hemorrhagic colitis) to hemolytic uremic syndrome (HUS), a life-threatening condition. HUS is characterized by hemolytic anemia, thrombocytopenia and renal failure, triggered by Shiga toxin (Stx). STEC are non-invasive bacteria that colonize the intestine where they release Stx that then reaches the blood stream; an event that might be facilitated by damages in the intestinal mucosa and promoted by inflammation. Given that neutrophils (PMN) are recruited to the intestine after the infection, our aim was to determine if these cells can contribute to the local inflammation by secreting IL-1β. We employed PMN isolated from peripheral blood of healthy donors. These cells were incubated with Stx-producer E coli O157:H7 (E coli Stx+) or with the Stx-non-producer bacteria (E coli Stx-) and 4hs later we determined IL-1β levels in the supernatants by ELISA. PMNs secreted IL-1β in similar levels in response to both strains when incubated at MOI of 0.05 (1232±51 pg/ml vs 1293±32 pg/ml; PMN+E coli Stx+ vs PMN+E coli Stx- respectively, n=4) and at MOI of 1 (n=3). IL-1β secretion by PMN incubated with E coli Stx- was not modulated by addition of Stx type 2 (Stx2; 0.1 μg/ml). In addition, there were no differences between the levels of IL-1β secreted in response to the culture supernatants of both strains (n=2). In accordance to these observations, Stx2 was unable per se of inducing IL-1β secretion or of modulating the secretion induced by PAM3CSK4 (a TLR2/1 agonist)+ATP. In conclusion, PMN secrete IL-1β in response to E coli O157:H7, but this property is neither dependent nor is modulated by Stx. These findings suggest that PMN might contribute to the pathology by increasing the local inflammation through the secretion of IL-1β.

572. (340) **EVALUATION OF PROS1, GAS6 AND TAM RECEPTORS EXPRESSION IN DUODENAL MUCOSAE OF CELIAC DISEASE PATIENTS**

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The tyrosine kinase Tyro-3, Axl and Mertk (TAM) receptors and their endogenous ligands Protein S (Pros1) and the Growth arrest-specific factor 6 (Gas6) have been shown to play an essential role in haemostasis, removal of apoptotic bodies, control of the inflammatory response as well as in the pathogenesis of different diseases. Scarce information regarding this axis and small intestine has been reported. We are interested in studying the TAM axis in human small intestine under physiological and pathological conditions, such as in Celiac disease (CD). CD is a chronic enteropathy characterized by epithelial cell death, loss of villus structure as well as others histological changes as consequence of an uncontrolled Th1 response specific for wheat proteins.
The aim of this work was to assess the expression of components of this axis in the human small intestinal mucosa.
Duodenal samples were collected during the routine procedure for CD diagnosis (CD patients=11, controls=9). Sections of paraffin-embedded tissues were analyzed by immunofluorescence. RT-qPCR analysis was carried out on whole duodenal biopsies. Non-parametric T-test was used.
We found higher expression of Gas6, at mRNA ($p<0.05$), and protein levels by IFI, ($p<0.05$) in CD duodenal mucosae and epithelium samples compared with control tissues ($p<0.01$). Gas6 was predominantly found in the nucleolus of Epithelia. While, Pros1 showed heterogeneous expression CD patients could be divided into two groups, with higher levels or similar to control samples. CD patients showed an up-regulation of Axl in the epithelium ($p<0.05$) while Tyro-3 and Mertk did not show significant expression difference compared to control samples.
In conclusion, this is the first description of the expression of this axis in the small intestine. We found that Gas6 and Axl were up-regulated in the epithelium of duodenal mucosae in CD patients. Epithelial expression of Gas6 suggest a potential role in the disease mechanism.

573. (344) **CHARACTERIZATION OF THE MECHANISM THAT TARGETS INTERLEUKIN-1 BETA (IL-1B) TOWARDS AUTOPHAGOSOMES FOR SECRETION**

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IL-1β is a major proinflammatory cytokine synthesized in the cytoplasm as an inactive precursor that is activated by proteolytic cleavage. It is a leaderless cytosolic protein that is secreted by unconventional mechanisms different from the classical ER-Golgi pathway. Our previous studies indicated that neutrophil cytoplasmic IL-1β is targeted to an autophagic compartment and is secreted by an unconventional secretory autophagic mechanism. The aim of this study was to gain insights into the mechanisms involved in IL-1β loading into the vesicles from which IL-1β is going to be secreted by human neutrophils. Neutrophils were isolated from healthy donors and stimulated for 5 h with LPS+ATP. We first investigated by ELISA if IL-1β is released inside extracellular vesicles. We found that 783±223 pg/ml of IL-1β were released to supernatants devoid of extracellular vesicles and 47±14 pg/ml were released inside extracellular vesicles (n=4; $p<0.05$). Additionally, neutrophils were cultured with LPS+ATP and the chaperon HSP90 inhibitor Radicicol (1μM) was added at 130 min post LPS-stimulation. By quantification with ELISA, we determined that inhibition of the chaperone reduced IL-1β secretion at 5 hs post-stimulation (X±SEM: 310±46 pg/ml vs 194±30 pg/ml, vehicle vs Radicicol respectively, n=11, $p<0.05$). Furthermore, by employing immunofluorescence staining and confocal microscopy we determined that after 4h post-LPS+ATP stimulation, the percentage of the vesicular colocalization between IL-1β and HSP90 (n=3; $p<0.05$), as well as the percentage of vesicular IL-1β decreased in the presence of Radicicol (n=3; $p<0.05$).
Our results suggest that HSP90 might contribute to translocation of IL-1β into neutrophil autophagosomes. They also suggest that IL-1β released from autophagosomes is primarily soluble rather than being in the lumen of EVs.

574. (348) **HUMAN CHORIONIC GONADOTROPIN LOWERS THE PRODUCTION OF PRO-INFLAMMATORY CYTOKINE TNF-ALPHA BY B AND T LYMPHOCYTES FROM MULTIPLE SCLEROSIS PATIENTS.**

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Multiple sclerosis (MS) affects mainly women in a 2:1 ratio. MS pathogenesis is complex and involves B and T lymphocytes in a pro-inflammatory self-reactive context. During pregnancy, disease relapse decreases in a 70%. Human chorionic gonadotrophin (hCG) is produced only during pregnancy and its immunosupresive role has been widely described. The aim of this study was to evaluate the effect of hCG treatment in cytokine production by T and B lymphocytes in blood samples from MS patients.

Women with MS during fertile life, treated only with IFN-β1, were recruited and blood samples were obtained. PBMCs were isolated and cultivated for 24 h in the presence/absence of urinary (U;100 IU/ml) or recombinant (r;5 μg/ml) hCG. The last 5h of culture, PBMCs were stimulated with PMA/Ionomycin and brefeldin when indicated. Supernatants were stored at -80°C. TNF-α and IL-10 were assessed by Cytokine Beads Array. Numbers of T (CD3,CD4,CD25,Foxp3) and B (CD19) lymphocytes, as well as, intracellular production of TNF-α and IL-10 were analyzed by flow cytometry. Data were analyzed by paired t-test.
UhCG treatment significantly reduced the production of TNF-α by PBMC (pg/ml;C=1969±420; UhCG=1441±297*;*p<0,01vsC,n=10) and the subsets involved in this reduction were both T (MFI;C=57±3;UhCG=50±4*;*p<0,05vsC,n=10) and B (MFI;C=146±75;UhCG=108±53*;*p<0,01vsC,n=10) cells. While UhCG increased IL-10 production by $CD3^+CD25^+CD4^+Foxp3^+$ Treg cells in a group of patients, the overall effect was not significant. rhCG treatment did not induce changes among the groups.
MS treatment with IFN-β1 is characterized by a shift towards an anti-inflammatory profile. In this context, UhCG was able to further reduce pro-inflammatory TNF-α production by T and B lymphocytes. The importance of co-treatment with hCG relies in the fact that it is a physiological hormone, commonly used in fertilization therapies and therefore, less toxic than other immune-modulators. This opens new avenues to explore the use of the hCG as co-treatment for the disease.

575. (367) **EFFECTS OF UV RADIATION ON KERATINOCYTE-MACROPHAGE DIALOG DURING AN INNATE IMMUNE RESPONSE**

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Cutaneous immune system comprises a complex network of cells, including keratinocytes (in the epidermis) and macrophages (in the dermis). Skin UVB irradiation affects mainly the epidermis, leading keratinocytes to release a wide variety of cytokines and soluble mediators that are significantly involved in immunoregulation.
The aim of the present work was to evaluate the influence of UV-induced keratinocytes' soluble mediators on macrophages response to a bacterial stimulus.
Human keratinocytes (HaCaT cells) were exposed to UVB (0, 12.5, 25 and 50 mJ/cm2) and cultured for 24h. HaCaT supernatants (HSN) were collected, TNF-α, IL-6, IL-1β and IL-10 production was measured (ELISA) and, finally, HSN were used to condition macrophage response. Human monocytic cell line (THP-1) was differentiated to macrophages (PMA), challenged with 0, 50, 100 and 200 μg/ml of heat-killed BCG, and cultured for 24h to measured cytokines production. Subsequently, THP-1 macrophages were challenged in the presence of HSN, and cytokine and ROS production (DCF-DA) were evaluated 24 and 2h after the challenge, respectively.
UVB-irradiated keratinocytes increased the production of the evaluated cytokines, reaching the maximum levels with a 25mJ/cm2 exposure: TNF-α, IL-6, IL-1β and IL-10 increased 2.5, 20, 2.5 and 2 times vs. non-irradiated controls, respectively, returning to basal levels with 50mJ/cm2. BCG stimulation of THP-1 cells did not change IL-6 and IL-10 production, but increased IL-1β (50, 61 and 80 vs. 29 pg/ml) and TNF-α production (163, 162 and 161 vs. 65 pg/ml). HSN did not affect TNF-α, IL-6 and IL-10 production by stimulated THP-1 cells, but significantly increased IL-1β production (50 and 100 μg/ml BCG: p<0.001, p<0.01, respectively). Moreover, HSN significantly increased THP-1 ROS production after BCG challenge (p<0.05).
These results suggest that UVB radiation can modulate keratinocytes' cytokine production, conditioning the macrophages response (ROS and cytokine production) to microbial challenges.

576. (421) **EFFECTS OF GLUTEN FREE DIET ON ADULTS WITH CELIAC DISEASE**

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Celiac disease (CD) is one of the most common causes of chronic malabsorption with the consequent alteration of micronutrient absorption. The only accepted treatment is lifelong adherence to a gluten-free diet (GFD). The aims of this study were to identify factors that are independently correlated with GFD adherence, to evaluate the levels of micronutrients, and the quality of life in celiac disease. Adults diagnosed with biopsy-confirmed CD participated in this study and followed by completion of three questionnaires and had blood drawn for IgA and IgG anti tissue transglutaminase (tTG) antibody titer, immunoglobulin A (IgA) level and micronutrients concentration. Quality of life was evaluated using the generic quality of life instrument the Short Form 12. Results: Twenty-eight patients participated and 47% of participants were found to adhere well to a GFD, registered as auto perception and 84% answered that they had positive symptoms when eating gluten. No significances differences were found in the serum concentration of folate, magnesium, iron and vitamin B12 between both groups (p>0.05). The degree of adherence to GFD was poor predictor of low levels of micronutrients (RR: 0.76; 95% CI: 0.38-1.51). Approximately 32.1% of patients presented positive serology (anti-tTG IgA). The percentages of patients with positive serology and more the 1 year of compliance with GFD was slightly higher in those patients with high adherence (23.1%) vs those with low adherence (13.3%). No significant different were found between gender and groups of age for the domain MCS (mental component score) and PCS (physical component score) (p>0.05). Conclusion: The presence of antibodies was independent of the perception of self-adherence. The evaluation of quality of life was acceptable for the PCS while for the MCS, the vitality and energy component indicated deterioration. The adherence to diet was associated with some factors but not with level of micronutrients.

577. (478) **THE ROLE OF MINTHOSTACHYS VERTICILLATA ESSENTIAL OIL IN THE IMMUNE RESPONSE AND ITS POSSIBLE APPLICATION FOR THE PREVENTION OF BOVINE INTRAMAMMARY INFECTIONS**

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In a previous study we have demonstrated that *Minthostachys verticillata* essential oil (EO) modulate the pro-inflammatory cytokines expression in a mouse mastitis model at 72 and 96 h post-inoculation (p.i). The aim of this work was to determine the effect of EO on TLR2, TNF-α and IL-1β expression in a mouse mastitis model challenged with *Enterococcus faecium* at 24 and 48 h p.i. In addition, antibody production was evaluated at 360 h. Female Balb/c lactating mice were inoculated in mammary glands (MG) as follows: Group 1 (control): 100 μl of PBS/DMSO; Group 2: 100 μl of *Enterococcus faecium* (EF) ($1x10^8$ CFU/ml); Group 3: pretreatment with EO and challenge with EF. Gene expression levels in the MG by quantitative Real Time-PCR and EF-specific antibodies (IgM, IgG, IgA) in serum and MG homogenates by indirect ELISA, were evaluated. TLR2 and TNF-α expression were not detected in treated groups at the times assayed. No IL-1β expression was detected in group 2 at 24 h p.i. However, this expression was increased respect to data obtained in group 1 at 48 h p.i (p<0.05). In group 3, IL-1β expression was increased at 24 h p.i. compared with the data obtained in group 1 and 2 (p<0.05). However, this expression decreases significantly respect to data obtained in group 2 at 48 p.i. (p<0.001). In group 2, EF-specific antibody levels in MG homogenates and serum, were increased respect to those observed in group 1 (p<0.001, p<0.05). In group 3, EF-specific antibody levels in MG homogenates were similar to those observed in group 1. Nevertheless, antibody levels in serum were increased respect to those observed in group 1 and 2 (p<0.001; p<0.05). The results demonstrated that EO modulates

the immune response in a mouse mastitis model and may serve as a natural product for prevention of bovine mastitis.

578. (568) **DEVELOPMENT OF A SURFACE DISPLAY SYSTEM ON BACTERIUM LIKE PARTICLES FOR MUCOSAL VACCINATION**

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Nasal vaccination is a promising alternative to parenteral vaccination as it is non-invasive and elicits antigen-specific responses both systemic and locally. Previous studies showed that bacterium-like-particles (BLPs) are effective stimulators of local and systemic immune responses when administered intranasally. Here we explored the use of BLPs, derived from the immunomodulatory strain *Lactobacillus rhamnosus* IBL027 (IBLP027) as a display platform of a model antigen, the Venus fluorescent protein using a novel lysin motif ($LysM_5$) as anchor.
The ability of the $LysM_5$-Venus-IBLP027 complex to induce specific systemic and respiratory humoral immune responses after intranasal immunization was evaluated. Six-week-old BALB/c mice were immunized with $LysM_5$-Venus-IBLP027 (40 and 20 ug of recombinant protein/mouse). Nasal administration of His-$LysM_5$-Venus alone or intraperitoneal administration of His-$LysM_5$-Venus with complete Freund's adjuvant were used as controls. Mice were primed at day 0 and boosted at day 14. The levels of specific serum IgG, IgG1 and IgG2a and the levels of broncho-alveolar lavage (BAL) total Ig (IgT) were evaluated ten days after priming or boosting. It was observed that His-$LysM_5$-Venus is immunogenic when it is administered with complete Freund's adjuvant, since specific IgT and IgG antibodies were detected after priming. In addition, the other groups did not induce an obvious respiratory or systemic humoral response (P<0.05). After boosting, the levels of IgG and IgT were improved in mice that received His-$LysM_5$-Venus intraperitoneally. Nevertheless, nasally administered His-$LysM_5$-Venus alone did not modify antibodies levels (P<0,05). However, when the complex $LysM_5$-Venus-IBLP027 was nasally administered, a significant increase in both IgT and IgG was observed (P <0.05). Moreover, the IgG2a/IgG1 ratio in mice was 0.6 while immunization with high-dose His-$LysM_5$-Venus-IBLP027 induced an IgG2a/IgG1=1.1 ratio, suggesting an improved Th1 response. These results demonstrate that the $LysM_5$-IBLP027 system could be a useful tool for the development of mucosal vaccines.

579. (645) **B-CELL ACTIVATING FACTOR (BAFF) AND PERIPHERAL B CELL POPULATIONS IN TEGUMENTARY LEISHMANIASIS**

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BAFF is crucial regulating peripheral B cell functions. However, elevated serum levels can be detrimental and are associated with clinical activity in several autoimmune diseases as systemic lupus erythematosus. Recently, elevated BAFF levels were found in visceral leishmaniasis but less is known about tegumentary leishmaniasis which is prevalent in our region and composed by cutaneous (CL) and mucosal (ML) forms.
The aim of this work was to characterize B cell factors in CL and ML, which is more aggressive and present longer duration. We found diminished non class switched ($CD27^+IgD^+$) memory B cells (MBC) in CL (7.75±5.91%) and more significantly in ML (5.90±4.17%) compared to healthy subjects (HS, 12.13±6.58%, *p=0.0043*). In relation to BAFF receptors (BAFF-R and TACI) on classical CD27+ MBC, both receptors were diminished in ML comparing with HS (19.28±13.28% vs. 36.4±12.7%, *p=0.0294*; 8.10±6.68% vs. 29.99±10.86%, *p=0.0002,* respectively). We also found elevated levels of BAFF and IgG in plasma in ML in contrast to HS (2224±1298 pg/ml vs. 1280±416 pg/ml, *p=0.0075*; 12.66±4.05 mg/ml vs. 9.36±1.76 mg/ml, *p=0.0175,* respectively) while CL showed no differences. We next performed paired analysis of seven ML cases before treatment administration and 10 (1-12) months [median (range)] after recovery and found significant decrease of BAFF in the latter (1444±573 pg/ml vs. 901±268 pg/ml, *p=0.0312*). Moreover, independent ML cases which reached clinical improvement showed decreased BAFF levels in comparison with active disease cases (1004±511 pg/ml vs. 2496±1340 pg/ml, *p=0.0008*).
Our results show that B cells alterations occur in tegumentary leishmaniasis, which are emphasized in the aggressive mucosal form, related to elevated BAFF levels, hypergammaglobulinemia, decrease BAFF receptors and diminished non switched MBC. Moreover, as BAFF returns to normal values after recovery, we suggest its possible implication during mucosal infection. *Statistical tests used: Kruskal-Wallis; Wilcoxon; Mann Whitney. Data expressed as Mean ± SD.*

580. (655) **PROBIOTIC LACTOBACILLUS INCREASE THE MICROBICIDAL ACTIVITY OF THE INTESTINAL FLUIDS IN INFLAMMATORY BOWEL DISEASE**

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We demonstrated that probiotics oral administration increase Paneth cells, the main intestinal cell responsible of antimicrobial peptides (AMPs) production. Currently we explore, in a murine model of inflammatory bowel disease (IBD), whether lactobacillus modify the altered production of AMPs reported in the disease, in order to preserve intestinal barrier.
IBD were induced in Balb/c mice by subcutaneous doses of 7.5mg/kg/day indomethacin, on days 6 and 7. Animals were supplemented with: Group 1: Lactobacillus casei CRL 431 (LC 431), G2: Lactobacillus paracasei CNCM I-1518 (LP 1518), or G3: water, upon 7 and 5 days, respectively. Additional controls fed with LC 431, LP 1518 or water, respectively, received 2 injection of PBS. At day 8, mice were sacrificed.
A 2.27± 1.32% body weight loss was observed in G3 after indomethacin injection, while gains of 1.30±0.98, and 2.14±0.80 were observed for G1 and G2, respectively, and 3.79 ±1.84 for control animals. Fur appearance of each mouse showed a Score of: 3 (smooth Coat) for G1 and G2, and Score: 1 (majority of back is piloerected) for G3.
In vitro, an increase microbicidal activity against S. aureus and S. thyphimurium were observed in the intestinal fluids of G1 and G2 comparing animals treated with indomethacin that received water in their beverage (G3). Hematoxylin-eosin studies showed severe mononuclear cell infiltrates in the lamina propria and thickening of small intestinal wall on G3. Besides, serum markers of inflammatory process (LDH and PCR) were increase in G3 comparing with G1 and G2 ($p^<0.05$).
Finally, a slightly increase of anaerobes and lactobacillus and decrease of total enterobacteria were observed as part of colon microbiota in G1 and G2 animals compared to G3.
Probiotics can be promising microorganisms for the stimulation of intestinal antimicrobial activity in pathologies such as IBD, contributing to preserved intestinal barrier and protect against pathogens.

581. (670) **ISPA IMPROVE PROTECTION OF EXPERIMENTAL FOOT-AND-MOUTH DISEASE VACCINE IN MICE. IMMUNOGENICITY STUDY IN MURINE MODEL AND CATTLE.**

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ISPA are lipid cage-like particles. In this report, we demonstrate an increase of immune response induced by FMDV-ISPA vaccine. The murine model predicts the quality of FMD vaccines in bovines.
BALB/c mice were immunized with: iFMDV-A2001 (0.3µg); iFMDV-ISPA (10µg); Commercial vaccine; ISPA and PBS, then 21 dpv were challenged. iFMDV-ISPA group presented α-iFMDV Ab titers: 5.06±0.07, higher than iFMDV (3.8±0.3) and commercial vaccine

(4.62±0.02) groups (p<0.05). When animals were challenged with FMDV, 100% of mice vaccinated with iFMDV-ISPA and commercial vaccine were protected, and only 40% in iFMDV group. A significative increase of Ab α-iFMDV IgG1, IgG2a, IgG2b and IgG3 types was found in iFMDV-ISPA group
At 21 dpv, splenocytes were stimulated in vitro with iFMDV; iFMDV-ISPA group showed greater proliferation than iFMDV, ISPA or PBS groups (p <0.05), and similar that induced by commercial vaccine. A slight increase was observed in the CD4 +/IFNγ+ population in iFMDV-ISPA and commercial vaccine groups compared with iFMDV.
Mice inoculated with ISPA (sc), at 18 hpv, showed a significant increase in granulocytes in axillary lymph nodes and a decrease of granulocytes, DCs, monocytes and LT-CD8+ in spleen.
Calves (n=4) were vaccinated with iFMDV or iFMDV-ISPA, at 30 dpv there was an increase in total α-iFMDV Ab (3.7±0.6) in iFMDV-ISPA group in comparation with iFMDV group (p<0.05). On the other hand, Seroneutralizing antibodies were 1.8± 0.3 (these SN levels are correlated with protected animals) and 1.02±0.02 respectively (p<0.05).
Bovine Dendritic Cells incubated with ISPA showed an increase in the expression of CD40 and IL6.
Inclusion of ISPA in an experimental FMD vaccine induced an increase in humoral and cellular immunity. In mice, we observed a greater protection against viral challenge. In cattle, the titers reached are linked to a Percentage Expectation of Protection higher than 80%.

582. (675) **LIVER FIBROSIS IN HIV/HCV CO-INFECTED INDIVIDUALS: NK REDUCED CYTOTOXICITY IS NOT ASSOCIATED TO A DYSFUNCTIONAL LT-CD4+ STIMULATION**

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Natural killer (NK) cells ameliorate liver fibrosis by killing activated hepatic stellate cells. We have demonstrated in HCV/HIV co-infected patients with advanced liver fibrosis that functionality of peripheral blood NK cells is significantly affected. Since LT-CD4+ modulate NK cell activity, we aimed to evaluate LT-CD4+ regulation of NK cell functionality in HCV/HIV coinfected patients with different stages of liver fibrosis.
LT-CD4+ cells were purified from cryopreserved PBMCs from 30 HCV/HIV+ patients with mild or advanced fibrosis (METAVIR F0/F1 and F4, respectively). Similarly, NK cells were purified from one healthy volunteer. LT-CD4+ were stimulated with anti-CD3/CD28 beads at three different bead-to-cell ratios (1:1 48h, 1:5 and 1:35 24h), and conditioned medium (CM) were collected. Healthy NK cells were pre-activated with CM or vehicle overnight, followed by co-culture with K562 cells. CD107a externalization, and intracellular IFN-γ and TNF-α were measure by FC. Data was analysed using non-parametric statistics.
Frequency of CD107a+ NK cells progressively increased as the intensity of LT-CD4+ stimulation was incremented (p<0.05). Nevertheless, NK cell degranulation was similarly induced whether CM was produced by LT-CD4+ from patients with minimal or advanced fibrosis. Median CD107a+ NK cell percentages following F0/F1 or F4 CM pre-stimulation were 30.5 and 26.8 (1:35), 42.1 vs. 40.0 (1:5) and 65.0 vs. 64.8 (1:1), respectively. Finally, we did not find significant differences regarding cytokine secretion depending on the activation state of LT-CD4+ or the fibrosis levels of the patients. Median IFN-γ+ and TNF-α+ NK cell percentages after F0/F1 or F4 CM pre-stimulation were 7.3 and 10.8 vs. 7.6 and 11.3, respectively.
Results suggest that reduced cytotoxicity of NK cells may not be related to a deficient LT-CD4+ modulation. Lower percentage of NK cells, and/or an impaired NK cell functionality could act as markers of advanced liver fibrosis in HCV/HIV+ patients.

583. (676) **EFFECT OF L. CASEI CRL 431 AND ITS CELLULAR WALL ON THE THYMUS AT DIFFERENT AGES IN A MICE MODEL**

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Thymus is responsible of ontogeny and maturation of T lymphocytes, however, it begins to regress with time.Aim: to study the effect of *L. casei 431* and/or its cell wall in the thymus at different ages. BALB/c mice were divided into groups of age: 21, 28, 45, 90 and 180 days(d), which were subdivided according to the supplement received: Normal control(NC) received the conventional balanced diet and water *ad libitum*; *Lactobacillus case*i(Lc) group received the conventional balanced diet and oral suspension of probiotic bacteria. Mice were sacrificed at the corresponding age. Initial and final weight of the animal, weight of the thymus was taken. Samples were: large intestine for microbiota analysis, serum(S), small intestinal fluid(IF) and thymus for cytokine analysis (IFNγ, IL-6, IL-10, IL-12 p70, TNFα, IL-3). Cytokines determination in culture supernatant of thymus stimulated with the bacteria (B) or its cell wall (W). Lymphocyte population in thymus and in mesenteric lymph nodes (MLN) were analyzed by flow cytometry.Results: probiotic supplementation increased body weight in all groups of age. Body weight/thymus ratio decreased at 28, 45 and 90d in Lc. Supplementation showed decreased population of enterobacteria and increased population of lactobacilli, without significant differences between NC and Lc in the total anaerobic population.Cytokines in cell culture supernatant showed a decrease in IFNγ and TNFα, increased values of IL-10, IL-12 and IL-3, IL-6 values were near to NC in TB and Lc. Decreases in the CD4+ population in thymus showed in Lc group at 45d and significantly increased at 90d respect to NC. In NC CD4+/CD8+ ratio a significant increase at 90d (p <0.05) respect to Lc (NC = 80.72 ± 0.32, Lc = 0.45 ± 0.06).Lc supplementation and in vitro stimulation with bacteria or its W induce effect in thymus at different periods of time in mice.

584. (690) **CLOSTRIDIUM DIFFICILE INFECTION: MURINE MODEL DEVELOPMENT AND IMMUNOLOGICAL CHARACTERIZATION.**

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Clostridium difficile-associated disease is caused by a gram-positive, anaerobic, spore-forming bacterium *Clostridium difficile* (*C. difficile*) and is the most common cause of antibiotic associated diarrhea. BI/NAP1/027, an emerging strain of *C. difficile*, has dramatically changed the epidemiology of *C. difficile* infection (CDI) with outbreaks of severe disease in North America and Europe. Antibiotics treatment is the standard method of therapy but also the major cause of susceptibility to CDI. Therefore, identification of key effector molecules of host immune response against CDI may provide novel immunotherapies.
After induction of dysbiosis, mice were infected with spores from the hypervirulent BI/NAP1/027 strain. Post-infected mice evidenced typical CDI symptoms such as diarrhea, hunched posture, and weight loss (p<0.01). Mice were euthanized 8 days post-infection and colon was removed to isolated lamina propria mononuclear cells (LPMC). First, we evaluated the expression of pro-inflammatory cytokines on LPMC by flow cytometry. No differences were observed in $CD3^+IFN\text{-}\gamma^+$ cells after CDI, but we detected an increase of $CD3^+CD4^+IFN\text{-}\gamma^+$ cells (p<0.05) and a decrease of $CD3^+CD4^-IFN\text{-}\gamma^+$ cells (p<0.05) in infected mice. We also observed a positive modulation of $CD3^+CD4^+IL\text{-}17A^+$ cells (p<0.05) and in $CD3^+CD4^-IL\text{-}17A^+$ cells (p<0.05) from *C.difficile*-infected mice. Next, we determined the levels of SLAM and ICOS, two costimulatory molecules that regulates the innate and adaptive immune response. LPMC from *C. difficile*-infected mice showed a downmodulation of $CD3^+SLAM^+$ (p<0.01) and $CD3^+ICOS^+$ (p<0.01) cells compared to control mice.
Our results suggest that *C. difficile* infection regulates IFN-γ and IL-17A production by T cells, while inducing a downmodulation of the costimulatory molecules SLAM and ICOS on $CD3^+$ cells. Understanding cytokine-mediated responses and molecular drivers impli-

cated on inflammation and disease's severity could be crucial for the discovery of new therapeutic targets and biomarkers that could improve the quality of existing treatments and diagnoses.

585. (702) **ALLOGENEIC MELANOMA AND NON-MELANOMA TUMOR CELL VACCINES PROVIDE PROTECTION AGAINST MELANOMA IN A MURINE MODEL**

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Vaccination in cancer aims to generate a long-lasting response, however, one of the major obstacles in the development of cancer vaccines has been the inability to clearly identify relevant immunogenic antigens. In particular, dendritic cell (DC)-based cancer immunotherapy can be achieved by loading DCs with syngeneic tumor cells, in which case tumor self-antigens, including cancer testis antigens (CTA), other tumor-associated antigens (TAA) and neoantigens generated through mutations during tumor progression, are provided. On the other hand, DCs loaded with allogeneic tumor cells could only supply shared CTA and other TAA. To assess the advantages of each system, we have analyzed in a murine model the effect on anti-melanoma protection of loading DCs with irradiated syngeneic B16-F1 melanoma, allogeneic Cloudman melanoma, or allogeneic 4T1 mammary carcinoma cells (DC-ApoNec vaccines), which were characterized by whole exome sequencing and RNA-seq. DC-ApoNec vaccines were administered to C57Bl6 mice, followed by B16-F1 tumoral challenge. Allogeneic melanoma cells induced effective anti-melanoma protection ($p<0.05$), but syngeneic melanoma cells established a more potent ($p<0.001$) and long-lasting protection. Interestingly, when allogeneic mammary carcinoma cells were used to load DCs, short-term ($p<0.001$) and long-lasting anti-melanoma protection were also obtained. We observed both induction of anti-B16-F1 humoral response and a cellular IFN-γ response triggered in response to B16-F1 lysate by CD-ApoNec B16-F1 and 4T1 vaccines. Using high throughput sequencing data and MHC-I binding tools we predicted immunogenic epitopes provided by the different antigen sources, and determined that B16-F1 neoantigens were not present in allogeneic cells, while they shared CTA and other TAA. This work shows that the use of allogeneic cells triggers both humoral and cellular anti-melanoma responses and provides anti-melanoma protection, suggesting the immunotherapeutic potential of CTAs and other TAAs shared between tumor cells of different origin.

586. (714) **HUMORAL IMMUNE RESPONSE INDUCED BY NOVEL LIPOSOMAL FORMULATIONS USING AN HYDROPHOBIZED MANNAN FROM SACCHAROMYCES CEREVISIAE AS IMMUNOSTIMULANTS.**

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Liposomes are vaccine adjuvant systems able to transport hydro- and liposoluble molecules, allowing the co-incorporation of antigen and different immunostimulants. Our aim was to evaluate the adjuvanticity of a cationic liposomal formulation (Lip) with the addition of an hydrophobized mannan from *Saccharomyces cerevisiae* (Man) and/or CpG oligodeoxynucleotide (CpG-ODN) as immunostimulants, in the immunization against recombinant clumping factor of Staphylococcus aureus (rClf). Balb/c mice were immunized with: Lip+CpG-ODN+rClf, Lip+Man+rClf, or Lip+Man+CpG-ODN+rClf (n=6/group). Mice received two subcutaneous doses, every 3 weeks of 10 μg of rClf and were bleed 10 days after every dose. Control groups received Lip+Man or Lip+Man+CpG. Anti-rClf IgG, IgG1 and IgG2 levels were assessed by indirect ELISA in plasma. No specific rClf antibodies were found in pre-immune serum. Control groups didn't show specific humoral response. Lip+CpG-ODN+rClf, Lip+Man+rClf and Lip+Man+CpG-ODN+rClf (OD_{450nm}: 1.196±0.303, 1.199±0.259 and 1.064± 0.330) all induced higher IgG levels than control groups after the second dose ($p<0.001$, Bonferroni test). Furthermore, the three formulations led to high IgG titters at the end of the protocol, being the mean for each group: $0.8x10^6$, $1.2x10^6$ and $1.5x10^6$, respectively. IgG subclasses also presented high titters in all experimental groups. For IgG1, the titters were $1.8x10^6$, $1.2x10^6$ and $1.3x10^6$ for the groups Lip+CpG-ODN+rClf, Lip+Man+rClf and Lip+Man+CpG-ODN+rClf, meanwhile the IgG2 titters were $2.9x10^5$, $1.9x10^5$ and $2.0x10^5$, respectively. No significantly differences were found between experimental groups for any titration. The results suggest that both hydrophobized mannan and CpG-ODN act as immunostimulant. Furthermore, their combination with cationic liposomes results in an efficient induction of specific humoral immune responses to recombinant antigens in mice, even with production of IgG2.

587. (730) **MONOCYTE-DERIVED IL-18 ENHANCE PD-L1 EXPRESSION ON TUMOR-EXPERIENCED HUMAN NK CELLS.**

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Natural killer (NK) cells play an important role in the elimination of tumor and virus-infected cells, however, recently evidence of a regulatory role is emerging in different models of autoimmunity, transplants and viral infections. In the tumor context, we have demonstrated that NK cells from tumor bearing mice showed an up-regulated expression of the inhibitory molecule PD-L1 and restrict $CD8^+$ T cell priming. Moreover, in human NK cells, direct tumor recognition through NKG2D receptor induced the expression of PD-L1, which was further up-regulated in the presence of peripheral blood mononuclear cells (PBMCs) through IL-18. However, the underlying mechanisms that control these interactions are unknown. Thus, the aim of this work was to identify the IL-18-producing cell population and the signals and cells involved in its induction, which results in PD-L1 expression on human NK cells. To distinguish between NK cell-derived factors versus tumor cell-derived factors, we generated conditioned medium (CM) from NK cells cultured with K562 tumor cells and CM from serum-deprived apoptotic K562 cells. Then, PBMCs were stimulated with these CM or with K562 cells in the absence or in the presence of an IFN-γ neutralizing antibody. K562 cells and both CM induced IL-18 secretion by PBMCs, measured by ELISA, which was partially reduced by IFN-γ blockade. Next, to identify the IL-18-producing population, we cultured NK cells with K562 cells, with or without syngeneic monocytes. The addition of monocytes resulted in a higher IL-18 secretion evaluated by ELISA ($p<0.05$), and an up-regulated expression of PD-L1 on NK cells ($CD56^+$ $CD3^-$ cells) ($p<0.01$) which was abrogated by IL-18 blockade using a neutralizing antibody ($p<0.05$). Our results suggest that after tumor recognition, K562-derived factors and NK cell-derived IFN-γ induced the production of IL-18 by monocytes, which enhanced PD-L1 expression on tumor experienced NK cells.

588. (774) **4-METHYLUMBELLIFERONE (4MU) OVERCOMES TUMOR PROGRESSION MEDIATED BY HYPOXIA AND IMPROVES THE EFFICACY OF IMMUNOTHERAPY IN PRECLINICAL MODELS OF GASTROINTESTINAL TUMORS**

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Hypoxia drives essential adaptations for cancer cell survival including metabolism alteration, pH regulation, epithelial-mesenchymal transition, angiogenesis and evasion of the immune response. Hypoxic cells increase their survival by regulating pH at least in part through modulation of extracellular carbonic anhydrases (CAIX and CAXII). Hypoxia also induces immune suppressor cells and

tumor-associated macrophages (TAMs) infiltration, and stimulates the expression of Programmed Death-Ligand 1 (PD-L1) in tumors, suppressing T cell the activation. We have shown that the coumarin 4-methylumbelliferone (4Mu) decreases intratumoral pressure and modify the balance of angiogenic/antiangiogenic factors. Our aim was to evaluate the expression of hypoxia-inducible factor 1alpha (HIF-1α), CAIX, CAXII and changes on tumor microenvironment in preclinical models of gastrointestinal cancer.
We analyzed in vitro expression of CAIX, CAXII and HIF-1α by qPCR in colorectal carcinoma (CRC) and hepatocellular carcinoma (HCC) cells. We also analyzed the effect of 4Mu on CAIX, CAXII, HIF-1, Arginase 1 (Arg1), inducible Nitric Oxide Sintetase (iNOS), indoleamine dioxygenase (IDO), transforming growth factor beta (TGF-β), interleukin 10 (IL-10) and interleukin 1b (IL-1b) and F4/80 + cells in tumor samples. Then, we evaluate the in vivo efficacy of 4Mu plus the checkpoint inhibitor monoclonal antibody antiPD-1.
4Mu reduced the expression of CA on CRC and HCC cells ($p<0.01$). 4Mu also induces a decrease of CAIX and CAXII in tumor samples. We found reduced F4/80, Arg1, iNOS and IDO in treated CRC and HCC tumors. The levels of TGF beta and IL-10 were diminished ($p<0.001$) while IL-1b was increased ($p<0.01$). Although 4Mu alone slightly inhibited tumor progression, 4Mu + antiPD1 showed a potent antitumor effect in mice ($p<0.05$). We suggest that modulation of HIF1 and CA induced by 4Mu contributes to microenvironment remodeling, changes the profile of immune cell populations, and increases the efficacy of immunotherapy leading to tumor growth inhibition.

589. (779) **DOWNREGULATED EXPRESSION OF THE CANCER STEM CELLS MARKER CD47 INDUCED BY 4-METHYLUMBELLIFERONE PROMOTED PHAGOCYTOSIS BY ANTIGEN PRESENTING CELLS AND STIMULATED SPECIFIC T CELL RESPONSE AGAINST HEPATOCELLULAR CARCINOMA.**

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The tumor microenvironment (TME) represents a complex interplay between different cellular components, including tumor cells and cancer stem cells (CSCs), with the associated stroma; such interaction promotes immune escape and sustains tumor growth. We previously demonstrated that the hyaluronan synthesis inhibitor 4-methylumbelliferone (4Mu) combined with an adenovirus encoding interleukin-12 genes (AdIL-12) showed a potent antitumor effect, increased animal survival and achieves a successful antitumor immune response in an orthotopic HCC established in fibrotic livers. Our aim was to investigate why 4Mu boosted the immune response and induces antitumor activity in HCC. We evaluated if 4Mu facilitates tumor recognition and phagocytosis by in vitro phagocytosis assays using intraperitoneal macrophages. We also analyzed the expression of CSCs markers, particularly CD47+, and isolated CSCs by magnetic separation of CD133+ cells. We found that 4Mu downregulated the expression of the CSCs marker CD47+ within HCC tumors, promoted phagocytosis by antigen presenting cells and, combined with Ad-IL12, induced a potent cytotoxic specific T cell response against HCC. We also observed a significantly decrease in hepatic mRNA levels of CD133+, CD90+, EpCAM+, CD44+ and CD13+ CSCs markers ($p<0.01$). The in vitro studies on isolated CSCs revealed that 4Mu significantly reduces CD133+ and increases mice survival in a metastatic HCC model. Our results showed that combined strategy modifies TME and ameliorates HCC aggressiveness by targeting CSCs.

590. (788) **STUDY OF THE IL-17 FAMILY CYTOKINES AND RECEPTORS EXPRESSION ON DIFFERENT TUMOR CELL LINES AND THEIR IMPACT ON TUMOR PROGRESSION.**

Constanza Rodriguez, Jimena Tosello Boari, Cintia Araujo Furlan, Fernando Canale, Santiago Boccardo, Sabrina Bossio, Cristian Gabriel Beccaria, Adriana Gruppi, Carolina Lucia Montes, Eva Virginia Acosta Rodriguez
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While relatively well-defined in infectious diseases and autoimmunity, the roles of IL-17 cytokines in cancer remain controversial. Thus, IL-17 may play a pro-tumoral role by directly sustaining cancer cell growth or may support antitumoral immunity potentiating CD8+ T cell (CTL) and NK cell responses. Our aim is to determine the role of IL-17 in the overall progression of different cancer types, dissecting pro-tumoral and anti-tumoral effects. Using different murine tumor cell lines of melanoma (B16-SIY), fibrosarcoma (MC57-SIY and MCA-OVA), lymphoma (EL4-SIY) and acute myeloid leukemia (C1498-SIY), we first quantified by real-time PCR the transcripts encoding IL-17A and IL-17F and receptors subunits IL-17RA, IL-17RC and IL-17RD. IL-17A transcript was detected only in EL4-SIY while IL-17RA, IL-17RD and IL-17F mRNA were expressed in all the cell lines assayed. IL-17RC transcripts were expressed in MCA-OVA, B16-SIY and MC57-SIY but not in EL4-SIY nor in C1498-SIY. Important variations in transcript amounts were observed among cell lines evaluated: IL-17F mRNA expression was higher in C1498-SIY ($p< 0.001$) and B16-SIY ($p<0.05$); IL-17RC mRNA augmented in B16-SIY ($p<0.001$) and MCA-OVA ($p<0.05$); IL-17RA and IL-17RD transcripts were augmented in B16-SIY ($p<0.01$ and $p<0.001$, respectively). These differences suggest dissimilar sensitivities of the cell lines to IL-17-mediated growth signals. We next investigated tumor progression and antitumoral immunity after injection of cells in WT (B6) mice in comparison to mice deficient in IL-17RA (RKO) or IL-17A and IL-17F (DKO). Injection of B16-SIY and MC57-SIY in RKO and DKO mice resulted in increased tumor volume and reduced tumor-specific CTL in comparison to WT mice while injection of MCA-OVA showed no differences in tumor progression among all experimental groups. These results indicate that IL-17 cytokines have dissimilar impact in the overall progression of different tumors. Further research will establish the relative influence of pro- and anti-tumoral effects according to the cancer phenotype.

ONCOLOGÍA / ONCOLOGY ORAL SESSION 4

591. (350) **THE OXIDIZED AMINOACID M-TYROSINE RESHAPES THE METABOLIC FATE OF TUMORS CELLS IMPAIRING METASTASES.**

Emiliano Ortiz[1, 2], Nicolas Anselmino[1, 2], Estefanía Labanca, Sofía Lage Vickers[1, 2], Nora Navone, Javier Cotignola[1, 2], Raúl Ruggiero, Elba S. Vazquez[1, 2], Geraldine Gueron[1, 2]
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Interest in cancer energetics has now become a cornerstone of cancer biology. The accelerated aerobic glycolysis with an increase in lactate production in cancer cells has been first postulated by Warburg. Although aerobic glycolysis is inefficient in terms of energetic resources, the capacity to produce intermediates must be a key driver to rule metabolism in cancer cells. The acquired metabolic signature contributes to the failure of therapy. Previous results from our laboratory have shown for the first time the phenomenon of concomitant resistance (CR) in human solid tumors, effect mediated by m-Tyrosine (m-Tyr), a potent anti-tumoral oxidized aminoacid exhibiting undetectable toxic-side effects. In this work we explored the capacity of i.v m-Tyr administration to impair metastasis. We used spontaneous (naspharingeal and lung) and experimental (prostate) metastases models. Results show a significant reduction in both, spontaneous metastasis derived from murine mammary carcinomas (4T1, C7HI, and LMM3) and prostate cancer (PCa) experimental metastases.
Further, we explored in parallel the effect of m-Tyr on the energetic balance of prostate cancer cells. Our results performed on PC3 cells demonstrated for the first time that m-Tyr is able to reshape the metabolic fate of PCa cells due to the down-regulation of the LDHA gene (RT-qPCR), which encodes for the lactate dehydrogenase, an

enzyme involved in the last step of glycolysis. The shift to a less glycolytic phenotype was evidenced by a decrease in the extracellular acidification rate (ECAR), in oxygen consumption rate (OCR) and in ATP production, reflecting an impairment of mitochondrial function. Same results were obtained using the C42B cell line. Altogether, these data support a key role of m-Tyr controlling prostate energetic status, thus ascertaining it as a potential option to boost the therapeutic efficacy of the current treatment for metastasis.

592. (256) **DIFFERENTIAL EFFECTS OF HV1 PROTON CHANNEL INHIBITION ON INTRACELLULAR PH AND CELL CYCLE PROGRESSION OF TUMORIGENIC AND NON-TUMORIGENIC HUMAN BREAST CELLS. 2D AND 3D STUDIES.**

Clara Ventura[5, 4], Ignacio Leon[3], Agustin Asuaje[2], Nicolas Enrique[2], Pedro Martín[2], Mariel Núñez[5], Claudia Cocca[5, 1], María Verónica Milesi[2]

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In tumoral cells, metabolic reprogramming conduces to a large production of acidic substances which must be extruded to avoid the intracellular acidification. We previously reported that the inhibition of the proton channel (Hv1) by ClGBI [2-(6-chloro-1H-benzimidazol-2-yl)guanidine] differentially inhibited cell proliferation in tumorigenic (MCF-7 and MDA-MB-231) and non-tumorigenic (MCF-10A) human breast cells. Here, we show the effect of ClGBI on intracellular pH (pHi), cell viability and cell cycle of these cell lines using 2D and 3D cultures. In monolayers, the pHi (BCECF ratiometric assay) and the percentage of mitotic cells (immunofluorescence using DAPI and anti-α-tubulin antibody) were determined. The effect of ClGBI on cell viability of 3D culture MCF-7 cells (hanging drop method) was assayed by flow cytometry using propidium iodide. Results: The pHi was tested in the three cell lines after 0.5, 24 and 48h of Hv1 inhibition. Significant pHi alterations were observed only after 48h of 10μM ClGBI exposure, achieving a greater acidification in MDA-MB-231 (-0.61±0.04 pH units, p<0.0001 vs. C), than in MCF-7 (-0.23±0.03 pH units, p<0.01 vs. C) and MCF-10A (-0.09±0.02 pH units, p<0.05 vs. C) cells. In the same way, we observed that ClGBI in a concentration and time-dependent way increased the percentage of mitotic cells in MDA-MB-231 and MCF-7 cell lines (p<0.05 vs. C), without changes in MCF-10A cells. This effect could be reverted by removing the inhibitor of the culture. Finally, ClGBI reduced cell viability of MCF-7 cells growing in a 3D culture in a concentration dependent manner (IC50=59.46±1.25μM). Our results show that the inhibition of Hv1 channel offers a good strategy to breast cancer treatment, since it differentially alters pHi and cell cycle distribution in tumorigenic and non-tumorigenic human breast cells. The reduction on cell viability observed in a more complex system such as the 3D MCF-7 culture strengthens this idea.

593. (172) **P63 DEPLETION REDUCES 3D AND TUMOR GROWTH CAPACITY IN BLADDER CANCER CELLS**

Macarena Zambrano, Marianela Sciacca, Yanina Langle, Denise Belgorosky, Eduardo Imanol Agüero, Eduardo Sandes, Ana Maria Eijan, Catalina Lodillinsky

Área de Investigación-Instituto de Oncología Ángel H Roffo

Based in the need to develop more precise therapies, bladder cancer has been classified related to its gene expression pattern in luminal like and basal like bladder tumors. The biological and clinical significance of these signatures remains unclear. p63 characterize basal like bladder tumors. Nevertheless the p63-related molecular pathways in this pathology are not well known yet. Our hypothesis is that p63 acts as a protumoral factor, by activation of invasive signaling programs. Our previous results showed that p63 depleted bladder cancer cells have lower extracellular matrix degradation and migratory capacity associated with the lost of MT1-MMP expression. Knocking down for p63 in human bladder cancer cell lines MGHU3 and UMUC14 was developed using a TET-ON system where p63 depletion is induced externally under doxycycline (DOX). Both cell lines were grown in 3D conditions during 30 days treated with o without DOX and spheroids diameter was analyzed. p63 depleted spheroids (DOX) growth was significantly lower than untreated controls (CRL) (ANOVA with Tukey comparisons, p<0.001 DOX vs CRL).
Nude mice were injected with MGHU3 shp63 or shNT inducible cells. Mice received or not DOX in drinking water. Tumor volume was significantly lower in p63 depleted tumors compared CRL. (p<0.05 shp63 DOX vs shp63 CRL; p<0.01 shp63 DOX vs shNT DOX.)
Based in all data previously exposed we can conclude that p63 is an essential protein involved during tumor progression by modulating invasiveness, migration, and 3D and tumor growth in bladder cancer. It indicates p63, either direct or indirectly, could be considered as a possible target for new antitumor therapies.

594. (306) **ANTITUMORAL ACTIVITY OF NEW CHEMICAL COMPOUNDS IN TUMOR MODELS**

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The development of new antitumor agents is, presently, a demand for the public health due to the demonstrated side effects of the known antineoplastic drugs, to the development of drug resistance and, also, to the lack of effective therapies for some types of cancer and for the treatment of metastasis. Our aim was to evaluate the antitumor activity of two families of new synthesised compounds: 12 oxadiazoles and 13 propargylamines. The in vitro effect of the compounds on viability was initially evaluated on 4T1 mammary adenocarcinoma, and MC3T3e1 normal osteoblastic murine cell lines, at 100 μM. There were selected 2 propargylamines (MMA06 and MMA4210f3) that showed more than 50% inhibition of cell viability, with respect to the non-treated control without affecting normal cells, while the oxadiazoles exhibited more than 50% decrease on viability of tumor and normal cells. Then, it was tested the antiproliferative effect of the selected compounds on cells with human background: MDA MB 231 (breast cancer) and PANC-1 (pancreas carcinoma) cell lines in a wide range of concentrations. MDA MB 231 cells were inhibited more than 50% by MMA06 75 μM and MMA4210f3 300 μM (IC50: 74.05 and 291.0 μM, respectively), whereas for PANC-1 cells the concentrations were 35 μM and 10 μM, respectively (IC50: 21.59 and 13.56 μM, respectively). MMA06 increased apoptosis (flow cytometry) of MDA MB 231 cells at 50μM (P<0.05) and 300 μM (P<0.001), while MMA4210f3 did it at 300μM (P<0.01). 1) The selected propargylamines, a kind of compounds rarely suggested as antitumor agents, showed in vitro antitumor activity, being devoid of toxic effects on normal cells. 2) MMA06 and MMA4210f3 decreased viability and increased apoptosis of two different human tumor cell lines. 3) The high sensitivity to the compounds of the pancreas cell line, opens a new therapeutic pathway that deserves deeper investigation.

595. (383) **COOPERATION BETWEEN EPITHELIAL AND MYOEPITHELIAL CELLS IN BREAST CANCER PROGRESSION**

Marianela Sciacca, Macarena Zambrano, Denise Belgorosky, Yanina V. Langle, Eduardo Imanol Agüero, Eduardo Sandes, Ana Maria Eijan, Catalina Lodillinsky

Instituto de Oncología Ángel H Roffo

Normal ductal of mammary gland is lined by two main cell types, the luminal (LEP) facing the lumen and the myoepithelial (MEP) facing the stroma. Most of breast tumors are generated from LEP, but the role of MEP in tumorigenesis is not clear. The cellular model LM38 consists in three cell lines: LM38-LP (epithelial and myoepithelial), LM38-HP (epithelial) and the LM38-D2 (myoepithelial).
Previously we demonstrated that only the bi-cellular LM38-LP cell line was able to develop intraductal tumors, suggesting that cell interaction could confer an advantage for tumor formation and progression. In this work, we examined different cell mechanism like

survival, adhesion and invasion.
The viability of three cell lines was analyzed by MTS assay after 24 and 48 hours, in different concentrations of bovine serum (SFB). LM38-LP cells had the highest viability with 1% and 5% of SFB compared to LM38-HP and LM38-D2 lines (p<0,001 LP vs HP and D2). Gelatin degradation assay was performed. LM38-LP had higher degradative activity than LM38-HP and LM38-D2 (p<0,01; p<0,05) and this activity was associated with MEP cell population. Three cell lines had similar adhesion ability to collagen IV, one of components of the basal membrane.
Furthermore, we evaluated the contribution of soluble factors in cell interaction. A significant increase in the cell viability was observed when the conditioned medium (CM) of LM38-D2 was added to LM38-HP and LM38-LP cells (p<0,05). Also, LM38-LP cells in the presence of CM of LM38-D2 showed an increase in proliferation nuclear antigen PCNA-expression compared with control.
Finally, the LM38-LP cell line presented better survival and higher invasive activity. Both mechanisms could be used by the cells to achieve tumor formation and progression. These results, based on a bi-cellular model, show the importance of cellular cooperation to achieve tumor growth in the mammary ductal environment.

596. (151) **4-METHYLUMBELLIFERONE ENHANCES TEMOZOLOMIDE ANTI-TUMORAL EFFECT ON GLIOBLASTOMA CELLS WITHOUT AFFECTING NORMAL BRAIN CELLS.**

Matías Pibuel[2], Yamila Molinari[1], Mariángeles Díaz[2], Daniela Poodts[2], Élida Álvarez[2], Lucas Silvestroff[1], Silvina Lompardía[2], Paula Franco[1], Silvia E. Hajos[2]
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Glioblastoma is the most common primary tumor of central nervous system, in which migration and invasion are strongly associated with high mortality. After first-line treatment, surgical resection, radiotherapy and temozolomide, the median survival of patients is 14 months. Therefore, new drugs are required for glioblastoma therapy. 4-methylumbelliferone (4MU) is a coumarin derivative widely used as a hyaluronan (HA) synthesis inhibitor, although, HA-independent-effects of 4MU have been reported. While 4MU anti-tumoral activity was described on several carcinomas, its effects on glioblastoma were not reported yet. We hypothesized that 4MU, alone or in combination with temozolomide, could inhibit growth and migration of glioblastoma cells, providing a potential therapeutic alternative. The aim of this work was to determine the anti-tumoral effect of 4MU on glioblastoma cells by evaluating its toxicity and selectivity. Murine glioblastoma cell line (GL26), mouse normal brain primary cultures (MNBPC) and GL26/MNBPC co-cultures were used. We evaluated metabolic activity by XTT assay, migration by wound healing assay, metalloproteases activity by zymography and cell death by FDA/PI and Annexin-V-PE/7AAD using flow cytometry. In GL26 cells, 4MU reduced metabolic activity, gap closure and metalloproteases activity in a dose dependent manner (p<0.05) and increased the percentage of PI+ and Annexin-V-PE+ cells (p<0.05). HA co-treatment restored normal metalloproteases activity but could not revert other 4MU effects. 4MU+temozolomide combination notably augmented the percentage of PI+ and Annexin-V-PE+ GL26 cells, respect to individual treatments (p<0.01). Remarkably, in MNBPC, 4MU did not affect the percentage of PI+ cells respect to untreated condition (p>0.05). At the highest 4MU dose, metabolic activity of MNBPC was slightly reduced. Moreover, when both GL26 and MNBPC were co-cultured, 4MU selectively increased the percentage of PI+ GL26 cells without affecting MNBPC cell death. These results demonstrate an important anti-tumoral and selective effect of 4MU as a promising new drug for glioblastoma treatment.

TRANSDUCCIÓN DE SEÑALES Y MECANISMOS MOLECULARES DE ENFERMEDAD / SIGNAL TRANSDUCTION ORAL SESSION

597. (123) **3D BIOCOMPATIBLE MATRIX FOR THE BIOCHEMICAL ANALYSIS OF COMMERCIAL CANCER CELL LINES AND THEIR METABOLISM REWIRING IN RESPONSES TO THERAPEUTIC DRUGS.**

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One of 6 deaths worldwide is cause by cancer. The early diagnoses and treatment resulted in decreases in some countries however the statistic worldwide is maintained. Drug screening has been one of the few tools with success. Generally preclinical studies use 2D cell cultures as the first strategy in the screening of compound for cancer therapy however cancer research approaches need improvement by models which could create the biological and natural architecture of the tumours; 3D cell culture is a promising approach when they emulate natural tumour environment.
Here we implement a 3D biocompatible printed matrix as commercial cell lines support for drug testing.We evaluate a colorectal cancer cell line (HCT116) and kidney embryonic cancer cell line (HEK293) as cell laden on 3D printed matrix by a 4th Generation Envisiontec Bioplotter. We compared drug response on 2D and 3D models. 2 drugs were tested on cell lines (chloroquina, and lithium chloride) by cytotoxicity assay (Sulforhodamine B -SRB assay) and Live/dead viability assay on 3D constructs. Our results showed in addition to different architectural organization in 3D matrix for the cell lines , drug response differences between type of cultures, for lithium chloride HEK293 was more than 8 fold IC50 more resistant in 3D culture than in 2D cultures (p <0.02) while CCR cell line showed the same IC50 in both culture types. In addition for chloroquine both cell lines were more sensitive in 3D culture than in 2D cell culture (p<0.01).
Our results demonstrated that dimensional and architectural matrix differences not only affect drug response but cell organization and metabolism where 3D matrix prone differently the cell lines to be more or less dependent to catabolic metabolism.

598. (187) **VEGF AND BFGF MODULATE ANTI-ANGIOGENIC TREATMENT RESPONSE IN EXPERIMENTAL PROLACTINOMAS**

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Anti-angiogenic therapies as Bevacizumab (Bvz) are administered as salvage therapy in some cases of aggressive pituitary tumors. Our preliminary data shows that MMQ xenografted prolactinomas present a refractory response to Bvz treatment when it is applied in late stages of tumor development. Therefore, the present study aimed to elucidate the molecular mechanisms that account for the observed resistance. For this purpose, we studied the *in vitro* effect of Bvz treatment on MMQ tumoral lactotrophs and HMEC endothelial cells. Both cell lines were treated with Bvz (25, 100 and 250 µg/ml) or control (medium) for 48 hours. We found that Bvz did not impact on MMQ and HMEC cell viability (MTS; ns) or apoptotic rate (FC; ns). In order to study Bvz effect on the angiogenic properties of both cell types, we measured VEGF release to culture supernatants and found a decrease in both tumoral and endothelial cells (ELISA; p<0,05), although no differences were found in bFGF production (ELISA; ns). Furthermore, a dose-dependant decrease in secreted matrix metalloproteinases was observed (Zymography; p<0,05 MMQ and HMEC), supporting its anti-angiogenic effect. Moreover, we treated HMEC cells with MMQ conditioned media and we found that lactotrophs treated with Bvz induced a trend of reduction of HMEC viability (MTS; ns), migration (WHA; ns), and *Vegf* synthesis (RT-qPCR; p<0,05). However, when we quantified the proan-

giogenic factor synthesis under direct Bvz action, we observed that *bFgf* expression was increased both in MMQ and HMEC, while *Vegf* was not modulated in HMEC but significantly increased in MMQ cells (RT-qPCR; ns and p<0,05, respectively). These results suggest that compensatory mechanisms involving VEGF and bFGF could be triggered within the tumor and its microenvironment and could explain a late refractory response to Bvz treatment despite of its initial beneficial effect.

599. (463) **SIRT1-ACTIVATING POLYPHENOLS LIMIT SECRETOME AGGRESSIVENESS IN SENESCENT RETINAL PIGMENT EPITHELIAL CELLS**

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Cellular senescence triggers the expression of a wide variety of inflammatory factors named the senescence associated secretory phenotype (SASP). The SASP contributes to diseases of aging by disrupting tissue structure and function. Age-related macular degeneration (AMD) is a progressive disease which leads to irreversible loss of vision. Cell senescence of the retinal RPE is suggested to play a central role in the etiology of AMD. We have previously showed that Caffeic acid (CAF) and Chlorogenic acid (CLO) control the SASP of oxidative-stress induced senescent RPE cells. Now, we aim to determine the molecular mechanism underlying these beneficial effects. Methods: Human RPE cells (ARPE-19 line) were incubated with 150 µM H_2O_2 for 90 minutes during 3 days and then maintained for nine days to establish senescent cultures (SEN). These cultures were exposed to CAF 30 µM or CL 50 µM for the last 6 days in the presence or absence of SIRT1 inhibitor, Ex-527. RNAm and protein levels for IL1-β, IL-6 were analyzed by western blot. Senescence was determined by positive staining for Senescence Associated β-gal activity (SA-β-gal+) and increased expression of p21 and p16. γH2AX, a DNA damage marker, was tested by IFI. Results: Senescent cells showed activated phospho - p38 (Thr180/Tyr182) and phospho-NF-κB p65 (Ser536). Polyphenols inactivated these pathways. CAF and CL diminished p16 expression. In contrast, no changes were found in p21, γH2AX or SA-β-gal+ staining. SIRT1 inhibition prevented CAF and CLO effects on p38 and p65 phosphorylation as well as on IL-1β and IL-6 expression. Conditioned medium (CM) from SEN cultures promoted γH2AX foci and SA-β-gal+ activity in control cells. CAF and CL suppressed SASP-mediated paracrine effects. Conclusions: CAF and CL treatments control SASP expression in a SIRT1-dependent manner. Therapeutic interventions based on polyphenols might block senescence detrimetal effects in AMD and other age-related pathologies.

600. (566) **DIFFERENTIAL ROLES OF MITOGENIC PATHWAYS IN ANGIOGENESIS INDUCED BY PTHrP EMPLOYING IN VITRO AND IN VIVO MODELS OF COLORECTAL CANCER**

Natalia Graciela Calvo, Pedro Carriere, María Belén Novoa Díaz, María Julia Martín, Claudia Gentili
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Parathyroid Hormone-related Peptide (PTHrP) is involved in various human cancers such as colorectal cancer where angiogenesis plays a critical role. In previous studies we observed that PTHrP stimulates the production of VEGF in Caco-2 and HCT 116 colon cancer cells which acts promoting the angiogenesis. The objective of the present work was to gain insight into the signaling events that link PTHrP to tumor angiogenesis studying the possible role of ERK1/2 MAPK and PI3K/AKT. qPCR assay and the use of the corresponding inhibitors revealed that PTHrP increases mRNA levels of the angiogenic factors VEGF, HIF-1α and MMP-9 in Caco-2 and HCT 116 cell lines via ERK1/2 and PI3K/AKT signaling pathways. Then, to determine if ERK1/2 and AKT mediate the angiogenic potential of colon cancer cells induced by PTHrP, we employed conditioned media from Caco-2 or HCT116 cells pretreated with the corresponding inhibitors, following with the incubation with the hormone. The inhibition of ERK1/2 and PI3K/AKT signaling pathways in colon cancer cells abrogated the stimulatory effects of Caco-2 and HCT 116 cells induced by PTHrP on endothelial HMEC-1 cell migration. According to the results obtained *in vitro*, we showed increased protein and mRNA levels of VEGF in nude mice xenografts of HCT116 cells by immunohistochemistry and qPCR analysis, respectively. Furthermore, we also observed that the localization of VEGF was near the nuclear membrane and cytoplasmic, suggesting also the possible involvement of this factor in an intracrine signaling. As previous studies revealed that PTHrP activates ERK 1/2 but not AKT in the *in vivo* model, these findings suggest that ERK1/2 is involved in tumor angiogenesis induced by PTHrP, while AKT may not participate in this process. This study clearly illustrate different responses according to the model used, reinforcing the importance of the use of *in vivo* models.

601. (612) **RSK IS INVOLVED IN ANGIOGENESIS INDUCED BY HYPOXIC CONDITIONS IN CERVICAL CANCER**

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Angiogenesis which plays a critical role in tumor growth and metastases is a very early event in the development of premalignant lesions induced by HPV in the cervical cancer (CC). It involves multiple processes including the secretion of angiogenic factors by tumor cell which increases in hypoxic conditions. The objective of this work was to gain insight into the signaling events involved in angiogenesis induced by hypoxic conditions in CC studying the possible role of p90 ribosomal S6 kinase (RSK). Beside the emerging role of RSK in cancer, few studies have investigated its implication in the cell response to hypoxia. We employed HMEC-1 cells, an immortalized cell line of human microvascular endothelial cells, and conditioned media (CMs) from cervical cancer Hela cells, to evaluate the effect on endothelial cells of the factors released by cervical cancer cells treated with cobalt chloride (CoCl2) as a hypoxia mimic in the presence and absence of RSK inhibitor SL0101. We first evaluated the number of endothelial cells by crystal violet staining and trypan blue dye exclusion assay. We observed that CMs from Hela cells treated with CoCl2 increases the number of endothelial cells. However, the inhibition of RSK signaling pathway in Hela cells attenuated the stimulatory effects of these cervical cancer cells under hypoxic conditions in the proliferation of HMEC-1 cells. In addition, we performed tube formation assays using Geltrex. HMEC-1 cells were seeded on geltrex and treated with the corresponding CMs. CMs from Hela cells incubated with CoCl2 induce the formation of tube-like structures in endothelial cells seeded on geltrex whereas the pre-treated of the cervical cancer cells with the inhibitor SL0101 reversed partially this effect. These results suggest that RSK has a novel role in angiogenesis induced by hypoxic conditions in CC.

602. (630) **MODULATION BY PTHrP OF MOLECULAR MECHANISMS ASSOCIATED WITH THE INDUCTION OF CANCER STEM CELL PHENOTYPE AND EPITHELIAL TO MESENCHYMAL TRANSITION IN HUMAN COLORECTAL CANCER CELLS.**

María Belén Novoa Díaz, Pedro Carriere, Natalia Graciela Calvo, María Julia Martín, Claudia Gentili
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Parathyroid hormone-related peptide (PTHrP) is expressed in many colorectal cancer (CRC) patients. This disease is the second most common cancer in Argentina. In Caco-2 and HCT116 cell lines, both derived from human CRC, we found that PTHrP exerts proliferative and protective effects, induces cell migration, and promotes tumor-associated angiogenesis. PTHrP also attenuates the sensitivity of these cells to the chemotherapeutic drug Irinotecan through ERK MAPK and β-catenin pathways. Herein we further investigated the mechanism modulated by PTHrP leading to a more aggressive phenotype of CRC cells employing HCT116 cells and HCT116 tumor xe-

nografts in nude mice. In vitro we found that PTHrP induces Ser552 phosphorylation of β-catenin and its subsequent nuclear translocation. Once in the nucleus, β-catenin can activate the expression of molecular markers associated with other events of CRC progression such as cancer stem cell (CSC) phenotype and epithelial to mesenchymal transition (EMT). In both experimental models we observed that PTHrP regulates protein levels of two CSC markers, CD44 and CD24 and also modulates protein expression of the EMT markers, CK-18, E-cadherin and ZEB-1. Met is a receptor tyrosine kinase (RTK) with aberrant expression and signaling in advanced CRC. We found that PTHrP decreases Met protein levels being this effect reverted by ERK1/2 and p38 MAPK specific inhibitors, suggesting that in PTHrP-treated HCT116 cells this RTK is degraded after its activation via MAPK signaling. According with our hypothesis, PTHrP increases Met protein levels in the murine model. Finally, the specific Met inhibitor reverted β-catenin phosphorylation and EMT markers expression induced by PTHrP suggesting that Met signaling is involved in these molecular events. Advances in the knowledge of the characteristics of aggressive CRC, such as the induction of CSC or EMT, will provide valuable information in understanding this disease and will facilitate the development of new therapeutic approaches

GENÉTICA / GENETICS ORAL SESSION

603. (231) **DESIGN OF A LENTIVIRAL VECTOR TRANSCRIPTIONALLY REGULATED FOR SPECIFIC NEURON THERAPEUTIC GENE DELIVERY**

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Alzheimer's disease is a neurodegenerative disorder characterized by a progressive loss of cognitive functions. One hallmark is the formation of amyloid plaques, composed mainly by Aß peptide oligomers (AβOs). Neprilysin (NEP) is the main endopeptidase for the degradation and clearance of Aß in the brain. Strategies aiming to increase NEP levels should contribute to decrease the amount of AβOs and could have a therapeutic effect. In this work we developed a LV coding the NEP cDNA under SYN promoter (LV-SYN-NEP) for the delivery of transgenic NEP. The objective is to evaluate its performance in neuron-like cells at different days of differentiation and correlate vector-encoded NEP expression with Synaptophysin, which is a marker of neuronal differentiation.

Neuronal progenitor cell line SH-SY5Y was transduced with LV-SYN-NEP and LV-CMV-NEP. 72 hours post transduction SH-SY5Y cells were put under neuron differentiation conditions (Retinoic Acid 10 uM). Expression of NEP and Synaptophysin was measured at day 0, 5 and 7 of differentiation by flow cytometry and immunofluorescence, respectively.

We observed in SH-SY5Y transduced with LV-SYN-NEP 0%, 16% and 33% of NEP+ cells at days 0, 5 and 7 of differentiation, respectively, while SH-SY5Y transduced with LV-CMV-NEP resulted in more than 90% of NEP+ cells at all time points assessed. NEP was not expressed in mock-transduced cells. We also found that LV-delivered NEP expression correlates with Synaphophysin, demonstrating that LV-SYN-NEP is able to express in differentiated neurons. We also demonstrated that SYN promoter activity increases with the degree of neuronal differentiation.

In conclusion, we developed a neuron-specific lentiviral vector to deliver NEP transgene to neuronal cells which is expressed as neurons become differentiated. In the future, we will test its protective potential against toxic aβ peptides.

604. (235) **EFFICIENT GENOME EDITING AND GENE ADDITION USING BABOON ENVELOPE GP PSEUDOTYPED VIRAL DERIVED "NANOBLADES" LOADED WITH CAS9/SGRNA RIBONUCLEOPROTEINS AND AAV6 FOR DONOR DNA CASSETTE DELIVERY.**

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Programmable nucleases have enabled rapid and accessible genome engineering in cells and living organisms. However, their delivery into target cells can be challenging into primary cells. Here, we have designed "Nanoblades", a new technology to deliver a genomic cleaving agent into cells. These are murine leukemia virus-derived virus like particle (VLP) loaded with Cas9 protein through fusion with the gag viral protein and guide RNAs. Cas9 together with gRNAs introduces site specific double strand break (DSBs) in target genes which can be repaired by non-homologous end-joining (NHEJ) or by homology-directed repair (HDR) introducing a new sequence from an exogenous template DNA bearing homology to the sequences flanking the DSBs (donor-DNA).

Previously, we demonstrated that Nanoblades were extremely efficient in delivery of Cas9/sgRNA cargo into K562 cell line and human T, B , HSCs and HSC-derived progenitors T-cells (pro-T cells), thanks to their surface co-pseudotyping with baboon retroviral and VSV-G envelopes.

The objective of this work was to edit Wiscott Aldrich Syndrome (WAS) gene locus by HDR using Nanoblades and AAV6 carrying a donor-DNA, consisting in GFP reporter gene flanked by homologous arms of the WAS gene.

AAV6 was added to K562 cells at different time points with respect to Nanoblades addition, in order to find the optimal addition dynamic that maximizes HDR. Different multiplicities of infection (MOIs) of AAV were tested. HDR-mediated gene editing was determined by PCR and GFP expression by flow cytometry 7 days after Nanoblades addition.

We show that HDR-mediated edition of WAS gene occurred in 50% of the cells adding nanoblades and AAV6 (MOI 100000 vg/cell) simultaneously. We are currently testing this protocol in HSCs and pro-T cells.

In summary, Nanoblades in combination with AAV6 carrying donor-DNA are efficient tools for gene editing and have important prospects for basic and clinical translation for gene therapy.

605. (259) **IDENTIFICATION OF HYPOPITUITARISM RELATED VARIANTS IN ARGENTINEAN PATIENTS BY MOLECULAR INVERSION PROBE SEQUENCING (MIPS), A NOVEL MOLECULAR APPROACH FOR LOW COST SEQUENCING**

Sebastian Vishnopolska, Marta Ciaccio, Marcelo Martí, Sally Camper, Jacob Kitzman, Adriana Seilicovich, Alicia Belgorosky, Maria Isabel Di Palma, Natalia Perez Garrido, Miranda Lucas, Pablo Ramirez, Roxana Marino, Bergada Ignacio, Keselman Ana, Braslavsky Debora, Mortensen Amanda, María Inés Pérez Millán

IQUIBICEN, CONICET, *Dpto. de Química Biológica, FCEN-UBA*

Pituitary hormone deficiency occurs ~1:4,000 live births. Over 30 genes have been implicated in isolated and/or combined pituitary hormone deficiency (IGHD/CPHD). Mutations are estimated to account for ~16% of patient cases, thus the majority of familial and sporadic cases have no known genetic origin. We recently implemented a novel and cost-effective approach based on Molecular

inversion probe sequencing (MIPS) to identify novel variants and candidate genes in sporadic trios and familial cases of CPHD and IGHD. We captured 693 coding exons of 30 known genes and 37 candidate genes. We captured genomic DNA from 176 pediatric patients from Argentina with CPHD or IGHD and 133 relatives and conducted next generation sequencing. We obtained a 600X average coverage per sample over targeted regions. We discovered 10 likely pathogenic variants; 8 of them are novel. We classified each variant following the ACMG-AMP guidelines, which is so far the most detailed and quantitative system for variant interpretation in genetic testing. We identified heterozygous variants in 6 genes: GH1 (p.Arg209His), GLI2 (p.Leu761Phe, p.Ser1048fs and p.Lys-1162Arg), LHX3 (p.Pro187Ser, p.Leu220Met), LHX4 (p.Gln100His, p.Trp204Leu), PNPLA6 (p.Thr1115Pro) and HESX1 (p.Ile26Thr and p.Gln117*). Mutations in PROP1 are the most common known cause of CPHD, accounting for 11% of total cases worldwide. The frequency of PROP1 mutations varies widely by population group, and the rate was previously unknown for Argentina. We found no cases of PROP1 mutations. We are testing the effects of these variants on the activity of the transcription factors in cell culture. Identification of disease causing mutations in CPHD is complicated by phenotypic variation and incomplete penetrance. Identifying potential pathogenic variants will make it feasible to predict clinical outcomes from genetic data, which is necessary for patient diagnosis and prognosis, and for assessing the risk of future affected individuals.

606. (738) **ANALYSIS OF DIFFERENTIALLY EXPRESSED GENES AFTER EX VIVO X-RAYS IRRADIATION OF HUMAN PERIPHERAL WHITE BLOOD CELLS**

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The understanding and characterization of the radio-induced response at molecular level is pivotal for developing new approaches on practices that employ Ionizing Radiation (IR). Currently, gene expression signatures are being developed for radiation biodosimetry and as predictive biomarkers for personalizing radiotherapy. In order to detect potential radiation-exposure biomarkers, we performed a bioinformatic meta-analysis of public microarrays of ex vivo X-rays-irradiated human peripheral white blood cells and a validation of the resulting differentially expressed genes (DEGs) by qPCR. Gene expression of five datasets from Gene Expression Omnibus were analyzed with R software and Bioconductor packages. DEGs functional enrichment was performed with Over Representation and Gene Set Enrichment Analysis while iRegulon was used to detect master regulators transcription factors (TF) from DEGs. Human peripheral blood samples from six healthy human donors were X-rays-irradiated at 1-4Gy or left unirradiated. Dicentric Chromosome Assay was performed as biodosimetry control while mRNA from samples was obtained 24 h after exposure for qPCR validations with GAPDH as a reference gene (p-values<0.05 were considered significant). Bioinformatic analysis identified a total of 452 DEGs after X-rays exposure (parameters: lfc=0.7, p-value<0.05). While some of them are well known to be involved in radiation response, others resulted as novel. The DGEs showed enrichment in biological processes such as regulation after IR-exposure, DNA damage checkpoint, signal transduction by p53 and mitotic cell cycle checkpoint. PCNA, TIGAR, DRAM, PLK2 and NUDT15 expressions levels significantly increased at 1-4 Gy vs controls, demonstrated by qPCR. Meanwhile, TCF4 exhibited a significant decrease post-irradiation. This gene was previously detected as a master regulator TF by the bioinformatic analysis. Therefore, the detection and validation of this six DEGs can provide potential candidates as radiation-exposure biomarkers. These findings could also reveal novel insights about molecular networks involved in radio-induce response.

607. (789) **IMPLEMENTATION OF ARRAY-CGH IN PATIENTS WITH INTELLECTUAL DISABILITY , MULTIPLE CONGENITAL ANOMALIES AND SELECTED CHROMOSOMAL ABNORMALITIES.**

Lucía Espeche[1], Marisol Delea[1], Soledad Massara[2], Andrea Solari[1], María Paz Bidondo[1], Pablo Barbero[1], Jaen Oliveri[2], Mónica Ritler[3], Paloma Brun[2], Viviana Cosentino[4], Rubén Martin[5], María Ángeles Mori[5], María Palomares[5], Julián Nevado[5], Liliana Dain[1], Sandra Rozental[1]
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Introduction: arrayCGH is a significantly high resolution method to scan the genome for gains and losses of chromosomal material. Although in several countries it is a first-tier test for patients with intellectual disability (ID) or with multiple congenital anomalies (MCA), in our country it represents an expensive technique and a very few laboratories have begun to implement it. Karyotyping remains to be the routine study in public health.
Aim: To report our experience in the use of ArrayCGH for patients with ID, MCA and selected chromosomal abnormalities.
Materials and methods: we studied 176 patients with two arrayCGH platforms (Agilent ISCA-v2-60K and KaryoArray®v3.0-8x60K): 128 had ID, 40 had MCA and 7 presented a cytogenetic abnormality detected by karyotype. Patients with ID and MCA did not show a cytogenetic anomaly or had a failed karyotype test.
Results: We found that 14/128 (11%) ID patients had causal or potentially causal CNVs: 12 were recognizable syndromes. One patient had an imbalance associated with a microdeletion syndrome accompanied with other pathogenic imbalance. Besides, 10/40 (25%) MCA patients presented causal or potentially causal CNVs: 2 were chromosomal abnormalities, 3 were recognizable syndromes and 1 had a microdeletion syndrome accompanied with other pathogenic imbalance. Moreover, we fully characterized all the cytogenetic abnormalities analyzed.
Conclusion: ArrayCGH was useful to perform an accurate analysis of microdeletions or duplications that could not be detected by standard karyotyping. The percentage of pathogenic imbalances was higher in patients with MCA than in those with ID in accordance with the data reported in the literature.

INMUNOLOGÍA / IMMUNOLOGY ORAL SESSION 6

608. (142) **STAPHYLOCOCCUS AUREUS PROTEINS SPA AND SBI SIGNIFICANTLY CONTRIBUTE TO THE EARLY RECRUITMENT OF NEUTROPHILS TO THE SITE OF INFECTION DURING SKIN ABSCESS FORMATION.**

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Neutrophils play a prominent role in the formation and resolution of skin abscesses. This study was aimed at determining the contribution of *S. aureus* proteins SpA and Sbi to the early recruitment of neutrophils during skin infection. Three hour after Intradermal inoculation of 1x108 CFU of S. aureus increased neutrophils in circulation were found. Interestingly, the percentage of neutrophils found in blood of mice inoculated with the SpA- mutant were significantly higher than that found in the wild type inoculated group (40 % vs 65 %, $p<0.05$). Circulating neutrophils are elicited from the vasculature to the site of infection in response to pro-inflammatory factors. At three hours post-inoculation the expression of SpA and Sbi significantly contributed to TNF-α production, a critical cytokine for the activation of the endothelium. To characterize the early influx of neutrophils to the site of infection in real time we used intra-vital microscopy. A significant increase in the number of neutrophils attracted to the area was observed at two hours post inoculation with wild type S. aureus compared with the control group ($p<0.05$). On the contrary, neutrophil recruitment was not observed in mice challenged

with the SpA- or the Sbi- mutants. In vivo imaging also revealed that neutrophils from mice inoculated with wild type S. aureus were preferentially rolling whereas those from the control group were free. One hour later, neutrophils in the control group were mostly free or rolling with some adherent whereas neutrophils from S. aureus inoculated mice were found rolling, adhering and extravasating. Accumulation of neutrophils in the skin tissue was only observed with wild type S. aureus at 5 hours post-inoculation. These results demonstrate that SpA and Sbi are staphylococcal proteins critical for the early recruitment of neutrophils to the skin and should not be blocked during *S. aureus* cutaneous infections.

609. (388) **TRYPANOSOMA CRUZI INFECTION ALTERS ADIPOCYTE HOMEOSTASIS AFFECTING THE EXPRESSION OF METABOLIC ENZYMES, PPARS AND ADIPOKINES PROMOTING AN INFLAMMATORY PHENOTYPE.**

Florencia Belén Gonzalez[1], María Florencia Pacini[1], Silvina Raquel Villar[1], Cecilia Farré[1], Luciano D'Attilio[1], Oscar Bottasso[1], Graciela Piwien Pilipuk[2], Ana Rosa Pérez[1]
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Adipose tissue (AT) is a target of *T. cruzi* (Tc) infection, being a parasite reservoir in mice and humans. Previously, we reported that acute murine Tc infection is linked to a severe AT loss, probably triggered by inflammation, as well as by the parasite itself. Here, we evaluated how infection affects AT homeostasis, considering adipocyte anabolic/catabolic pathways and immune-endocrine patterns in C57BL/6 mice (n= 5-6/group) . During infection, both catabolic and anabolic pathways are profoundly affected, since mRNA expression of lipolytic (HSL and ATGL) and lipogenic (LPL, FAS and DGAT) enzymes measured by qPCR was intensely downregulated (all enzymes, Co vs Tc p<0,05). Infected AT reveals a pro-inflammatory profile, with increased leucocyte infiltration (H&E) and TNF-α overexpression (Co vs Tc p<0,05). Also, AT-derived adipokines leptin and adiponectin were downregulated (both, Co vs Tc p<0,05). Strikingly, peroxisome proliferator-activated receptor (PPAR)-α and PPAR-γ (transcription factors that have key regulatory functions in lipid metabolism and anti-inflammation) were strongly decreased in AT (both, Co vs Tc p<0,05), while PPAR-δ tended to diminished. AT is conformed not only by adipocytes but also by stromal vascular fraction cells (SVFCs) including CD4+ and CD8+ T lymphocytes, macrophages and dendritic cells; which were increased during infection (all, Co vs Tc p<0.05) as shown by flow cytometry. Adipocytes and SVFCs were separated by collagenase digestion and the expression of different immune-metabolic factors was assessed independently. The same pattern of expression than observed in whole AT was detected in isolated adipocytes from Tc animals. Coincidently, SFVCs showed an inflammatory profile with TNF-α overexpression and PPARs downregulation (all, Co vs Tc p<0.05). These results suggest that acute Tc infection disrupts both adipocyte catabolic and anabolic metabolism secondary to PPARs robust downregulation, tipping the balance towards to an adverse status compatible with the AT atrophy and the acquisition of an inflammatory phenotype.

610. (513) **IMMUNOGENICITY AND PROTECTIVE EFFICACY OF ANTI-T. CRUZI NASAL VACCINE PROTOTYPES BASED ON TRANSIALIDASE**

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Currently there is not available a prophylactic vaccine for Chagas disease. Therefore, in this work we evaluated whether the administration of different nasal vaccine prototypes could prevent the development of T. cruzi (Tc) infection through the induction of specific and systemic immune response. A fragment from the immunodominant parasite antigen called transialidase (TSf), containing both B and T epitopes was selected by bioinformatics. TSf was expressed in a L. lactis recombinant strain and then purified, avoiding the presence of endotoxins like LPS in vaccine formulations. Thus, female Balb/c mice (n= 5-6/group) were immunized by intranasal route (three doses, one every two weeks) with different vaccine formulations combining TSf with different adjuvants (c-di-AMP or ISPA). We also assayed the whole recombinant L. lactis expressing TSf as delivery system plus c-di-AMP (LL-TSf+c-di-AMP). Non immunized mice were used as control group (Co). In immunized mice, humoral and cellular immune responses were assayed prior to infection. After oral infection with 2500 Tc/mice (Tulahuen strain), the parasitemia and the clinical score were determined. Intranasal immunized mice with TSf+c-di-AMP showed enhanced levels of IgG2a and IgG1 compared to TSf and Co groups (in all cases, p<0.05). TSf+ISPA and LL-TSf+c-di-AMP immunized mice also tend to increase both TSf-specific-antibodies compared to Co and TSf. TSf+c-di-AMP immunized mice and, in a lesser extent TSf+ISPA, elicited a higher TS-specific cellular mediated immune response after 24 and 48 h (DHT in footpads). Parasite load after 14 and 28 days post-infection was less evident in TSf+c-di-AMP animals compared with the rest of the groups. Moreover, TSf+c-di-AMP animals exhibited less clinical affectation (clinical global score, p<0.05 vs. all groups). Taken together, these results indicate that TSf+c-di-AMP and TSf+ISPA formulations tested in this work would be good candidates for the development of a prophylactic mucosal vaccine against T. cruzi.

611. (772) **MOLECULAR AND METABOLIC MECHANISM INVOLVED IN THE IMPARIMENT OF CD4 T CELL RESPONSE DURING TRYPANOSOMA CRUZI INFECTION**

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We have previously shown an increased mitochondrial ROS (ROSm) production, mitochondrial alterations and a decreased glucose uptake in CD4 T cells during the acute phase of *Trypanosoma cruz*i infection. In order to determine the energy balance during infection we evaluated AMPK phosphorylation (p-AMPK) in CD4 T cells, purified from spleen of BALB/c mice infected with 500 trypomastigotes (tp) of Tulahuen strain at different times post infection (p.i) compared to CD4 T cells of control mice. CD4 T cells from acute phase of infection exhibit p-AMPK increase indicating unfavorable metabolic conditions. Besides, it has been demonstrated that CD11a and CD49d can be used as strategy to evaluate "specific" CD4 T cells. We observed an increase in the frequency of the population CD4+CD11a+CD49d+ in infected mice (p<0.0005), which presented mitochondrial alterations during acute phase of infection (p<0.0005), evaluated by FACS with potential-dependent and potential-independent mitochondrial dyes. To address if these alterations are related to infective dose, BALB/c mice were infected i.p. with 500, 1000 and 5000 tp and sacrificed during acute phase. We observed increase in ROSm production (p<0.05), mitochondrial alterations (p<0.0005) and reduced glucose uptake (p<0.005) in CD4 T cells from infected mice compared to control cells. Although, infective dose did not have a differential effect in these parameters, apoptosis evaluated by FACS in these groups of animals was increased compared to control cells and was higher in BALB/C mice infected with 5000 tp (p<0.005). Moreover, we evaluated apoptosis in ROSm+ CD4 T cells and have observed that was an increased during chronic phase of infection (p<0.0005). These results may indicate that *T. cruzi* induces mitochondrial alteration leading to metabolic dysregulation in CD4 T cells during acute phase of infection, which are not influenced by initial infective dose and may influence the outcome of these cells at chronic phase of infection.

REPRODUCCIÓN / REPRODUCTION 4

612. (441) **URIC ACID LEVELS IN GESTATIONS COMPLICATED WITH PREECLAMPSIA AND FETAL GROWTH RESTRICTION**

Ana Irene Corominas[1], Silvia Balconi[1], María Ortiz[1], Bernardo

Maskin[1], Nora Martinez[3], Alicia Damiano[2, 3]
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Preeclampsia is a pregnancy disorder characterized by the onset of hypertension after 20 weeks of gestation. Although its etiology is unknown, inadequate placentation and maternal vascular dysfunction were associated with multiple complications and increased maternal and fetal morbimortality.
Preeclampsia is subdivided into early (before 34+0 weeks) and late (after 34+0 weeks) onset, with or without intrauterine fetal growth restriction (IUGR).
The identification of pregnant women at risk for preeclampsia is still difficult and current biomarkers fail to differentiate preeclampsia associated to IUGR with pure IUGR without preeclampsia.
In other tissues, it was reported that an increase in serum uric acid may be related to endothelial dysfunction. Previously, we demonstrated that uricemia increased in preeclamptic pregnancies, and the uricemia ratio after and before the 20th week of gestation may be useful to discard those women who are not at risk of developing preeclampsia.
Our aim was to evaluate the behavior of the uricemia ratio in pregnancies complicated by IUGR associated or not to preeclampsia.
A retrospective descriptive-quantitative study was carried out in all women who attended their pregnancy at the Hospital Posadas during 2014. Uricemia ratio (uricemia after 20th week /uricemia before 20th week) was calculated in women who presented IUGR in simple pregnancies associated or not to preeclampsia.
In 3794 simple gestations analyzed, 214 presented IUGR. In gestations complicated with preeclampsia associated to IUGR 58% of the newborns were under percentile 3, whereas in pregnancies with pure IUGR without preeclampsia, only 33% of the newborns were under percentile 3. Uricemia ratios were higher in women with preeclampsia and IUGR than in those who only presented IUGR (2.03±0.67 vs 1.23±0.30, p<0.001).
Our findings showed that uricemia increase may be linked to hypertensive pregnancies and more severe IUGR, suggesting that the etiology of the insufficient placentation in both syndromes may be different.

613. (678) **STUDY OF THE EFFECTS OF LACTOFERRIN, AN OVIDUCTAL PROTEIN, ON PREGNANCY AND IN VITRO FERTILIZATION RATES IN RATS**

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Our previous results indicate that lactoferrin (LF) is present in the human oviduct secretion and binds to human gametes. We have reported that the presence of LF was able to reduce *in vitro* human gamete interaction. Our aim was to study the effect of LF on reproductive process parameters both *in vivo* (effect on birth and pregnancy rates) and *in vitro* (effect on *in vitro* fertilization –IVF- rate) in Wistar rats. Female rats (80 days old) were randomly assigned to one of four treatment groups and received a daily injection during an estrous cycle (100, 200, 400 mg LF/kg or 0.9% w/v sodium chloride -controls-). On proestrous day, treated female rats were kept with a male rat. After gestation period the number of pregnant animals and born pups was registered. For IVF assessment, cauda motile sperm were obtained from male rats (100-140 days old). On the other hand, female gametes were recovered from female rat oviducts (80 days old) after hormonal ovarian stimulation. Oocytes were inseminated with capacitated sperm and incubated in the presence of LF (0 and 100 μg/ml). Oocytes were stained with Hoechst 33258 and examined in a fluorescence microscope. The number of fertilized oocytes was registered and IVF rate was estimated. *In vivo* studies revealed that none of the animals treated with 100 mg LF/kg (n=5) resulted pregnant and the statistical analysis showed a significant reduction (p<0.05) of the pregnancy rate respect to control (n=7). Additionally, the administration of 200mg LF/kg was able to reduce the mean number of pups (11.6+/-0.5 vs. 7.5+/-2.0; p<0.01). Results indicate that the presence of 100 μg/ml of LF tend to reduce the mean IVF rate <(51.5+/-7.5)% vs. (25.3+/-9.2)%, n=5; p=0.06>. Results suggest that the administration of LF could affect the fertilization process and pregnancy in rats.

614. (354) **ENRICHED ENVIRONMENT STRATEGY IMPROVES THE PREGNANCY OUTCOME AND THE OFFSPRING'S ADULT HEALTH FOLLOWING AN IMMUNE CHALLENGE DURING GESTATION.**

Julieta Aylen Schander, Julieta Aisemberg, Carolina Marvaldi, Federico Jensen, Ana Maria Franchi
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The enrichment of the environment has been associated to a general wellbeing of animals and we have previously demonstrated that the exposition to an enriched environment (EE) reduces (41%) the preterm delivery rate induced by the administration of bacterial lipopolysaccharide (LPS) in a mouse model. The objective of the present study was to evaluate the effects of LPS and EE treatment in the offspring. Our EE protocol consisted in housing a group of ten six week old BALB/c females in larger cages containing a variety of objects, such as stairs, tunnels and wheels that provided optimal conditions for social interaction, further exploration, visual, cognitive, and voluntary exercise activity. Standard conditions (control environment, CE) consisted of standard laboratory cages, that housed 4 animals. After 6 weeks females were mated with males in regular cages and pregnant females returned to EE (or CE) cages till day 15th of pregnancy, when LPS (or saline solution) was administered. Eight hours after LPS administration, females were killed to evaluate fetal and placental weight. We found that placental weight was significantly diminished in EE mothers, whereas fetal/placental ratio was not different between groups. Another group was allowed to continue the gestation to term, when litter size, perinatal death, weight gain during lactation and some physical landmarks of rodent development were evaluated. We did not found differences in the weight gain, but CE-LPS group presented 66,7% of perinatal death, which was not observed in EE-LPS-treated group. Also a group of pups from CE-LPS-treated mothers presented a delay in physical landmarks. Furthermore, preliminary results showed that LPS increases triglyceride and cholesterol levels in the offspring adulthood and it seems to be prevented by the maternal exposition to EE. Collectively, our results shown that EE prevents some of the deleterious effects of LPS exposition during gestation.

615. (215) **METALLOPROTEINASE EXPRESSION AND ACTIVITY IN FETAL CARDIOPATHY AFTER PERIGESTATIONAL ALCOHOL CONSUMPTION UP TO ORGANOGENESIS, IN MOUSE.**

Martin Ventureira, Elisa Cebral
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Previously we observed that perigestational alcohol consumption up to day 10 of mouse gestation (D10) produces abnormal atrial and ventricular myocardial wall, elevated apoptosis, diminished proliferation and altered heart rate in fetuses at day 13 of gestation (D13) after cessation of alcohol intake at D10. Given the role of metalloproteinases (MMPs) in fetal cardiogenesis and that their expression and/or activity are regulated by oxidative stress, we hypothesize that fetal cardiac alterations after perigestational alcohol intake are associated with disrupted MMP expression/activity. Ethanol 10% in drinking water was administered to murine CF-1 females for 15 days before and up to D10, and gestation continued with water until D13 (treated females (TF)). Control females (CF) were administered with drinking water without ethanol. Fetuses and fetal hearts were dissected and prepared for transmission electron microscopy, immunohistochemistry, zymography and NADPH-diaphorase reaction (assay of nitric oxide synthase activity). Neither MMP-2 nor MMP-9 protein expressions were altered, but both Pro-MMP-2 and MMP-2 gelatinolitic activities were elevated in hearts from TF-fetuses compared to the controls (p<0.05). Dissected hearts from TF-fetuses had increased NADPH-diaphorase reaction in toto revealing augmented NOS activity. By immunohistochemistry, the TF-cardiac tissues presented elevated 3-nitrotirosyne protein expression compared to con-

trols (p<0.01). Cardiomyocytes from TF-fetuses had disorganized myofibrils and reduced mitochondrial diameter (p<0.01, Image J). In conclusion, perigestational alcohol consumption induced high fetal cardiac Pro- and active MMP-2 activities and protein nitrosilation related to the increase of nitric oxide reactive species, suggesting that intracellular activation of Pro-MMP-2 could be one of the main mechanism that lead to disruption of myofibrils and mitochondrial structure. Despite the cessation of alcohol intake at D10, the alcohol induced-D13-fetal cardiopathy probably leads to typical congenital cardiopathy of the Fetal Alcohol Spectrum Disorder.

616. (331) **EFFECTS OF A SINGLE POST-OVULATORY DOSE OF ULIPRISTAL ACETATE (UPA) ON IMPLANTATION IN MICE**

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UPA is a selective progesterone receptor modulator used as the most effective emergency contraceptive pill in several countries. Although its primary mode of action is to inhibit or delay ovulation, this effect is limited to its intake before LH peak. Therefore, considering its high effectiveness in humans, it is possible that this drug prevents pregnancy by other mechanisms as well. In this regard, despite previous results in mice discarding effects on gamete transport and interaction, we have recently observed that a single post-ovulatory dose of UPA produces a decrease in the number of mouse developing fetuses. Based on this, the aim of this work was to evaluate potential effects of UPA on embryo-uterine interaction in mice. Females in proestrus stage were caged with male breeders and successful mating was confirmed by the presence of copulatory plug (embryonic day 0.5, E0.5). UPA (40mg/kg) or vehicle (sesame oil) was administered i.p. on day E2.5. Detection of early implantation sites was performed on day E5.5 by injecting i.v. Evans blue dye. Results showed that administration of UPA produced a dramatic decrease in this parameter compared to control (p<0.001). To study uterine receptivity, we performed histological examination of the uterine horns on day E3.5. Uteri from UPA-treated females exhibited a dyssynchronous growth between glands and stroma as well as non-physiological combinations of inactivity, mitosis, secretory change, and apoptosis. Finally, artificial decidualization was induced in treated pseudopregnant females by intrauterine injection of sesame oil, and the decidual reaction was evaluated on day E7.5. Deciduoma of UPA-treated females was significantly smaller than control (p<0.05), suggestive of impaired decidualization. Altogether, these results suggest that post-ovulatory administration of UPA may exert a contraceptive action by impairing embryo-uterine interaction due to effects on endometrial morphology and functionality.

617. (370) **MOUSE PATERNAL ALCOHOL EXPOSURE AFFECTS SPERMATOZOA AND COMPROMISES THE SURVIVAL OF ITS OFFSPRING**

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Previously we observed that male alcohol consumption delayed embryo differentiation by deregulation of peri-implantation events and alteration of embryo morphogenesis in vitro. Aim: To evaluate the effect of paternal alcohol consumption on spermatozoa, embryo and litter survival on a mouse model. Methods: CF-1 male mice were exposed (treated group, T) or not (control group, C) to 15% (v/v) ethanol in drinking water ad libitum for 15 days. Spermatozoa from cauda were obtained by swim-out to determine sperm concentration, motility, head decondensation (15, 30 and 60 min) and apoptosis by Annexin V-FITC on both groups. Control and treated males were mated with non-treated CF-1 females (1:1). Pregnant females (positive vaginal plug: day1) were sacrificed at day 2 of gestation to obtain 2-cell embryos which were cultured in vitro for 7 days and apoptosis was determined. Pregnancy outcomes were also evaluated and litter mortality, weight and size registered. Results: Male alcohol consumption did not alter the number of mated female but significantly increased sperm head decondensation compared to controls (p<0.05). There were not differences in sperm concentration and motility. Besides, we observed a higher number of oocytes at day 2 of gestation from females mated with treated males than control ones (p<0.01). Apoptosis was detected in peri-implantation embryos at 7 days of culture in vitro from treated vs. control groups. However, no differences were observed when we evaluated apoptosis on spermatozoa. After 21 days of pregnancy we registered born mice from both groups and observed during the first week of age an increased number of deaths from treated group (p<0.001) compared to control group. We observed less activity and poor fur in those pups from treated group vs. control males. Conclusion: Short-term paternal alcohol consumption impairs sperm head decondensation altering the embryo survival and pups behavior at an early age.

618. (148) **MELATONIN TREATMENT PREVENTS THE PROGRESSION OF EXPERIMENTAL AUTOIMMUNE ORCHITIS IN RATS**

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Testicular inflammation is frequently associated to infertility. We developed an experimental model of autoimmune orchitis (EAO) in rats. In EAO interstitial lymphomonocytes increased from focal (50-60days, d) to severe EAO (70-80d), concomitantly with apoptosis of post-meiotic germ cells (GC) and pre-meiotic GC cycle arrest, leading to aspermatogenesis. Melatonin (MLT) is a hormone with anti-oxidant, anti-inflammatory and anti-apoptotic functions. The aim of this study was to evaluate whether MLT prevents the progression of testicular damage and inflammation of rats with focal EAO. Adult Wistar rats were immunized with testicular homogenate and adjuvants or not treated (Normal group, N). 60d after the first immunization, EAO rats were semicastrated and treated daily with MLT (10mg/kg i.p, MLT group) or vehicle (1% methanol in saline, S Group) for 20d. Testicular histopathological analysis was performed to determine the inflammation and orchitis score (EAO=V+T, where V is a factor from 0 to 9 depending on the % of sloughed seminiferous tubules (ST) and T a factor that considers the severity of ST damage). The incidence of orchitis at 60d in the S group was 100% (7/7) and in the MLT group 89% (8/9). N group did not develop orchitis (EAO score:0.0). EAO and inflammation score was only determined in rats that developed EAO at 60d. EAO score significantly increased in the 100% (7/7) of the S group rats (mean±ESM 60d:3.071±0.820, 80d: 5.786±1.367, p<0.05). EAO score was similar at 60 and 80d in the MLT group (mean±ESM 60d: 3.750±0.366, 80d: 3.750±1.065); in MLT group, EAO score was reduced in 63% (5/8), was similar in 12% (1/8) and was increased in 25% (2/8) of the animals. Inflammation score significantly decreased in MLTvsS group at 80d (mean±ESM S80d: 2.875±0.459, MLT80d: 1.333±0.408, N80d: 0.500±0.500, p<0.05). We demonstrate that melatonin treatment prevents the progression of orchitis and reduces the inflammatory microenvironment.

619. (202) **TROPHOBLASTIC CELL SURVIVAL REGULATION BY LEPTIN UNDER HYPOXIC CONDITION**

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Leptin is a pleiotropic hormone produced by the placenta where it plays important functions. We have previously demonstrated that leptin promotes proliferation and survival of trophoblastic cells. In this work we aimed to study the mechanisms that mediate the effect of leptin in placental apoptosis induced by cobalt chloride (CoCl2), a hypoxia mimicking agent that stabilizes HIF-1α transcription factor. For this study we use Swan-71 cells, a first trimester trophoblastic human cell line, cultured under standard conditions, as well as human term placenta explants. Swan-71 cells and placental explants

were treated with CoCl2 (50, 100 and 250 μM) to induce cellular stress with or without 100 ng/ml of recombinant leptin. The expression of HIF-1α, p53, Ki67 was determinate by Western blot or immunofluorescence (IF). All procedures were approved by ethical review committee at the Alejandro Posadas National Hospital. The role of leptin on proliferation and survival in Swan-71 cells treated with CoCl2 same concentration was also determined. The expression of the proliferation marker Ki67 was determined by IF and the viability was assessed by the MTT assay. We studied HIF-1α expression and we confirmed that the treatment with 100 μM of CoCl2 induced its expression in a time and dose dependent way (p<0,05) and leptin seems to reduced HIF-1α levels. On the other hand, leptin increases cell proliferation and HIF-1α stabilization blocked this effect. These findings suggest that leptin is capable to protect placental cells under hypoxia conditions.

620. (722) **A MATERNAL DIET ENRICHED IN SATURATED FAT PROGRAMS ALTERATIONS IN INTESTINAL PERMEABILITY RELATED TO METABOLIC PATHOLOGIES IN RAT FETUSES AND OFFSPRING**

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Maternal programming of metabolic alterations is a cause for the worldwide increase in obesity. The intestine plays an important role in metabolic homeostasis. It regulates the absorption of nutrients and precludes microbiota and small-inflammatory substances from entering the organism, thus preventing systemic infection and inflammation. Moreover, intestine integrity prevents low-grade inflammation to enter the porta system, inducing fatty liver disease.
Our aim was to evaluate potential alterations in the expression of Claudin-3, a protein involved in tight junctions and therefore in epithelial barrier integrity, in the intestine of fetuses and offspring, and to analyze intestinal permeability in the offspring from rats fed with a saturated fat-enriched diet.
Methods: Female Wistar rats were fed with standard or saturated fat diet (28% fat) since they were 6 week-old (SFD rats). After 8 weeks, they were mated with control males. Control and SFD rats were euthanized at 21 days of gestation or allowed to deliver for further evaluation of their offspring at 140 days of age. Fetal and offspring intestines were obtained. Protein levels and localization of Claudin-3 were assessed in intestines from fetuses by immunohistochemistry and by Western Blot in the offspring. Permeability was assessed by the ability of precluding 40KDa-FITC from crossing the intestinal lumen. Circulating transaminases levels as markers of liver function, were assessed by a commercial colorimetric assay.
Intestine claudin-3 levels were reduced in the fetuses (30% p<0.05) and offspring (25%, p<0.05) from SFD rats. An increase in intestinal permeability (30% p<0.01) and in plasma transaminases levels (20%, p<0.05) were found in the offspring from SFD rats.
Conclusions: Maternal saturated fat-enriched diet induced an increase in intestinal permeability probably due to a decrease in tight junction protein expression in fetuses and offspring that probably induces anomalies in liver function, thus prompting the offspring to the development of metabolic pathologies.

REPRODUCCIÓN / REPRODUCTION 5

621. (646) **ANDROGEN INSENSITIVITY AFFECTS TESTICULAR MACROPHAGE POPULATION AT EARLY PREPUBERTY**

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In rodents, testicular macrophages play an important role in regulating steroidogenesis of Leydig cells and maintain homeostasis within the testis, including the germ cell niche. Even though germ cells never express the androgen receptor, and Sertoli cells express it after the 4th year of life, patients with androgen insensitivity syndrome (AIS) start losing their germ cells as young as 2 years old (Aliberti P. et al, 2018).
Our objective was to describe the distribution of macrophages throughout normal testis development and compare it to the observed in AIS patients.
Twenty-three normal testicular samples were grouped in 3 developmental stages: Infancy (n=9, mean age 3.4 months, range 1.5mo-8mo), Childhood (n=7, 3.3 years, 1.3y-8y), and Puberty (n=7, 13.8 years, 9y-15.6y). Nine AIS samples were divided in 2 groups: Childhood (n=5, 6.4 years, 1.8y-10.3y) and Puberty (n=4, 19 years, 16.2y-23y). Expression of CD68, a macrophage marker by immunohistochemistry was performed and calculate the percentage ratio between them and interstitial cells.
In the normal testis samples, a lower percentage of macrophages was observed at Infancy (Mean±SD, 11.1%±4.3) than at Puberty (19%±4.8), without differences with Childhood (16.4%±7.7). The percentage of macrophages did not differ between both AIS groups, Childhood (4.1%±2.3) and Puberty (8.2%±5.5). A significantly lower percentage of macrophages in AIS than their normal counterparts was found. Macrophage histology also differed within both conditions, with spindled but mostly rounded macrophages present in the normal samples while in the AIS samples most were spindle shaped.
In addition to the histopathological changes observed in testicular patients with AIS, a decreased subpopulation of macrophages was found.
These results suggest that, in humans, androgen action is necessary for the normal development and distribution of testicular macrophages. Furthermore, testicular macrophages could have an important role in germ cell survival.

622. (673) **EFFECTS OF A HIGH FAT DIET ON MALE NEW ZEELAND RABBITS PROSTATE**

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The addition of fat to the normal diet of adult male rabbits leads to hypercholesterolemia (HC) with multiorganic/systemic consequences. The reproductive consequences are seminal and sperm disorders. HC was associated with decreased semen volume and morphological-functional sperm disorders. The first could be attributed to changes in the physiology of the sex glands. Interestingly, olive oil (OO) added to the fat diet improved the altered parameters. The aim of this study was to analyze the prostatic histology and quantify the content of prostatic acid phosphatase in the seminal plasma of male rabbits under different - protective or high fat - diets. The level of testosterone in the seminal plasma was also tested. New Zealand white rabbits were fed commercial rabbit pellets (normocholesterolemic rabbits: NCR), plus 14% bovine fat (HCR) or 7% bovine fat plus OO (7%) (½HCR + ½OO). In CRH, the height of the prostatic epithelium and the length of its microvilli decreased significantly (p<0.05) compared to NCR. In the ½HCR + ½OO group, only the length of the pro- prostate microvilli was recovered. Therefore, cholesterol intake mainly affects pro-prostate microvilli and prostate epithelium. Levels of prostatic acid phosphatase and testosterone showed a tendency to decrease in fat-consuming animals compared to controls. In conclusion, a high-fat diet affects prostate morphology and physiology, with consequences on semen. At the histological level, it was observed that the addition of olive oil to the diet prevents the damage associated with HC.

623. (721) **STUDY OF SPERMATOGONIA POPULATION IN INFERTILE PATIENTS WITH IMMUNE CELLS INFILTRATES**

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Azoospermia is linked to local testicular microenvironment deregulation, with inflammatory cells present in the 15% of testicular biopsies of infertile patients. The aim of this study was to study the kinetics be-

havior of spermatogonia (SP) population in infertile patients displaying testicular immune cell infiltrates. Biopsies obtained from infertile patients (Hospital. Clínicas, CABA) with obstructive and non-obstructive azoospermia were classified according to morphological criteria in: normal spermatogenesis (N, n=7), hypoespermatogenesis (Hypo, n=5) and severe hypospermatogenesis (SHypo, n=5). The number of immune cells was quantified by immunohistochemistry employing the leukocyte common antigen marker (CD45) and the number of SP (A dark, A pale and B) by morphological criteria in the same slides. The number of CD45 cells increased significantly in Hypo and SHypo biopsies vs N (Mean±SEM N:22.150±2.510, Hypo:69.090±9.398, SHypo:95.180±12.54, p<0.001). The total number of SP decreased significantly in SHypo vs N (Mean±SEM N:27.820±0.972; Hypo:23.800±1.420; SHypo:9.339±0.699, p<0.001). The number of SP A dark decrease significantly in Hypo and also in SHypo compare to N (Mean±SEM N:4.716±0.2759; Hypo:2.994±0.2599; SHypo:1.016±0.1732, p<0.001); the number of SP A pale and B SP decreased only in SHypo (Mean±SEM, SP Apale, N:17.220±0.811; Hypo:15.950±1.088; SHypo:5.746±0.457; SPB, N:1.630±0.181; Hypo:1.055±1.156; SHypo:0.164±0.051). The hormonal profile showed that serum LH, testosterone did not change, while FSH was increased in SHypo vs N (Mean±SEM N:6.614±1.889; Hypo:19.200±6.003; SHypo:25.200±7.185, p<0.05). We demonstrated that inflammation is associated to spermatogenesis impairment and alter SP kinetic behavior. SP A dark, a quiescent population, consider as "the true stem cells" of the testis is reduced in both Hypo and SHypo, suggesting that is a population very sensitive to the inflammatory microenvironment.

624. (107) **ASSESSMENT OF THE ROLE OF L-GLUTAMINE IN THE REGULATION OF SERTOLI CELL PROLIFERATION**

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The final number of Sertoli cells (SC) reached during the proliferative periods determines sperm production capacity in adulthood. On the other hand, it is well known that nutrient availability plays a pivotal role in the decision of a cell to commit to cell proliferation. Crucial to the decision process is the mammalian target of rapamycin (mTOR), whose activity is controlled by, besides hormones such as FSH, the presence of amino acids (aa). L-glutamine (gln), the most abundant aa in the blood, plays an important role in proliferation in many cell types. However, the role of gln in the regulation of SC proliferation has not been studied yet. The aim of this work was to investigate whether SC depends on the presence of gln to proliferate. SC obtained from 8-day old rats were maintained in the absence or presence of gln (2.5 mM) and stimulated with FSH 100 ng/ml. BrdU incorporation, cyclin D2 (CCND2) expression by RT-qPCR and phosphorylated mTOR (P-mTOR) levels by western blot were evaluated. Results are expressed as mean±SD of three independent experiments (different letters indicate statistically significant differences, p<0.05). It was observed that gln increased BrdU incorporation (0 mM gln: 1.4±0.3^{a}; 2.5 mM gln: 4.1±0.6^{b}; 0 mM gln + FSH: 4.0±0.6^{b}; 2.5 mM gln + FSH: 13.0±2.6^{c} % BrdU-positive cells) and also enhanced FSH stimulus on CCND2 expression. In addition, gln increased P-mTOR levels (2.5 mM gln: 2.7±0.3^{b}; 0 mM gln + FSH: 3.6±0.4^{c}; 2.5 mM gln + FSH: 4.7±0.5^{d} fold variation P-mTOR/T-mTOR vs. 0 mM gln). These results suggest that gln might be necessary for the regulation of Sertoli proliferation possibly through the modulation of mTOR pathway. PICT2014-0945.

625. (222) **REGULATORY ACTIONS OF MELATONIN IN RODENT SERTOLI CELLS**

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Melatonin acting through the hypothalamus and pituitary regulates testicular function. In addition, direct actions of melatonin at testicular levels have also been detected. Recently, we described a protective role of melatonin against free radical damage in testicular mast cells. The aim of this study was to investigate the participation of melatonin in the regulation of the oxidative state and lactate dehydrogenase (*Ldh*) expression in Sertoli cells. For this purpose, we used Sertoli cells isolated from 21 days old Syrian hamsters and the murine TM4 Sertoli cell line, both expressing the melatonergic receptor subtype 1.

After a 2 h incubation in the presence of the oxidizing agent tert-butyl hydroperoxide (TBHP; 500μM), reactive oxygen species (ROS) production from TM4 cells was significantly increased. This action was prevented by 1µM melatonin (flurometric assay, arbitrary units, a.u.: control: 6761 ± 501^{a}; TBHP: 13084 ± 686^{b}; melatonin + TBHP: 9085 ± 326^{c}; X ± SEM; P<0.05).

Melatonin (1μM) also inhibited H_2O_2 generation in TM4 Sertoli cells (fluorometric assay, a.u.: control: 4032 ± 130.13^{a}; melatonin: 2965 ± 39.61^{b}; X ± SEM; P<0.05).

Moreover, after a 5 h incubation, melatonin (1μM) significantly induced protein expression of the antioxidant enzymes catalase and peroxiredoxin 1 in TM4 Sertoli cells (determined by Western blot; P<0.05).

In immature hamster Sertoli cells, while melatonin seems to decrease lipid peroxidation (TBARS assay), it significantly increases catalase and peroxiredoxin 1 protein expression (P<0.05).

One of the most important Sertoli cell functions is to provide energy substrates (i.e. lactate) to germ cells. *Ldh a* gene expression was significantly increased in the presence of melatonin in both TM4 Sertoli cells and immature hamster Sertoli cells (quantified by qPCR; P<0.05).

Our data, although preliminary, reveal that melatonin exerts an antioxidant effect in Sertoli cells. In addition, this indolamine positively influences *Ldh* expression thus promoting germ cell development.

626. (650) **OLIVE OIL IMPROVES THE ALTERATIONS OF THE TESTICULAR CHOLESTEROL METABOLISM REGULATED BY SREBP-2 IN HYPERCHOLESTEROLEMIC RABBITS**

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The relationship between hypercholesterolemia and reduced male fertility has been reported previously. Hypercholesterolemic rabbits (HCR) were associated with deleterious changes in semen and sperm cells: semen volume decreased and the sperm membrane became overloaded with cholesterol. Olive Oil (OO) supplementation (7% v/p) improved semen parameters affected by high fat diet. The increase in membrane cholesterol may be due to changes in the intracellular metabolism of this lipid. New Zealand White rabbits, 4 rabbits from each group, were fed commercial rabbit pellet (normocholesterolemic rabbits: NCR), plus 14% bovine grease (HCR) or 7% bovine grease plus OO (7%) (½HCR + ½OO). Within molecular regulation, SREBP (Sterol- Regulatory-Binding-Protein) is an essential protein for cholesterol homeostasis and membrane biogenesis. At 3 months of diet, SREBP-2 mRNA expression showed no significant changes between NCR and HCR assessed by RT-PCR. In contrast, protein expression, detected by western blot showed a significant increase in HCR testis. The target molecules of SREBP2: HMGCoAR (3-hydroxy-3-methyl-glutaryl-coenzyme A reductase) R-LDL (low-density lipoprotein receptor) and ABCA-1 (ATP-binding cassette, sub-family A member 1) followed the same pattern. Interestingly, ½HCR + ½OO showed a recovery in the expression of the mentioned proteins. In addition, the SREBP2 protein was sub-cellular detected in the testis by indirect immunofluorescence. Taken together, the changes in expression and location of the SREBP and downstream proteins in testis of HCR animals could be related to high fat diet and seminal consequences. Instead, OO was able to improve the altered parameters in dietary acquired hypercholesterolemic rabbits.

627. (474) **EFFECT OF BIOACTIVE NATURAL COMPOUNDS**

ON OXIDATIVE STATUS AND ESTROGENIC ACTIVITY IN AN IN VITRO EXPERIMENTAL MODEL OF ENDOMETRIOSIS

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Endometriosis is a benign gynecological disease with no effective long-term treatment due to adverse side effects. This is one of the reasons why research has focused on natural compounds, which some beneficial effects have already been demonstrated on cancer as well as on endometriosis. Carnosic acid (CA) and rosmarinic acid (RA) are the most abundant bioactive compounds found in the leaves' extract of rosemary. Wogonin (WG) is a flavonoid isolated from the root of Scutellaria baicalensis, one of the fundamental herbs used in traditional Chinese medicine. In previous studies we demonstrated that these compounds inhibit endometrial stromal cell proliferation. The aim of the present study was to evaluate their effect on reactive oxygen species (ROS) generation, on estrogen receptor (ER) expression and on estradiol (E2) secretion in a human endometrial stromal cell line (t-HESC). For ROS detection by fluorometry, t-HESC were first stimulated with different concentrations of CA, RA or WG during 24hs. For Western blot of ERα and ELISA of E2, stimulation of the cells was done for 48hs. A strong antioxidant effect was demonstrated after 50μg/ml of RA, while 80μM of WG caused a pro-oxidant effect on the cells. The only compound that provoked a change on ERα expression was WG, causing its reduction after 48hs of 40μM and 80μM stimulation; nevertheless, WG had no effect on E2 secretion to the conditioned media. Even though apparently contradictory, RA's antioxidant and WG's pro-oxidant effect can both be beneficial in endometriosis treatment. Moreover, WG's pro-oxidant effect and decreased ERα expression could be in part responsible for the inhibition of endometrial stromal cell proliferation previously reported. The present results are only the latest obtained in a long journey we have taken evaluating these compounds in endometriosis and are encouraging to continue exploring this path.

628. (238) **METABOLIC SYNDROME, MELATONIN AND AGOMELATINE IMPROVE METABOLIC FACTORS AND FOLLICLE DEVELOPMENT IN THE FEMALE ADULT RAT .**

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Metabolic syndrome (MS), is characterized by hypertension, hyperglycemia, overweight and ovarian failure. Melatonin (MT) produced by the pineal gland, has important effects on metabolism and on ovarian function. Agomelatine, (AGOM), a melatonin agonist, may improve some metabolic parameters altered in MS. The purpose of the present work is to evaluate biochemistry and ovarian changes produced by fructose administration to female adult rats and the possible benefit effects of melatonin and agomelatine on them. Wistar rats received the following treatment during sixty days: FRUC, fructose 10% p/v; MT, 25ug/ml ; AGOM, 50ug/ml. Groups studied: (n= 8): CONTROL, MT, AGOM, FRUC, FRUC-MT, FRUC-AGOM. Blood pressure, was measured and glucose tolerance curve (TC-Gip) was performed (2g glucose/kg). Truncal blood was collected to determine: triglycerides (TG), cholesterol (CHOL), c-HDL. The day of sacrifice, ovaries were dissected and histological studies were performed. Blood pressure was increased by FRUC (148±2mm Hg) vs CONTROL (118±1mmHg, p<0,001) and was decreased by FRUC-MT (132±2, p=0.001) y FRUC-AGOM (128±3, p<0.001). TC-Gip, showed a trend to decline of glucose at 30 minutes in FRUC group (319±26mg/dL) and a significantly decrease in AGOM group (259±26) vs CONTROL (365±37). TG were increased by FRUC (127±24mg/dL, p=0,037 vs CONTROL 85±13) and MT reversed this effect (FRUC-MT, 66±7, p=0,004). FRUC , p<0.001), MT and AGOM produced a global increase of CHOL. Meanwhile, FRUC, MT and AGOM increased c-HDL in comparison with control, FRUC (45±2, p=0,004), MT (44±1), AGOM (43±2), CONTROL (37±1mg/dl). Follicle development, was analyzed: FRU, decreased primary follicle number (4.5±0.25 vs control 3.0±0.41, p<0.01) and AGOM improve them (3.5±0.87, p<0.01). Secondary and antral ones were decreased by fructose (1.75±0.25 vs 3.5±0.9, p<0.001), 1±0.41 vs 4±0.58, p<0.001), no changes were observed by the other treatments. Fructose alter many biochemical parameters and follicular development in female rats. Some of them were improved by MEL and AGOM.

TRANSDUCCIÓN DE SEÑALES Y MECANISMOS MOLECULARES DE ENFERMEDAD / SIGNAL TRANSDUCTION 3

629. (732) **SIGNALING PATHWAYS INVOLVED IN THE REGULATION OF THE GPRC5A GENE IN T84 CELLS**

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The G protein-coupled receptor 5A (GPRC5A), also known as Retinoic acid-induced gene 3 (RAI3) or Retinoic acid-induced gene 1 (RAIG1). GPRC5A was first cloned in our laboratory with the name PEG-1 (phorbol ester induced gene-1), because it was found to be a 12-O-tetradecanoyl phorbol 13-acetate (TPA)-inducible gene. TPA, also called phorbol 12-myristate 13-acetate (PMA), is a small molecule drug that activates the signal transduction enzyme protein kinase C (PKC) by directly binding to its C1 domains. Commonly, TPA is employed as a tumor-promoting agent. GPRC5A dysregulation is associated to diverse types of cancer in humans and it was originally reported as a tumor suppressor in non-small cell lung carcinoma and in oral squamous cell carcinoma. In contrast, it has also been reported that GPRC5A could act as an oncogene in breast cancer, colorectal cancer and pancreatic cancer. This dual behavior makes GPRC5A an interesting gene to study. However, little is known about the function of this protein and its regulation. The aim of this work was studying the regulation of GPRC5A in human colon cancer. To determine the signaling pathways involved in this regulation, T84 cells (human colon adenocarcinoma cells) were stimulated with TPA for 4 h, in presence or absence of different pathway inhibitors, and the gene expression was analyzed by real-time PCR. PKC, PKA, MEK inhibitors and BAPTA treatment (intracellular calcium chelator) decreased significantly the GPRC5A mRNA expression. On the contrary, the SGK1, NF-κB, JNK and P38 inhibitors increased significantly GPRC5A mRNA levels. Interestingly, the AKT inhibitor in absence of TPA stimulation increased significantly the GPRC5A expression but did not show effect in presence of TPA. In conclusion, GPRC5A is regulated by multiple common pathways. Supported by PIP 2015-2017, PUE 22920160100129CO, and PICT-2015-1031 to AGV.

630. (776) **MICRORNA 148A ROLE IN HUMAN EMBRYONIC STEM CELLS AND PLURIPOTENCY**

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Fundacion para la Lucha contra Enfermedades Neurológicas de la Infancia. (FLENI-CONICET)

In mammalian organisms, miRNA 148a is expressed in germ cells, before and after implantation of the blastocyst. Although its role remains unknown during embryogenesis, others and we have detected miR-148a in global microRNA sequencing experiments in pluripotent stem cells.
The aim of this work is to study in human embryonic stem cells (hESC) the function of miR-148a, by downregulating its expression. As we have seen that miR-148a CRISPR knockout seems lethal to hESC, we have prepared an inducible CRISPR interference to measure pluripotency maintenance in these cells. We have designed RNA guides (sgRNA) targeting selected transcription start sites (TSS) found in hESC, in the miR-148a promoter region. To find the two putatives TSS, we have processed mirSTP software

results over nascent microRNA sequencing published on-line in two different hESC.
The inactivated (d)cas9 fused to the transcription inhibitory protein KRAB, under the control of doxycyclin, can specifically and conditionally inhibit transcription with adequate sgRNA. We are now preparing lentiviruses to express those sgRNA in H3-KRAB in-house hESC line, with two different plasmids. These lentiviruses selected by puromycin and hygromycin will allow to target both TSS in the same time. We will then monitor miR148a and pluripotent markers expression by quantitative PCR and immunofluorescence.
We will use a four day differentiation scheme (FBS 20%) to focus on the early shift from pluripotency to multilineage differentiation. We will then follow the entry into G1 phase of the cell cycle by flow cytometry with iodure of propidium staining.
We have confirmed that miR-148a is expressed in pluripotent stem cells. To analyze pluripotency we are now preparing cellular clones where miR-148a can be induced to decreased. The next step will be to use an early differentiation protocol with these hESC. The decrease of miR148a should strengthen both the entry into G1 phase and concomitant differentiation.

631. (787) **REGULATION OF ACYL-COA SYNTHETASE 4 (ACSL4) EXPRESSION BY TRANSCRIPTIONAL AND POST TRANSCRIPTIONAL MECHANISMS IN BREAST CANCER CELLS**

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Acyl-CoA synthetase 4 (ACSL4) is an enzyme that catalyzes acyl-CoA synthesis from long chain fatty acid, being arachidonic acid its preferred substrate. ACSL4 levels correlate with aggressive phenotype in breast cancer. Its expression promotes tumor aggressiveness by increasing migration, proliferation and invasion. The aim of this work is to describe transcriptional and post traductional mechanisms that could regulate ACSL4 expression in breast cancer cell lines. We demonstrated by ChIP and by the use of a specific inhibitor that Estrogen-related receptor alpha is involved in ACSL4 transcriptional regulation. We studied the participation of proteosomal degradation. ACSL4 protein stability was tested on MCF-7 breast cancer cells with cycloheximide (CHX) treatment. Immunoblot showed a time-dependent decrease in ACSL4 levels after CHX treatment, significant at 2h of CHX (***p<0.0001). The results show that ACSL4 is less stable in MCF-7 than in MDA-MB-231 (studied in previous works); in agreement with higher ACSL4 expression levels in MDA-MB-231 than in MCF-7 cells. Treatment with MG-132 (a potent proteasome inhibitor) promoted a clear increase of ACSL4 levels in MCF-7. We identified ubiquitination sites in ACSL4 protein sequence by bioinformatic analysis and performed site directed mutagenesis on Lysine 702. We transfected MCF-7 cells with pCMV6-FLAG ACSL4-LYS-lys702 or the control vector containing wild type ACSL4. We observed that mutated ACSL4 levels are increased respect to wild type protein (*** p< 0,001). To analyse a possible mechanism in ACSL4 ubiquitination, we tested Parkin, that is an E3 ubiquitin ligase involved in cellular aggressiveness in breast tumors. Parkin displays particularly lower expression in MDA-MB-231 than in MCF-7 cells. We performed co-immunoprecipitation experiments in HEK293 cells and observed that Parkin interacts specifically with ACSL4. These results support the role of proteosome degradation in the regulation of ACSL4 levels and suggest that Parkin could be involved in ACSL4 degradation in breast cancer cells.

632. (798) **RACK1 SILENCING REDUCES PROLIFERATION AND SENSITIZES MELANOMA CELLS TO PI3K AND BRAF INHIBITORS**

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Receptor for activated C kinase (RACK1) is a scaffold protein that interacts with many protein kinases and membrane-bound receptors playing an important role in a wide range of biological processes. Many studies have shown that RACK1 plays opposed roles in different types of cancer, where it has either pro- or anti-oncogenic effects. The function of RACK1 in melanoma has not been investigated in depth.
We have observed that RACK1 overexpression increases Akt phosphorylation (at T308 and S473) and Cyclin D1 levels and significantly increases proliferation of SK-Mel28 and A375 melanoma cell lines. However, since overexpression of scaffold proteins might induce non physiological alterations we decided to study the role of RACK1 in melanoma by knocking-down RACK1 expression using RNAi. Transduction of both A375 and SK-Mel28 cell lines with lentiviral particles encoding two RACK1-specific short hairpin RNAs efficiently silences RACK1 expression. In agreement with our overexpression data, RACK1 silencing significantly decreased A375 and SK-Mel28 proliferation after 96h by 36.6 and 34.83% respectively (p<0.0001) compare to control cells. We found that RACK1 silencing markedly decreased the phosphorylation of AKT (at T308 and S473), ERK1/2 and PKCα. Of note, these three protein kinases were activated by RACK1 overexpression. Then we studied the role of RACK1 in cell viability in A375 cells. These cells are mutant for BRAF but are partially resistant to PLX-4032 at low micromolar concentrations. In line with our previous results, RACK1 silencing reduced the viability of melanoma cells upon treatment with PI3K and BRAF inhibitors LY294002 and PLX-4032, producing a leftward shift of the IC50 from 14.05 to 7.01μM and from 2.31 to 0.41μM respectively in comparison with control cells. These results reveal that RACK1 is required for MAPK/ERK and PI3K/Akt activity and contributes to cell resistance mediated by both pathways.

633. (800) **FUNCTIONAL CROSS-TALK BETWEEN THE GLUCOCORTICOID AND PROGESTERONE RECEPTORS IN MAMMARY EPITHELIAL CELLS**

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IFIBYNE - UBA - CONICET

Glucocorticoid (GR) and progesterone (PR) receptors are members of the steroid receptor family. Active PR is associated with cell proliferation and the progression of mammary tumors while GR promotes cell differentiation. Therefore, their relative abundance can modulate the proliferative response of the mammary epithelium. In view of these precedents, the objective of this work was to test the capacity of both receptors to be part of the same complex and study the differences in recruitment of chromatin remodelers to specific response regions where both receptors share binding sites. To assess PR and GR nuclear dynamics in vivo T47D cells were transfected with expression vectors encoding both receptors fused to fluorescent proteins (eGFPPR and mCherryGR) and incubated with their R5020 and/or Dex ligands, respectively. Then, fluorescence correlation spectroscopy (FCS) was used, which allows obtaining quantitative parameters related to the mobility of fluorescent molecules and their interaction with fixed targets in living cells. Results showed that when both receptors are activated, they move in the nucleus, simultaneously. Both, the GR ligand binding domain and the DNA binding domain are involved in the interaction with the PR. Upon activation with their respective ligands, both receptors are also recruited to a large fraction of specific binding regions. This result led us to investigate which chromatin remodelers are recruited in these regions and what differences exist between them when both receptors are treated simultaneously with their specific hormones. ChIPs assays were performed for p300, BRG1 and Foxa1 in T47D A1-2 cells expressing GR and PR and in T47D cells expressing PR only. We analyzed specific regions located in GREB1, STAT5A, SNAI1 and ELF5 genes. Taken together, these results suggest that PR and GR could be part of the same protein complex and play an important role in the cellular response of the mammary epithelium.

634. (97) **AKAP350 RECRUITS CIP4 TO THE LYTIC IMMUNE SYNAPSE IN NATURAL KILLER CELLS, CONDITIONING ACTIN REORGANIZATION**

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Lytic Immune synapse (LIS) is defined as the contact of the natural killer (NK) or cytotoxic T cells with its target cell and the subsequent directional secretion of lytic granules necessaries for target cell death. The development of the IS in NK cells involves, after an initial interaction with the target cell, actin remodeling and NK integrin LFA-1 clustering at the interaction site. CIP4 is a regulator of actin polymerization, which is recruited to the MTOC at the LIS in NK cells, having a crucial role in the lytic response. AKAP350 is an A-kinase anchoring protein involved in the regulation of microtubule dynamics, which recruits CIP4 to the MTOC in migratory cells. Our previous results indicate that AKAP350 expression conditions LFA clustering and overall lytic response in NK cells. The aim of our work was to evaluate AKAP350 participation in the recruitment of CIP4 to the MTOC and the LIS and actin reorganization in NK cells. YTS NK cells with decreased expression of AKAP350 (AKAP350KD) were exposed to erythroleukemia derived KT-86 cells (2:1 ratio) for 30 minutes and CIP4 and actin distribution was analyzed by immunofluorescence confocal microscopy. AKAP350 knock down lead to decreased CIP4 localization at the MTOC (-40%*) and at the LIS (-35%*). Concomitantly, actin accumulation at the LIS was impaired in AKAP350KD cells (-66%*). Western blot analyzis of MTOC enriched NK fractions also showed decreased CIP4 localization in AKAP350KD cells (-43%). Our results suggest that, in NK cells, AKAP350 is involved in the reorganization of the actin cytoskeleton at the LIS by regulating CIP4 localization at the MTOC and its polarization during the LIS development. *p<0.05

635. (163) **AMINO ACIDS DEPRIVATION INCREASES NADPH OXIDASE 4 EXPRESSION IN MOUSE HEPATOCYTES IN CULTURE**

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Protein malnutrition occurs when there is insufficient protein to meet metabolic demands. Although it is well known that recurrent protein deprivation settles metabolic and ultrastructure changes distinctive of a preneoplastic profile, it has not been completely understood how it can influence the early liver transformation stages. The aim of this work was to deep into the molecular mechanisms that support preneoplastic alterations in newborn mouse hepatocytes that survive under amino acids (Aa) deprivation in culture (Sel line), focusing on TGF-β- and EGF-mediated cell signaling, and NADPH oxidase (NOX) 4 in the cross-roads of these pathways. We found that the Sel line showed higher levels of phospho-AKT and phospho-ERKs ($p < 0.001$) in relation to the control (Par line), which correlated with diminished caspase 3 ($p < 0.01$) activity and overactivation of the EGFR pathway ($p < 0.05$). Lack of Aa induced upregulation of NOX4 ($p < 0.05$) which was coincident with increased expression of phospho-SMAD-2 ($p < 0.05$) via TGF-β canonical pathway, and strikingly with a lower production of ROS ($p < 0.05$). However, cells that survived showed a reduced glutathione content ($p < 0.001$) and higher Catalase ($p < 0.05$) and γ-GSC ($p < 0.01$) protein levels, interpreted as an adaptation of cells to counteract oxidative stress. EGFR and TGF-β pathways were implicated in the NOX4 increment seen in Sel line. Inhibition of TGF-β receptor diminished NOX4 protein ($p < 0.05$) and strikingly, after EGF receptor inhibition, NOX4 protein levels also decreased ($p < 0.01$). Therefore, both TGF-β and EGF pathways are shown to be involved in the upregulation of NOX4 in the Sel line. This work provides novel results regarding to the regulation of NOX4 in the preneoplastic transformation of hepatocytes in the absence of Aa, and in the context of TGF-β and EGFR pathways.

636. (390) **RESPONSE TO EXTRACELLULAR ATP IS DEPENDENT ON THE EXPRESSION OF AQP2 IN RENAL CELLS**

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Extracellular ATP is a physiological signal that can activate P2 receptors and trigger intracellular Ca^{2+} signals to influence cellular behavior, including migration. We have previously described in renal cells that intracellular Ca^{2+} signals can be modulated by the interaction between AQP2 and the Ca^{2+} channel TRPV4. Previous works from our laboratory have also shown that AQP2 expression accelerates cell motility and proliferation. The objective of the present work was to study the influence of AQP2-TRPV4 interaction in Ca^{2+} signals elicited by extracellular ATP and its consequences in cell migration. We used two rat cortical collecting duct cell lines expressing AQP2 (AQP2-RCCD$_1$) or not (WT-RCCD$_1$). We studied Ca^{2+} signals using FURA-2 in response to 20 μM of extracellular ATP. We found that Ca^{2+} signals in WT-RCCD$_1$ cells are greater than in AQP2-RCCD$_1$ cells (Area Under the Curve; AUC_{WT}: 401 ± 17 vs. AUC_{AQP2}: 272 ± 10; n=318 cells of 7 independent experiments. $p<0.001$). This response depends on both intra- and extracellular Ca^{2+} and can be abolished by general P2 inhibitors (suramin 100 μM + PPADS [pyridoxalphosphate-6-azophenyl-2›,4›-disulfonic acid] 100 μM). HC-067047 (1 μM), a specific TRPV4 inhibitor, only decreases the response of WT-RCCD$_1$ cells. We also studied cell migration in the presence of extracellular ATP using the wound healing assay. We found that ATP stimuli augments migration only in cells that do not express AQP2 (% of wound closure area; WT_{CTRL}: 34.9 ± 1.2 % vs. WT_{ATP}: 47.6 ± 3.8 %; 3 independent experiments. $p<0.05$). Therefore, we could suggest that the increased motility of WT-RCCD$_1$ cells elicited by ATP might be related to an enhanced TRPV4 signaling. These results, together with our previous works let us conclude that AQP2 can function as a positive or negative TRPV4 modulator depending on the cellular context with significant physiological consequences.

637. (666) **FUNCTIONAL CHARACTERIZATION OF A POLYCYSTIN-2-CALCIUM SENSING RECEPTOR COMPLEX PRESENT IN LLC-PK1 RENAL EPITHELIAL CELLS**

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Polycystin-2 (PC2, TRPP2) is a Ca^{2+}-permeable nonselective cation channel encoded by the PKD2 gene whose mutations cause autosomal dominant polycystic kidney disease (ADPKD). Recent studies from our laboratory (Dai et al, Exp Cell Res, 2017), determined that the expression of plasma membrane PC2 in LLC-PK1 renal epithelial cells is regulated by changes in external Ca^{2+} concentration through the Calcium-Sensing Receptor (CaSR). The present study explored the existence of a structural coupling between PC2 and CaSR in LLC-PK1 cells. The presence of a PC2-CaSR complex was explored as follows. Cell lysates with either anti-CaSR (CaSR elution) or PC2 antibody (PC2 elution), were added to Pierce Protein A/G coupled to magnetic beads (Thermo Scientific) for elution of the complexes. CaSR elution co-immunoprecipitated with PC2 as indicated by dot-blot labeling with anti–PC2 antibody. Conversely, PC2 elution co-immunoprecipitated CaSR. In the presence of high Ca^{2+} (6 mM), CaSR-PC2 colabeling was at least 60% lower for either elution sample. A BLM reconstitution system was used to test for the presence of ion channel activity in the Co-IP materials. Reconstitution was carried out in a KCl gradient (150:15 mM). Both Co-IP materials showed spontaneous cation-selective ion channel activity whose I/V relationship rendered a single channel conductance of 128 ± 11 pS (n = 3) and 104 ± 28 pS (n = 3) for the CaSR and PC2 materials, respectively. The data indicate that the Co-IP technique using either anti-PC2 or anti-CaSR antibodies, results in the elution of a functional and structural PC2-CaSR complex. Further studies will be required to assess the interaction between the CaSR and PC2, including physical interactions with cytoskeletal connections.

638. (667) **EFFECT OF THE CAMP PATHWAY ON THE LENGTH**

OF THE PRIMARY CILIUM OF LLC-PK1 RENAL EPITHELIAL CELLS

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The primary cilium is a sensory organelle whose dysfunction leads to the onset of cystic renal disease. The primary cilium may contribute to the regulation of Ca^{2+} transport in renal epithelial cells. Little is known, however, as to how renal epithelial cells control primary cilium length and in particular how Polycystin-2 (PC2) function in the primary cilium may contribute to this regulation. In this study, we explored the effect of the cAMP pathway on ciliary length in LLC-PK1 renal epithelial cells. The length of the primary cilium was obtained by labeling with a specific antibody against α-acetylated tubulin and tracing the immunochemical signal with the ImageJ software. Under Control conditions (1,2 mM Ca^{2+}) the ciliary length was 4,72 ± 0,05 μm (n = 510). Exposure of cells to 8-Br-cAMP (1 mM) in the presence of normal Ca^{2+} increased primary cilium length by 16.31 ± 1,46% (n = 157, $p < 0.001$). Similar results were obtained after exposure of cells to arginine-vasopressin (AVP, 10 μM). In high calcium (6,2 mM), however, where ciliary length is 13,5 ± 1,47% shorter than Control, exposure of cells to 8-Br-cAMP induced an increase in the length of the primary cilium of 13,77 ± 2,23% (n = 136, $p < 0.001$). The data indicate that maneuvers that lead to activation of the cAMP pathway feedback Ca^{2+} signals and control ciliary length in LLC-PK1 cells. Dysregulation of this mechanism may be essential in the onset of autosomal dominant polycystic kidney disease.

639. (748) **THE BEHAVIOR OF WATER PORE IN HUMAN AQUAPORIN-1 IS INFLUENCED BY DIFFERENT FACTORS AT THE CELL MEMBRANE LEVEL**

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Studies of the structural changes in proteins and how the environment influences on proteins during cell life are becoming more relevant to understand their dynamics and their regulation. The aquaporins are no exception. Working with human Aquaporin-1 (hAQP1) our group has studied the behavior of the protein at different levels by computational methods (mathematical modeling, molecular dynamic simulations (MDS), and protein-protein docking) and experimental methods (video-microscopy, internal pressure measurements and voltage- clamp). On in-vitro experiments using protein expression in X.laevis oocytes, we found that different challenges can modified water transport across hAQP1. We have reported that a decrease on water permeability (Pf) occurs when increasing the membrane tension due to cell swelling and that this behavior is reversible and occurs in a cooperative manner among monomers. Now we studied the dynamic behavior of the hAQP1 water pore by MDS experiments. We examine different molecular descriptors (RMSD, SASA, etc.) in the spatial, temporal and in the frequency domain. 214 variables describing water pore of each of 9 monomers of 3 systems (M, FT and RT) under different conditions of "lateral restriction" were used to perform PCA and PARAFAC, both multivariate statistical procedures. The results show that restrictions in α-Carbons that face to the lipid bilayer, affect subtly but significantly the behavior of non-restricted sites inside the water pore. On the other hand, our in-vitro experiments show that co-expression of AQP1 plus ENaC makes the cell membrane more elastic than AQP1 expression alone ($p<0,0001$ in ε values) and therefore the osmotic water flux and Pf increase ($p < 0.05$). These effects are more pronounced in the presence of aldosterone and disappear in the presence of amiloride, an inhibitor of ENaC. In computational experiments, protein–protein docking tested shows a hypothome, with a possible interaction at level of transmembrane domain of both proteins.

HEMATOLOGÍA / HEMATOLOGY

640. (51) **STEROIDOGENIC PATHWAY IN PERIPHERAL BLOOD MONONUCLEAR CELLS (PBMC) OF HEALTHY INDIVIDUALS**

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There is growing evidence that cells of the immune system can synthetize and secrete products not associated with their traditional functions, such as hormones and neuropeptides. In a previous work, we have demonstrated the presence of several enzymes associated with sex steroid synthesis in PBMCs from healthy individuals. Our goal now was to complete the study of the steroidogenic pathway in these cells. We separated mononuclear cells from males (n=36) and females (n=55) of ages 25 – 90 using a Ficoll gradient. RNA was obtained with Trizol reagent, and reverse transcribed to obtain cDNA. We designed primers for aromatase (Aro), 5α-reductase (5aR) 1 and 3, 3β hydroxysteroid dehydrogenase (3bHSD), 3α hydroxysteroid dehydrogenase (3aHSD) 1 and 3, P450 side-chain-cleavage (P450scc), cytochrome P450c17, and 17β hydroxysteroid dehydrogenase (17bHSD) 1, 3 and 5. Real-Time PCR was performed using L19 as a housekeeping gene. Statistical analysis were carried out using R software. We found expression of all of the enzymes studied in PBMCs, except for Aro and 3αHSD1. In particular, there was a significantly higher expression of 5aR1 in men older than 50 years versus younger than 50, and when we compared both sexes, there were higher levels of this enzyme expression in males older than 50 versus females of the same age (ANOVA p=0,043). Regarding 5aR3, we found a higher expression in men older than 50 years when comparing them to women older than 50 years (p=0,035). These results show that PBMCs have the required machinery to produce sex steroids, and that in some cases the expression varies regarding sex and age. The transcription of enzymes involved in androgen production appears to be more active in male PBMCs.

641. (52) **EXPRESSION OF STEROIDOGENIC ENZYMES IN PERIPHERAL BLOOD MONONUCLEAR CELLS (PBMC) OF PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA.**

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Chronic lymphocytic leukemia (CLL) is a malignant neoplasm that occurs in elderly individuals. It is characterized by proliferation of immunologically immature lymphocytes, which accumulate in blood, bone marrow and lymphatic tissues. Given that this disease displays a gender difference towards the male sex, several authors have studied the possible underlying mechanisms in which sex hormones mediate their effects in lymphocytes. The presence of androgen and estrogen receptors is well established both in healthy individuals and patients with CLL, yet little is known about endogenous synthesis of sex steroids. Previously we have demonstrated the presence of several enzymes required for steroid synthesis in PBMC of healthy individuals. Our goal was to analyze the expression of those enzymes in PBMC from patients with CLL (n=16), and to compare them with that of normal subjects (n=17). We separated PBMC from males age 45 to 90 using Ficoll gradient. RNA was obtained with Trizol reagent, and reverse transcribed to obtain cDNA. We designed primers for aromatase (Aro), 5α-reductase (5aR) 1 and 3, 3β hydroxysteroid dehydrogenase (3bHSD), 3α hydroxysteroid dehydrogenase (3aHSD) 1 and 3, P450 side-chain-cleavage (P450scc), cytochrome P450c17, and 17 hydroxysteroid dehydrogenase (17bHSD) 1, 3 and 5. Real-Time PCR was performed using L19 as a housekeeping gene. Statistical analysis were carried out using R software. We found no expression of Aro and 3αHSD1 in patients

with CLL, as it occurred in controls. Regarding P450scc, P450c17, 5αR2 and 17βHSD3, the expression showed no difference with healthy subjects. On the other hand, we found decreased expression of 5αR1, 5αR3, 3βHSD, 3αHSD3, 17βHSD1 and 17βHSD5 in patients with LLC when compared to healthy individuals (p=0,0002; p=0,0011; p=0,0361; p=0,0014; p=0,0070; p=0,0019, respectively). These findings show that PBMCs from patients with LLC have diminished expression of several enzymes required for sex steroid synthesis, which could compromise their normal functions.

642. (258) **THE ALTERNATIVE NF-KB PATHWAY ACTIVITY IN HODGKIN LYMPHOMA.**

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Refractory and relapsed disease (RRD) is currently the challenge when treating Hodgkin Lymphoma (HL) patients. There is no specific therapy rather than rescue chemotherapy schemes, which fails in 50% of the cases and associates with high risk toxicity. This highlights the need to deeper understand the HL molecular biology and the screening for therapeutic directed-targets.

We have previously reported that HL relies on the alternative NFkB pathway, mediated by Rel-B and NIK, to survive. Its constitutive activation seems to be involved in the RRD.

We aimed to determine the specific Rel-B target genes in HL. We performed ChIP-Seq in U-H01 human HL cell line for Rel-A, Rel-B, cRel, p50 and p52. We analyzed the +/- 2kb peaks from the gene transcription start site. The +/- 2kb Rel-B peaks were distributed on 4,509 genes, meanwhile the +/- 2kb cRel peaks were on 1,994 and the Rel-A ones on 830 genes. Only 6% of Rel-B peaks overlapped with Rel-A and 11% with cRel. The data was merged with gene expression arrays that compared U-H01 shRel-B transduced-induced vs transduced-uninduced cells. Genes that were up- or down-regulated more than 2-fold and with a $p<0.001$ were considered significant.

One of the exclusively Rel-B target genes was BCL2. We showed that exogenous BCL2 was able to partially rescue HL cell lines from dying in response to Re-B depletion. We also found that BCL2 is useful as a predictive marker for overall survival in a cohort of 96 HL patients [Log Rank Test (p=0.002)].

We found that Rel-B, among NF-kB transcription factors, plays an important role in refractory and relapsed HL disease, being BCL2 a key downstream target. We consider BCL2 could be a potential therapeutic target in the subset of HL patients that shows constitutive activation of the alternative NF-kB pathway.

643. (385) **GENE REGULATION OF THE REDOX BALANCE IN BETA TALASEMIA MINOR**

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B-Thalassemia minor is a common inherited red cell disorder characterized by ineffective erythropoiesis and production of reactive oxidative species (ROS). The aim of the present study was to evaluate the production of ROS and the expression of cytoprotective mechanisms in this pathology to contribute to the knowledge of outcome global redox balance in this environment. Methodology: Sixteen individuals with β-thalassemia minor and 12 apparently healthy was analyze in the Universidad Nacional de Tucumán between of 2016-2017. A complete blood count, hemoglobin electrophoresis in alkaline pH and hemoglobin A2 levels were quantified. Moreover, thiobarbituric acid reactive species and SOD activity were evaluated. Beta-thalassemia mutations were determined by real-time polymerase chain reaction. FoxO3 (Forkhead Box O3), Nrf2 (Nuclear Factor Erythroid 2-related factor), SOD (superoxide dismutase),CAT(catalase), Prx2 (peroxiredoxin-2), IL-6 and TNF-α genes expressions was investigate by real-time reverse transcription-polymerase chain reaction using mononuclear cells from peripheral blood.

Results: The levels of thiobarbituric acid reactive species were significantly increase in β-thalassemia trait group compared with controls, which would indicate higher peroxidation of the membrane lipid in these patients. We showed higher expression of Nrf-2, SOD, Prx-2, IL-6 and TNF-α in the β-thalassemia group. FoxO3 and catalase genes expression no revealed significant differences between both groups. SOD activity was significantly higher in the β-thalassemia trait group than in the control group.

Conclusion: The results obtained suggest an increase in ROS and inflammatory state, which are important and influential factors in the behavior and severity of anemia in subjects with β-thalassemia minor. Our findings shed new light on the mechanisms of adaptation against oxidative stress mediated through the Nrf-2 pathway and the expression of Prx-2 together with SOD to minimize the oxidative damage present in this pathology.

644. (491) **THE EFFECT OF ERYTHROPOIETIN ON HEPCIDIN EXPRESSION IN HEPATIC CELLS INVOLVES PI3K/MTOR SIGNALING**

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Iron (Fe) homeostasis is essential for living organisms, since cells rely on this micronutrient for a variety of metabolic processes. This is particularly relevant for stress erythropoiesis, when Fe stored in hepatocytes must be mobilized for its uptake by bone marrow erythroblasts and the subsequent synthesis of hemoglobin. Hepatic stores are maintained by the peptide hepcidin (Hamp), which impairs Fe release by inducing degradation of the ferroportin transporter. *In vivo*, erythropoietin (Epo) has been shown to decrease Hamp levels, but questions arise about this being a direct effect on hepatocytes, and about the possible mechanisms involved.

In order to study a possible direct action of Epo on Hamp production in hepatic cells, we assessed the ability of the cytokine to induce signaling in the human hepatoblastoma cell line HepG2. EpoR receptor expression was detected in this cell type (RT-PCR, flow cytometry). Epo (160 ng/mL) stimulated phosphorylation of the EpoR-associated kinase JAK2 (flow cytometry, Control: 20.9±3.1%, *Epo 5': 38.4±5.2%, *P<0.05, n=4). Incubation of cells with Epo significantly decreased Hamp mRNA levels (a.u., real-time PCR, Control: 1, *Epo 6 h: 0.4±0.2, *P<0.05, n=4), an effect also observed in Fe deficit (deferoxamine 100 μM) and in Fe overload (Fe-citrate 3 μM). The PI3K inhibitor LY294002 (LY) significantly abrogated the effect of Epo on Hamp basal expression, and the mTOR kinase inhibitor rapamycin (R) partially impaired its effect (a.u., real-time PCR; Control: 1, Epo: 0.5±0.1, *LY+Epo: 1.2±0.2, R+Epo: 0.7±0.2; *P<0.05 vs. Epo, n=4). In this regard, phosphorylation of mTOR was detected upon exposure to Epo (10 min, flow cytometry).

These results show a direct effect of Epo on hepcidin expression in a hepatic cell line under different Fe conditions, and suggest that the involvement of PI3K/mTOR signalling is required.

645. (539) **MECHANISMS INVOLVED IN THE STIMULATORY ACTION OF ERYTHROPOIETIN ON THE PROMIGRATORY EFFECT OF TUMOR NECROSIS FACTOR-ALPHA**

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The proliferation and migration of endothelial cells are vascular outcomes of inflammation and in this sense, proinflammatory cytokines may enhance the effects of other promigratory factors. Previously, we found that a stimulatory effect of TNF-α on endothelial cells (migration, adhesion, VCAM, ICAM) was enhanced by the si-

multaneous treatment with erythropoietin (Epo). Based on this, we were interested in investigating the mechanisms involved. Migration of endothelial EAhy.926 cells induced by TNF-α was enhanced by the interaction of Epo+TNF-α, despite no effect of Epo alone was detected at 10 IU/mL (E vs. ET, P<0.001, ET vs. T, P<0.01, n=7). Initially, we found an increased expression of TNF-α and Epo receptors induced by the proinflammatory cytokine. Since scratching assays showed a decreased effect of Epo+TNF-α in the presence of an antioxidant (NAC) (ET vs. ETN, P<0.01, n=7), we then analyzed whether reactive oxygen species (ROS) could be involved (flow cytometry; Gm: C 296±25, T 336±20, E 271±32, *ET 465±29, ETNac 312±7, *P<0.001 vs. E and ETNac, n=8). Given that the increased effect of Epo in the presence of TNF-α may be associated to Epo signaling deregulation, we assessed the participation of the tyrosine phosphatase PTP1B. Compared with Epo alone, the combination of TNF-α and Epo decreased phosphatase activity (a.u.: C 0.5±0.1, T 2.5±0.1, E 9.4±2.4, *ET 1.0±0.5; *P<0.01 vs. E, n=3), and expression of PTP1B (flow cytometry, Gm: C 2424±103, T 2632±285, E 3524±225, *ET 2751±302, *P<0.05 vs. E, n=5).
The results suggest that the oxidative stress generated by the inflammatory environment may cause PTP1B inactivity, therefore increasing the period of cell activation by Epo, justifying the stimulatory action of Epo on the migratory effect of TNF-α.
The proangiogenic ability of proinflammatory factors, enhanced in the presence of erythropoietin, might favor the action of this growth factor as a vascular protectant in ischemia.

646. (729) **EFFECT OF ERYTHROPOIETIN IN BRONCHIAL CELLS IN AN IRON EXCESS MOUSE MODEL.**
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Imbalances of iron homeostasis are implicated in acute and chronic lung diseases. However, the mechanisms involved in pulmonary iron deposition and its role in the pathogenesis of lung diseases remains unknown.
The aim was evaluate the effect of erythropoietin on bronquial cells in an iron excess mouse model studying the regulatory proteins of the iron cycle CF1 mice(25±5g; 3 months-old) were divided into 4 groups(n=4/group):1)Control; 2)Iron-overload(iron saccharate;-days0,4,8,12 ip;1800mg/kg); 3)EPO(days17,18,19) ip;20000UI/kg);4)Iron-overload+EPO. Immunohistochemistry: anti-prohepcidin, L-ferritin, DMT1(divalent metal transporter1) and ZIP14(Zrt-Irt-like Protein14) followed by Perl´s staining. The Protocol was approved by the CICUAE; UNS.
We observe that the DMT1 localization in bronchial cells was cytoplasmatic in iron overload+EPO, control and EPO while in overload the importer was in the apical zone and in membrane cells.
ZIP14 expression in bronchial cells was evident in iron overload while it was slight iron overload+EPO, control and EPO.
In control and EPO hemosiderin was absent while in Iron overload and iron overload+EPO it was abundant in alveoli.
The L-ferritin expression in iron overload was intense in alveoli and apical in bronchial cells. However it expression in iron overload+EPO was cytoplasmic in bronchial cells. It expression was slight in alveoli and cytoplasmatic in bronchial cells of control and EPO.
The prohepcidin expression was similar in all conditions.
The decrease of ZIP14 expression, and the change in the DMT1 and L-ferritin localization in Iron overload+EPO compared to iron overload, could be reflecting a lower iron uptake and storage in bronchial cells in EPO presence, suggesting a protective mechanism EPO-DEPENDENT.

647. (490) **INVOLVEMENT OF HOMOCYSTEINE AND ADENOSINE IN ERYTHROPOIETIN RESISTANCE IN HUMAN ERYTHROLEUKEMIA CELLS**
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End Stage Renal Disease (ESRD) is associated with the anemia detected in inflammatory conditions, and negative prognosis arises when hiperhomocysteinemia (HHcy) and accumulation of adenosine (Ado) enhance TNF-α cytotoxicity. Previously, we found that the presence of Ado and Hcy increased the sensitivity of undifferentiated erythroleukemia K562 cells to TNF-α-induced apoptosis, which could not be prevented by erythropoietin (Epo). In this study, we investigated whether differentiated cells could be protected by Epo in an inflammatory environment with Hcy and Ado accumulation. Cells were pre-treated with Epo (10 U/mL, 2 h) and differentiated with hemin (C, 30 μM, 48 h). Hcy (500 μM), Ado (250 μM) and TNF-α (T, 30 ng/mL) were added in the last 24 h of differentiation. Contrary to our observations in undifferentiated cells, Epo prevented TNF-α-induced apoptosis (Hoechst staining: C 17.1±0.9; *T 33.9±1.6; *EpoT 25.2±0.5; *EpoHcyAdoT 35.0±1.5,*P<0.05, n=8). The higher sensitivity of these cells to TNF-α could be explained by a lower expression of c-FLIP (caspase 8 inhibitor, Real-Time PCR). However, Epo was unable to protect the cells against the proinflammatory cytokine when Hcy and Ado were present. We propose that mitochondrial depolarization is involved, since the mitochondrial membrane potential was lower in treatments with Hcy and Ado than in assays in the absence of these compounds (MitoTracker dye, MMP: C: 13.8±2.2%; *ET: 13.5±1.8 *EHcyAdoT 7.6±0.5%, *P<0.05, n=5).
In conclusion, unlike the behaviour of undifferentiated cells, erythroid differentiation increases the sensitivity of K562 cells to TNF-α, which can be prevented by Epo. However, this protective effect of Epo is inhibited when Ado and Hcy are present. This may represent a new explanation for the resistance to human recombinant erythropoietin treatment observed in patients with anemia and hyperhomocysteinemia.

648. (736) **INTEGRATIVE RESPONSE OF IRON CYCLE PROTEINS BY IRON EXCESS IN NEUROBLASTOMA CELLS.**
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Many of the known neurodegenerative diseases have been shown to be influenced by changes in brain iron. Unlike other diseases where the association with iron metabolism has been established, the link between iron and neurodegenerative diseases remains unclear The aim of this study was study the iron effect in neuroblastoma cells(SH-SY5Y) evaluating the expression of iron cycle proteins,ZIP14(Zrt-Irt-like Protein14), DMT1 (divalent metals transporter1) and TfR1(Transferrin receptor1)(importers); H/L-ferritin(storage) and prohepcidin(regulatory protein).
The cellular viability was observed by a dose-response curve (neutral red) with low(30-80μM) or high(200-600μM) FAC(ferric ammonium citrate) concentrations. Iron cellular uptake was measured in the culture medium of cells treated with FAC30μM/72hs and 600μM/24hs(FerColor kit). In cells treated with FAC30μM/72hs and/or pretreated with NAC(N-acetylcystein)2mM/12hs the proteins expression and localization were determinated by immunocytochemistry and immunofluorescence.
The cellular viability decreased in cells incubated 72hs and 24hs with low or high FAC concentrations(p<0,05). Iron uptake was confirmed by its reduction in the culture medium (25%FAC600μM/24hs;42%-FAC30μM/72hs). ZIP14,prohepcidin,H/L-ferritin expressions were intense in SH-SY5Y+FAC respect to the control. DMT1 immunoexpression was lower in SH-SY5Y+FAC than control. In SH-SY5Y+NAC+FAC the change in the expressions of our studied proteins induced by iron were reversed by NAC pretreatment.
TfR1 was in the cellular membrane and cytoplasmatic in basal conditions and only cytoplasmic in FAC presence.The colocalization of TfR2 and HFE was restricted to FAC presence.
The increase of the storage proteins H/L-ferritin evidence the SH-SY5Y cells ability to uptake iron from the extracellular medium, it could explains by the change of TfR1 location (internalization) and the increase in ZIP14 expression.
These iron importers could be the responsible for the neuronal death induced by oxidative stress being the importer DMT1 a minor rol.

In the proposed regulatory integrative via would participate ZIP14, TfR1 and prohepcidin like a proteins responsibles of iron deposits like ferritin, inducing the cellular death.

649. (765) **REGULATION OF IRON IMPORTERS IN REGIONS OF THE CENTRAL NERVOUS SYSTEM IN IRON ACCUMULATION MODELS**

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Introduction: In neurodegenerative diseases is frequently detected the iron accumulation in brain. Therefore, understanding the brain iron proteins regulation may shed further light on that process for the prevention and treatment of neurodegenerative disorders. Objective: study the effect of iron excess on divalent metal transporter1 (DMT1) and Zrt-Irt-likeProtein14 (ZIP14) expressions in mice brain and in human neuroblastoma cells. Materials and Methods: In vivo studies: CF1 mice(25±5g) were divided into 2 groups (n=6/group;paired design):1)Iron-overload: Fe-Saccharate ip (days0,4,8,12;1800mg/kg),2)Iron-adequate. The Protocol was approved by the Committee on Experimental Animal Use and Care-UNS. In vitro studies: SH-SY5Y cells were treated with FAC30μM/72hs. Immunohistochemistry of mice brain and immunocytochemistry of cells was made for DMT1 and ZIP14. Perl`s staining. Results: Brain: In control mice, DMT1 expression was observed in striatum, hippocampus and cerebellum. In iron-overload, DMT1 expression in striatum, hippocampus and cerebellum was slight respect to control. In cerebellum, DMT1 was strongly expressed in granule cells, with slight immunoreactivity in Purkinje cells of control and iron-overloaded mice. ZIP14 was weakly expressed in striatum, hippocampus and cerebellum in control mice, while, in iron-overload, ZIP14 expression was strong. In cerebellum, ZIP14 was intensely expressed in granule cells and molecular layer control and iron-overloaded mice. Hemosiderin was absent in striatum, hippocampus and cerebellum of control mice, while in iron-overload abundant iron deposit was found. Neuronal cells: DMT1 immunoreactivity was lower in cells with FAC than that observed in control, while ZIP14 was higher. Conclusions: The increased ZIP14 expression, could be the responsible of iron accumulation that occurs in striatum, hippocampus and cerebellum being while DMT1 would not have a prominent role. Therefore ZIP14 and DMT1 importers could be part of a mechanism of cellular control of iron uptake

ONCOLOGÍA / ONCOLOGY 8

650. (565) **IN VITRO ANTICANCER ACTIVITY OF A NOVEL COPPER COMPOUND AGAINST 2D AND 3D BREAST CANCER CELLS**

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CEQUINOR-CONICET-UNLP

Coordination complexes have been extensively studied as antitumor agents, offering a potential alternative for cancer treatment. In this sense, copper compounds are attracting increasing interest as alternatives to traditional platinum drugs. Moreover, Schiff bases have been broadly investigated due to their antitumor properties, and can act as polydentate ligands in interaction with metal cations.
The aim of this work is to evaluate the antitumoral activity of a novel copper complex cation with a tridentate Schiff base ligand (SBT), containing thiophen $[Cu(BTS)(H2O)]^+$, in 2D (monolayer) and 3D (multicellular spheroids) cancer models.
The cytotoxic activity was tested against a panel of human cell lines including Jurkat (leukemia), MG-63 (osteosarcoma), A549 (lung), MDA-MB-231 (breast) and MCF7 (breast), using MTT assay. The complex significantly reduced the cell viability in all cell lines tested (Jurkat IC_{50}: 2.64μM; MG-63 IC_{50}: 3.49μM; A549 IC_{50}: 2.46μM; MDA-MB-231 IC_{50}: 1.89μM; MCF7 IC_{50}: 1.58μM) ($p<0.001$).
The putative cell death mechanisms triggered by complex in more sensitive cell lines (MCF7 and MDA-MB-231) were investigated. The induction of apoptosis and cell cycle were analysed by flow cytometry. The compound conveyed cells to apoptosis and induced DNA fragmentation (sub-G1 peak) on both cancer cell lines. Furthermore, the analysis of proteasomal activity showed the complex has an inhibitory effect on the chymotrypsin-like activity.
Additionally, MCF7 and MDA-MB-231 spheroids were cultured by the hanging drop technique and the effect of the compound on cell viability was evaluated by resazurin reduction assay. The compound diminished cell viability on spheroids (MCF7 IC_{50}: 8.69μM; MDA-MB.231 IC_{50}: 3.11μM), affecting the spherical shape and the capability to re-attach on the surface and to migrate. Moreover, pre-treatment of MCF7 cells with the compound lead to a decrease in normal development of spheroids.
Altogether, these results suggest that the copper compound is a good candidate to evaluate on in vivo assays.

651. (573) **IN VITRO ANTITUMOR EFFECTS OF TWO COPPER COMPLEXES WITH PHENANTROLINES DERIVATIVES TOWARD 2D AND 3D BONE, LUNG AND BREAST CANCER CELL MODELS**

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Several findings provide evidence that copper ions are capable of interacting directly with proteins and DNA, causing site-specific damage. It has been reported that copper compounds increase cell death in different cancer cell lines and both, the ligand and the metal play an important role in the pharmacological properties of the complex. During this work, we attempt to evaluate the biological activity of two different copper complexes, $Cu(II)(dmp)_2(CH_3CN)](ClO_4)_2$ (1) and $Cu(II)(phen)_2](ClO_4)$ (2) against cancer cells lines: A549 (lung), MG-63 (osteosarcoma), MCF7 and MDA-MB-231 (breast cancer). On monolayer, cell viability was analyzed by MTT, the cell migration was evaluated by wound healing assay, the ROS production was quantified using DHR123 and the genotoxic activity was determinate using comet assay. Besides, the cell viability of the spheroids (A549, MCF7, and MG-63) was evaluated by the Alamar blue assay. Complex 1 proved to be more active than complex 2 in all the cell lines tested, showing the following IC50 values of complexes 1 and 2 respectively, A549 1.94 μM vs 3.34 μM, MG-63 3.07 μM vs 3.11 μM, MDA-MB-231 1.95 μM vs 5.11 μM and MCF7 2.99 μM vs 6.11 μM. ($p<0.001$).Furthermore, a greater inhibitory effect was observed in the inhibition of migration by compound 1 and an increase in ROS levels too. On 3D results the complex 1 was more active than complex 2, observing the following IC50 values of complex 1 and 2, MCF7 6.98 μM vs 31.39 μM, A549 8.31 μM vs 10.56 μM and MG-63 11.24 μM vs 30.12 μM,($p<0.001$). Furthermore, complex 1 induces morphological changes and decreases the spheroid-cell invasion in collagen. Taken together, these results show that the anticancer activity of the complex 1 was more pronounced, it would be interesting to test this compound in further in vivo studies for cancer treatment.

652. (747) **SYNERGISTIC ANTIPROLIFERATIVE EFFECT OF C6-CERAMIDE AND 2´NITROFLAVONE COMBINATION IN MURINE MAMMARY TUMOR CELLS**

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Flavonoids are polyphenolic compounds that exhibit multiple biological activities, such as antiproliferative, antiallergic, antiangiogenic and antioxidant actions. We have previously demonstrated that the synthetic flavonoid 2´-nitroflavone (2NF) inhibited tumor growth in vitro and in vivo in a breast cancer murine model. On the other hand, existing evidences have suggested that sphingolipid metabolites are key molecules in regulating a number of cancerous behaviours. Thus, while sphingosine-1-phosphate promotes cancer cell survival and proliferation, ceramide and sphingosine accumulation could promote cell death. Moreover, certain flavonoids have been reported to increase the levels of ceramides, by inhibiting sphingosine kinase-1 (SphK-1), an enzyme involved in ceramide catabolism.
The aim of our study was to investigate the effect of the combination of exogenously added short-chain cell-permeable ceramides and 2NF in LM3 murine mammary tumor cells. It was initially demon-

strated by western blot analysis that LM3 cells over-expressed SphK-1 compared to normal murine NMUMG cells. In addition, the incubation of LM3 cells with different concentrations of 2NF or ceramides alone for 48 h inhibited cell growth, with IC50 values of 21±2 μM (2NF), 46±0.5 μM (C2-ceramide) and 35±1 μM (C6-ceramide). When 2NF and ceramides (C2 or C6) were simultaneously added in concentrations that individually inhibited cell growth approximately 30% (5 μM for 2NF, 40 μM for C2-ceramide and 30 μM for C6-ceramide) we found no significant differences in antiproliferative activity for the combination 2NF+C2-ceramide, but cell viability was drastically reduced for the combination 2NF+C6-ceramide. In order to quantitatively characterize the interaction between 2NF and C6-ceramide, dose-effect curves were analyzed by Compusyn software, being combination index of 0.68±0.1, indicative of synergism. In conclusion, our results demonstrated a synergistic antiproliferative effect of C6-ceramide and 2NF combination in LM3 tumor cells, suggesting that 2NF could affect ceramide catabolism, probably by SphK-1 inhibition.

Keywords: flavonoids, ceramides, mammary tumor, antiproliferative effect, synergism

653. (644) **BEYOND THE CLASSIC EFFECTS: ANTITUMOR ACTION OF RANITIDINE**

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Previously we demonstrated that histamine (HA) produces an increase in growth of human pancreatic xenografts of PANC-1 and BxPC3 cells in nude mice and in lung metastases. In vitro, HA hindered the activation of mesenchymal epithelial transition (a process associated with invasion and metastasis) by ionizing radiation. Literature describes the acquisition of an invasive phenotype in different tumor cells that survive gamma irradiation. The aim of this work was to evaluate the in vivo action of ranitidine (Ran) on the growth PANC-1 and BxPC3 tumors irradiated or not, as well as the appearance of metastases.

PANC-1 and BxPC3 tumors were irradiated or not with 2 Gy of gamma radiation, transplanted to non-irradiated mice, and treated or not with Ran (150 mg/kg/day, p.o.). Non-irradiated PANC-1 tumors growing in RAN treated animals showed longer doubling times ($p<0.05$). Histological studies showed that Ran produced a more differentiated phenotype and decreased the expression of the proliferation marker PCNA and the endothelial marker CD34 in tumors ($p<0.05$) while a lower number of lung metastatic foci was detected ($p<0.05$). Cell death markers as necrosis, apoptotic index and, pro and antiapoptotic proteins expression (Bax/Bcl-2) did not vary significantly. BxPC3 xenografts showed similar results to PANC-1, except for lung metastases that were not evident.

Irradiation of PANC-1 tumors increased tumor growth rate ($p<0.01$). A rise in tumor anisocytosis, in PCNA and in CD34 expression was observed together with an increase in lung metastases ($p<0.05$). In all cases, Ran blocked the increments ($p<0.01$). Irradiated BxPC3 tumor growth rate was similar to controls while some lung metastatic foci were present. However, Ran reduced irradiated BxPC3 tumor growth, lung metastases, PCNA and CD34 expression ($p<0.05$).

Ran, a drug of low toxicity widely used in clinics, demonstrated antitumor and antimetastatic capacity in two in vivo experimental models of pancreatic ductal adenocarcinoma

654. (635) **DESMOPRESSIN AS A REPOSITIONING DRUG WITH POTENTIAL ANTI-TUMOR EFFECT IN AGGRESSIVE TUMORS OF SMALL CELL LUNG CANCER, PROSTATE CANCER AND NEUROBLASTOMA.**

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Drug repositioning offers the possibility of founding new molecular targets using safe and effective known compounds. Desmopressin is a synthetic peptide agonist for vasopressin V2 receptor (V2r), used since decades for the treatment of urinary and hemostatic diseases. Our laboratory have been studying desmopressin as a repositioning drug in oncology, showing their hemostatic and anti-tumor properties in several tumor types. Neuroendocrine tumors (NET) comprise a heterogeneous group of neoplasms with a wide range of morphological and functional characteristics. Interestingly, a NE transdifferentiation associated to the treatment has been detected in prostate and lung tumor cells, showing therapy resistance, enhanced aggressiveness and poor prognosis. Similar features have been reported in neuroblastoma, a tumoral type that shares characteristics with NET, such as specific neuropeptides and a variable aggressiveness. Moreover, the incidence of aggressive tumor with NE features is increasing and only few therapeutic alternatives are available. The aim of this work was to evaluate desmopressin effect on key events of the tumor development in human tumor cell lines with NE features as PC-3 (PCa) and NCI-H82 (Small Cell Lung Cancer), and in cell lines of neuroblastoma as CHP-212 and SK-N-AS. All cell lines express V2r and desmopressin showed in all cases a cytostatic effect measured by MTS assay. It also regulates the in vitro expression of the genes Bcl-2, Bcl-xL and BAX associated to apoptosis, which were evaluated by RT-qPCR, promoting a pro-apoptotic balance in PC-3 and NCI-H82. Desmopressin also inhibited the PCa tumor growth in vivo by a 40% in a mouse xenograft model. These results evidence the desmopressin anti-tumor properties on aggressive human NE cell lines and show, for the first time, the in vitro effect of desmopressin on neuroblastoma cell lines, recognizing it as a repositioning drug for the treatment of this type of tumors with few therapeutic alternatives.

655. (626) **AN ANTITUMOR TRIAZOLYL PEPTIDYL PENICILLIN DERIVATIVE INHIBITS WNT/B-CATENIN PATHWAY AND ANGIOGENESIS**

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In a previous work, we demonstrated that TAP7f, an antitumor penicillin derivative formed by penicillin linked to the dipeptide Leu-Phe through a triazole group, exhibited antimetastatic properties through the inhibition of cell adhesion, migration and invasion of highly metastatic melanoma B16F10 cells. This penicillin derivative also inhibited MMP-2 and 9 expression and activity. In order to extend the research on TAP7f antimetastatic properties, we investigated the effect of this derivative on Wnt/β-catenin pathway. By Western blot analysis, we found that a 10 μM concentration of TAP7f inhibited 51±11% ($p<0.001$) and 70±12% ($p<0.0001$) the expression of β-catenin after 18 h and 24 h of treatment, respectively. Additionally, the same concentration of TAP7f significantly reduced the expression of β-catenin downstream targets c-myc and cyclin-D after 24 h of treatment ($p<0.01$ and $p<0.001$, respectively). We also observed that TAP7f significantly decreased the expression levels of vimentin (39±4%) and the transcription factor Snail (50±10%), both epithelial-mesenchymal transition (EMT) markers. As it has been reported that Wnt/β-catenin pathway is implicated in the promotion of angiogenesis, we studied the possible antiangiogenic effect of TAP7f employing the in vivo matrigel plug assay in C57/BL6 mice. Results obtained showed that a 20 μM concentration of TAP7f significantly inhibited the neovascularization induced by 250 ng/ml of fibroblast growth factor (FGF-2) 8 days after plug implantation. Thus, histological analysis revealed a significant reduction of endothelial cells infiltration of plugs containing TAP7f. In conclusion, our results demonstrated that TAP7f affects EMT and angiogenesis probably by down-regulating the Wnt/β-catenin pathway. These findings, together with the inhibitory effect on melanoma cell adhesion, migration and invasion suggest the potential of TAP7f as an effective antimetastatic agent.

656. (442) **COMBINATION BETWEEN 5-FLUOROURACIL AND**

YERBA MATE EXTRACT INHIBITS COLORECTAL CANCER CELL GROWTH.

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Yerba Mate (Ilex paraguariensis) is a native plant from southern South America. Many experimental studies have shown antioxidant, anti-inflammatory and antitumoral properties of Yerba Mate. Colorectal cancer (CRC) is the third most common cancer in both men and women. Previously, we have evaluated the effects of Yerba Mate extract on specific events of tumor progression and we demonstrated that the extract inhibits tumor cell proliferation, adhesion and migration and reduces invasiveness capacity in vitro. In the other hand, in vivo results suggest that the extract reduced tumor vascularization, increased tumor latency and decreased tumor growth. The aim of this study was to evaluate the effects of the combination of Yerba Mate extract with 5-fluorouracil (5-FU), which is widely used as a first-line regimen in adjuvant chemotherapy for colorectal cancer.
We evaluated the effect of the combination (YM + 5-FU) using a tumor cells panel extending to CT26, COLO 205, HCT116 and HT-29. In addition, we conducted an in vivo animal experiment to assess the influence of Yerba Mate extract on the sensitivity to 5-FU in CRC. The extract was administrated to male and female Balb/C mice via the drinking water before and after the subcutaneous inoculation of CT26 tumor cells. Four to six days after implantation of tumor cells the skins were palpated to evaluate tumor latency. After 15 days of cells inoculation, 5-FU was administered via i.p (50 mg/kg/week). Survival was registered during the experiment, unless the tumor volumes reached 2000 mm3, in which case the animals were sacrificed. The results suggest that the combination of Yerba Mate extract with 5-FU increased susceptibility of the colon cancer cells to the cytotoxicity of 5-Fu in Balb/C mice. We hypothesize that the treatment with the Yerba Mate extract could amplify the anti-tumor effects of 5-FU.

657. (454) **ANTITUMOR EFFECTS OF ALOYSIA POLYSTACHYA EXTRACT IN COLORECTAL CANCER**

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Aloysia Polystachya (AP) is an aromatic native plant of Verbenaceae family which is widely distributed in subtropical regions of South America and the North of Argentine. Uses in medicine of Aloysia species include diuretic, sedative, antispasmodic activities. We have previously demonstrated that AP extracts exert cytotoxic effects in several human tumor cell lines, including apoptosis. The aim of this work was to investigate if cytotoxic effects of AP extract could be extensive to cancer stem cells (CSC), and also, the possible sensitization or potentiation of chemotherapeutic drugs, using colorectal cancer model. Therefore, the colorectal cancer cell lines CT26 (mouse) and HCT116 (human), were stimulated with AP extract or vehicle, with or without 5-fluorouracyle (5-FURA) and then, the surviving and CSC properties were determined. The surviving was measured after crystal violet staining at 570nm and CSC phenotype was determined by measuring the CSC marker CD133 (PCR), colony formation (clonogenic assay) and the HOESCHT efflux capacity (chemotherapeutic drugs efflux via ABCG2 transporters). We found that AP extract (0,0004625 mg/ml of flavonoids) decreased the CD133 expression (98%), the colony formation ($p<0,01$) and the efflux capacity of chemotherapeutic drugs ($p<0,05$) respect to control cells. In addition, AP (0,0004625 mg/ml flavonoids) increased the cell death induced by 5-FURA (3,5uM) treatment (20%) respect to cells stimulated with 5-FURA alone. Our results demonstrate that AP increases the 5-FURA effect and CSC population may be a target for AP-induced cell death. Being CSC the most resistant to chemotherapeutic drugs, responsible of cancer progression and perpetuation, the autochthonous plant derivatives could be attractive tools to be investigated as future oncologic therapeutics.

658. (445) **SYNTHETIC HSP90 INHIBITORS AS PROMISING PROSTATIC CANCER THERAPEUTIC AGENTS**

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According to current bibliography, the biological activity of the heat shock protein of 90 KDa (Hsp90) is dependent of its ATPase activity. Hsp90 is not only associated to the maintenance of cellular proteostasis, as the traditional chaperone role implies; it also plays a crucial role in many cellular process and mechanisms. Therefore, it is not surprising to find it associated to cell proliferation, migration, invasion and almost all the named “hallmarks of cancer”. Cancer cells are thought to be “addicted” to this molecular chaperone showing high sensitivity to Hsp90 inhibitors compared to non-tumoral cells. Consequently, Hsp90 inhibitors are interesting for the development of new antitumoral therapies. Geldanamycin (GA) is a known Hsp90 inhibitor, however it is not used in clinical treatments for its harmful side effects. Nonetheless, GA was taken as base structure for the development of potential non-toxic therapeutic agents. In previous works we tested synthetic drugs design in silico as potential Hsp90 ATPase inhibitors. In this study, we focused on a group of compounds (named as series 4), which had shown an interesting inhibition of the ATPase activity, to evaluate their capability to affect a prostatic cancer model. In particular, compounds 4C, 4D, 4F show a reduction of the viability and migration of prostatic tumoral cells, with comparable effects to GA. However, while cell treatment with GA prevented steroid receptor nuclear import, all the synthetic drugs were not active in this regard. These properties could have pharmacological relevance since the lack of side effects such as steroid receptor inactivation could not be desirable. Interestingly, our results contradicts the current dogma, i.e. drugs that affect the ATPase activity of Hsp90 show no effect in all the chaperone biological activities. Consequently, these compounds could be used as base for the development of potential new oncological therapies.

659. (452) **EFFECT OF NOVEL INHIBITORS ON THE BIOLOGICAL ACTIVITY OF HSP90 IN PROSTATE CANCER**

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Hsp90 is a molecular chaperone that stabilizes in an ATP-dependent manner, the active conformation of a large number of proteins with stable tertiary structure. Several substrate proteins of this chaperone are related to tumor development and progression, hence making Hsp90 an attractive target for antitumor therapy. Inhibition of Hsp90 ATPase activity shows strong antitumor effects, and Hsp90 inhibitors seem to be the only chemotherapeutic agents capable to affect all cancer hallmarks. However, drug side effects are still an important concern. The aim of the present work was the study of novel compounds as potential antitumoral therapeutic agents. To its effect, drugs were designed and analyzed by in silico molecular docking simulations. Next, we assessed the effects of the drugs on the Hsp90 ATPase activity in vitro, viability and migration of prostate cancer cell models, and their inhibitory action on glucocorticoid receptor (GR) nuclear translocation. Geldanamycin (GA), a known Hsp90 inhibitor, was used as a positive control in all experiments. A total of 5 compounds (named N15, A15, C3, C6 and P1) were assayed. all of them confirmed in silico predictions regarding their ability to inhibit Hsp90 ATPase activity. As we expected, cell treatment with GA prevented steroid receptor nuclear import, and showed a negative effect on the viability and migration of PC3 cells. All the synthetic drugs showed an inhibitory action on cell migration, but none had effect on the GR nuclear import. Pyrazoline-derivative compounds (C3 and C6) were the only compounds that showed inhibition of cell viability. These properties could have pharmacological relevance, given the lack of

side effects such as steroid receptor inhibition, which is a desirable characteristic for pharmacological applications. Moreover, the study provides novel insights that could contribute to the design of more active and less toxic drugs.

660. (583) **AXIN2 GENOMIC PROFILE AS A PREDICTIVE MARKER OF EARLY STAGE RIGHT-SIDED COLORECTAL CANCER TUMORS WITH MICROSATELLITE INSTABILITY**

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It has been previously demonstrated that AXIN2 gene behaves as an oncogene promoting colorectal cancer development by activating the WNT signaling. However, AXIN2 has also been categorized as a tumor suppressor. With the aim of defining the genomic and transcriptomic profile of AXIN2 in colorectal cancer we performed an in silico analysis on data obtained from four comprehensive studies: Nature 2012 (n=276); Nature Medicine 2013 (n=390); Cell Reports 2016 (n=619); and Cancer Cell 2018 (n=1134). Bioinformatic tools and R packages from Bioconductor were employed for data integrative analysis and visualization. Results indicate that the frequency of alteration/mutation of the gene is usually not higher than 10% of the patients, and it is associated with the alteration of genes related with the WNT pathway. A significant association between AXIN2 mutated patients and the Instability Microsatellite (MSI) molecular subtype was determined. Also, there was an association with the tumor location, being AXIN2 more frequently mutated in tumors derived from the right colon. Staging and sample type were evaluated and the group of AXIN2 mutated showed an association with earlier stages compared with the other group. Overall survival analysis indicated that patients who carry mutations in the AXIN2 gene have a worse prognosis ($p<0.05$). However, patients that harbor AXIN2 mutation were, in turn, more responsive to chemotherapy treatment ($p<0.01$). Likewise, we determined a negative association between the AXIN2 mutation and the gene expression, positioning it as a tumor suppressor gene rather than as an oncogene. The association of AXIN2 mutation with poor prognosis and its appearance in early stages, position it as a prognostic and predictive marker in the defined molecular subtype of right-sided colorectal tumors with MSI. At this regard, it has been defined that colorectal cancers with MSI are predominantly located on the right-sided and had an early pathological stage.

661. (620) **EVALUATION OF THE ANTITUMOR ACTIVITY OF THE SYNTHETIC PEPTIDE [V4Q5]DDAVP IN ADDITION TO STANDARD CHEMOTHERAPY IN PRECLINICAL COLORECTAL CANCER MODELS**

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Colorectal cancer (CRC) stands as a major problem for public health, mainly due to related mortality caused by the metastatic progression. [V^4Q^5]dDAVP is a second generation vasopressin analog with reported antimetastatic activity in breast and lung cancer models, which acts as a specific vasopressin type-2 receptor (AVPR2) agonist in tumor and microvascular cells. Our aim was to explore for the first time the potential benefits of the addition of [V^4Q^5]dDAVP to an established standard-of-care chemotherapy as 5- fluorouracil (5-FU), in preclinical CRC models using mouse CT-26 and human COLO-205 cell lines. Results were statistically analyzed by Student T-test or ANOVA, using GraphPad Prism 6.0 and Compusyn software. In vitro, the addition of [V^4Q^5]dDAVP (1 μM) to the treatment with 5-FU (0,25-5 μM) exerted a synergic inhibitory effect on proliferation of CRC cells expressing AVPR2 ($p<0.01$), as well as a partial cell cycle arrest at the G0/G1 checkpoint ($p<0.001$). In vivo, i.v. treatment with [V^4Q^5]dDAVP interfered with CRC metastatic spread in the syngeneic experimental lung colonization model CT-26, while the addition of weekly cycles of 5-FU (50 mg/kg i.p.) did not provide further therapeutic benefit. In animals bearing growing CRC s.c. syngeneic tumors or xenografts, the addition of [V^4Q^5]dDAVP (0.3 μg/kg i.v. thrice-weekly) to weekly cycles of 5-FU (50 or 80 mg/kg i.p.) reduced tumor progression ($p<0.05$), exhibiting modulation of local tumor aggressiveness and reducing tumor growth rate. As a conclusion, according to the preliminary results obtained in this work and taking into account the need for novel CRC therapies with enhanced efficacy and reduced associated-toxicity, [V^4Q^5]dDAVP is a promising candidate for further preclinical testing as a co-adjuvant therapy with potential application in combination to standard chemotherapy regimens.

TOXICOLOGÍA / TOXICOLOGY 2

662. (39) **MECHANISM OF NEONICOTINOID TOXICITY IN HUMAN TROPHOBLAST**

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Neonicotinoid (NEO) insecticides are a relatively new class of pesticides developed to replace other insecticides. NEO were thought to have limited toxicity to mammals, since their higher specificity toward insects. Nevertheless NEO toxicity in non-target organisms is not widely studied. We have demonstrated that the NEO acetamiprid (Ace) and its commercial formulation (CFace) are cytotoxic to human trophoblast cell lines, and trigger reactive oxygen species production. Moreover, CFace is more toxic to trophoplasts than Ace. The aim of this study was to investigate the toxicity mechanisms of Ace and CFace in a human trophoblast cell line.
Human trophoblst HTR-8/SVneo cells were exposed to Ace or CFace (0.1-100 μM) for 4 and 24 h. Antioxidant enzyme activities (catalase, superoxide dismutase and glutathione s-transferase), as well as protein oxidation –PO– (advanced oxidation protein products method) and genotoxic damage –GD– (comet alkaline assay) were determined under these conditions.
Exposure to Ace and CFace modulated antioxidant enzymes: Ace reduced activities at high concentrations (10-100 μM) at 24 h ($p<0.05$), whereas CFace altered activities at all assayed conditions. Similar results were observed for oxidative damage. Ace and CFace increased levels of PO in these conditions ($p<0.05$), CFace damage started at lower concentrations than Ace. GD was observed after exposure to CFace, increasing along with incubation times and concentrations ($p<0.05$). In order to determine the participation of oxidative stress in cell toxicity, cells were treated with the antioxidant N-acetyl cysteine (NAC, 2 mM) prior to insecticide exposure. NAC protected trophoblasts from cell death and reverted protein oxidation ($p<0.05$).
The neonicotinoid acetamiprid is toxic to human trophoblasts, oxidative imbalance may be a mechanism underlying NEO toxicity. CFace is more toxic to trophoblasts than its active principle. In this sense, CFace contents 70% of Ace and other constituents not declared which are detrimental for cells and improve toxicity.

663. (64) **NEONATAL EXPOSURE OF EWE LAMBS TO A GLYPHOSATE-BASED HERBICIDE ALTERED THE EXPRESSION OF GENES INVOLVED IN OVARIAN FOLLICULAR DEVELOPMENT INDEPENDENTLY OF ADMINISTRATION ROUTE**

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The exposure of ewe lambs to endocrine disruptor compounds may alter female fertility. Previously we demonstrate that glyphosate-based herbicides (GBH) alter ovarian follicular development. Our hypothesis suggests that exposure of ewe lambs during an early postnatal development to GBH may modify the ovarian gene

expression even after exposure has ended. The aim of the present study was to compare the effect of oral and subcutaneous exposure to a GBH from postnatal day (PND) 1 to PND14 on the expression of ovarian genes at PND45. Ewe lambs were exposed to subcutaneous (n: 5) or orally (n: 5) using a low dose of a GBH (glyphosate at 2 mg/Kg/day) and controls (n: 12) with saline solution. On PND45, the ovaries were sectioned and immediately frozen in liquid nitrogen and stored at -80 °C for RNA extraction. Levels of mRNA of genes involved in follicular development such as steroid receptors (ESR1, ESR2 and PR), follicle-stimulating hormone receptor (FSHR), bone morphogenetic protein 15 (BMP15), BMP receptor 1B (BMPR1B), growth and differentiation factor 9 (GDF9), insulin-like growth factor 2 (IGF-2) were evaluated. The mRNA expression of β-Actin protein was used as housekeeping gene. Levels of mRNA were expressed relative to control group (C=1). Lambs exposed to GBH showed a reduction of FSHR (scGBH=0.45±0.06; oGBH=0.51±0.09) and GDF9 (scGBH=0.54±0.05; oGBH=0.53±0.1). Interestingly, the GBH effects on ovary gene expression were similar in both routes of exposure. No changes were observed in mRNA expression in the other genes assayed (ESR1, ESR2, PR, BMP15, BMPR1B and IGF-2). Our results demonstrated that after the neonatal exposure to low doses of GBH, the expression of two genes involved in ovine follicular development are altered independently of administration route. These results provide mechanistic evidences related the altered follicular development in lambs exposed to GBH and raise concern about ovarian function in adults.

664. (80) **EFFECTS OF DEVELOPMENTAL EXPOSURE TO GLYPHOSATE AND A GLYPHOSATE-BASED HERBICIDE ON THE MALE RAT MAMMARY GLAND**

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Glyphosate exposure during critical periods of development induces adverse effects on the reproductive system of male rats. Previously, we have shown that glyphosate-based herbicides (GBHs) produce endocrine-disrupting effects on the male rat mammary gland, and that these effects could be due to the active principle (glyphosate), the adjuvants or both. Here, we evaluated in postpubertal rats, whether the effects on male mammary gland development are caused by glyphosate alone or its combination with adjuvants. Pregnant rats were exposed orally through the diet to vehicle (saline solution), 4 mg/kg/day of glyphosate (GLI) or 4 mg/kg/day of GBH from gestational day 9 until weaning. On postnatal day 60, the male offspring were sacrificed and mammary gland samples were collected. Total area, perimeter, longitudinal growth, mammary density and the number of terminal end buds (TEBs) were measured in mammary gland whole-mounts (WMs). Also, estrogen (ESR1) and androgen receptor (AR) mRNA and protein expression were evaluated and mRNA expression of ESR1 alternative transcripts, cyclin D1 (CCND1), epidermal growth factor receptor (EGFR) and prolactin receptor (PRLR) were assessed. In addition, the methylation status of ESR1 promoters was analyzed. GLI and GBH reduced mammary gland total area and GBH also decreased the perimeter of the gland and increased the number of TEBs. ESR1 mRNA and protein expression and ESR1-OS mRNA levels were reduced in both exposed groups, which was accompanied by hypermethylation of ESR1-OS promoter. AR mRNA levels were also reduced in both groups; however, its protein expression was higher in GBH-exposed animals. Besides, CCND1, EGFR and PRLR mRNA expression were lower in GLI and GBH exposed animals. Our results suggest that GBH effects on male rat mammary gland growth and gene expression could be mediated mainly by the active principle of the herbicide.

665. (233) **NEONATAL EXPOSURE TO A GLYPHOSATE-BASED HERBICIDE ALTERS UTERINE CELL PROLIFERATION IN EWE LAMB**

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Glyphosate based herbicides (GBH) are one of the most extensively used pesticides in agriculture all over the world. Recently, we showed that in rats a brief exposure to a low dose of GBH during the first week of life alters uterine development, induces epithelial hyperplasia and causes post-implantation embryo loss. The present study investigates whether a brief postnatal exposure to a low dose of GBH using two different administration routes alters the uterine differentiation of prepubertal ewe lamb. Ewe lambs (Frisone breed) were sc or orally exposed from postnatal day 1 (PND1) to PND14 to saline solution (vehicle) or a low dose of a GBH (glyphosate at 2 mg/Kg/day). At PND45, uterine horns samples were collected for paraffin-embedding or stored at -80°C until mRNA extraction. Cell proliferation was assessed in all uterine compartments by quantifying the expression of Ki-67 protein using immunohistochemistry. RT-PCR was performed to evaluate the expression of genes related with uterine development and differentiation, such as: steroid receptors (ESR1, ESR2 and PR) and insulin-like growth factors (IGF-1, IGF-2 and its receptor IGF-1R). GBH treatment decreased the rate of proliferation in the luminal epithelium (scGBH 6.4±0.9**, oGBH 5.7±0.7** vs C 12.4±2.0, **p<0.01); glandular epithelium (scGBH 5.0±0.7*, oGBH 4.5±0.6* vs C 11.7±2.2, *p<0.05); the subepithelial stroma (scGBH 0.9±0.1**, oGBH 1.1±0.1** vs C 1.7±0.1, **p<0.01); and in the myometrium (scGBH 0.6±0.1*, oGBH 0.5±0.1* vs C 1.5±0.3, *p<0.05). Exposure to GBH did not alter the expression of sex steroid receptors and IGF family members, thus abnormal cell proliferation could not be ascribed to deregulation of these pathways. Postnatal exposure to an environmental relevant dose of GBH disrupts the development of prepubertal sheep uterus by decreasing cell proliferation, which could compromise future reproductive performance.

666. (525) **PERINATAL EXPOSURE TO GLYPHOSATE OR A COMMERCIAL FORMULATION DISRUPTS THE WINDOW OF UTERINE RECEPTIVITY REDUCING THE IMPLANTATION SITES IN RATS**

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Glyphosate is the active principle of the most globally used herbicides. The toxicity of commercial formulations (glyphosate-based herbicides, GBHs) vs. glyphosate alone is a matter of intense debate. Present study investigates the effects of perinatal exposure to glyphosate (Gly) or a GBH on female reproductive performance. Pregnant rats (F0) were exposed to Gly or a GBH through food, in a dose of 2 mg of glyphosate/kg/day, from gestational day (GD) 9 until weaning (lactational day (LD) 21). Glyphosate levels were measured in the serum of F0 dams during gestation and lactation by UHPLC–MS/MS. When F1 females reached the sexual maturity, they were bred and submitted to a fertility test to evaluate the pregnancy rate, and on GD19, the number of corpora lutea and the implantation and resorption sites. Other pregnant control or exposed females were sacrificed on GD5 to assess the hormonal and molecular milieu during the preimplantation period by determining: the serum levels of 17β-estradiol (E2) by radioimmunoassay, and in uterine samples, the protein expression of estrogen receptor alpha (ERα) and Ki67 proliferation marker by immunohistochemistry. Glyphosate serum concentrations in F0 dams were similar over the treatment period, and levels reached were 13.4 ± 2.3 ug/L and 38.7 ± 5.6 ug/L for Gly and GBH groups, respectively. Perinatal exposure of F1 female rats to Gly or GBH induced subfertility. The pregnancy rate was not affected, but we detected a decreased number of implantation sites. Moreover, both treatments increased the serum levels of E2 (Control: 21.4 ± 2.6 pg/ml; Gly: 30.4 ± 0.6 pg/ml; GBH: 30.4 ± 0.9 pg/ml) at the preimplantation period, which was associated with higher

uterine ERα expression and cell proliferation. We propose that estrogenic-proliferative alterations might disrupt the window of uterine receptivity, reducing the number of implantation sites.

667. (424) **USE AS A PARAMETERS OF THE TOXICITY OF FUMONISIN, THE ERYTHROCYTE SENESCENCE MARKERS**

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In vivo aging of erythrocytes (EC) is associated with increased cellular density, which corresponds to increased cell age. A change in the overall redox status toward a more oxidized state has been reported during cellular aging. Fumonisin B1 (FB1) is a mycotoxin that occurs as a frequent contaminant of corn and corn-based foods in Argentina, has an inhibitory effect on ceramide synthetase, key enzyme in the biosynthesis of sphingolipids, essentials for membrane composition. Our objective was to assay the techniques to determine the aging of EC and apply to the exposure study to fumonisins. Changes of EC shapes were studied by flow cytometry. EC were resuspended in PBS (isotonic solution) and in solution 3mM Tris, 0.65% NaCl (hypotonic solution), and processed immediately (t0) and in PBS incubated 24 (t24) or 48 hs (t48) at 37 ºC (senescent condition). The M2: M1 ratio, termed the spherical index and the asymmetry of the global histogram (PCD) express the erythrocyte sphericity asymmetry. Two gates in FSC histogram: R1 and R2 were determined. Two median values (M1 and M2) were calculated for each gate of interest. Erythrocyte malondialdehyde (MDA) was measured as index of lipid peroxidation (TBA). Results: The TBA studies showed significant increase in senescent EC (t48) (p:0.003, <0.05) vs EC in PBS (t0) , (6,9±1,6 vs 3.5±0,79 nmol/ml respectively). The ratio M2/M1 decreased in hypotonic solution and in senescent EC vs EC in isotonic solution (3.39 vs 5.29); PCD increased in hypotonic condition vs isotonic condition (0.0827 vs -0.0018), which indicates a tendency towards sphericity. These parameters serve to study the senescence of red blood cells and membrane alteration induced by exposure to fumonisins. The application of these methods will provide us with tools to study the effects of exposure to fumonisins.

668. (579) **ABOUT PESTICIDES AND OTHER DEMONS: INDUCTION OF TUMOR ANGIOGENESIS IN OUR BREASTS**

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Breast cancer is the most frequent tumor in women worldwide. Pesticides that act as endocrine disruptors have been shown to affect normal mammary development. In this study, we examined the action of Hexachlorobenzene (HCB) and Chlorpyrifos (CPF) on angiogenesis in mammary carcinogenesis in vivo and in vitro. We analyzed the levels of proangiogenic factors such as vascular endothelial growth factor (VEGF) and cyclooxygenase-2 (COX-2), as well as the expression of the nitric oxide synthases (NOS) by WB and their production of nitric oxide (NO) by Griess reagent. In a xenograft model with MCF-7 cells (+ERα), HCB (3 mg/kg b.w.) and CPF (0.1 mg/kg b.w.) enhance angiogenic switch (number of vessels/mm2) and increase VEGF expression in mice skin (p<0.05). For in vitro time-course studies, HCB (0.005 μM) at 3 h increases VEGF, COX-2 and NOS protein levels (p<0.05), while 5 μM enhances VEGF and COX-2 levels (p<0.001) at 24 h, but decreases NOS expression at different times. CPF (0.05 and 50 μM) at 6 and 24 h, increases all involved protein expression (p<0.05). Exposure to each pesticide enhances NO production (0.005 μM HCB at 3 h and 0.05 μM CPF at 3 and 6 h; p<0.05). To demonstrate that VEGF and COX-2 induction occurs through NO-dependent mechanism, we inhibited (L-NMMA) the production of NO in the presence of pesticides at low doses. We found that L-NMMA prevents the increase in VEGF and COX-2 expression in MCF-7 induced by HCB or CPF (p<0.001). The environmental doses of HCB and CPF stimulate angiogenic switch and VEGF expression in vivo. Pesticides induce VEGF, COX-2 and NOS expression in MCF-7 at similar doses that promote proliferation and angiogenesis. These alterations could contribute to the formation of preneoplastic lesions in the healthy mammary gland, as well as to the progression in human breast tumors.

669. (611) **THE PESTICIDE CHLORPYRIFOS INDUCES CELL MIGRATION AND MODULATES MMPS VIA MAPKS PATHWAY IN BREAST CANCER CELL LINES.**

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Chlorpyrifos (CPF) is one of the insecticides most used in agriculture in our country. We have previously demonstrated that 0.05 uM CPF induces cell estrogen receptor mediated-proliferation in MCF-7 estrogen dependent cell line. However, the proliferation was inhibited by Reactive Oxygen Species (ROS) increment induced by 50 uM CPF in MCF-7 and in the estrogen independent MDA MB-231 cells. Moreover, 50 uM CPF was able to promote invasiveness in both cell lines. Objective: In this work we analyzed the signals involved in migration and invasiveness CPF-promoted and the role of the metalloproteases in this action. Methods: MCF-7 and MDA MB231 cells were exposed to CPF (0.05-50 uM). Wound assay was performed to study cell migration, zimmography to determine gelatinolase activity and Western Blot for phosphorylation protein levels. Results: In MDA MB-231 cells, CPF induced cell migration (300% over control, p< 0.01) after 24 h of exposure. This action was reverted by p38 inhibitor (SB202190 10uM) and ERK 1/2 inhibitor (PD98059 5uM; p<0.01). 0.05 and 50 uM CPF produced an increment of MMP2 activity and MMP2 secretion that were found dependent of p38, ERK1/2. 0.05 uM CPF induced these actions also in a ROS-dependent way. In MCF-7 cells both 0.05 and 50 uM CPF induced an increment in MMP2 secretion (p<0.01) which could be reverted by NAC (p<0.05), SB202190 (p<0.05) and ICI 10 nM (p<0.05). Furthermore, an increment in of p-cSRC and p-GSK3b were observed after 1 h of exposure. This action was prevented by adding c-SRC inhibitor PP2 (2 uM) (p<0.05). The increment of p-GSK3b was also reverted by SB202190 (10uM). Conclusions: CPF induces the increment of p-cSRC, p-p38 and pGSK3b and this pathway could be related with the promotion of migration and invasion that we reported previously. The increment of MMP2 secretion could be also related with those processes.

670. (693) **NEW INSIGHTS ON PESTICIDE EXPOSURE AS A RISK FACTOR IN BREAST CANCER**

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The incidence of breast cancer is increasing and exposure to pesticides like hexachlorobenzene (HCB) and chlorpyrifos (CPF) has become important as a potential risk factor. These compounds may alter proangiogenic ability and promotes tumor growth. Regional studies show the presence of HCB and CPF in human serum and breast milk. We demonstrated that these compounds induce proliferation, migration and invasion in human breast cancer cells. Angiogenesis plays a role in local tumor growth and metastasis.

There is a correlation between elevated levels of Hypoxia Inducible Factor-1α (HIF-1α), tumor metastasis and angiogenesis. HIF-1α induces genes like Vascular Endothelial Growth Factor (VEGF), Nitric Oxide Synthase-2 (NOS-2) and Cyclooxygenase-2 (COX-2). VEGF acts on tumor endothelial cells to increase their proliferation, survival and migration. COX-2 and NOS-2 promote carcinogenesis and angiogenesis.
The aim of our work was to examine the HCB or CPF action on breast cancer angiogenesis. We studied the effects of in vitro exposure to HCB (0.05 and 5 μM) or CPF (0.05 μM), for 6 and 24 hours in MDA-MB-231 breast cancer cells on: a) HIF-1α expression, b) VEGF secretion, c) VEGF expression levels, d) COX-2 and e) NOS-2 protein levels (Western Blot). Our results showed that MDA-MB-231 exposed for 6 hours to CPF increases HIF-1α ($p<0.001$) and NOS-2 ($p<0.01$) expression, as well as VEGF ($p<0.01$) secretion. Besides, CPF stimulates VEGF ($p<0.01$) and COX-2 ($p<0.01$) levels in cell lysates only at 24 hours. Moreover, HCB (0.05 and 5 μM) for 6 hours enhances NOS-2 ($p<0.05$) expression and VEGF ($p<0.01$) secretion; meanwhile, at 24 hours only HCB (5 μM) stimulates VEGF levels in cell lysates, and both HCB doses increase COX-2 levels ($p<0.01$). In conclusion, our results demonstrate that HCB and CPF stimulate proangiogenic factors in MDA-MB-231. Altogether, these data highlight that pesticide exposure could promote the angiogenic processes contributing mammary carcinogenesis.

671. (226) **METABOLIC ALTERATIONS DUE TO CHRONIC EXPOSURE TO ACOUSTIC STRESS**

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Our previous results showed that acoustic stress (AS) induces severe intestinal inflammation and affects glucose homeostasis in the mouse. In this study, we investigated the mechanisms involved in the altered glucose metabolism due to AS. Two-month old CBA/J male mice were subjected to basal oral glucose tolerance tests (OGTT) and then randomized into AS (n=6) and control (n=6) groups. AS mice were subsequently exposed to 24hs of noise (300Hz-70dB) at 3mo old (1 AS/week, 5 weeks), at 6mo old, and finally at 13mo old (each one, 2 AS/week, 5 weeks). They were further exsanguinated for determining plasma levels of glucose (GLU), total and HDL cholesterol, triglycerides (TGL) and hepatic transaminases (TGP/TGO). Fasting insulin, glucagon, GLP-1 and GIP concentrations were also determined. Main results showed: I- AS group displayed a trend toward an increased incidence of hepatic adenomas than controls (66.7% vs 16.7%, p=0,07). II- Blood glucose was roughly 70 mg/dl higher in the AS group vs. control mice (GLU = 267.4 vs. 201.0 mg/dl p=0.18). This effect was associated with higher insulin resistance (IR) assessed by TGLxGLU (p=0.0253) and TGL/HDL (p=0.0339) indexes. III- AS mice showed a diminishing in glucagon levels (p=0.0495) and a trend in insulin secretion (HOMA-beta 28.7 vs. 78.6%, p=0.15). IV- Pancreas from AS mice showed signs of hypertrophy and hyperplasia determined by fractal geometry analysis. V- AS group showed an important increase of TGL levels (976.4 vs. 123.0 mg/dl, p=0.0253). VI- Guts from AS group showed damage and alterations of villi and increased expression of TNFα. This observation is associated with lower GIP levels detected in the AS group (p=0.0275). In sum, chronic AS promotes the generation of hepatic tumors, chronic intestinal inflammation and several metabolic alterations, especially hypertriglyceridemia, that lead to pancreas disfunction in part due to lipotoxicity and reduced incretin secretion.

672. (413) **IMMUNOMODULATORY EFFECT OF THE ENDOCRINE DISRUPTOR BISPHENOL-A (BPA) IN HUMAN KERATINOCYTES AND MACROPHAGES: INTERACTION WITH DOPAMINERGIC PATHWAYS**

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BPA, an endocrine disruptor used in the manufacture of plastics and epoxy resins, could modulate the inflammatory immune response. It is known that BPA may interfere with dopaminergic transmission at CNS but there are less evidences at peripheral level. We previously demonstrated that the treatment with BPA for 24 h did not modify the production of IL-6 and IL-8 or the response to dopamine in human keratinocytes and macrophages. In this work we evaluate the effect of BPA, at long times, alone or in combination with dopamine (DA) on the cytokine production (IL-6, IL-8, IL-1β, ELISA), the NFκB pathway (Western blot) and the metalloproteinase (MMP) activity (Zymography) in a cell line of human keratinocytes HaCaT and THP-1 macrophages (PMA-differentiated cells). The cells were cultured with BPA (10-7 M) for 10 days and then stimulated with DA (10-5 and 10-4 M) for 24 h. In HaCaT keratinocytes, during the treatment with BPA no changes were observed in the levels of IL-6 nor in the activation of NFκB induced by dopamine in the absence of the disruptor. However, the presence of BPA alters the effect of DA on the production of IL-8 and the MMP-9 activity ($p<0.05$), compared to the results obtained in absence of endocrine disruptor. In THP-1 macrophages the prolonged stimulation of BPA modified the levels of IL-8 ($p<0.05$), but not IL-1β, compared to that observed in the absence of BPA. In addition, the activation of the NFκB pathway was not induced in the absence or presence of DA. Moreover, the increase in MMP-9 activity induced by DA in the absence of the disruptor was not modify in presence of BPA. In conclusion, we demonstrated, for the first time, that the treatment with BPA for long periods could affect the peripheral dopaminergic pathways in human keratinocytes and macrophages.

673. (115) **DOPAMINE ENHANCES ARSENIC-INDUCED CELL DEATH WITH INCREASED MITOCHONDRIAL MASS IN HUMAN KERATINOCYTES**

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Chronic exposure to arsenic, an environmental toxicant, is associated with skin alterations. Arsenic affects the normal balance of keratinocyte proliferation and death by compromising mitochondrial function. Mitochondrial damage is compensated by mitochondrial biogenesis and increased mitochondrial mass (MM). Dopamine (DA) is a neurotransmitter that modulates the immune system. Keratinocytes synthesize and degrade DA and express DA receptors. Objective: Here we explored the effect of arsenic trioxide (ATO) and DA on mitochondrial membrane potential (MMP), mitochondrial mass (MM), and cell death in the human keratinocyte cell line HaCaT. Methods: HaCat cells were exposed to increasing doses of ATO or/and DA for 48h and stained with nonyl-acridine orange (NAO) to evaluate MM, tetramethylrhodamine-ester (TMRE) to evaluate MMP, and propidium iodide (PI) to evaluate cell viability. The samples were analysed by flow cytometry. Results: ATO caused a dose-dependent linear increase in MM within the range 5μM to 30μM (slope =0.147; R2=0.982), a dose-dependent linear decrease in MMP (slope=-9.955; R2=0.979). At 30μM the cell death rate was 35% (SE=1.57%). When cells where exposed to DA alone a linear dose-dependent decrease in MM was observed, particularly within the range 0.1nM-1μM (slope=-0.24: R2=0.73). No changes were observed in cell viability and MMP up to 100 μM DA. When ATO was combined with 100μM DA a similar pattern of change was observed with a dose dependent increase of MM, decrease of MMP and increased keratinocyte death. At 30 μM the cell death rate was 40% (SE=0.18). Conclusion: At doses above 5μM, ATO caused mitochondrial damage, increased MM and cell death in HaCat keratinocytes. The increase in MM may indicate that mitochondrial biogenesis was a protective response against ATO toxicity. DA decreased MM with no effect on viability, but increased ATO-induced cell death in keratinocytes.

INFECTOLOGÍA / INFECTOLOGY 2

674. (58) **RECOMBINATION RATES ALONG THE ENTIRE EPSTEIN BARR VIRUS GENOME DISPLAY A HIGHLY HETEROGENEOUS LANDSCAPE**

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Epstein Barr virus (EBV) has a large DNA genome assumed to be stable, but also subject to mutational processes such as nucleotide substitution and recombination, the latter explored to a lesser extent. Moreover, differences in the extent of recombination events across herpes sub-families were recently reported. Given the relevance of recombination in viral evolution and its possible impact in pathogenesis, we aimed to fully characterize and quantify its extension in all available EBV complete genome by assessing global and local recombination rate values (ρ/bp).

Our results provide the first EBV recombination map based on recombination rates assessment, both at a global and gene by gene level, where the mean value for the entire genome was 0.035 (HPDI 0.020-0.062) ρ/bp. We quantified how this evolutionary process changes along the EBV genome, and proved it to be non-homogeneous, since regulatory regions depicted the lowest recombination rate values while repetitive regions the highest signal. Moreover, GC content rich regions seem not to be linked to high recombination rates as previously reported.

At an intragenic level, four genes (EBNA3C, EBNA3B, BRRF2 and BBLF2-BBLF3) presented a recombination rate above genome average. We specifically quantified the signal strength among different recombination-initiators features previously described (Brown et al, Genomics 2014) and concluded that those which elicited the greatest amount of changes in ρ/bp, TGGAG and CCCAG, were two well characterized recombination inducing motifs in eukaryotic cells. Strikingly, although TGGAG was not the most frequently detected DNA motif across the EBV genome (697 hits), it still induced a significantly greater proportion of initiation events (0.025 events/hits) than other more represented motifs, P = 0.04; one tailed proportion test.

Finally, our results support that idea that diversity and evolution of herpesviruses are impacted by mechanisms, such as recombination, which extends beyond the usual consideration of point mutations

675. (560) **INDIVIDUALIZED ANTIRETROVIRAL THERAPY. IMPACT OF PHARMACOGENETIC AND THERAPEUTIC DRUG MONITORING IN THE SAFETY AND EFFICACY OF FIRST LINE ANTIRRETROVIRAL THERAPY IN PATIENTS WITH HIV INFECTION. PRELIMINARY REPORT**

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Although the life expectancy of HIV patients and access to treatment today resembles that of the general population, more than 20% of these patients discontinue treatment at standard doses, mainly due to adverse effects.

The incorporation of therapeutic individualization in daily practice could help to identify the most appropriate dose for each patient of Atazanavir and Efavirenz, thus preventing some known toxicities and avoiding their eventual early discontinuation.

In this prospective and randomized study the objective is evaluating the usefulness of individualization treatment guided by FG and TDM in the selection of the most adequate initial dose of efavirenz and atazanavir in patients with HIV infection in Argentina.

Currently, the protocol is being carried out by 4 centers in Buenos Aires and has 95 patients enrolled, of which 47 are Pharmacological Adequacy (TDM + FG) and 48 of the control group (usual practice)

Three patients belonging to the Control group had to abandon the treatment at standard doses due to the presence of relevant adverse effects.

Among the patients in the Pharmacological Adaptation group (47), five subjects whose enzyme impact was low according to the pharmacogenomic analysis, were instructed to reduce the dose of Atazanavir, tolerating them acceptably and maintaining the efficacy of the treatment.

The pharmacological adaptation of initial and ongoing doses of first-line antiretroviral drugs seems feasible and useful for the individualized therapeutic approach of patients with HIV infection who begin treatment.

676. (638) **DEVELOPMENT OF A DENGUE NS1 ANTIGEN EXPRESSION SYSTEM IN MAMMALIAN CELLS.**

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The nonstructural protein 1 (NS1) of dengue virus is a multifaceted glycoprotein known to play a fundamental role in virus replication, immune evasion and pathogenesis. It can be detected in the bloodstream of patients, with both primary or secondary infections, and its levels correlate with viremia and disease severity. These characteristics point it as an interesting target for the development of diagnostic and therapeutic tools. Most attempts to express this protein in Escherichia coli or in a yeast system have resulted in insoluble aggregates with low protein yield and loss of biological activity. In addition, refolding of the aggregates requires time consuming optimization steps.

In this work an expression system based on mammalian cells was developed to produce the protein in its native conformation and with intact biological and antigenic characteristics through a simple purification step from the cell culture supernatant.

DNA fragments encoding NS1 protein from the four dengue serotypes were cloned in pCAGGS vector by the infusion PCR cloning system, downstream of the interleukin 2 secretion sequence and fused to a hexa histidine tag. Protein expression in HEK293-T cells culture supernatant and lysate was analyzed by Western Blot 48 and 72 hours after transfection. Subsequently, the assay was scaled up to larger production volumes and the protein was purified by affinity chromatography using a Ni-NTA resin (Amintra™). In order to assess antigenicity of serotype 1 purified protein, an ELISA with serum samples from infected and healthy individuals was carried out.

In conclusion, it was demonstrated that the recombinant NS1 proteins of the four serotypes are correctly expressed and released to the culture medium with proper antigenicity. These tools are promising for use in basic research as wells as for the production of antigens for diagnostic kits.

677. (771) **DETERMINATION OF THE FREQUENCY OF MYELOID-DERIVED SUPPRESSOR CELLS DURING HUMAN ACTIVE TUBERCULOSIS.**

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Tuberculosis (TB), together with HIV infection, is the leading cause of death from an infectious disease worldwide. In fact, *Mycobacterium tuberculosis* (Mtb) causes almost 10 million of new cases per year. Myeloid-derived suppressor cells (MDSCs) display an immunosuppressive function during several pathological conditions such as cancer and hepatitis. MDSC-mediated suppression of host immunity during chronic inflammation is crucial for immune regulation and tolerance to limit immunopathology. Furthermore, the unfavorable effects of MDSCs are evident in tumor biology where they accumulate and suppress cytokines Th1 responses. The aim of this work was to study the expression of MDSCs during active TB. Thus, we investigated the frequency of granulocytic MDSCs (g-MDSCs) and

monocytic MDSCs (m-MDSCs) in peripheral blood mononuclear cells (PBMCs) from TB patients classified as high (HR-TB) or low (LR-TB) responders on the basis of their immunological response to Mtb-antigen. Using flow cytometry, we observed that both g-MDSCs ($CD14^{-}$ $CD11b^{+}$ $CD15^{+}$) and m-MDSCs ($CD14^{+}$ $CD11b^{+}$ $HLA\text{-}DR^{-/low}$) showed significantly higher levels ($P<0.05$) in PBMCs from TB patients as compared to healthy donors (HD). Moreover, we detected elevated percentages of m-MDSCs in LR-TB as compared to HR-TB ($P=0.044$) or HD ($P=0.002$), without significant differences between these last two groups of subjects ($P=0.1932$). Besides, no significant differences in the levels of g-MDSCs were found among the groups of individuals under study. Interestingly, m-MDSC frequency was decreased in LR-TB after two weeks of anti-TB drugs treatment ($P<0.05$). Together, our data reveal that MDSC are highly induced during active TB. In addition, LR-TB patients showed the highest levels of m-MDSCs, whereas TB patients with strong immunity and HD had similar levels of those cells. Therefore, our findings indicate a correlation between the percentage of MDSCs and TB severity, suggesting that the frequency of MDSCs could be a marker of treatment evolution during active disease.

678. (372) **MIF EXPRESSION IS INVOLVED IN THE ACTIVATION OF CD4+ T-CELLS AND FACILITATES THE INFECTION BY HIV-1.**

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Macrophage Migration Inhibitory Factor (MIF) plasma levels are increased in HIV+ individuals compared to healthy donors. Our group described a novel function of MIF in inflammatory processes when acting on primary HIV-1 infected MDMs. Additional evidence suggests that MIF expression might play a relevant role in the activation of CD4+ T lymphocytes (CD4TL). Aim: to identify the role of MIF in CD4TL activation and subsequent HIV-1 infection.
Primary CD4TL from healthy donors were activated with PHA with or without a MIF-blocking antibody. Also, the effect of exogenous-added MIF on unactivated CD4TL was assessed. Activation of CD4TL was evaluated by flow cytometry. Permissiveness to HIV-1 infection was evaluated. Two reporter lines (CEM and Ghost) were stimulated with MIF to evaluate GFP expression downstream the LTR viral promoter.
In primary CD4TL, MIF neutralization inhibited PHA-driven activation. A decrease in the frequency of blast-like cells (16.88%) compared to PHA-activated CD4TL (50.71%) ($p=0.017$) was observed. Also, significant lower expression of HLA-DR, CD25 and CD28 was found.
Number of infected cells was significantly lower when blocking MIF activity (1.32%), compared to the PHA-alone control (6.58%, $p=0.0026$), resembling results from unactivated CD4TL (0.85%).
MIF-stimulated unactivated CD4TL showed a higher viral production after infection (95.82 ng/ml) compared to unstimulated cells (11.84 ng/ml) ($p=0.022$). No differences were detected in infection percentage, cell viability or activation among all conditions. Finally, LTR promoter-driven expression of GFP in reporter cell lines was unaffected by MIF.
Our work depicts an important role of MIF in CD4TL activation. Intracellular MIF activity drives CD4TC activation which, in turn, promotes permissiveness to HIV-1 infection. Conversely, exogenous MIF triggered higher viral production without affecting cell activation, proliferation or infection percentage.
These differences could be related to signalling pathway. Intracellular expression could trigger mechanisms unavailable to exogenous MIF due to a possible low incorporation into the cell.

679. (462) **CHANGES IN BACTERIAL MORPHOLOGY AND HOST DAMAGE AFTER INTRA-PERITONEAL INFECTION BY ESCHERICHIA COLI IN MICE.**

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Escherichia coli (E.coli) is a main cause of sepsis in humans after intra-abdominal infections. The pathogenesis of this disease is far from clear. In a previous communication we reported the budding of vesicles from enteroaggregative E. coli under specific conditions, some of them containing bacterial DNA. The purpose of this work was to study if E.coli was different after provoking sepsis than before, along with the description of the pathology induced by this bacterium in mice. C3H/BiDa mice were intra-peritoneally infected with 4 x 106 cfu of wild type E.coli and observed until dead. Organs were studied by histology and transmission electron microscopy (TEM) and bacteria by Gram staining and TEM. Mouse blood and plasma were aditionally inoculated in vitro with E.coli, and studied with the same methods. Mice died within 24 h and showed spread, lacunal bleeding in the liver, intravascular red blood cells lysis and hepatocytes vacuolation. Also, there were shape changes in the glomeruli of the kidneys as well as P.A.Schiff positive secretion in renal tubules. Changes in the shape, length and aggregation of E.coli were found in bacteria obtained from intraperitoneal fluid and in vitro inoculated blood and plasma, with a higher expression of 25-80 nm budding vesicles, as compared with the controls. Our conclusions are that E.coli appears to be different after having induced the infection than before and that the main changes in the host locate in the kidney and the liver. Further biochemical studies are ongoing.

680. (803) **CHARACTERIZING THE EXPRESSION PROFILE OF THE LYMPHOCYTE SCAVENGER RECEPTORS CD5 AND CD6 ON B CELLS FROM MOUSE STRAINS EXHIBITING DIFFERENT SUSCEPTIBILITY TO ECHINOCOCCUS GRANULOSUS INFECTION.**

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CD5 and CD6 are scavenger receptors mainly expressed on T- and B1a-cells, involved in the fine-tuning of activation/differentiation signals delivered by their TCR/BCR, respectively. Additionally, they sense the presence of conserved pathogen-associated structures from bacteria, fungi and/or viruses; and more recently, we described their interaction with tegumental antigens from Echinococcus granulosus. Interestingly, B1a-cells seemed to be relevant in susceptibility/resistance phenomena during murine infection by E.granulosus, and B1a-cells counts differ between Balb/c and C57Bl/6 mouse strains, which exhibit high- and low-susceptibility to E.granulosus infection, respectively. Herein we performed experimental infections in Balb/c and C57Bl/6 mice and characterized the expression profile of CD5/CD6 on peritoneal and splenic B-cells during early infection (n=5 mice/strain/time-point). Regarding peritoneal cells, a significantly lower number of basal CD5+ B-cells was observed in C57Bl/6 mice ($p<0.05$). Then, although no numeric differences were shown among strains during infection, CD5 intensity decreased with similar kinetics in both strains. On the other hand, although no basal numeric differences were observed in CD6+ B-cells, C57Bl/6 mice exhibited significantly lower CD6 expression densities ($p<0.05$). In addition, the number of CD6+ B-cells increased during infection in both strains, concomitantly with a decreased intensity in CD6 expression. Regarding the spleen, we observed a numeric increase of CD5+ B-cells in both strains, again concomitantly with decreased receptor intensity. No relevant differences/variations were observed for CD6+ B-cells. Finally, comparisons of expression profiles between anatomical sites showed -for both strains- that basal levels of CD5 were significantly higher in the spleen than in the peritoneal cavity, while the inverse situation was observed for CD6 ($p<0.05$). During infection, such a difference disappeared only for CD5. Summing up, our results pave the road for further functional studies on CD5/CD6 roles during E.granulosus infection, and they represent the first report on CD5/CD6 characterization in B cell during a helminth-driven infection.

681. (804) **THE ECTODOMAINS OF THE LYMPHOCYTE SCAVENGER RECEPTORS CD5 AND CD6 INTERACT WITH ECHINOCOCCUS GRANULOSUS TEGUMENTAL ANTIGENS AND PROTECT MICE AGAINST SECONDARY HYDATIDOSIS.**

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Scavenger Receptors (SRs) from the host's innate immune system are known to bind multiple ligands to promote the removal of non-self or altered-self targets. We report herein the interaction of CD5 and CD6 -two highly homologous class I SRs- with Echinococcus granulosus. CD5 and CD6 are lymphocyte surface receptors mainly expressed on the T and B1a cell subsets, and involved in the fine-tuning of activation and differentiation signals delivered by the antigen-specific receptors (TCR and BCR, respectively), to which they physically associate. CD5 and CD6 have been shown to interact with and sense the presence of conserved pathogen-associated structures from bacteria, fungi and/or viruses. Binding studies shown here indicate that both soluble and membrane-bound forms of CD5 and CD6 bind to intact viable protoscoleces from E. granulosus through recognition of metaperiodate-resistant tegumental components. Further in vitro assays demonstrate that membrane-bound or soluble CD5 and CD6 forms differentially modulate the pro- and anti-inflammatory cytokine release induced following peritoneal cell exposure to E. granulosus tegumental components. In this sense, while the soluble CD6 ectodomain down-regulates -in a dose dependent manner- the secretion ratesof IL-10, TNF-α and IL-6 ($p<0.05$, n=4), the soluble CD5 ectodomain up-regulates -also in a dose dependent way- the production of TNF-α and IL-6 ($p<0.05$, n=4) without affecting IL-10 secretion ($p>0.05$, n=4). Importantly, prophylactic infusion of soluble CD5 or CD6 significantly ($p<0.05$) ameliorated the infection outcome in the murine model of secondary cystic echinococcosis. For example, lower infection rates (CD5: 30%, CD6: 50%, and control: 95%, median values) and fewer number of developed hydatid cysts (CD5: 5, CD6: 10, and control: 25, median values) were observed in treated mice respect to control littermates. Taken together, our results expand the pathogen binding properties of CD5 and CD6 and provide evidence for their therapeutic potential in human hydatidosis.

682. (81) **NF200 EXPRESSION IN RATS WITH EXPERIMENTAL DIABETES AFTER NATIVE PLANTS DECOCCTIONS CONSUMPTION**

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Numerous studies indicate that 85% of world population uses "medicinal plants" to improve its health. Oxalis erythrorhiza(Oe) and Tessaria absinthioides(Ta) growth in Cuyo region(Argentine), and are popularly consumed to regulate the glucose and cholesterol levels, even when it does not exist a scientific support. Diabetes mellitus represents a major problem for health systems. These patients have a chronic hyperglycemia, an oxidative stress state (through the Oxide Nitric Sintase (NOS) induction), and can also develop degenerative changes in neurons and glial cells. Thus the search of new alternative therapies remains in force. We had previously, shown effects of Oe and Ta decoctions(10%W/V) on metabolism and hypothalamic LXRs expression. In this study, 42 days old male rats(SD), diabetics(Ed, i.p.STZ30mg/Kg) or controls(C,i.p.vehicle) received (5% W/V) decoctions of Oe(EdOe/COe) or Ta(EdTa/CTa), or water(EdW/CW) as drink for 4 weeks. Glucose, cholesterol and triglycerides were measured on blood samples by colorimetric kits. Hypothalamic neural NOS(nNOS) and Neurofilaments 200(200 kDa(NF200); 160kDa(NF160) and 68kDa(NF68)) expression levels were evaluated by WB. Glucose levels in EdW were significantly higher than CW(300%;$p<0.05$) and the decoctions did not produce any change. Among the groups no significant differences were observed in cholesterol and triglycerides levels. In EdW, COe and CTa the nNOS levels were higher than in CW(28%; 21% and 50% respectively; $p<0.05$), but the decoctions did not produce any change in Ed condition. All NF bands values were higher in EdW, COe and CTa respect to CW(23%; 20% and 20% respectively; $p<0.05$).However, Oe and Ta significantly reduced the NF200 expression in Ed compared to W(25% and 29% respectively; $p<0.05$), with similar values to CW. The obtained results suggest that these plants could have neuroprotective effects. Therefore, a more extended treatment will be necessary to elucidate the research objective, with the aim to propose Oe and Ta like new therapeutic tools (PIP0243,PIO-SECITI2250,CICITCA UNSJ,CONICET).

683. (401) **IMPROVEMENT OF THIOL REDOX HOMEOSTASIS IN GLAUCOMATOUS PRIMARY VISUAL CORTEX AFTER LIPOIC ACID ADMINISTRATION**

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Lipoic acid has been used to ameliorate oxidative stress in glaucoma as it scavenges reactive oxygen and nitrogen species, repair oxidized proteins and lipids, and regenerate endogenous antioxidants. The aim of this work was to evaluate if thiol redox homeostasis improved after lipoic acid administration in visual cortex in a glaucoma rat model. Wistar rats (3 months) were divided in four groups (n=24): glaucoma in which rats were operated under a microscope by cauterized two of the episcleral veins (G), glaucoma treated with lipoic acid 100 mg/kg i.p. (LG), control which received a sham procedure (C) and control treated with lipoic acid 100 mg/kg i.p. (LC). Seven days after surgery, rats were euthanized, brains were removed and visual cortex was separated. The following markers were evaluated: reduced glutathione (GSH), oxidized glutathione (GSSG), redox index (GSH/GSSG), thioredoxin reductase (TrxR), glutathione reductase (GR), glutathione peroxidase (GPx), and glucose-6-phosphate deshidrogenase (G6PD).
Comparing G to C: GSH decreased 46 % ($p< 0.001$), GSSG increased 26 % ($p< 0.05$), redox index diminished 57 % ($p< 0.001$), TrxR decreased 34 % ($p<0.001$) GR diminished 52% ($p<0.001$), GPx increased 56 % ($p<0.05$) and G6PD decreased 45 % ($p<0.01$). Comparing LG to G: GSH increased 78 % ($p< 0.001$), GSSG decreased 26 % ($p< 0.05$), redox index increased 140 % ($p<0.01$), TrxR increased 26 % ($p<0.05$) and GR increased 81 % ($p<0.01$). No changes were found in GPx and G6PD.
Thiol redox homeostasis is altered in visual cortex in glaucoma. Lipoic acid plays a protective role in oxidative processes since it increases GSH, redox index, the activities of GR and TRxR, as well as decreases GSSG. The improvement in GSH recycling supports that lipoic acid could be used as a novel therapy for reducing oxidative damage in glaucoma since it improves thiol redox homeostasis in visual cortex.

684. (607) **MITOCHONDRIAL FUNCTION IN THE BRAIN CORTEX OF RATS IN AN EXPERIMENTAL GLAUCOMA MODEL**

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Glaucoma is a disease characterized by a specific pattern of optic nerve head damage, retinal ganglion cells death and traditionally associated with high intraocular pressure. The understanding of the role of mitochondria in the onset and progression of glaucomatous damage is important to the comprehension of this pathology since the function of the mitochondria exerts an important impact in the neuronal life or death pathway and is essential for neurotransmis-

sion.
The aims were to evaluate the alterations of mitochondrial function and antioxidant defenses in an experimental glaucoma model.
Three-month female Wistar rats were divided in two groups (n=4): glaucoma group in which rats were operated under a microscope by cauterized two of the episcleral veins (GG) and control group which received a sham procedure (CG). Seven days after surgery rats were euthanized, brain cortex was separated, and mitochondria were isolated. The local committee for animal care (CICUAL-FFyB) approved the experimental model. The following markers were evaluated: oxygen consumption (OC), ATP production, hydrogen peroxide production, protein carbonylation (PC), and the activities of the enzymes superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione S-transferase (GST) and thioredoxin reductase (TrxR).
In GG the ATP production decreased 38% compared to CG (C 52.0 ± 4.4 nmol ATP/ min mg prot $p<0.05$) and protein carbonylation increased 60% compared to CG (C 4.28 ± 0.74 nmol/mg prot $p<0.05$). No significant differences were found in OC, hydrogen peroxide production, SOD, GPx, GR, GST, and TrxR.
The results suggest that glaucoma produces an alteration of brain cortex mitochondrial function characterized by a decrease in its capacity to produce ATP. Since no changes were observed in OC, this situation could be a consequence of mitochondrial membrane potential alterations. Additionally, protein oxidation could contribute to altered mitochondrial function, leading to changes in protein function or inactivation.

685. (658) **GALECTIN-1 IMPROVES COGNITION IN AN ANIMAL MODEL OF ALZHEIMER'S DISEASE AND PREVENTS BLOOD-BRAIN BARRIER DISRUPTION CAUSED BY EXPOSURE TO AMYLOID-B IN VITRO.**

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Alzheimer's disease (AD) is a neurodegenerative disorder associated with an imbalance of production and clearance of amyloid-β peptides (Aβ), generating amyloid plaques in the brain. Neurofibrillary tangles and inflammation in cortex and hippocampus are recognized hallmarks of the disease, in addition to microvascular alterations and dysfunction of the blood-brain barrier (BBB). The glycan-binding protein galectin-1 (Gal1) was demonstrated to have neuroprotective effects in other neurodegenerative diseases, associated with inflammatory modulation. It can act on immune and endothelial cells in peripheral and central nervous system. We have shown that Gal1 treatment improved cognition in PDAPPJ20, mouse model of AD , assessed by novel location recognition test ($p<0.05$), and caused a marked decrease of amyloid plaques in the hippocampus. Here, we propose that Aβ impacts negatively on the components of the BBB, particularly on endothelial cells, affecting their function and phenotype, and that Gal1 could modulate those effects. For this purpose, we assessed the deposition of perivascular Aβ, which are known to disrupt microvascular function, on the brain of AD mice. Using tomato lectin to label the vasculature coupled with immunofluorescence against amyloid-β, we found a 30% reduction of vascular Aβ ($p<0.05$) without affecting vascular density after Gal1 treatment. We employed an in vitro approach as a BBB model to determine whether fibrilar Aβ affects the endothelial function. This essay consisted in a completely sealed monolayer of human brain microvasculature endothelial cells, verified by a low permeability to Evans Blue (EB), on a transwell membrane. We found that Aβ exposure disrupted the barrier integrity, indicated by augmented permeability to EB ($p<0.05$) and loss of specific localization of tight junction protein occludin on the plasma membrane. We found that Gal1 treatment preserved BBB integrity against Aβ exposure, suggesting a vascular protective effect.

686. (664) **HIGH SODIUM DIET SELECTIVELY AFFECTED OSMOTIC SENSITIVE BRAIN AREAS IN RATS WITH RENAL DENERVATION: AT1 RECEPTOR MODULATION**

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The sympathetic nervous system (SNS) has a key role in renal sodium equilibrium in normal and pathological conditions in close relation with circulating and central angiotensin-II. Moreover, vasopressin release is under angiotensin-II control. It is known that high sodium intake activates brain and renal angiotensin system and inhibits the circulating angiotensin-II. Our aim was to evidenciate the brain AT_1 receptors (AT_1-R) role under high sodium diet conditions without SNS influence, for this purpose male Wistar rats with renal nervous ablation and implanted with bilateral cannulae in lateral ventricle, received 4 % sodium diet for 5 days . The surgical procedures were performed under ketamine/xylaxine (75/5 mg/kg i.p.) anesthesia, after one day recovery period, the animals were housed in metabolic cages during five days with normal/high salt diet. On day 6 the animals were injected with saline/losartan (AT_1-R antagonist 4ug/1 μl) intracerebrally. The parameters analyzed were: -brain c-Fos expression in Paraventricular nucleus (PVN), subfornical (SFO) and organum vasculosum lamina terminalis (OVLT) by inmunohistochemistry; -water intake and food intake; -urine excretion. Two-way ANOVA analyses followed by Student-Newman-Keuls posttest, $p<0.05$ difference was consider significant. Results: The diet reduced c-Fos expression in OVLT and induced an increase in c-Fos positive cells number in SFO and PVN. Interestingly, the increased neuronal activation induced by the high sodium diet in PVN was prevented by AT_1-R antagonism. Moreover, losartan increased the urine excretion and water intake only in high sodium exposed animals. No differences were found between any groups respect food intake. Conclusions: AT_1-R mediate the PVN neuronal activation induced by high sodium diet independently of SNS. Since angiotensin-II through AT_1-R mediates the vasopressin release, the increased diuresis induced by losartan in high sodium diet conditions could be explained by a possible decrease in vasopressin neurons activation due to AT_1-R blockade in PVN.

687. (703) **VALIDATION OF LASER-INDUCED CHOROIDAL NEOVASCULARIZATION (CNV) MOUSE MODEL.**

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Age-related macular degeneration (AMD) in its neovascular form is the leading cause of vision loss among adults above the age of 55. In the present study, we validate an established procedure for induction of choroidal neovascularization (CNV) in mice. This procedure consists in the perforation of Bruch's membrane by laser-induced photocoagulation, mimicking neovascular form of AMD.
Thus, this study was performed in this model, in order to characterize the neovascular process and its impact on the retinal functionality and the inflammatory profile. Due to, we previously demonstrated that α2M, and its receptor (LRP1) participate during retinal NV we also focalized in the participation of α2M/LRP1 system.
C57BL/6 mice, 3–6 months of age, previously anesthetized were treated with four spots of argon green laser photocoagulation per eye. After 7 days of laser burn, we analyzed the NV on choroid/RPE flatmounts by isolectin B4 staining using confocal microscopy. Using specific cell markers we characterize cells in the lesion area: CD105 (ECs), NG2 (pericytes), F4/80 (microglia) by immunostaining, as wells as LRP1 cell distribution. In addition, the levels of α2M and LRP1 were analyzed by WB and the inflammatory profile, as IL1β, IL6, and TNFα using qPCR assay. The retinal functionality was assessed by scotopic ERG.
We had able to standardize the size of the lesion. An important number of ECs and pericyte was observed around the lesion. Microglia

was localized in central and peripheral area of the lesion. While α2M and LRP1 showed increase level of expression on cells close to the lesion. Accompanied with high pro-inflammatory and pro-angiogenic factors. The scotopic ERG a- and b-wave were decreased ($p<0.05$) in the eyes of this animals.
In conclusion, we were able to reproduce and validate the CNV model in our laboratory, which offer the opportunity to evaluate the participation of α2M/LRP1 system.

688. (375) **PRELIMINARY EFFECTS OF MELATONIN ADMINISTRATION ON FE-DEPENDENT RESPONSE TO OXIDATIVE STRESS IN RAT BRAIN**
Marcelo Pablo Hernando, Natacha E. Piloni, Joaquín Cogo Pagella, Susana Puntarulo, Claudio O. Cervino
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Melatonin (ML) is considered as a quencher of free radicals generation. Its secretion is related to the photoperiod. The effect of ML on rats' brain was studied after acute Fe-dextran exposure. Rats were ip administrated with Fe-dextran (500mg/kg) or saline solution (control), and after 6 h ML (0, 50 and 100 mg/kg) was ip injected and the brain was removed 2 h later. Endogenous ML production was changed by exposing the rats to normal photoperiod, 12:12h (control), 15 days of 24h light (L), or 24 h darkness (D). At the end of each photoperiod, the Fe-dextranor saline solution was administrated. After 8 h, thiobarbituric acid reactive substances content (TBARS), catalase activity (CAT) and glutathione content (GSH) were evaluated. In presence of Fe, no changes on TBARS and CAT were observed at any doses of ML administrated. GSH decreased by 60 and 40% after the injection with 50 and 100 mg/kg ML, respectively, as compared to control. In the absence of Fe, when the increase in ML content was due to exposure to D, no alterations were observed in TBARS, an increase in GSH and a decrease in CAT activity was determined. Furthermore, in rats supplemented with Fe, TBARS increased, GSH was not affected, and CAT activity decreased, as compared to control photoperiod. However, GSH was significantly lower compared with absence of Fe. In the presence of L, no changes were detected. These results suggested that ML effects on oxidative status showed a different profile depending of Fe overload. Keywords: Melatonin, Fe acute exposure, TBARS, catalase, reduced glutathione.

689. (382) **EFFECTS OF THE COMBINATION OF MELATONIN AND KETAMINE HYDROCHLORIDE ON STANDARD ANAESTHESIA REGIMENS IN RATS**
Claudio O. Cervino, Natalia Bello Caraballo, Joaquín Cogo Pagella, Omar Iodice
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Aim. Assess melatonin administration as an anaesthetic agent and establish whether combining this drug can decrease the required dosage of ketamine and/or enhance its effects. *Materials and Methods.* Single and combined doses of melatonin (15 to 100 mg/kg) and ketamine (35, 50 and 70 mg/kg) are tested in 54 rats. Duration and depth of anaesthesia are assessed through recording righting and withdrawal reflexes. Four times of anaesthesia are defined (induction, general, surgical and full recovery). *Results.* Nine treatments are tested: a) control and doses of 15 mg/kg melatonin and 35 mg/kg ketamine do not produce anaesthetic effects; b) doses of 50 and 70 mg/kg ketamine and combination melatonin-ketamine 15+50 mg/kg only produce general anaesthesia; c) melatonin 100 mg/kg and melatonin 100 mg/kg combinations with ketamine 50 and 70 mg/kg produce general and surgical anaesthesia. *Discussion.* Anaesthetic effects of melatonin are verified. These effects are enhanced when combined with ketamine. Our results are compared with those obtained with combinations of ketamine and other anaesthetic drugs. The possible effect of the ketamine-melatonin combination on the GABAergic system and the NMDA receptors for glutamate is discussed. *Conclusion.* The melatonin-ketamine combination causes a rapid onset of anaesthesia and prolongs the effect of ketamine alone in rats. So, this study suggests that melatonin modulates mechanisms involved in the induction of anaesthesia by ketamine.

690. (497) **A SYNERGISM BETWEEN HYPOXIC EPISODES AND LOW ETHANOL DOSES TRIGGERS DELETERIOUS EFFECTS UPON NEONATAL BREATHING PATTERNS.**
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Effects of early ethanol exposure upon neonatal respiratory plasticity have received progressive attention given a multifactorial perspective related with sudden infant death syndrome or hypoxia-associated syndromes. In this preclinical study we examine how low doses of ethanol potentiate the effects induced by sequential exposures to hypoxia in 3–9-day-old pups, period equivalent to the 3rd human gestational trimester. At postnatal days (PDs) 3, 5 and 7, pups received 1.0 g/kg ethanol (ip) or vehicle and later were exposed to normoxia or hypoxia (8%O2) during 15min. At testing day (PD9), pups were exposed to normoxia/hypoxia/recovery-normoxia under the effects of 0.0, 1.0 or 2.0 g/kg EtOH. Breathing frequencies and apneas were recorded by plethysmography.
The duration of hyperventilation induced by hypoxia progressively increased. At PD7 pups were able to maintain hyperventilation along the entire hypoxic-test. At PDs 5-7, hyperventilation was altered by ethanol intoxication. While vehicle-pups hyperventilate efficiently, intoxicated-pups were only able to maintain hyperventilation during a short temporal interval. Immediately after these pups showed a significant respiratory depression comparable to the breathing rates obtained during normoxia. At PD3, hypoxia generated a significant number of apneic episodes. At PD9, the hyperventilation was significantly mediated by the state of intoxication. Pups treated with 2.0 g/kg EtOH exhibited a very low capability in terms of generating adequate hyperventilation. During recovery-normoxia, intoxicated-males (2.0g/kg) presented an abrupt respiratory depression coupled with heightened levels of apnea.
These results indicate that a relatively low ethanol dose coupled with a hypoxic event disrupts early respiratory plasticity. Furthermore, ethanol intoxication paired with hypoxia alters the capability of the organism to exhibit compensatory breathing patterns when defied with the lack of ambient oxygen. The results also indicate that males are more vulnerable to the deleterious effects of the synergism comprising hypoxia and ethanol particularly during the phase of recovery-normoxia.

691. (361) **MONO- OR BIPARENTAL CARE AFFECTS ADOLESCENT ETHANOL INTAKE AND BEHAVIOR**
Eliana Ferreyra[2], Abraham Ramirez[2], Lucila Pasquetta, Juan Molina[2, 1], Roberto Miranda Morales[2, 1]
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Social attachment plays an important role in progeny development. Different social experiences during early development and throughout life can affect ethanol use and abuse. In the present study we aimed to analyze if different rearing conditions (mono- or biparental care), in a non-monogamous mice strain, may have a differential impact on adolescent behavior and initiation to ethanol consumption. C57BL/6 adolescent mice were reared in a monoparental (MP, only mother) or biparental (BP, cohabitation of father-mother since copulation) condition until weaning (postnatal day, PD, 21). At PDs 28-30, animals were evaluated in a 4-hr daily, double-bottle ethanol consumption test (10% ethanol vs. water) during three weeks and four days per week. In this test animals were not food or water deprived and have access to food during the test. A different group of animals were evaluated at PDs 34-37 in a modified version of the concentric square field. This test allows simultaneous measurement of different behavioral patterns. Some of the areas evoke shelter-seeking behavior, whereas others evoke exploration, risk assessment and risk taking. Results from consumption test indicated a strong effect of parental care condition. Since the fifth day of test, MP adolescents consumed significantly more ethanol than BP counterparts. No difference were observed in water intake. When analyzing the behavior of the adolescents, we found that MP subjects displayed more anxiogenic-like behavior than BP adolescents. The first group

spent significantly more time in a dark shelter area and less time in a brightly illuminated bridge. Taking these results together, it seems that absence of the father during lactation increases anxiety responses in the litter that could in turn increases initiation of ethanol consumption during adolescence. Further research is being conducted aimed to analyze the neurobiology corresponding of this phenomenon and parents' behavior during this period.

692. (801) **EXCITOTOXIC ROLE OF ENDOGENOUS GLUTAMATE AS A MEDIATOR OF THE DAMAGE INDUCED BY COBALT CHLORIDE IN THE ZEBRAFISH RETINA**

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Zebrafish (Danio rerio) represents a useful vertebrate model for the investigation of human diseases and for studying mechanisms involved in tissue growth and regeneration. We have previously characterized a cytotoxic retinal damage in adult zebrafish by the injection of cobalt chloride (CoCl2) into the vitreous cavity (VC), which injures mainly the photoreceptors. This causes a strong induction of the proliferative activity of multipotent Müller glia and derived progenitors that are activated for retinal repair.
We divided 6 groups of zebrafish by injecting into the VC CoCl2 (injury) or saline solution (injury control). Injured and uninjured zebrafish were also injected 18 h before and 6 and 30 h after lesion with vehicle or glutamate ionotropic receptors antagonists (0.25 mM DNQX –AMPAR antagonist– and 1.5 mM APV –NMDAR antagonist–). We performed immunohistochemistry to label different kind of retinal cells and Hoechst staining to observe retina morphology after injury in presence or absence of the antagonists. We also performed behavioural studies to correlate locomotor behaviour with visual acuity of the experimental groups videotaping them during 1 min for further analysis.
We demonstrated CoCl2 deleterious effect on photoreceptors and progenitor cell proliferation were significantly reduced by treatment with DNQX and APV. At different intervals post-injury zebrafish in the different groups showed variable behavioural responses to visual stimuli, indicating different degrees of visual deficit. The morphological level of retina degeneration was correlated with the deficit observed in visual acuity for each group. Cone photoreceptor regeneration and visual acuity recovery occurred by 25 days after injury.
In conclusion, our results indicate that glutamate receptor antagonists showed strong neuroprotective effects that were evidenced both at the morphological as well as the behavioural level. We have also demonstrated CoCl2 toxic effects for retinal cells are importantly mediated by glutamate cytotoxicity.

INMUNOLOGÍA / IMMUNOLOGY 6

693. (144) **MODULATION OF NEUTROPHIL EXTRACELLULAR TRAPS RELEASE BY KLEBSIELLA PNEUMONIAE IN HUMAN NEUTROPHILS**

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Klebsiella pneumoniae (Kp) carbapenemase (KPC)-producing bacteria are a group of emerging highly drug-resistant Gram-negative bacilli, causing infections associated with significant mortality, especially in immunocompromised patients. Neutrophil (PMN)-mediated response is essential for fighting bacterial infections. The release of neutrophil extracellular traps (NETs) is a major immune mechanism intended to capture and destroy pathogens. Since the evasion of NETs may be an advantage for pathogen proliferation and dissemination, our aim was to investigate whether Kp was able to modulate NETs formation in human PMN. Therefore, we determine NETosis on purified PMN in response to a Kp-KPC-producer strain (Kp-KPC), and used Escherichia coli (Eco) for comparison. PMN-bacteria were incubated for 3 hours in a 1:10 ratio. NETs were observed and quantified using confocal microscopy after DNA and Elastase staining. Additionally, NETs-associated Myeloperoxidase (MPO) and DNA were released by nuclease treatment and measured in the supernatants of PMN-bacteria co-cultures. PMN failed to produce NETs when challenged with Kp-KPC, while Eco was a potent NET inducer (NETs area, μM^2: Ctrl=1094±698, Kp-KPC=1480±845, Eco=20222±7854*; Released DNA, ng/mL: Ctrl=217±26, Kp-KPC=327±39, Eco=631±77*; Released MPO, O.D: Ctrl=0.13±0.02, Kp-KPC=0.11±0.03, Eco=0.96±0.15*, *p<0.05 vs. Ctrl and Kp-KPC; n=8). This data was in line with PMN-bacterial killing, determined by colony forming unit (CFU) quantification after 3 hours of PMN-bacteria co-cultures (% bacterial survival: Kp-KPC=139±21, Eco=32±5, p<0.05; n=4). Moreover, Kp-KPC was able to decrease NETosis induced by other stimuli, such as glucose oxidase (GO) or Eco itself (GO, NETs area, μM^2: GO= 37485±7992, Kp-KPC+GO=16644±4926; Released DNA, ng/mL: GO=341±37, Kp-KPC+GO=167±34, p<0.05; n=5) (Eco: NETs area, μM^2: Eco=27018±6223, Eco+Kp-KPC=7051±1324; Released DNA, ng/mL: Eco=387±50, Eco+Kp-KPC=144±13; Released MPO, O.D: Eco=1.5±0.1, Eco+Kp-KPC=0.8±0.1; p<0.05; n=5). Our results indicate that Kp is able to subvert one of the most relevant bactericidal mechanisms of PMN, NETs formation, and this can be related to a higher survival of Kp compared to other bacteria.

694. (160) **THE MICROENVIRONMENT OF M. TUBERCULOSIS INFECTION MODULATES THE METABOLIC PATHWAYS OF M1 MACROPHAGES**

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Since metabolic pathways regulate macrophage biology, they may represent a target for pathogens to circumvent this leukocyte's effector functions. Macrophage activation towards the pro-inflammatory and microbicidal (M1) program is accompanied by a metabolic shift towards glycolysis and away from oxidative phosphorylation (OXPHOS), a switch that is governed by HIF-1a. Herein we hypothesize that the microenvironment generated during infection with Mycobacterium tuberculosis, the etiological agent for tuberculosis (TB), leads to changes in the metabolic pathways of M1 macrophages and impairment of microbicidal activity. Our approach was to activate M1 human macrophages using IFN-γ/LPS in the presence of the acellular fraction of tuberculous pleural effusions (PE), a bona fide TB-associated microenvironment. We found that the release of lactate, the final glycolysis product, augmented in M1 macrophages, is reduced in the presence of tuberculous PE ($p<0.05$) compared to cells exposed to PE from other etiologies (e.g., cancer, pneumonia). Likewise, HIF-1a levels were also reduced in PE-treated M1 macrophages ($p<0.05$). Based on this, we inferred that HIF-1a stabilization reverts the effect of tuberculous PE. However, the addition of Prolyl Hydroxylase Inhibitors (HIF-1a protein stabilizers) did not revert the levels of HIF-1a protein or lactate release in tuberculous PE-treated M1 macrophages, suggesting that the loss of HIF-1a is not due to proteolysis. Instead, we found that HIF-1a mRNA levels were diminished in tuberculous PE-treated M1 macrophages ($p<0.05$). Additionally, these cells displayed an increased mitochondrial respiration, estimated as OXPHOS-associated oxygen consumption, compared to non-conditioned M1 macrophages ($p<0.05$). Tuberculous PE also promoted lower pro-inflammatory functions in M1 macrophages including reduced production of mitochondrial radical oxygen species ($p<0.05$) and IL-1β ($p<0.05$), as well as a poor capacity to control the bacillary load ($p<0.05$). In conclusion, we demonstrate that a TB-associated microenvironment alters the metabolic reprogramming of M1 macrophages resulting in an impaired ability to control M. tuberculosis infection.

695. (185) **ROLE OF IL-10 AND ITS RELATIONSHIP WITH GLUCOCORTICOIDS IN DIFFERENT ENDOTOXIC CONTEXTS THAT RESEMBLE TO THE SEPSIS PHASES**

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In sepsis caused by Gram-negative infections the membrane lipopolysaccharides (LPS) have a central role, initially inducing an exacerbated production of pro-inflammatory cytokines followed by an anti-inflammatory state known as endotoxin tolerance (TOL). The inflammation resolution and TOL induction involves effectors as IL-10 and glucocorticoids (GC). The aim of this study was to evaluate the role of IL-10 in the pro/anti-inflammatory events induced by LPS and its relationship with GC. For this, wild-type (WT) BALB/c and IL-10 knockout (KO) mice were challenged with one doses of LPS (endotoxic shock) or inoculated daily with different doses of LPS (TOL). Corticosterone levels were determined by RIA, cytokines by ELISA, histopathology by periodic acid Schiff (PAS). After LPS challenge, WT showed high levels of plasma IL10 whereas it was not detectable (nd) in KO mice (IL-10 (pg/ml): WT= 6374±1505; KO= nd; $p<0.001$). The corticosterone increased in both strains being this significantly higher in KO mice (corticosterone (pg/ml): WT= 1331±243; KO=3513±799; $p<0.001$)). Histological analyses revealed more tissue damage in KO mice after LPS challenge, exhibiting remarkable glycogen depletion by PAS staining in liver (PAS+ hepatocytes (% of staining /area): WT= 39.7 ± 2.7; KO=2.5 ± 0.5; $p < 0.01$). Tolerant WT mice showed an increase in the plasma IL-10 levels (IL-10 (pg/ml): WT TOL=70±16; WT=nd; $p<0.01$)), however was possible to establish tolerance in KO mice. Corticosterone levels during tolerance establishment showed a sustained increase in both strains being significantly higher in KO mice (corticosterone (pg/ml): WTTOL= 1725±218; KOTOL=9992±2492; $p<0.001$), correlating with a fasciculata zone increased versus WTTOL ($p<0.05$). However, when GC receptors were blocked by RU486 partially prevented the tolerance establishment only in KO mice reaching a mortality rate of 40%. Finally, our results demonstrate the importance of the complementary action between IL-10 and GC, which may help to understand the complexity in sepsis.

696. (203) **ROLE OF INNATE CD8+ T CELLS IN CANCER**

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Innate CD8+ T cells were discovered about 10 years ago. These cells have particular phenotypic features (CD44hi CD122hi CD49dhi) and exert cytotoxic activity through NKG2D without specific antigen recognition. Innate CD8+ T cells express high levels of the transcription factor Eomesodermin, known to be induced by IL-4, and low expression of T-bet. They rapidly produce IFNγ after IL-12 and IL-18 stimulation due to constitutive expression of their receptors. Moreover, an antitumor role of innate CD8+ cells has been recently postulated both in mice and human. Our experiments using murine tumor cell lines demonstrate that systemic expression of IL-12+IL-18 (by hydrodynamic injection of its cDNAs) significantly increased the number of innate CD8+ cells in SLO (spleen and lymph nodes) and attenuate tumor growth in OT-I mice compared to control group (injected with an empty cDNA) ($p<0.05$) suggesting that innate CD8+ T cells could exert tumor growth control in an Ag-independent manner. Accordingly, phenotypic analysis (based on the mentioned markers) demonstrate a significant increase in CD8+CD44hiNKG2D+ innate cells in SLO of OT-I mice ($p<0.05$). Our data demonstrate that the absence of IL-4 (IL-4 KO mice) did not alter the increased number of innate CD8+ cells in SLO after IL-12+IL-18 expression compared to WT mice demonstrating that generation of these population could be redundantly driven by other signals. Interestingly, IFNγ KO mice show both lower number and lower antitumor effects in OT-I mice after IL-12+IL-18 expression ($p<0.05$). This result suggests that IFNγ could be involved in both generation and cytotoxic mechanisms of these cells.

All together this data suggest that innate CD8+ cells have a critical role in tumor growth control by mechanisms that involve NKG2D and IFNγ and could represent an important antitumor mechanism against cancer cells when TCR specific immune response is bypassed by down-regulation of MHC-I expression in cancer cells.

697. (230) **EFFECT OF THE BRUTON TYROSINE KINASE (BTK)-INHIBITORS SPEBRUTINIB (CC-292) AND ACALABRUTINIB (ACP-196) ON MACROPHAGE´S PHENOTYPE AND FUNCTIONS.**

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Ibrutinib is a first-in-class Btk inhibitor used in the treatment of Chronic Lymphocytic Leukemia (CLL). Besides its effects on leukemic B-cells, ibrutinib also affects functions on T cells, NK cells and macrophages.Second generation Btk-inhibitors with higher selectivity have been developed and are being evaluated in clinical trials. Here we aimed to evaluate the effect of second-generation Btk inhibitors, spebrutinib and acalabrutinib, on macrophages´ phenotype and functions.

Macrophages were differentiated by culturing human monocytes with M-CSF. For M1 polarization GM-CSF+IFN-ɣ was used. Phagocytosis of CFSE labeled rituximab-coated CLL cells and M1/M2-associated markers were evaluated by flow cytometry. Glucose and lactate concentration in culture supernatants was determined using commercial kits and TNF-α secretion by ELISA. Statistical significance was determined using the Friedman test and the Dunn's post-test.

While we confirmed that ibrutinib reduces rituximab-coated CLL cells phagocytosis, we found that spebrutinib and acalabrutinib did not ($n=7$, $p<0.05$). We also found thatacalabrutinib impaired M1-polarization by up-regulating M2-associated markers(CD206, CD163, CD14 and CD16), by down-regulating M1-associated markers (CD86 and HLA-DR) and by affecting glucose metabolism, with a decrease in glucose consumption and lactate production ($n=6$, $p<0.05$). In contrast, we found that spebrutinib did not modify M1 markers while glucose consumption and lactate production were diminished ($n=6$, $p<0.05$). Finally, we found that ibrutinib and acalabrutinib, but not spebrutinib, significantly decreased TNF-α secretion in response to Pam3CSK4, LPS and irradiated-Mtb ($n=10$, $p<0.05$).

Our results suggest that spebrutinib, acalabrutinib and ibrutinib have different effects on macrophages, probably due to their differences in kinase-selectivity.Second-generation Btk inhibitors did not interfere with macrophage-phagocytosis of rituximab-coated CLL cells suggesting that they could be better options for combination strategies with anti-CD20 antibodies. Nevertheless, we found that acalabrutinib impaired M1 polarization and macrophage response to microbial-stimulation, which could have a detrimental effect on the immune system of treated patients.

698. (341) **HYALURONAN ACTION ON MONOCYTES/MACROPHAGES: A LINK BETWEEN TUMOR ANGIOGENESIS AND TSG-6 EXPRESSION LEVELS**

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Hyaluronan (HA) is a glycosaminoglycan able to modulate immune and angiogenic responses. At homeostasis, high-molecular-weight(HMW) HA is predominant whereas the low-molecular-weight(LMW) is present in inflammation. HA binds to several receptors as well as HA-binding protein, TSG-6. It is involved in ECM remodeling affecting HA function and inflammation. HA in tumor recruits associated-macrophages and regulates their angiogenesis action by unclear mechanisms.
Aim: To evaluate the TSG-6 levels and angiogenic behavior of human monocytes/macrophages (MO) preconditioned with HA (HMW or LMW) in breast and colorectalcarcinoma.
Methods: MDA-MB 231 breast or LoVo colorectalcarcinoma tumor lysates(TL) were prepared by freeze-thaw cycles. MO from PBMCs were pulsed with TL plus HA(20ug/ml) LMW($1,5x10^6$Da) or HMW($2x10^5$Da) for 24h. MO were characterized with CD14, HLA, CD80 and CD206 by flow cytometry. VEGF levels were evaluated by ELISA. IL-8, FGF-2 and TSG-6 expression levels were evaluated by RT-qPCR. TSG-6 was analyzed through western blot. For the xenograft mouse model MDA-MB-231 or LoVo cells were inoculated. After 9days, MO pulsed or/not with HA were inoculated sc within the tumor. Animals were euthanatized at day 29 , tumors were fixed and stained with: i)Lectin GSLI-FITC for vasculature detection and ii)HA-binding protein and Ab-anti-TSG-6.
Results: HA treatments did not modify the expression of MO cell surface markers. MO treated with MDA TL plus HA-HMW: i)VEGF(329,5±12,79pg/ml), IL-8(NRQ:5,761±1,461) and FGF-2(NRQ:2,972±0,8020) levels increased and ii)TSG-6(NRQ:5,860±2,711) mRNA levels diminished significantly($p<0,05$). However, these factors showed no significant difference in MO treated with LoVo TL with/without HA. Mice inoculated with MO plus HA-HMW increased its vasculature(1,759±0,1173AU) and diminished TSG-6(1,820±0,4308AU) in MDA model. While in the LoVo model no differences were found among treatments.
Conclusion: HA-HMW modulates MO angiogenic behavior and TSG-6 levels in breast carcinoma, but not in colon carcinoma. Our results indicate that MO HA- modulation depends of its molecular weight but also tumor factors, as TSG-6.

699. (410) **CD32 EXPRESSION PROMOTES T CELL ACTIVATION IN SEVERELY RSV-INFECTED CHILDREN**

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Background: Respiratory syncytial virus (RSV) is the leading cause of bronchiolitis in young children worldwide. CD32 is a low-affinity Fc receptor with specificity for IgG antibodies commonly expressed on most myeloid cells. Preliminary studies of our group have shown an activating role for CD32 in CD4+ T cells from healthy adults. Here we aim to investigate the expression and function of CD32 in CD4+ T cells from RSV-severely infected children.Specific aims: 1- To analyze the expression of CD32 on CD4+ T cells from RSV-infected. 2- To examine whether CD4+ T cells bind aggregated IgG. 3 - To determine whether the expression of CD32 is associated with phenotypic markers. 4- To explore the biologic response of CD4+ T cells upon ligation with anti-CD32 antibody.Results: We observed an increased frequency of CD32+CD4+T cells in RSV-infected (16%±1.2; n=44) compared with healthy infants (5%±0.5; n=8), $p<0.0001$. Consistent with this finding, we also found that resting CD4+ T cells bind aggregated IgG (2%±0.2; $p<0.0001$) that it is significantly reduced in the presence of a blocking anti-CD32 antibody (1%±0.4; $p<0.05$). Moreover, CD32 expression preferentially occurred on CD4+ T cells displaying activation markers (CD25, HLA-DR and Tim-3). When purified CD4+ T cell were stimulated with anti-CD32, we observed that most RSV-infected children (n=13) showed an increased frequency of Ki67+CD4+T proliferating cells (27.2%±4.2 vs 20.1%±3.8; $p<0.001$). However, a small group of patients (n=4) noted a decreased frequency of Ki67+CD4+T cells (p NS). Interestingly, we detected large amounts of IL-2 in the supernatant culture of CD4+ T cells upon CD32 stimulation from all patients included ($p<0.001$). Data represent mean (%) ± SEM of n donors.Conclusions: Our observations indicate that RSV infection induce the up-regulation of CD32 in CD4+ T cells modifying their function. Further studies are needed to characterize the inhibitory or activating profile of CD32+.

700. (431) **EFFECT OF BLS IN THE MURINE BREAST ADENOCARCINOMA 4T1 MODEL**

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Brucella lumazine synthase (BLS) is a stable homodecameric protein. BLS signaling via Toll-Like Receptor 4 (TLR4) regulates innate and adaptive immune responses, inducing dendritic cell maturation and CD8+ T-cell cytotoxicity. In the B16 melanoma model, BLS delays tumor growth via tumor TLR4 when administered at day 2. In this work, we studied the effect of BLS in the metastatic breast adenocarcinoma 4T1 model. Firstly, we characterized the cell line evaluating the expression of TLR4 in 4T1 cells by flow cytometry. Lower levels of TLR4 were detected on 4T1 cells compared to B16 cells. Different growth curves were analyzed by inoculating different amounts of 4T1 cells s.c. (5,105-1,106 cells) into the mammary gland. Metastases and lung damage were observed in all mice, however tumors were not measurable. In order to evaluate if BLS has an effect in tumor growth, 5x104 4T1 cells were s.c. inoculated in the right flank and 200μg of BLS was administered at day 2. Tumor growth is not affected by BLS. At day 21 tumors were analyzed by flow cytometry and lungs were examined. Lung metastases were seen in both groups. Furthermore, lungs from mice treated with BLS show more damage and reduced size. Regarding to the tumor microenvironment, a higher percentage of CD45+ cells (4,130%±1,498 vs 42,21%±39,27; $p<0.005$) is detected in tumors from BLS treated mice compared to controls. Within this population, BLS increases the percentage of CD4+ (7,753%±1,559 vs 27,73%±21,66, $p<0.05$), but not CD8+ cells, contrary to what we have described in the B16 model. These results show that BLS does not affect 4T1 tumor growth but enhances lung metastases and damage. Moreover, BLS increases tumor infiltrating lymphocytes without any therapeutic effect. This may be due to the lack of higher levels of CD8+ cells in the tumor microenvironment

701. (492) **EPAC2 AND PROSTAGLANDIN-E2 SINTASA INVOLVEMENT IN GLUCOCORTICOID STEROIDOGENESIS DURING EXPERIMENTAL TRYPANOSOMA CRUZI INFECTION**

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The hypothalamic–pituitary–adrenal (HPA) axis is a dynamic system that regulates glucocorticoid (GC) synthesis in the adrenal glands. The classic GC-releasing reflex is mainly mediated by ACTH. Many key factors, such as cytokines, prostaglandin-E2 (PGE2) and Epac2 have been identified as intra-adrenal enhancers of steroideogenesis independently of systemic ACTH levels. However, little is known about how these factors collectively influence GC synthesis during Trypanosoma cruzi (Tc) infection.
Previous studies in Tc-infected mice showed after 14 days post-infection (dpi) increased circulating levels of GC, while ACTH remains unchanged. At the same time, intra-adrenal expression of pro-inflammatory cytokines is augmented.
In this study, we decided to evaluate the ACTH-dependent and independent pathways of GC synthesis during Tc infection. Thus, we determined at different dpi the expression of PKA-p/PKA (as a measure of ACTH-dependent pathway activation) and also PGE2-synthase (PGE2-S) and Epac2 as ACTH-independent modulators of adrenal steroideogenesis. C57BL/6 mice were infected with Tc or inoculated with saline (Co). Data are showed as mean±SEM (n=3-5/day/group). Assessments were made between 10 to 21 dpi. The PKA-p/PKA ratio was increased only at 11 dpi [Western blot, PKA-p/

PKA: Co=1±01; Tc(10dpi)=1.1±0.1; Tc(11dpi)=2.4±0.7*; Tc(13dpi)=1.0±0.2; Tc(14dpi)=1.1±0.2; Tc(16dpi)=0.8±0.1; *p<0,05 vs Co]. Tc mice displayed significantly intra-adrenal elevation of PGE2-S after 11 dpi [RT-qPCR, PGE2-S: Co=7.8±1.2; Tc(10dpi)=13.1±5.4; Tc(11dpi)=29.1±14.5*; Tc(13dpi)=22.4±1.9*; Tc(14dpi)=31.7±6.6*; Tc(19dpi)=47.7±17.7*; *p<0,05 vs Co]. Moreover, Epac2 increased after 14dpi (RT-qPCR and Western, p<0,05 vs Co), decreasing after 16dpi. In parallel, no statistical differences were observed regarding IL-1R expression (Western blot and RT-qPCR).
These results suggested that Tc infection activates both steroidogenic pathways, resulting in a remarkably increased GC production. Also, our results suggest that early in the course of infection, GC secretion may be triggered by the ACTH-mediated response, but in the late phase of acute infection, GC secretion may be sustained by other factors including PGE2 and Epac2.

702. (621) **COMESAL RESPIRATORY BACTERIA DOLOSIGRANULUM PIGRUM 040417 BENEFICIALLY MODULATES IMMUNE RESPONSE MEDIATED BY ACTIVATION OF TLR3**

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The human commensal respiratory bacterium *Dolosigranulum pigrum* 040417 (DP) increases the resistance of infant mice to Respiratory Syncytial Virus infection. The immunological mechanisms involved in the beneficial effect of DP were not investigated before. In this work, the effect of DP on the innate antiviral respiratory immune response triggered by TLR3 activation was investigated. Infant BALB/c mice (3-week-old) were nasally treated with 10^8 DP cells for two consecutive days. On the third day, treated mice were nasally challenged with poly(I:C) (250 µg/mouse) for three consecutive days. Untreated control mice were challenged with poly(I:C) similarly. Lung damage and the respiratory and systemic immune response were studied two days after the last poly(I:C) administration. TLR3 activation induced a marked lung damage that was accompanied by pro-inflammatory factors production and inflammatory cells recruitment into the respiratory tract. However, lung tissue injury was significantly lower in DP-treated mice. Lower levels of albumin concentrations and LDH activity (control=101.9±4.1, DP=67.3±2.5 UI/ml) were found in the bronchoalveolar lavage (BAL) of DP-treated mice compared to controls, indicating a lower alteration of the alveolar-capillary barrier and reduced cellular damage. Poly(I:C) increased neutrophils and macrophages numbers in the lung, and TNF-α and IL-6 in serum and BAL in both experimental groups. However, the DP group had significantly higher levels of TNF-α (control=115.6±3.2, DP=163.1±2.9 pg/ml) and IL-6. DP also increased lung $CD3^+CD4^+IL\text{-}10^+$ T cells and $CD11c^+SiglecF^+IFN\text{-}\beta^+$ alveolar macrophages, with the consequent increases in respiratory levels of IL-10 (control=379.1±12.1, DP=451.4±11.5 pg/ml) and IFN-β (control=121.7±3.1, DP=152.9±3.4 pg/ml). No differences were observed between the groups when evaluating $CD3^+CD4^+IFN\text{-}\gamma^+$ T cells or IFN-γ levels. These results suggest that nasal DP administration differentially modulate the respiratory immune response triggered by TLR3 activation, improving antiviral immunity and reducing inflammatory damage.

703. (641) **INTERLEUKIN-10 PRODUCTION AND DNA POLYMORPHISMS IN PATIENTS WITH HEMOLYTIC UREMIC SYNDROME.**

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Development of Hemolytic Uremic Syndrome (HUS) is associated with Shiga toxin and inflammatory response. However, anti-inflammatory mechanisms could also be triggered to limit inflammation in vivo, such as production of the regulatory cytokine IL-10. Individual differences in IL-10 secretion associated with IL10-promoter single-nucleotide polymorphisms (SNP), such as rs1800896 (Legacy notation: -1082A>G), in which A- and G-alleles show low and high IL10 expression, respectively. The aim of this work was to evaluate the production of IL-10 and the specific SNP -1082A>G in patients with HUS.
Blood samples were collected from HUS patients during the acute period and from Healthy Children (HC). Plasma and mononuclear cells (MNC) were isolated, and MNC were cultured with Medium or LPS (100ng/ml) for 20h. In parallel, absolute number of Monocytes (Mo) in MNC was calculated by flow cytometry. Protein expression of IL-10 was evaluated in plasma and MNC-supernatants by ELISA. DNA was obtained from MNC and IL10 SNP -1082A>G analysis was performed by allele specific-PCR (as-PCR) using a PCR-control template on CTLA4.
Plasmatic IL-10 concentration was increased in HUS patients (Mean±SEM (pg/mL): HUS=270.9±117.8*, HC=82.1±7.1, n=15, *p<0.05). The absolute number of Mo from HUS and HC was calculated (Mean±SEM= (0.12±0.03/0.07±0.02).106 Mo/mL). However, the levels of IL-10 secreted by MNC of both groups were similar after LPS stimulation (Mean±SEM (pg/mL: Basal/LPS (HUS=235±100/6470±1056*; HC=189±70/7630±1959*, n=5, *p<0.05 vs Basal). HUS(HC) DNA samples (n=8(8)) showed genotypes: [A/A]=2(4), [G/G]=1(0), [A/G]=5(4), These preliminary results show that plasmatic IL-10 is increased in HUS during acute period. Monocytes in MNC population suggests a tendency to be higher in HUS than HC according to previous reports, however preliminary results show similar IL-10 production. Although carrying the at-risk-allele [G] of IL10 SNP rs1800896 prefigures about twice risk of HUS vs HC, larger populations are needed to confirm this association.

704. (651) **PROTEOME ANALYSIS OF HUMAN BRONCHIAL EPITHELIAL CELLS INFECTED WITH BORDETELLA PERTUSSIS.**

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Bordetella pertussis (*Bp*), the causative agent of whooping cough, survives within host cells potentially contributing to its ability to persist within hosts and populations. Pertussis toxin (PT) plays a major role in the modulation of macrophage responses and intracellular survival. The airway epithelium is also considered central to the orchestration of the immune responses, yet their responses to *Bp* have not been fully investigated. In this study, we examined the molecular responses of the human bronchial epithelial cell line 16HBE14o- to *Bp* infection by shotgun proteomics. To this end, 16HBE14o- cells were infected with *Bp* wild type (wt) or an isogenic mutant lacking PT (ΔPT) during 4.5 h and 8 h at a multiplicity of infection of one and the host response was compared to a similarly treated uninfected control. Among 2425 identified proteins, the abundance levels of 745 proteins were significantly altered by *Bp* infection compared with the control (p<0.05). GO biological term and pathways enrichment analysis of the proteins displaying altered levels by the DAtabase for Visualization and Integrative Discovery (DAVID) revealed a significant enrichment of proteins involved in cell adhesion mediated by integrin, phagosome, AMPK signaling pathway, receptor internalization, and regulation of immune response, among others. A role of PT in the up-regulation of integrin mediated cell adhesion and in the regulation of immune response was observed. PT was found involved in the upregulation of VLA-5, an integrin implicated in *Bp* invasion, suggesting that *Bp* promote its own internalization. Moreover, proteins related with the biological process antigen processing and presentation showed a decreased abundance after ΔPT infection compared to wt infection, suggesting that PT influences the efficiency of a T cell-mediated immune response. Overall, this study describes for the first time human protein alterations induced by *Bp* infection, thus providing new clues for understanding pertussis pathogenesis.

705. (659) **INNATE IMMUNE EVASION MECHANISMS OF A HYPER-EPIDEMIC CLONE OF KLEBSIELLA PNEUMONIAE RESISTANT TO CARBEPENEMS**

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The emergence and rapid dissemination of *Klebsiella pneumoniae* (Kpn) carbapenem resistant has become a relevant problem in health care units, because it has been associated with higher mortality in susceptible patients, mainly attributed to Kpn Carbapenemase (KPC), an enzyme that hydrolyze carbapenems, one of the last resources in antibiotic treatment. As Kpn-KPC producers have been successful in terms of dissemination and persistence, we ask if Kpn-KPC could express mechanisms to avoid the innate immune response, focusing on their interaction with neutrophils (PMN). In this sense, we evaluated if one local isolate of Kpn-KPC, sequence type 258 (a hyper-epidemic clone), induces a differential response in healthy human isolated PMN, compared to another opportunistic pathogen as *Eschericia coli* ATCC 25922 (Eco). After evaluating some parameters of PMN activation, no differences were observed in the up-regulation of CD11b expression. However, reactive oxygen species (ROS) generation, measured by flow cytometry using dihidrorhodamine-123 (DHR) was lower for Kpn-KPC (ROS, % DHR+ PMN: Kpn-KPC=5.2±0.1; Eco=39.8±11.2, p<0.01). PMN chemotactic migration was also diminished (N° of PMN migrated: Kpn-KPC=25±2; Eco=45±4, p<0.05) and Kpn-KPC was more resistant to PMN-mediated killing after 1-hour incubation (%Bacterial Survival: Kpn-KPC=88.8±4.3; Eco=31.3±7.0, p<0.05). When phagocytosis was evaluated using 5% fresh human serum, the percentage of phagocytosis for Kpn-KPC was lower compare with Eco (% phagocytosis: Kpn-KPC=31±2.1; Eco=57±2.6, p<0.05). In line with this result, the percentage of bacteria that binds C3 determined by flow cytometry and the percentage of complement-mediated killing was lower for Kpn-KPC, compared with Eco (p<0.05), after 30 min of incubation with fresh human serum. Our results revealed that Kpn-KPC ST258 showed a survival advantage compared to Eco, by poorly triggering PMN responses and by escaping complement-mediated killing. In conclusion, our results suggest that Kpn possess bactericidal evasion mechanisms that could be a potential advantage for the dissemination/persistence of Kpn infections.

706. (694) **PRELIMINARY STUDY OF PRODUCTION AND IMMUNOMODULATORY ACTIVITY OF POLYSACCHARIDES DERIVED FROM WOOD DECAY FUNGI OF PATAGONIA**

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Many species of fungi have medicinal properties. Among bioactive compounds of these organisms, polysaccharides (PS) can modulate the immune system. Several species from native forests of Patagonia may represent candidates to produce bioactive PS. Our aim was to evaluate PS production from Ganoderma australe, Inonotus crustosus and Laetiporus portentosus; and assess PS ability to activate mice dendritic cells (DCs), which drive adaptive immune responses. G.lucidum was used as reference species. Fungi were cultivated in different liquid culture media (AG, M1, M2) and their ability to produce extracellular PS (exo-PS) and PS from mycelium (structural-PS and basic-PS) was analyzed. Structural-PS production was higher than basic-PS and exo-PS production in all species. Ganoderma species were the best structural-PS producers. G.lucidum cultured in AG produced the highest amount of structural-PS (p<0.05), but regarding μg PS/mg mycelium, all species had a similar performance. In M2, G.australe produced the highest levels of structural-PS (p<0,0001) and showed the highest ratio μg PS/mg mycelium (p<0.001). In M1, G.australe and L.portentosus performed better than the rest of the species. Regarding PS production in different media, G.lucidum and I.crustosus showed the highest production, in total mass (p<0.005) and in μg PS/mg mycelium (p<0,0001) in AG, whereas in the other species the production was similar in all media. Considering that Ganoderma species produced the highest structural-PS levels, these fractions were tested in their ability to activate DCs. Mice bone marrow derived DCs were exposed to PS (10-300ng/ml) for 18-48h (activation control: lipopolysaccharide). Preliminary results indicated that PS from G.australe in M1 increased IL-12 production from DCs (vs basal) at 24h (p<0,05), CD86 at 18-24h (p<0,0001), and the expression of the maturation marker MHCII at 48h (p<0,0001), all parameters in a concentration-dependent manner. This is the first study reporting the potential ability of structural-PS from G.australe to activate DCs.

707. (696) **MULTIVARIATE STUDY OF IMMUNOLOGICAL FACTORS IN PATIENTS WITH GENITAL INFECTIONS AND ITS RELATIONSHIP WITH INFERTILITY**

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Chlamydia trachomatis, *Mycoplasma hominis* and *Ureaplasma urealyticum* genital infections are frequent among men and women and often associated with infertility. Our aim was to study immune factors induced by these infections and their relation to infertility. The expression of cytokines (IFNγ, TNFα, IL-17A, IL-6, IL-8, TGFβ1, IL-10) and master transcription factors (MTF: Tbet, RORγt, GATA3, FOXp3) were analysed by RT-qPCR in vaginal fluid and semen of: infertile female patients with infection (IWI-f, n=30), and without infection (IWoI-f, n=14), fertile women without infection (FWoI, n=17), infertile men with infection (IWI-m, n=15) and without infection (IWoI-m, n=19). A principal component analysis of the expression levels of cytokines and MTF was made. Sixty to 87% of the total variance was represented by biplot graphs. This analysis showed that 76% of the FWoI-f and 57% of the IWoI-f were associated to low levels of cytokines. However, 36% infertile women were associated with moderate to high levels of IFNγ, IL-17A, TNFα and IL-10, and 57% with MTF in study. FWoI-f and IWoI-f groups showed a positive association between IL-6 and TGFβ1, significant for IWoI-f (p^c0.05). Thirty-one percent of the IWI-f *Mh* was associated to high IL-8 levels, while another 31% was associated to moderate to high IFNγ expression. An increased number of IWI-f with *Ct* or *Uu* patients had high levels of cytokines and MTF, without a trend to particular profile according to the infection. Regarding men, 58% from the IWoI-m group had low levels of cytokines. Contrarily, 33% of the IWI-m was associated to IFNγ, and another 33% with IL-6. In all groups, IL-10 and IL-17A were positively associated, significant for FWoI-f (p^c0.001), IWoI-f (p^c0.01), IWI-f *Ct* (p^c0.05), IWoI-m (p^c0.01) and IWI-m *Uu* (p^c0.05). These findings demonstrate that genital infections disrupt the normal balance of cytokines and in some cases could compromise fertility.

708. (697) **INCREASED SERUM LEVELS OF GALECTIN-1 DURING HIV INFECTION PROMOTES VIRAL RELEASE AND LATENCY REACTIVATION.**

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Galectin-1 (Gal-1) is an endogenous lectin with important immunomodulatory functions acting on both innate and adaptive immune cells. HIV-1 infects manly CD4+ T lymphocytes and macrophages causing CD4+ T cell depletion, ultimately leading to acquired immunodeficiency syndrome (AIDS). Implementation of combination antiretroviral therapy (cART) reduces viral replication and CD4+ T cell loss, thus significantly improving the prognosis of HIV-infected individuals. Nevertheless, chronic inflammation and the maintenance of latently infected cells-viral reservoir- represent two major barri-

ers to achieve the cure of HIV infection. Herein, we analyzed the role of Galectin-1 in HIV-1 replication and latency reactivation. We show that in vitro infection of CD4+ T cells induces the expression and secretion of Gal-1. In addition, we demonstrate that HIV-infected patients have increased serum levels of Gal-1 as compared to healthy donors ($p<0.001$) independently of their CD4+ T cell count and viral load. Fractionation of sera from HIV-infected individuals by size exclusion chromatography revealed that an important proportion of circulating Gal-1 is associated with extracellular vesicles. In vitro treatment of macrophages with serum-derived extracellular vesicles induces the secretion of Gal 1 as well as a proinflammatory profile, as evidenced by the secretion of IL-6 ($p<0.01$). Remarkably, we show that extracellular Gal-1 reverses HIV latency in latently infected Jurkat T cells (J-LAT), suggesting a role of Gal-1 in reservoir dynamics. Moreover, we observed that binding of extracellular Gal-1 at sites of viral assembly in HIV-infected cells promotes HIV release and cell-to-cell transmission. Altogether, our results show that during HIV infection there is an increase in circulating Gal-1, which may play a role in viral replication and in the reactivation of reservoirs in HIV-infected patients undergoing cART treatment.

709. (700) **VIP CONTRIBUTION TO SUSTAIN IMMUNETOLERANCE DURING THE PERI-IMPLANTATION PERIOD: FOCUS ON TREGS RECRUITMENT**

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Uterine receptivity and embryo implantation are two main processes that need a finely regulated balance between pro-inflammatory and tolerogenic mediators in order to allow a successful pregnancy. The immunopeptide VIP is a key regulator and is involved in the recruitment of regulatory T cells (Tregs) which are crucial in both periods. Here, we analyzed, using two mice models, the relevance of VIP associated with the recruitment of Tregs.

During the day of estrous we found histological differences between the uterus of WT mice vs VIP-KO mice (-/-) or VIP deficient mice (+/-) accompanied with undetectable expression levels of Foxp3, higher expression of RORγt and a decrease in IL-10 and VEGFc detected by RT-PCR ($p<0.05$ Mann-Whitney).

To study the implantation process we mate Foxp3-knock-in-GFP females with WT males and found at 4,5d a peak in Tregs in the uterus and draining lymph nodes. At 5,5d we found high levels of VEGFc that were significantly lower in VIP (+/-) ($p<0.05$ Mann-Whitney). Therefore, to study Tregs effects and trafficking in VIP (+/-), we performed adoptive transfer of Tregs (sorted from Foxp3-GFP females) to VIP (+/-) that haven't got pregnant during 6 months. Foxp3-GFP cells were mainly recruited into the uterus in relation to all other tested tissues accompanied with an increase in IL-10 expression ($p<0.05$ Mann-Whitney). Finally, we performed ex vivo migration assays using CD4+ sorted cells towards conditioned media from WT-explants or VIP (+/-) at 5.5d cultured ± VIP or ± VIP-antagonist for 24hs. VIP induced an enrichment of CD4+Foxp3+ and a decrease in CD4+ cells and the antagonist prevented this effect.

In conclusion, VIP may contribute to the selective recruitment of Tregs to the uterus during the estrous cycle and in embryo implantation contributing to a successful pregnancy.

710. (708) **IN VIVO ROLE OF IFN-Y-MEDIATED SKIN IMMUNE RESPONSE IN EXPERIMENTAL DERMATOPHYTOSIS IN IL-17RA DEFICIENT MICE**

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Microsporum canis is a dermatophyte fungus that causes superficial infections and is highly prevalent among immunocompetent children. Previously, we demonstrated that *M. canis* induces a type 17 immunity *in vivo* that controls fungal proliferation in skin and down-modulates an antigen-specific IFN-γ response.

Objective: To evaluate the role of IFN-γ in the experimental dermatophytosis outcome in absence of IL-17RA signaling.

Wild type (WT) and IL-17RA-deficient C57BL/6 (KO) mice were epicutaneously infected with *M. canis* hyphae. On different days post-infection (dpi), histopathology, CD11b+Ly6G+ population (neutrophils, FACS), cytokine analysis (ELISA, FACS) and fungal burden (colony forming units/ g skin) were determined in epidermis. For IFN-γ blocking, anti IFN-γ antibody (BioXCell, 400 μg/mice) or isotype control (IC) was injected (intraperitoneally) on 3 and 6 dpi.

We observed that by 6 and 8 dpi, infected KO showed an increase in fungal burden ($p<0.01$), neutrophil recruitment ($p<0.0001$), CXCL1 expression ($p<0.05$) and TNF ($p<0.002$) in the skin, compared to WT. After IFN-γ blocking in KO and by 8 dpi, unexpectedly, there was a reduction in fungal burden ($p<0.005$ vs WT; $p<0.05$ vs IC treated-KO) with a decrease in neutrophil infiltration ($p<0.0001$) and TNF production ($p<0.001$ vs WT, $p<0.0001$ vs IC-KO). In contrast, these mice showed an increased production of IL-17 pathway-related cytokines such as IL-23 ($p<0.001$ vs WT and IC-KO) and IL-22 ($p<0.005$ vs WT, $p<0.05$ vs IC-KO), among others, by epidermal cells.

Our results suggest that, in the absence of IL-17 signaling, there is a deregulated Th1 response that promotes M. canis skin infection.

711. (712) **IMMUNOHISTOLOGICAL EVALUATION OF ADJUVANT TREATMENT WITH LACTOBACILLI IN DIABETIC FOOT**

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Introduction: Patients suffering from Diabetes Mellitus (7.8% of the population) frequently present with infected diabetic foot ulcers (15%). Surgical debridement and antibiotic therapy is the conventional treatment. Its relative efficacy requires the testing of adjuvant treatments. In our hospitals, this treatment is consistent in the topical application of Lactobacillus plantarum cultures. The treatment decreases the bacterial load and promotes the generation of granulation and epithelial tissue. The histological modifications are being studied and in this work we focus on the semiquantification determination and its topographic connections of the two phenotypes of infiltrating macrophages M1 and M2 in the lesion.

Material and methods: Biopsies of Grade II and III diabetic foot ulcer of Wagner, from patients attending the Department of Traumatology of the AC Padilla hospital (Tucumán), treated with conventional treatment with (n = 14) and without (n = 8) adjuvant with Lactobacillus. Biopsies were taken at 10 and 20 days.Histopathological study: routine staining H-E. Masson's trichrome stain (collagen). Immunohistochemistry to quantify vascular area, number of vessels and endothelial cells (CD34 +), anti-actin and semi-quantify (score 0 to 3) Macrophages M1 (CD68 +) and M2 (CD 163+).

Results: in the biopsies of the patients treated with Lactobacillus plantarum we observed an increase in the collagen deposit, in the number of endothelial cells ($p<0.01$), and vascular area ($p<0.05$). Higher density of M1 completely surrounding each vascular element. M1 and M2 showed a diffuse distribution. However, greater disposition in the M1 band was observed in the bed of the ulcers, in early biopsies.

Conclusions: the adjuvant treatment promotes an intermediate quality granulation tissue. The M1 macrophages have a higher diffuse density. Its activity would be related to eliminating the infection and promoting angiogenesis making it possible to switch to M2 to initiate

the process of tissue repair.

712. (719) **CURCUMIN LOADED POLY LACTIC-CO-GLYCOLIC ACID NANOPARTICLES AS AN ANTIVIRAL FORMULATION AGAINST ZIKA VIRUS**

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Antiviral drugs in current use target only viral proteins; are, generally, specific for each type of virus and frequently induce the rapid emergence of resistant mutants, a particularly serious problem for RNA virus therapy due to its high mutation rate. The development of drugs that affect host factors necessary for viral multiplication that involve cellular pathways has proven to be attractive for chemotherapeutic intervention against viruses of the same genus, family and even unrelated, since it is not expected that the individual viral mutations compensate for the loss of a required factor of the host. One mechanism to control rapidly replicating viruses is to manipulate the levels of cellular nucleoside triphosphate pools. Inosine-5′-monophosphate dehydrogenase (IMPDH) is an enzyme involved in the de novo synthesis of guanine nucleotides. Inhibitors of IMPDH such as ribavirin, mycophenolic acid and merimepodib have shown activities against Chikungunya, Junín, Lassa, several flavivirus members and Ebola viruses. Other authors found that several polyphenols showed inhibitory effect on IMPDH, in particular, curcumin was the more active and exerted a competitive and uncompetitive actions to suppress the IMPDH activity. In the present work, the in vitro antiviral effect of curcumin against Zika virus was studied. Firstly, the toxicity was determined for 48 h in monkey Vero cells by de MTT assay. In order to minimize cytotoxicity and maximize the curcumin cell delivery, curcumin loaded poly lactic-co-glycolic acid (PLGA) nanoparticles were synthetized. No cytotoxic effects were observed until 150 μM. Through a viral yield inhibition assay, a dose-dependent viral replication inhibition was observed in a 0-100 μM curcumin concentration range. Our previous reports with other inhibitors and RNA virus, plus these results show that IMPDH is a promissory antiviral target to control pathogenic viral infections and curcumin loaded PLGA nanoparticles may be a good candidate drug formulation.

713. (752) **PATHOGEN-SPECIFIC T CELLS FROM PATIENTS WITH INFLAMMATORY BOWEL DISEASES CAN BE MODULATED BY PROBIOTICS FROM KEFIR**

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Kefir is a fermented milk with health-promoting properties. Lamina propria T cells (LPTC) play a central role in the pathogenesis of Inflammatory Bowel Diseases (IBD). These cells contribute to mucosal inflammation by secreting pro-inflammatory cytokines and being resistant to apoptosis. We aimed to modulate the proliferation and the pro-inflammatory response of pathogen-specific LPTC from IBD patients using microorganisms from kefir.
Colonic biopsies from IBD patients were incubated with probiotics from kefir and cytokines in supernatants were evaluated by ELISA afterwards. LPTC were isolated from biopsies (N=23; 7 from Crohn's Disease patients-CD and 15 from ulcerative colitis-UC patients) by collagenolytic digestion. In order to expand pathogen-specific T lymphocytes, cells were cultured with enteroadhesive (EA) *Escherichia coli* extract and IL-2 for 10 days. Thereafter, LPTC were incubated with anti-CD3/anti-CD28 and with microorganisms from kefir (*Enterococcus durans* and *Lactobacillus kefiri*), their conditioned media or 10 μM lactate for 96 hs. LPTCs proliferation (CFSE) and TNF-α, IFN-γ, IL-6, IL-10 and IL-13 cytokine secretion were evaluated (ELISA).
We found that *E. durans* and *L. kefiri* diminished the pro-inflammatory cytokine production by inflamed tissues (IL-6: 1800±670 and 576±3 vs 6900±2000 pg/ml basal production, respectively; IL-8: 38000±11000 and 32000±7300 vs 80000±27000 pg/ml basal production, respectively). EA E. coli specific LPTC lines were developed from all patients. Cellular proliferation of activated CD4+/CFSE-/PI- LPTC decreased significantly with *L. kefiri* , *E. durans* and lactate ($P<0.01$, N=15), but not with conditioned media. Moreover, TNF-α ($P<0.05$, N=10), IFN-γ ($P<0.05$, N=11) and IL-6 ($P<0.05$, N=8) secretion decreased with the presence of probiotics, their supernatants or lactate. No significant differences were observed for IL-10 and IL-13.
Our results showed that probiotic strains from kefir and their metabolites modulated pathogen-specific activated T cells from IBD patients. These results could contribute to future therapeutic approaches for IBD.

714. (767) **EVALUATION OF TRITRICHOMONAS FOETUS INFECTION CLEARANCE IN HEIFERS IMMUNIZED WITH A SINGLE INTRAVAGINAL DOSE OF FORMALDEHYDE FIXED CELLS**

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Tritrichomonas foetus is a flagellated parasitic of the urogenital tract of cattle that causes a sexually transmitted disease characterized by embryonic and fetal losses. Vaccines against *T. foetus* have shown to reduce time of infection after natural or experimental challenge. Usually, vaccine strategies are three systemic doses reinforced by a vaginal booster. The object of this study was to assess protection against *T. foetus* infection conferred by fixed hole cells given in a single vaginal instillation. *Aberdeen Angus* virgin heiffers were randomly assorted into 3 groups of 12 individuals to receive placebo, fixed cells with formalin and fixed cells with freshly prepared formaldehyde solution, challenged six weeks later with 106T. foetus motile cells. The median clearance rates among control heifers was 93.75 days while in animals immunized with formaldehyde fixed cells was 45 days. A single vaginal dose of cells fixed with fresh formaldehyde solution showed a rate of infection decay per unit of time of 2.54 (CI 95%=1.07;6.01). Further *in vivo* studies are necessary to confirm our observations and potentially change the current thinking about *trichomonas* infection/protection.

715. (768) **IMMUNOMODULATORY PROPERTIES OF PROSTAGLANDIN E2 ON NEUTROPHILS DURING HUMAN TUBERCULOSIS INFECTION.**

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During *Mycobacterium tuberculosis* (*Mtb*) infection, neutrophils are among the first cells that migrate to the infectious focus and represent the most abundant cell population harboring *Mtb* in samples from patients with active tuberculosis (TB). Neutrophilic infiltration and exuberant inflammation represent characteristic features of severe TB, but neutrophil biology during TB remains poorly understood. Prostaglandin E2 (PGE2), an active lipid compound, is a key mediator of immunopathology in chronic infections . Manipulation of PGE2 levels was proposed as an approach for countering the Type

I IFN signature of TB patients, but very limited information is available regarding this pathway in TB patients. The aim of this work was to investigate the role of PGE2 on the modulation of human neutrophil immune responses against *Mtb*. We detected a significantly higher number of neutrophils in patients with positive Acid Fast Bacilli (AFB) in sputum as compare to negative AFB TB patients ($p<0.01$). Besides, stimulation of neutrophils from healthy donors (HD) with an *Mtb* lysate (*Mtb*-Ag, 10 µg/ml) significantly enhanced CD11b expression, a marker of neutrophil activation. Interestingly, CD11b levels were markedly reduced by effect of PGE2 (2 µg/ml). Furthermore, for the first time to our knowledge, we observed that neutrophils from HD and TB patients express SLAMF1, a costimulatory molecule recently described as a microbial sensor. Also, *Mtb*-Ag stimulation significantly increased the percentage of SLAMF1+ neutrophils ($p<0.05$), but PGE2 treatment diminished SLAMF1 levels ($p<0.05$). Moreover, neutrophils stimulated with *Mtb*-Ag augmented LC3B-II levels as compared to non-stimulated cells. Importantly, PGE2 treatment significantly increased autophagy flux in human neutrophils stimulated with *Mtb*-Ag ($p<0.05$). Therefore, autophagy, a process involved in the defense against Mtb, might prevent excessive inflammation and tissue damage. Taken together, our findings extend the information about the mechanisms mediated by PGE2 that operate during the human immune response to *Mtb*.

716. (782) **MURINE ANTIGEN-PRESENTING CELLS INTERNALIZE BINARY ETHYLENIMIDE INACTIVATED FOOT-AND-MOUTH DISEASE VIRUS AND RELEASE EXTRACELLULAR VESICLES EXPRESSING VIRUS PROTEINS.**

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Extracellular vesicles (EVs) secreted by antigen-presenting cells (APC) play a crucial role in carrying and presenting major histocompatibility-peptide complexes, but can also spread pathogen and host-derived molecules during infections. Foot-and-mouth disease virus (FMDV) is a highly contagious disease of livestock worldwide and is economically important. The main strategy for the control is vaccination with virus chemically inactivated with binary ethylenimide (BEI-FMDV). The protection achieved is good, however, requires regular re-inoculations to produce sustained immunity over time.
In the present work we aimed to study whether APC differentiated from bone marrow cells are able to internalize BEI-FMDV and release extracellular vesicles. For this purpose, APC were differentiated from murine bone marrow cells with GM-CSF. On day 8, more than 85% of the cells expressed CD11c and MHC-II molecules. By flow cytometry we observed that 30% of APC internalized purified BEI-inactivated virions labelled with FITC (BEI-FMDV-FITC) after incubation for 60 min at 37°C with APC. EVs were isolated by centrifugation, ultrafiltration and ultracentrifugation from supernatant of APC pulsed with BEI-FMDV or LPS for 18 h and characterized by flow cytometry. EVs derived from APC pulsed whit FMDV (1, 5 and 10 µg/ml) demonstrated strong staining for the EVs marker CD9 (45%, 93%, 81%, respectively) and CD81 (9 %, 58% and 40 %). APC molecules such as MHC-II (>90%) and CD86 (>40%) were also expressed. Remarkably, FMDV antigens were expressed on EVs derived from APC incubated with 5 µg/ml FMVD.
Our results show that inactivated FMVD can be internalized by APC and these cells release EVs expressing FMDV molecules and APC cells markers.
The knowledge derived from this work will serve to deepen the knowledge of the interrelation between the FMDV and the immune system that will serve for the rational design of vaccines.

717. (784) **REDUCED TRP-IDO-AHR AXIS ACTIVITY IS ASSOCIATED WITH HIGHER LEVELS OF INFLAMMATORY CYTOKINES IN PATIENTS WITH SEVERE CHRONIC CHAGAS CARDIOMYOPATHY**

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After acute infection with *T. cruzi* approximately 30% of infected individuals develop Chronic Chagas cardiomyopathy (CCC) while the rest remain asymptomatic (Asy). Although the mechanisms underlying the differential progression to CCC are still not fully understood, CCC display a more intense inflammatory response than Asy patients, who appear to have a more regulated immune response. We have reported that the indoleamine 2,3 dioxygenase-tryptophan metabolites-aryl hydrocarbon receptor (IDO-Trp-AhR) axis is associated with both the development of a strong Th1 response able to control parasite replication and its regulation by inducing, depending on the levels of AhR activation, Treg or IL-10+ producing cells. AhR can be activated by several ligands many of them being derivatives of Trp, such as L-kynurenine (Kyn) generated by IDO activity. To determine whether IDO-Trp-AhR axis is associated with CCC development, we analyzed the levels of IL-6, TNF, Trp metabolites and AhR agonists in serum samples from healthy controls, CCC and Asy patients by using ELISA, HPLC, targeted LC-MS and AhR agonistic activity. CCC patients were subclassified as Mild (altered ECG without congestive cardiac failure) or Severe (altered ECG, congestive cardiac failure and other alterations). By using a luciferase plasmid reporter assay we detected a decreased global AhR agonistic activity in infected patient sera as compared to healthy controls ($p<0,0001$), with Mild patient's sera showing lower levels than Asy and Severe patients ($p<0.05$). Moreover, infected patients showed increased levels of circulating Kyn (HPLC, $p=0.01$; LC-MS, $p<0.0001$), IL-6 and TNF compared to healthy controls. In addition, Severe patients' sera presented lower levels of Kyn ($p<0.05$) and higher levels of Trp, IL-6 and TNF than those observed in Asy patients ($p<0.05$). Our results support an association between higher levels of inflammatory cytokines and reduced Trp-IDO-AhR axis activity in Severe patients. Analysis of targeted metabolomic data are underway in our laboratory.

718. (796) **MECHANISM OF THE EFFECTS OF 5,5-DIMETHYL 1-PYRROLINE N-OXIDE IN A MOUSE MODEL OF ACUTE RESPIRATORY DISTRESS SYNDROME**

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The nitrones spin trap 5,5-dimethyl-1-pyrroline N-oxide (DMPO) was developed for the study of free radicals. Later it was used to visualize protein- and DNA-centered radicals in cells, tissues and whole animals in animal models of diseases. Recently we found that DMPO blocks lipopolysaccharide (LPS)-triggered signaling in macrophages primed with LPS, traps protein- and DNA-centered radicals, and also decreases systemic inflammation and death in mice exposed to an overdose of LPS. Herein we aimed at determining the mechanism by which DMPO can protect the lung in a mouse model of acute-distress respiratory syndrome (ADRS) triggered by bacterial LPS. We hypothesize that these protective effects of DMPO can either be due to blocking quimiotaxis/homing and activation of neutrophils, myeloperoxidase (MPO) activity or other enzyme sources of reactive biochemical species o by trapping protein radicals and thus reducing their decay to end-oxidation products. We used male C57 mice (7 weeks-old, 9/group) and exposed them, under light anesthesia, to oropharyngeal aspiration of 50 ul of either vehicle (PBS) or 1 ug/ul LPS. Another group of animals (9/group) were pre-treated 30 min before LPS challenge with vehicle or vehicle containing 50 mM DMPO (2.5 nmol DMPO/mice). We found that LPS treatment increased ICAM-1 and iNOS expression, neutrophil in lung parenchyma (NIMP-14+ cells); and markers of tissue damage in BALF (LDH leakage); MPO protein and activity in lung parenchyma and BALF; and chlorotyrosine, nitrotyrosine and carbonyls in the lung parenchyma. These effects were prevented by pretreatment with

DMPO which trapped some protein radicals. DMPO did not affect MPO, iNOS or NOX-2 activity. Taking together our data are consistent with DMPO reducing LPS-induced neutrophil recruitment into the lung parenchyma, thus downstream effects are blocked. DMPO or its derivatives may prove to be effective anti-LPS treatments in ADRS and also sepsis. PICT-3369, PIP916 , PROICO 10-0218-PROICO 02-3418.

719. (799) **MYELOPEROXIDASE AND ADIPOCYTE DYSFUNCTION: MECHANISMS AND THERAPEUTICS**

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Inflammatory switch of macrophage is a key process involved in adipose tissue inflammation during a chronic positive energetic balance in obesity. However, in animals fed a high-fat diet neutrophils are found infiltrating the visceral adipose tissue, but their role in the overall dysfunction of the AT remains unclear. We hypothesize that activation of neutrophils into the AT, particularly the activation of myeloperoxidase (MPO) at that early step affects adipogénesis and adipocyte's physiology. To test this hypothesis we used a model of human preadipocytes and adipocytes and exposed them to human myeloperoxidase (MPO, 10 ng/ml) in the culture medium. Preadipocytes and adipocytes take up MPO from the culture medium, MPO produce HOCl intracellular. This HOCl kill the cells, thus conditions to non-cytotoxicity were adjusted. Intracellular produced HOCl increased triglyceride synthesis and thus the adipogenic process. In adipocytes, HOCl produced intracellularly reduced glucose-uptake, changed adipokine secretion (increased leptin, but reduced adiponectin); and caused chlorination and carbonylation of proteins. The major proteins involved in insulin signaling were identified as protein-DMPO adducts using immuno-spin trapping combined with tandem-mass spectrometry. These effects were prevented by either, inhibition of MPO with ABAH-a specific inhibitor of MPO, N-acetyl-cysteine-a source of intracellular GSH, resveratrol-a cell-permeable scavenger of HOCl; but taurine-a scavenger of extracellular HOCl did not have effect. DMPO reduced protein end-oxidation product accumulation, restored insulin sensitivity and improved the adipokine balance by reducing ER-stress and thus facilitating adiponectin secretion. MPO plays a key role in adipogénesis and adipocyte dysfunction by promoting oxidation of proteins involved in adipogénesis, insulin sensitivity and adipokine balance. Therapeutic interventions to reduce MPO activity or scavenging HOCl inside adipocytes may show to be effective to reduce their dysfunction in obesity. PICT3369, PIP916, PROICO 10-0218 and PROICO 02-3418