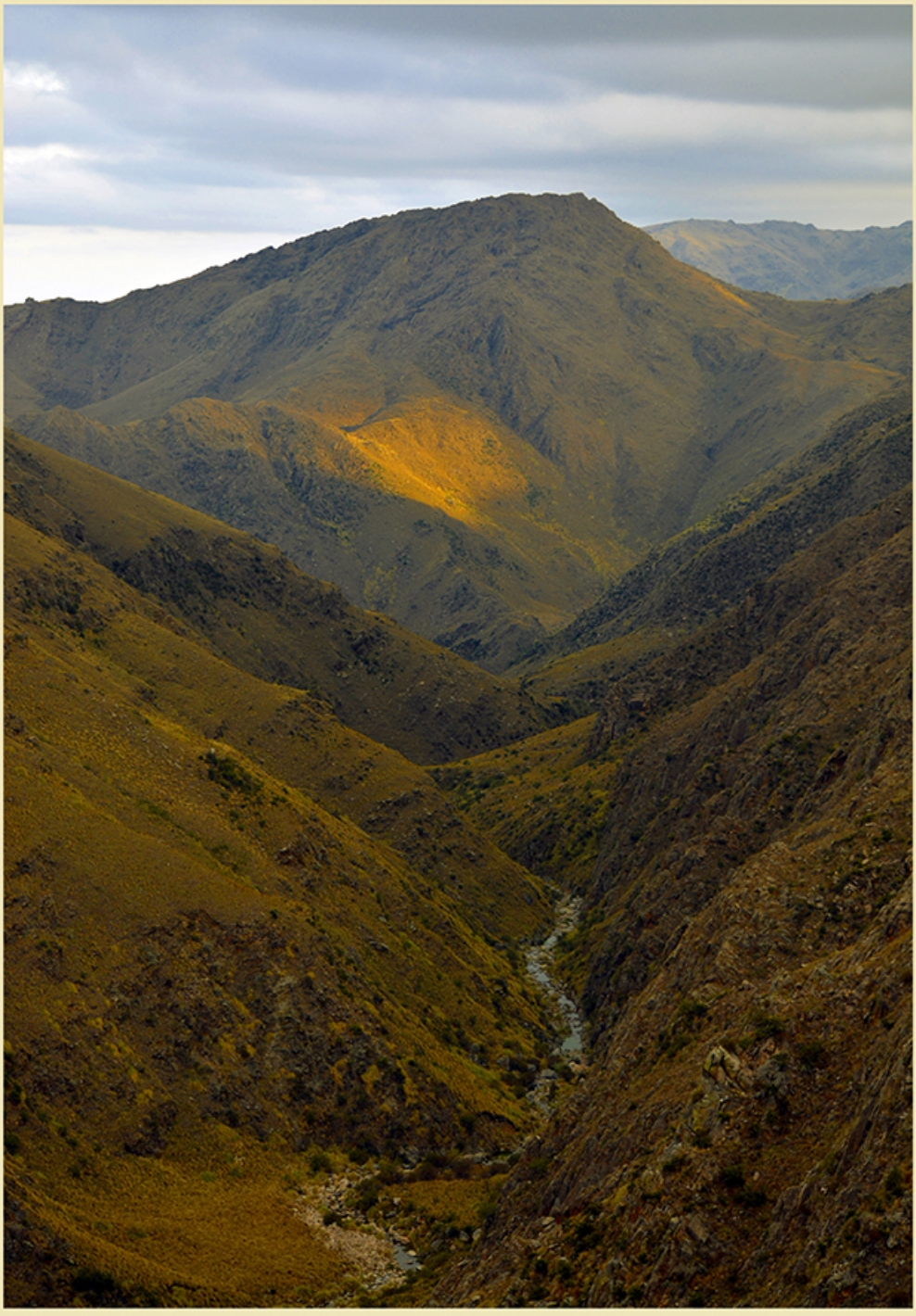
LXXI REUNIÓN ANUAL
DE LA SOCIEDAD ARGENTINA DE INMUNOLOGÍA

9 al 11 de noviembre de 2023 / San Luis



Sociedad Argentina de Inmunología

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50 años
Universidad Nacional de San Luis

LXXI REUNIÓN CIENTÍFICA ANUAL DE LA SOCIEDAD ARGENTINA DE INMUNOLOGÍA (SAI)

9 -11 de noviembre de 2023

Universidad Nacional de San Luis-San Luis

LXXI ANNUAL MEETING OF THE ARGENTINEAN SOCIETY OF IMMUNOLOGY (SAI)

November 9 - 11, 2023

Universidad Nacional de San Luis-San Luis

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María Silvia Di Genaro

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Universidad Nacional de San Luis-San Luis

RESPONSIBLE EDITOR
Comisión Directiva SAI
María Silvia Di Genaro

50 años
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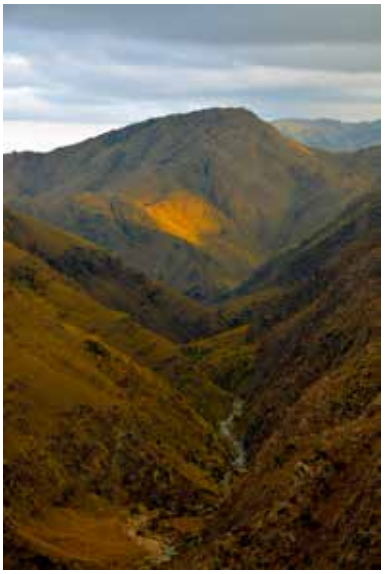
Camino del Macizo Central de San Luis
de Hebe Iriarte



Macizo de San Luis: cima de las Sierras de San Luis, a 2088m sobre el nivel del mar, con vistas inolvidables de cerros, quebradas, valles y pequeñas mesetas de altura. (Extraído de Ser Argentino.com).

Hebe Iriarte: Microbióloga, docente en el Área Microbiología e Inmunología de la Universidad Nacional de San Luis, personal técnica de apoyo de CONICET. Fotógrafa profesional, realiza fotografías de flora y fauna.

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LA SOCIEDAD ARGENTINA DE INMUNOLOGÍA QUE ORGANIZAN ESTA REUNIÓN AGRADECE LA PARTICIPACIÓN, APOYO Y COLABORACIÓN DE LAS SIGUIENTES ENTIDADES Y EMPRESAS

ENTIDADES OFICIALES

UNIVERSIDAD NACIONAL DE SAN LUIS
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DISCURSO DE BIENVENIDA DE LA PRESIDENTA SAI 2023

María Silvia Di Genaro

Sr. Rector de la Universidad Nacional de San Luis, Sr. Decano de la Facultad de Química, Bioquímica y Farmacia de la UNSL, autoridades invitadas, profesionales, estudiantes.

Queridos miembros de la Sociedad Argentina de Inmunología, en primer lugar, me gustaría agradecer la confianza depositada al haberme designado como Presidenta de la Sociedad Argentina de Inmunología (SAI), tarea que ha sido todo un honor y una responsabilidad enorme por el significado que siempre ha representado la Inmunología para mí y el pertenecer a esta Sociedad, desde que presenté mi primer trabajo como becaria hace ya casi tres décadas.

El Plan de Ciencia, Tecnología e Innovación 2030, convertido recientemente en ley, plantea como uno de sus grandes desafíos, el fortalecimiento de las capacidades científicas y tecnológicas de las instituciones de todo el país, con una perspectiva integradora y orientada a la descentralización del sector científico-tecnológico. Aunque se ha avanzado en la federalización de la ciencia en Argentina, los grupos de investigación del interior del país experimentamos todos los días la necesidad de seguir trabajando en este objetivo. En este sentido, la SAI propende al desarrollo y a la difusión de la Inmunología en todo el país. Apoyando este carácter federal de la SAI, hoy es todo un orgullo reunirnos y recibirlos en nuestra casa, en la Universidad Nacional de San Luis, siendo la primera vez que esta reunión se realiza en esta provincia.

Este año 2023, es un año muy importante para todos los argentinos al celebrarse 40 años de democracia. En particular es un año muy emotivo para nuestra querida Universidad Nacional de San Luis por cumplir sus 50 años desde su creación en 1973, ya que antes dependía de la Universidad Nacional de Cuyo. También ha sido un año muy significativo para nuestra Sociedad que ha experimentado el enorme orgullo del reconocimiento de nuestros socios por sus destacadas trayectorias científicas. Así, el Premio Konex de Brillante y de Platino: Microbiología e Inmunología 2023 ha sido otorgado al Dr. Gabriel Rabinovich y el Premio Konex de Platino 2023: Pandemia – COVID-19 al Dr. Jorge Geffner, ambos socios muy comprometidos con nuestra SAI. También, los Dres. Emilio Malchiodi, Norberto Zwirner y Ada Blidner, obtuvieron, por sus proyectos de investigación, el Premio y las Menciones Especiales César Milstein 2023 a la Investigación en Biotecnología con Impacto en la Salud. Felicitaciones a todos ellos. Nos enorgullece también que el reciente premio Nobel de Medicina 2023 haya sido otorgado a Katalin Karikó y Drew Weissman por sus descubrimientos sobre modificaciones de bases de nucleósidos que permitieron el desarrollo de vacunas de ARNm eficaces contra la COVID-19. También, tenemos la enorme satisfacción que el Laboratorio de la Dra. Juliana Cassataro, en la Universidad Nacional de San Martín (UNSAM), en conjunto con el laboratorio del Dr. Pablo Cassará han desarrollado una vacuna a subunidad para los refuerzos contra SARS-CoV-2, y que ya cuenta con la aprobación de ANMAT. Siendo la primera vez que una vacuna contra enfermedades infecciosas es diseñada y producida en nuestro país. Este importante avance visibiliza la capacidad del sistema científico y tecnológico y de la educación pública de Argentina.

En cuanto a las actividades llevadas a cabo en nuestra Sociedad este año, destacamos diversos cursos organizados por nuestros socios, incluyendo el curso sobre Inmunometabolismo organizado en conjunto por las Universidades Nacionales de Córdoba y San Luis, auspiciado por la SAI**,** y los seminarios Inmunometabolismo e Inmunología Clínica organizados por la Comisión Directiva de la SAI. Además, este año hemos obtenido el premio a mejor campaña 2023 del Día de la Inmunología otorgado por la Unión Internacional de Sociedades de Inmunología (IUIS, por sus siglas en inglés). Campaña que tuvo como objetivo difundir la importancia de la Inmunología en la Salud Pública. Agradezco a todos los socios que participaron, a los miembros de la Comisión Directiva de la SAI por su compromiso en estas actividades y al Comité de Docencia de la SAI. Por otra parte, nuestra Sociedad otorgó dos becas para asistir a IUIS 2023 a realizarse próximamente en Ciudad del Cabo, Sudáfrica.

Desde las últimas gestiones, continuamos integrando el Foro de Sociedades Científicas. También mantuvimos vigente el convenio con la Sociedad Brasilera de Inmunología, que nos permitió contar con dos destacas investigadoras en nuestra reunión.

Este año, los días 16 y 17 de junio se desarrollaron en la ciudad de Rosario las Jornadas de Invierno de la SAI, que fueron organizadas por el nodo de socios de Santa Fe (IDICER y UNL). En estas jornadas se expusieron trabajos de alto nivel científico. Además, para incentivar futuras colaboraciones, en las sesiones de posters, becarios y becarias expusieron sus proyectos de investigación. La Comisión Directiva agradece a la Dra. Ana Rosa Pérez y a su grupo por la iniciativa y por organizar estas Jornadas.

Este año sumamos 41 nuevos socios, de tal manera que hoy nuestra Sociedad cuenta con un total de 568 Socios, de los cuales 343 son titulares**,** 204 adherentes **y** 20 son honorarios.

Para esta reunión 2023 buscamos brindarles diferentes temas científicos de nuestra disciplina. El programa incluye cinco conferencias presenciales de destacados inmunólogos, como nuestros queridos Dres. Amigorena de Francia, Dr. Kalergis de Chile, Dr. O´Neill de Irlanda, Dr. Gagliani de Alemania y el Dr. Gabriel Rabinovich de Argentina. También, cinco simposios sobre Inmunidad de mucosas, Interacción Huésped-agente microbiano, Inmunometabolismo, Inmunología Clínica e Inmunología Traslacional, en los que participan importantes expositores argentinos y extranjeros. Además, el programa científico cuenta con las habituales sesiones de pósters en las cuales muchos de nuestros jóvenes investigadores muestran por primera sus recientes resultados. Este año tuvimos una excelente convocatoria, con 204 resúmenes aceptados, con trabajos de Argentina, Chile y Brasil, 7 postulaciones al Premio Leonardo Satz y 2 al de Inmunología Clínica. Además, proponemos este año el Premio Poster SAI, para el cual hubo 36 postulaciones, de las cuales se seleccionarán cuatro trabajos para ser expuestos en una sesión oral. Es de destacar que la SAI este año ha otorgado 96 becas de transporte para asistir a esta Reunión Anual.

La SAI, creada en 1972, cumplió el año pasado 50 años, sin embargo, las bases de esta Sociedad inician en los años 60 con reuniones informales en el llamado “Club de Inmunología”, organizadas por investigadores de diferentes lugares del país. A ese grupo de investigadores, pertenecía el Dr. Isaac Rivero de Mendoza, quien fuera presidente de la SAI desde 1996 hasta 1998, un pionero de

la Inmunología Clínica en Mendoza y a quien tenemos el enorme placer de otorgarle en esta reunión el Premio Trayectoria.

Finalmente, quiero expresar mi agradecimiento a los Presidentes SAI 2021 y 2022, Mariana Maccioni y Emilio Malchiodi, y al Vicepresidente actual, Martín Rumbo, por todo su apoyo durante este año. A los integrantes de la Comisión Directiva de SAI por el enorme trabajo realizado en la organización del programa científico. Al Secretario de SAI, Roberto Davicino, y al Tesorero de SAI, Antonio Carrera Silva, por su enorme compromiso y tan destacada labor. Un muy afectuoso agradecimiento al grupo del laboratorio de Inmunología de nuestra Universidad y socios de la SAI: Javier Elicabe, Juan Silva, Matías Distel, Marisol Velazquez, Carolina Gorlino, Samanta Funes, José Luis Arias y Marianela Leporati por todo su esfuerzo y colaboración en la organización de esta Reunión. Asimismo, mi agradecimiento a las Dras. Mariana Maccioni y Eliane Piaggio por organizar, y al Dr. Javier Elicabe, por coordinar, el curso precongreso, en el que participaron más de 40 estudiantes del todo el país. Un especial agradecimiento a Cytek y Gematec que otorgaron 30 becas de alojamientos a los participantes de este curso precongreso. Al CONICET, al FONCYT, The Company of Biology, EMBO y la Fundación Alexander von Humbold que nos han brindado su apoyo económico. Al Laboratorio de Hemoderivados de la Universidad Nacional de Córdoba, DIAGNOS MED y Científica San Luis que realizaron los aportes económicos para los premios que se otorgarán en esta Reunión. Mi más afectuoso reconocimiento y gratitud a la Universidad Nacional de San Luis y a la Facultad de Química, Bioquímica y Farmacia por su importantísimo apoyo económico y logístico para llevar a cabo esta Reunión de la SAI en nuestra casa.

Hago también extensivo mi agradecimiento a todos los invitados nacionales e internacionales, coordinadores, jurados y asistentes que desinteresadamente contribuyen a la excelencia de nuestra Reunión.

En nombre de la Comisión Directiva, dejo inaugurada la “LXXI Reunión Anual de la Sociedad Argentina de Inmunología”. Que esta reunión sea una nueva oportunidad de encuentros fructíferos, para iniciar y fortalecer colaboraciones entre los grupos de investigación en Inmunología, y para generar nuevas amistades entre los inmunólogos de nuestro país. Les doy la más cálida bienvenida a nuestra ciudad y a nuestra casa, la Universidad Nacional de San Luis.

CONFERENCIAS/LECTURES

I- THE EMBO LECTURE- *Thursday, November 9, 12-13 h*

Chairs: Andrés Alloati - Ignacio Cebrián

EPIGENETIC CONTROL OF ANTI-TUMOR IMMUNITY BY CAR T CELLS

Sebastián Amigorena
Institut Curie, Paris, France

After priming, naïve CD8+ T lymphocytes display heritable gene expression programs that define differentiation to memory or short-lived effector cells. Even if lineage specification in T cells is critical for protective immunity, the modalities of chromatin dynamics that contribute to the control gene expression programs along these differentiation paths remain unclear. We analyzed previously the role of Suv39h1, a histone H3 lysine 9 methyltransferases in heterochromatin dynamics during memory vs. effector differentiation in CD8+ T cells in response to *Listeria monocytogenes* infection. Suv39h1-dependent H3K9me3 deposition determines the silencing of a set of stem cell-related/memory genes. In conditions of chronic stimulation, including during anti-tumor immune responses, cells enter a distinctive differentiation program that ultimately leads to loss of effector functions (sometimes referred to as “exhaustion” or dysfunction”). We found that Suv39h1 promotes to this differentiation program through silencing of memory features in chronically stimulated intratumor T cells. Mice bearing Suv39h1-defective T cells showed delayed tumor growth and improved response to immune checkpoint blockade by anti-PD-1. Humans Suv39h1-KO CAR-T display increased memory features and prolonged persistence after tumor rejection in NSG mice. We conclude that epigenetic re-programing of CAR-T increases their therapeutic efficacy and confers long lasting protection in mouse pre-clinical models.

II- "LEONARDO SATZ" LECTURE- *Thursday, November 9, 18-19 h*

Chairs: Juan Pablo Marcker-Oberti - Samanta Funes

IMPAIRMENT OF IMMUNOLOGICAL AND NEUROLOGICAL SYNAPSES AS VIRULENCE MECHANISMS OF RESPIRATORY VIRUSES. IMPLICATIONS FOR VACCINE DESIGN DURING THE PANDEMIC

Alexis Kalergis

Millennium Institute on Immunology and Immunotherapy. P. Universidad Católica de Chile

Respiratory viral infections caused by pathogens, such as the Respiratory Syncytial Virus (hRSV), human Metapneumovirus (hMPV), and Severe Acute Respiratory Syndrome C2 (SARS-CoV-2) are major causes of disease in the pediatric and adult population in the world, due to an incomplete, delayed, or inefficient viral host immunity. Failure of immunity to clear infection by these viruses can even be seen after resolution of the disease, due to weakened innate and adaptive immune responses, preventing the establishment of protective immunity. The development of safe and effective vaccines to prevent the disease caused by those viral respiratory pathogens has become a real scientific challenge worldwide. Knowledge of the molecular virus-host interactions during these respiratory infections is essential for the development of vaccines and we have observed that some respiratory viruses impede the proper functioning of immune cells, such as T cells and dendritic cells, by altering the assembly of the immune synapse between these cells. Inhibition of the immune synapse could function as an important virulence factor by altering host immunity and increasing susceptibility to reinfection. Further, respiratory viruses can cause a learning impairment due to inflammation at the central nervous system. Due to alterations on the blood brain barrier after infection, elements of the immune system enter the CNS impairing the normal function of neurons and astrocytes in the host. Based on these data, we have generated novel vaccine approaches to strengthen the immunological synapse leading to protective immunity against these respiratory pathogens and preventing CNS damage. These data have contributed to novel vaccine approaches to strengthen the immunological synapse leading to a safe and efficacious immunity capable of protecting against respiratory pathogens, such as RSV and SARS-CoV-2. The immunity triggered by these vaccines can also reduce CNS damage caused by respiratory viruses.

III- LECTURE- *Friday, November 10, 12-13 h*
Chairs: Cristina Motrán - Carolina Gorlino

KREBS CYCLE IN IMMUNITY AND INFLAMMATION

Luke O'Neill
School of Biochemistry and Immunology, Trinity Biomedical Sciences Institute, Trinity College Dublin
Email: laoneill @tcd.ie

Metabolic changes triggered during innate immunity have become a particular focus for researchers interested in inflammation. Mitochondrial disturbance is a feature of inflammatory cells and we have been analysing the Krebs cycle intermediates succinate and fumarate, as well as the Krebs cycle-derived metabolite itaconate, in activated macrophages in response to signalling by Toll-like receptors. Itaconate derivatives are anti-inflammatory and have potential for the treatment of immune and inflammatory diseases. Fumarate is proving to be a very interesting metabolite. It is generated via repression of the enzyme FH and also induction of the argininosuccinate shunt. Fumarate suppresses IL10 production which in turn leads to increased TNF. The decrease in FH however also leads to mitochondrial disturbance, which involves release of double-stranded mitochondrial RNA. This is sensed by the RNA sensors RIG-I, MDA-5 and TLR-7 driving production of Type I Interferons. Metabolites like fumarate are therefore acting as signals and impacting on signalling pathways in unexpected ways. These insights are providing a new view of metabolism in immunity and inflammation and might indicate new therapeutic approaches.

IV- LECTURE- *Friday, November 10, 18-19 h*

Chairs: Antonio Carrera Silva - Dr. Carolina Amezcua

"IS OUR DIET OUT DESTINY?" EFFECTS OF SHORT-TERM DIETARY INTERVENTIONS ON IMMUNITY AND TUMOR THERAPIES

Nicola Gagliani

Hamburg Center for Translational Immunology (HCTI), Hamburg,Germany

Department of Medicine and Department of General, Visceral and Thoracic Surgery, University Medical Center Hamburg-Eppendorf, Hamburg, Germany.

When given the opportunity, we tend to shift away from balanced diets to energy-dense, fat-rich foods, often displacing fiber-rich diets, albeit briefly. The consequences of such sudden dietary shifts on health and disease have long been unclear.

Our research has unveiled that a short-term switch from a regular diet to a low-fiber, low-energy-dense diet had a noteworthy yet temporary adverse impact on mucosal and systemic immunity in mice. This heightened the mice's vulnerability to infections and compromised their capacity to respond to antigenic challenges. These effects were explained by decreased metabolic fitness and consequently cytokine production in CD4 T cells due to impaired mTOR activity, starving from the decreased provision of fiber metabolites by the gut microbiota. However, reintroducing dietary fiber effectively restored T cell metabolism, bolstered both mucosal and systemic CD4 T cell functions, and enhanced immunity. Ultimately, dietary intervention with human volunteers corroborated the impact of brief dietary changes on the functionality of human CD4 T cells (Siracusa F. et al Nature Immunology 2023).

In a separate study, we also discovered that a short-term diet rich in tryptophan or treatment with a microbiota-derived tryptophan metabolite called indole-3-acetic acid (3-IAA) amplified the response to cancer therapies in a mouse model of pancreatic cancer. This enhancement was attributed to neutrophil-derived myeloperoxidase, which oxidizes 3-IAA. When combined with chemotherapy, 3-IAA resulted in the accumulation of reactive oxygen species (ROS), reduced autophagy, compromised metabolic fitness, and ultimately decreased cancer cell proliferation. Importantly, our findings also revealed a substantial correlation between the levels of 3-IAA and therapy effectiveness in two independent cohorts of pancreatic ductal adenocarcinoma (PDAC) patients (Tintelnot J. et al Nature 2023).

Overall, our data illustrate the rapid and profound impact that different diets can have on immune responses and even on the outcomes of cancer therapies. This research underscores the fundamental role our daily food choices play in our health and opens the door to potential nutritional interventions, including the use of metabolites, as integral components of many therapies in the near future.

Disclosure: Similar abstract has been published at the Keystone Meeting, *Mechanisms of Microbiota-Immune Interactions-Towards the Next Decade*

V- LECTURE- *Saturday, November 11, 18-19 h*
Chairs: Claudia Sotomayor - Dr. Silvia Di Genaro

ADVENTURES OF AN IMMUNOLOGIST IN THE SUGAR LAND: FROM BASIC DISCOVERIES TO TECHNOLOGY TRANSFER AND BACK AGAIN

Gabriel Rabinovich
Laboratorio de Glicomedicina, Programa de Glicociencias, Instituto de Biología y Medicina Experimental (IBYME), CONICET, y Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Argentina. E-mail: gabyrabi@gmail.com

The rapid pace of glycobiology research, combined with the acute need for drugs to alleviate autoimmune inflammation and overcome resistance to anticancer therapies, is accelerating the translation of novel glycan- and lectin-based therapeutics into clinical practice. Galectins, a family of endogenous glycan-binding proteins, use both extracellular and intracellular mechanisms as well as glycan-dependent and independent pathways to reprogramme the fate and function of mmune cell types and control a variety of physiologic and pathologic processes. For more than two decades, we investigated the functions of galectins and their glycosylated ligands in the control of immune and vascular programs in both tumor and inflammatory microenvironments. We identified critical roles for galectin-1, a proto-type member of this family, in promoting tumor-immune escape, controlling host-pathogen interactions, inducing angiogenesis and influencing resolution of autoimmune and chronic inflammation. The mechanisms underlying these effects involve modulation of both lymphoid, myeloid and endothelial cell compartments. These findings inspired the creation of Galtec, a Start-up biotech aimed to translate galectin-based discoveries into novel technologies and immunotherapeutic modalities for cancer and autoimmune inflammation. During my talk, I will highlight the most important milestones, including pitfalls, of a long and sweet road starting from very basic discoveries to translational applications, and back again.

SYMPOSIUM 1-*Thursday, November 9, 10-12 h*
MUCOSAL IMMUNOLOGY

Chairs: Silvia Correa -Gabriela Perdigón

IMMUNOTHERAPY AS A PATH FOR TOLERANCE INDUCTION IN FOOD ALLERGY ON THE ROAD TO ALLERGY IMMUNOTHERAPY

Paola Lorena Smaldini

Instituto de Estudios Inmunológicos y Fisiopatológicos-(IIFP) CONICET-UNLP -asociado CIC PBA Departamento de Cs Biológicas, Facultad de Ciencias Exactas, La Plata, Argentina

Cow's milk allergy (CMA) is provoked by an aberrant immunological reaction to cow's milk proteins and its incidence and general awareness are increasing worldwide. Investigations into the potential factors contributing to its immunopathogenesis showed that deficiencies in the expression of FoxP3 and IL-10 promote an inadequate tolerance to food allergens. Oral and sublingual immunotherapies show promise as potential disease-modifying therapies to restore mucosal tolerance. The objective of our work was to restore mucosal mechanisms of tolerance through oral and sublingual immunotherapy (IT), and to study the cell populations and molecules involved using an IgE-dependent murine model of food allergy.

Balb/c mice were intra-gastrically sensitized with cow´s milk proteins (CMP) plus cholera toxin as mucosal adjuvant once a week for 6 weeks and orally challenged with CMP to evidence hypersensitivity. Thereafter, different oral or sublingual therapy strategies were carried out for immunomodulation through tolerance induction. Mice were orally challenged with the food allergen and treatment efficacy was in vivo (clinical score and cutaneous test) and in vitro (serum-specific antibodies IgE, IgG1 and IgG2a; IL-5, IL13, IFN-g, IL-10 and TGF-β secretion by splenocytes and in the intestinal mucosa by ELISA, and cell analysis by flow cytometry) evaluated.

The different immunomodulatory strategies promoted a decrease in clinical scores, specific serum levels of IgE and IgG1, IL-13 and IL-5 and negativization of skin tests in the treated groups compared to untreated and sensitized mice. In addition, an increase in IL-10 and TGF-β with an increase in IL-10-producing CD4+CD25+FoxP3+ LTs were detected in the lamina propria of treated mice. Furthermore, the transfer of Tregs, either induced in vitro or in vivo, demonstrated their ability to control and reverse the allergic response in sensitized animals. On the other hand, we found increased levels of tissue IFN-g ($p<0.05$) along with a higher frequency of both CD11c+CD11bCD8α+ lamina propria dendritic cells ($p<0.05$) and CD4+IFN-g+α4β7+ T cells in submaxillary lymph nodes in treated animals compared with sensitized mice.

In conclusion, the controlled mucosal administration of food allergens induced tolerance and immunomodulatory mechanisms that reversed the allergic reaction. These findings may pave the way for the development of mucosal immunotherapies that suppress the synthesis of IgE and intestinal inflammation.

STUDYING THE INTESTINAL MUCOSA INTERACTIONS TO UNDERSTAND THE INFLAMMATORY BOWEL DISEASES

Pablo Romagnoli

Instituto Universitario de Cs. Biomédicas de Córdoba (IUCBC), Centro de Investigación en Medicina Traslacional "Severo R. Amuchástegui" (CIMETSA); G.V al Instituto de Investigación Médica Mercedes y Martín Ferreyra (INIMEC-CONICET-UNC), Córdoba, Argentina

The intestinal mucosa is an amazingly complex internal surface that is continuously exposed to a multiplicity of microorganisms that constitute the microbiota. This microbiota influences a multiplicity of systems within our body. Most particularly, the entire immune system development is influenced by the interactions between the intestinal mucosa with the microbiota and the metabolites generated in response to the nutrient influx from the diet. To design possible interventions to treat the inflammatory bowel diseases (IBD) that increasingly affect our population, it is necessary to investigate how the microbiome composition as well as the metabolites that are generated in response to the diet impact in the intestinal mucosa homeostasis. First, we tested the hypothesis that indole-3-propionic acid (IPA), a metabolite derived from the tryptophan metabolism of commensal bacteria, provided signals that promote intestinal mucosa homeostasis. Using a mouse model of colitis caused by oral infection with *Citrobacter rodentium*, we found that IPA treatment prevented colitis and this effect was mediated by the nuclear receptor pregnane X receptor (PXR). We also found that the absolute numbers of lymphocytes producing IL-17A and IFN-γ in this colitis model are increased in the intraepithelial lymphocyte compartment of the colon after IPA treatment and that this effect is also dependent on the presence of PXR. On the other hand, we have conducted a clinical study involving healthy volunteers and IBD patients in which we detected that the amount of Trp in the diet is inversely proportional to the activity of IBD and that the individual metabolomic profile can discriminate between controls and different IBD diseases/stages. Second, we tested the hypothesis that luminal IgG identifies the microbiota involved in inflammatory processes in the intestinal mucosa. Preliminary results from a clinical study involving healthy individuals and IBD patients point to a set of particular species found in IBD. We believe the results from studying interactions that occur in the intestinal mucosa will provide critical insight to develop novel therapies needed for IBD.

CLOSTRIDIOIDES DIFFICILE INFECTION IN ARGENTINA: FROM EPIDEMIOLOGY TO IMMUNE PROTECTION MECHANISMS

Angela M. Barbero[1,2], Sabina Palma[1,2], Nicolás D. Moriconi[1], Gabriel Erbiti[3,4], Carlos Altamiranda[4], Martina Calvo Zarlenga[4], Mónica Machain[5], María G. Balbi[5], María G. Martínez[5], Jorgelina Suárez[5], Rodrigo E. Hernández Del Pino[1,2], Virginia Pasquinelli[1,2].

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Clostridioides difficile infection (CDI) is one of the most common health-care associated infection implicated in 15–25% of antibiotic-associated diarrhea episodes. *C. difficile* is a Gram+, obligate anaerobic and spore-forming bacterium that is widespread in the environment and has been identified by the CDC as an urgent antimicrobial resistance threat. The very few reports about the prevalence of CDI in Argentina, the low awareness and inconsistent diagnostic and surveillance protocols suggest that this infection is markedly underestimated. It is imperative to have a deeper knowledge of CDI epidemiology and the intricate interplay between *C. difficile* microbial factors and the host immune system. We analyzed 168 samples from hospitalized individuals with diarrhea in a retrospective study. We found a prevalence of CDI of 23,81% by using an algorithm that includes EIA, PCR and toxigenic culture. Through a meta-analysis that compares our results with 39 publications, no factors commonly associated with CDI were found as risk predictors in our study cohort. Parameters encompassing leukocyte, neutrophil, monocyte, lymphocyte, basophile, and platelet counts were established as predictors of CDI risk. We are also currently studying the immune response against *C. difficile*. We showed that both macrophages and platelets interact with *C. difficile*. Macrophages can internalize *C. difficile* by macropinocytosis and platelets improve this uptake. Our findings are the first evidence for the internalization of vegetative *C. difficile* by human macrophages and highlight the role of platelets in innate immunity during CDI. Furthermore, using an in vivo model of CDI, we observed that *C. difficile* modulates T cell functions. IL-17 is tightly regulated trough the course of infection. The decrease on IL-17 production and the increase of IL-10 could be associated with disease resolution. Moreover, we found a CD4-CD8- IL-17 producing population in lamina propria, which needs further exploration. Finally, CDI treatment in Argentina is based on oral administration of metronidazole or vancomycin. Failure rate of existing antibiotics in combating *C. difficile* seems to be high and increasing, and recurrent CDI is frequently observed. Alternative therapeutic approaches are undoubtedly needed. Aloe Vera (AV) has been used as an immunomodulatory and antimicrobial agent in the treatment of diseases. Interestingly, AV gel inhibits *C. difficile* growth and significantly increases vancomycin´s effect. On the other hand, we also evaluated the role of AV as a protector agent of the intestinal epithelia. AV has a protective effect on the monolayer's integrity treated with *C. difficile* (BI/NAP1/027) strain or TcdA/TcdB toxins. This first evidence positions AV as a potential promising combination therapy against *C. difficile*, reducing the antibiotics concentration treatment and the detrimental consequences on the commensal microbiota, protecting the epithelial barrier integrity.

THE INTERPLAY BETWEEN MICROBIOTA AND THE IMMUNE SYSTEM IN THE RESPIRATORY TRACT: ITS IMPACT ON THE RESISTANCE TO INFECTIONS

Julio Villena

Laboratory of Immunobiotechnology, Reference Centre for Lactobacilli (CERELA-CONICET), Tucumán, Argentina

Research in the decade has led to major advances in the characterization of the microbiota of the respiratory mucosa. The better understanding of the composition and variations of the respiratory microbiome in health and disease has provided important information regarding the specific respiratory commensal bacteria (RCB) with beneficial effects on the local immune system and the resistance to infections. Furthermore, epidemiological evidence as well as the evaluation of the microbiota in clinical trials and animal models has allowed the selection and characterization of RCB strains with the capacity to modulate the respiratory immunity in infant and adults. Bacteria from the genus *Corynebacterium* spp. and *Dolosigranulum* spp., have been strongly associated with respiratory health and the exclusion of pathogens. In this conference, the characterization of immunomodulatory RCB strains from the species *Corynebacterium pseudodiphtheriticum* and *Dolosigranulum pigrum* and their application for the protection against respiratory infections will be revised. Immunomodulatory RCB can modulate the innate immune response in the respiratory tract of infant and adult mice, improving their resistance to the pathogens Respiratory Syncytial Virus, *Streptococcus pneumoniae* and *Klebsiella pneumoniae*. Immunomodulatory RCB can stablish molecular interactions with respiratory epithelial cells improving their response to pathogen challenges. In addition, RCB stimulate alveolar macrophages increasing their phagocytic activity and their ability to produce cytokines that help to eliminate pathogens as well as to control the detrimental inflammation in the lungs. The role of the generation of trained immunity in alveolar macrophages in the long-term protection induced by immunomodulatory RCB will be discussed. In addition, the evaluation of functionality and safety of the new RCB strains through microbiological tests and comparative and functional genomic studies will be reviewed. These studies are a step forward in the positioning of immunomodulatory RCB strains as next-generation probiotics for the respiratory tract.

SYMPOSIUM 2 *-Thursday, November 9, 16-18 h*
HOST-MICROBE INTERACTION

Chairs: Eva Acosta Rodríguez - Ana Rosa Pérez

EVALUATION OF THE IMMUNE RESPONSE TRIGGERED BY AN INACTIVATED VACCINE AGAINST SARS-COV-2 IN CHILEAN ADULTS

Susan Bueno
Millennium Institute on Immunology and Immunotherapy, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile

The SARS-CoV-2 Omicron variant has challenged the control of the COVID-19 pandemic even in highly vaccinated countries due to its ability to partially evade the immune response induced by vaccines. Here we studied whether a second booster of CoronaVac®, an inactivated vaccine, enhances the cellular response against the ancestral SARS-CoV-2 and the Omicron variant. In the context of a phase 3 clinical study, SARS-CoV-2 cellular immunity was analyzed in stimulated peripheral mononuclear cells of 46 volunteers by flow cytometry and ELISPOT. We observed a significant increase of CD4+ T cell expressing activate inducing markers (AIM+) and IFN-gamma producing cells after stimulation with a pool of peptides derived from the Spike protein at 2 and 4 weeks after the second dose of CoronaVac. We observed that the response remained stable after first and the second booster dose and no significant differences in IFN-gamma secreting cells or AIM+CD4+ T cells were observed. Importantly, CD4+ T cell response was equally increased when stimulation with a pool of peptides derived from the Spike protein from the Omicron variant. Furthermore, we observed an increase in the number of responder individuals at 4 weeks after the second booster. Our results show that a second booster of CoronaVac® contributes to stabilizing spike-specific CD4+ T cell responses against the ancestral SARS-CoV-2 strain and the Omicron variant.

WITH A LITTLE HELP FROM MY FRIENDS: BYSTANDER ACTIVATION OF MICROGLIA BY *BRUCELLA ABORTUS*-INFECTED ASTROCYTES INDUCES NEURONAL DEATH

Guillermo Giambartolomei

Instituto de Inmunología, Genética y Metabolismo (INIGEM), CONICET, Buenos Aires, Argentina

Inflammation is a key contributor to pathogenesis in neurobrucellosis. Glial cell interactions are at the root of this pathological condition where neuronal demise is related to the neurological involvement that take place in neurobrucellosis. Here, we present evidence indicating that soluble factors secreted by Brucella abortus-infected astrocytes activate microglia to induce neuronal death. Culture supernatants (SN) from B. abortus-infected astrocytes induce the release of pro-inflammatory mediators and the increase of the microglial phagocytic capacity, two key features in the execution of live neurons by primary phagocytosis, a recently described mechanism whereby B. abortus-activated microglia kills neurons by phagocytosing them. IL-6 neutralization completely abrogates neuronal loss. IL-6 is solely involved in increasing the phagocytic capacity of activated microglia as induced by SN from B. abortus-infected astrocytes, and does not participate in their inflammatory activation. Both autocrine microglia-derived and paracrine astrocyte-secreted IL-6 endows microglial cells with up-regulated phagocytic capacity that allows them to phagocytose neurons. Blocking of IL-6 signaling by soluble gp130 abrogates microglial phagocytosis and the concomitant neuronal death, indicating that IL-6 activates microglia via trans-signaling. Altogether, these results demonstrate that soluble factors secreted by B. abortus-infected astrocytes activate microglia to induce, via IL-6 trans-signaling, the death of neurons. IL-6 signaling inhibition may thus be considered as a strategy to control inflammation and CNS damage in neurobrucellosis.

UNRAVELING THE ENDOCYTIC CONNECTIONS FOR ANTIGEN PRESENTATION AND TOXOPLASMA GONDII PROLIFERATION

Facundo Garrido[1], Cristina Croce[1], Sofía Dinamarca[1], Franco Nieto[1], Nicolas Blanchard[2], Luis Mayorga[1], Ignacio Cebrián[1]

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Dendritic cells (DCs) are the most powerful antigen-presenting cells of the immune system. They present exogenous antigens associated with Major Histocompatibility Complex (MHC) Class II molecules through the classical pathway or with MHC-I molecules through the cross-presentation pathway to activate CD4+ or CD8+ T lymphocytes, respectively. Moreover, DCs represent one of the main cellular targets during infection by *Toxoplasma gondii.* This intracellular parasite incorporates essential nutrients, such as cholesterol, to grow and proliferate inside a highly specialized organelle, the parasitophorous vacuole (PV). While doing so, *T. gondii* modulates the host immune response through multiple interactions with proteins and lipids. In this study, we focus on the interaction between the PV and two intracellular compartments that are relevant for the efficient transport of MHC-I molecules in DCs, multivesicular bodies (MVBs) and the endocytic recycling compartment (ERC).

In order to arrest MVBs biogenesis we used the inhibitor U18666A, a drug that interrupts cholesterol trafficking and changes the lipid composition of intracellular membranes. Upon bone marrow-derived DC (BMDC) treatment with U18666A, we evidenced a drastic disruption in the ability to present exogenous soluble and particulate antigens to CD4+ and CD8+ T cells. Strikingly, the presentation of *T. gondii*-associated antigens and parasite proliferation were hampered in treated cells. Since U18666A impairs the formation of MVBs, we analyzed in *T. gondii*-infected BMDCs the ESCRT machinery responsible for the generation of intraluminal vesicles. We observed that different MVBs markers, including ESCRT proteins, were recruited to the PV. Surprisingly, the main ESCRT-III component CHMP4b was massively recruited to the PV, and its expression level was upregulated upon BMDC infection by *T. gondii*. Finally, we demonstrated that BMDC treatment with U18666A interrupted cholesterol delivery and CHMP4b recruitment to the PV, which interfered with an efficient parasite replication.

We used a different approach to impair the ERC functionality in DCs by silencing the expression of Rab22a. We have previously shown that this experimental strategy leads to a drastic disrupture of the main intracellular pool of MHC-I molecules distributed at the ERC, and consequently it affects the cross-presentantion of *T. gondii*-associated antigens. Now, we deppened into the replicative capacity of this parasite inside Rab22a KD DCs. We studied the kinetics of Rab22a recruitment towards the PV, and how this GTPase modulates the size and number of lipid droplets in *T. gondii*-infected DCs. Finally, we found that after the initial infection, the parasite is unable to replicate efficiently inside Rab22a silenced DCs.

Altogether, our results highlight the importance of MVBs formation and the ERC integrity in DCs for optimal antigen presentation and *T. gondii* proliferation.

MODULATION OF HOST METABOLIC PATHWAYS ATTENUATES *LEISHMANIA* SPP INFECTION IN MACROPHAGES

Elaine Carvalho de Oliveira[1, 2], Rafael Tibúrcio[1, 2] Gabriela Duarte[1, 2], Léon Dimitri Melo [3], Amanda Lago[1,] Sara Nunes[1,2], Natalia Tavares[1, 2], Pedro M. M. de Moraes-Vieira[4,.5.7], and Cláudia Ida Brodskyn[1, 2, 6]

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In the settings of infection and inflammation, macrophages undergo a dynamic remodeling of their enzymatic activity to synthesize new building blocks and obtain of energy. These metabolic processes collectively support the immunological state acquired by macrophages to implement effective immune responses. Not surprisingly, several intracellular pathogens have developed means to manipulate macrophage metabolic machinery to survive and proliferate. Under resting conditions, macrophages rely on the oxidative phosphorylation (OXPHOS) of molecules (such as glucose, amino acid, fatty acids) to accommodate their energetic demands. However, upon activation, macrophages engage in the generation of ATP from the oxidative of glucose to pyruvate in the presence of oxygen (a metabolic pathway know as aerobic glycolysis). The metabolic rewiring from a quiescent (OXPHO-dependent) towards an activated (glycolytic) phenotype is a process known as Warburg effect. Shreds of evidence show that the elimination of intracellular pathogens such as viruses, bacteria, fungi, and protozoa depend on this metabolic switch. We reasoned that the reorganization of the metabolic program of macrophages is essential in protection against trypanosomatid infection. To further expand on this notion, we employed a model of parasite infection by exposing bone-marrow derived macrophages (BMDMs) harvested from C57/Bl6 mice to *Leishmania amazonensis* and *L. braziliensis*. Transmission of *Leishmania* spp, the etiological agent of leishmaniasis, figures as a major health concern, especially in the tropical zones of the world. Therefore, understanding the immunological and metabolic cues during *Leishmania* spp infection is critical to improve prophylactic and therapeutic strategies. We show that *Leishmania* spp-infected macrophages display augmentation of their energy metabolism in direct dependence to their mitochondria fitness. *Leishmania* spp infected macrophages exhibited higher levels of mitochondrial membrane potential and mass when contrasted to uninfected macrophages. Furthermore, pharmacological interference of key metabolic circuits results in blunted *Leishmania* spp infection irrespectively to the strain used. These observations can reveal a conserved immune evasion strategy shared by these two species of *Leishmania*. Altogether, our findings aid in the elucidation of important aspects surrounding the immunopathogenesis of leishmaniasis.

SYMPOSIUM 3 - *Friday, November 10, 10-12 h*
IMMUNOMEBOLISM

Chairs: Claudia Pérez Leriós - Pilar Aoki

EXPLORING THE IMMUNOMETABOLISM DURING TRYPANOSOMA CRUZI INFECTION

Cinthia Stempin
CIBICI-CONICET. Departamento de Bioquímica Clínica, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Córdoba, Argentina

Chagas disease (CD) is caused by the protozoan *T. cruzi.* Initially confined to Latin America, CD has now become a global concern due to globalization. It has a high morbidity and mortality rate and significant social impact posing a major public health threat. The disease progresses from an acute phase (AP) to a chronic one, with some individuals remaining asymptomatic while others develop progressive heart disease, leading to end-stage dilated cardiomyopathy in 20-30% of cases. Despite efforts by endemic and non-endemic countries to control, treat, and interrupt disease transmission, finding a cure or eradicating CD remains a pressing global issue.

The role of T cells-mediated immune response in controlling intracellular protozoan parasite infection is well established. However, *T. cruzi* exhibits numerous adaptations that enable it to establish a chronic infection, such as inducing T cell apoptosis delaying the onset of immune response. It is widely described that metabolic pathways dictate the effector functions of immune system cells and, therefore, can become targets of immune evasion for pathogens.

In this context, our lab has explored T cell metabolism during experimental T. *cruzi* infection. We focused on the metabolic and mitochondrial phenotype acquired by CD4 T cells during infection, and its functional consequences that could explain failures in this cell compartment. We found that CD4 T cells during AP of infection acquire an exacerbated glycolytic and oxidative metabolic profile, with high levels of mitochondrial reactive oxygen species (mROS) accumulating since early stages of the infection. High mROS levels observed during AP of infection correlates with increased mitochondrial damage. We observed that effector CD4 T cells are strongly affected by these alterations. Furthermore, these cells are less functional and more prone to apoptosis. Increased apoptosis could be related to deficient antioxidant systems, as well as mitochondrial dynamics alterations. In this sense, in vitro treatment with the antioxidant NAC, reduced the frequency of apoptosis in CD4 T cells with depolarized mitochondria. Moreover, in vivo treatment with the NAD precursor Nicotinamide Riboside, which is capable of inducing mitophagy, diminished the frequency of mROS-producing cells with depolarized mitochondria. Even more, we demonstrated that mitochondrial defects are TCR signaling-dependent and are related to the infection context, as they do not happen in an immunization model.

Altogether, these results are the first in demonstrating that *T. cruzi* acute infection triggers metabolic and mitochondrial alterations in CD4 T cells that are linked to a deficient function and apoptosis induction. Thus, these events would contribute to the host immune response's inability to clear the parasite. Then, these results provide new insights into understanding the molecular and metabolic processes that drive the dysfunction and the outcome of CD4 T cells during infection.

IMMUNOMETABOLIC CHARACTERIZATION OF MATERNAL MONOCYTES AND MACROPHAGES AT EARLY PREGNANCY

Daiana Vota

Universidad de Buenos Aires, Facultad de Ciencias Exactas y Naturales, Departamento de Química Biológica, Laboratorio de Inmunofarmacología. Buenos Aires, Argentina. CONICET, Instituto de Química Biológica de la Facultad de Ciencias Exactas y Naturales (IQUIBICEN), Buenos Aires, Argentina.

Immunometabolism has emerged as a central mechanism that determines the cellular fate and function during adaptive and innate immune responses. Accordingly, macrophages can reprogram their cellular metabolism to exhibit dynamic polarization changes associated to functional coordination for the effective establishment and resolution of immune responses. During pregnancy, soluble factors released by trophoblast cells in the maternal-fetal interface favor monocyte/macrophage recruitment and anti-inflammatory profiles. In line with this, defective placentation associates with altered immune and trophoblast interaction resulting in complicated pregnancies that threaten maternal and neonatal health and underlie metabolic programming of adult life. So far, the immunometabolic profiling of human maternal circulating monocytes at early pregnancy and whether the trophoblast has a conditioning role have not been reported. We aim to characterize the metabolic pathways active in circulating monocytes from pregnant women during early pregnancy and to deepen into the potential mechanisms of metabolic reprogramming induced by human first trimester trophoblast cells in the maternal fetal interface. Our results add an immunometabolic perspective to previous work on monocytes from early pregnant women, providing new clues to the understanding of the regulatory mechanisms that operate at this stage of pregnancy and founding future hypotheses regarding pregnancies complicated by placental insufficiency or metabolic disorders.

IMPACT OF THE CHOLINERGIC SIGNALING IN THE ADIPOSE TISSUE TYPE 2 IMMUNITY AND THE ONSET OF METABOLIC DISEASES

Luísa Menezes-Silva[1], Leonardo Mandu Gonçalves[1], Mirian Krystel de Siqueira[2], Jaqueline Marques Santos[1], Marina Caçador Ayupe[1], Caio Loureiro Salgado[1], Bárbara Cristina Pizzolante[1], Marcela Davoli-Ferreira[3], Bernardo de Castro Oliveira[1], Francielly Moreira[1], Walter Miguel Turato[4], Meire Ioshie Hiyane[1], Niels Olsen Saraiva Camara[1], Vania Ferreira Prado[5], Marco Antônio Máximo Prado[5], Jose Donato Jr[6], Jose Carlos Alves-Filho[7], Alexandre Kanashiro[7], Carla Máximo Prado[8], Denise Morais da Fonseca[1*]

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Although several neural mechanisms have been implicated in the control of immunity, little is known about the importance of acetylcholine (Ach) signaling in adipose tissue immune homeostasis. At the Laboratory of Mucosal Immunology, we are studying how cholinergic signaling interferes with adipose tissue immunity and host metabolism. Amongst the white adipose tissue depots, the mesenteric visceral adipose tissue (mesWAT) has the densest cholinergic innervation, as well as a higher number of Ach-producing CD45+ cells, specifically located at the fat-associated lymphoid clusters. The impairment of the cholinergic signaling resulted in a robust type 2 response, specifically in the mesentery but not in the other adipose tissue compartments. We showed that, in the mesentery, Ach controls IL-33 production and, consequently, the type 2 immune tonus. Therefore, blocking the Ach-signaling promotes IL-33 secretion by mesenteric non-hematopoietic stromal cells and shifts the immune tone toward type 2 immunity. By controlling the immune homeostasis in the mesenteric adipose tissue, Ach interferes in host metabolism and favors susceptibility to the development of metabolic syndrome.

SIMULTANEOUS INHIBITION OF MTORC1 AND PPAR GAMMA DRIVES THE DIFFERENTIATION OF HUMAN MONOCYTES INTO IMMUNOGENIC DENDRITIC CELLS

Fernando Erra Diaz [1], Lucía Bleichmar [1], Ignacio Mazzitelli [1], Claudia Melucci [1], Asa Thibodeau [2], Radu Marches [2], Tomás Dalotto-Moreno [3], Gabriel Rabinovich [3], Duygu Ucar [2] & Jorge Geffner [1]

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Monocytes are characterized by remarkable plasticity and can differentiate into macrophages (Mo-Macs) or dendritic cells (Mo-DCs). The mechanisms underlying this cell fate decision are still poorly understood. However, by culturing monocytes in the presence of GM-CSF + IL-4, large numbers of Mo-DCs can be generated in vitro (GMIL4 Mo-DCs) . Despite significant differences between these cells and DCs found in vivo, this remains the most widely used method to study human DC biology and to obtain DCs for use in the context of vaccines. We report that pharmacological inhibition of mTORC1 (Temsirolimus) or PPARγ (GW9662), in the presence of GM-CSF and in the absence of IL-4, induces differentiation of human monocytes into Mo-DCs in vitro. Moreover, compared to GMIL4 Mo-DCs, we observed that concomitant inhibition of mTORC1 and PPARγ pathways induced the differentiation of Mo-DCs with remarkable phenotypic stability, a stronger immunogenic profile and a higher capacity to induce the expansion of antigen-specific CD8 T cells (GMTGW Mo-DCs). Bulk RNAseq of monocytes undergoing differentiation (8h culture) and sorted Mo-DCs, revealed different transcriptional profiles between GMIL4 and GMTGW Mo-DCs, suggesting distinct differentiation pathways and cell identities. In this scenario, PCA analysis of the transcriptional profiles showed that GMTGW Mo-DCs have greater similarity to blood and tumor-associated cDC2 cells than GMIL4-Mo DCs. In addition, GMTGW-Mo DCs exhibit a strong type I interferon signature and lower expression of several inhibitory ligands and receptors, including PD-L1, and significant Langerin (CD207) expression in a subpopulation of cells compared to GMIL4-Mo DCs. In summary, we here describe a previously uncharacterized differentiation pathway that induces the generation of Mo-DCs, with potential implications for DC vaccination and cancer immunotherapy.

Symposium 4 - *Friday, November 10, 16-18h*
CLINICAL IMMUNOLOGY
Chairs: *Gabriela Simesen - Laura Pérez*

INDUCED AUTOIMMUNITY: ASIA SYNDROME AND AUTOIMMUNITY BY CHECKPOINT INHIBITORS

Gloria Rivero
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The concept of the “mosaic of autoimmunity” is a reference to multiple compounds that determine the development to autoimmunity, such as genes, hormones, infectious and other environmental stimuli. Regarding triggers of autoimmunity, in 2011, Shoenfeld et al described the ASIA Syndrome (Autoimmune-autoinflamatory Syndrome Induced by Adjuvants), a group of conditions that appear in genetically predisposed individuals who have exposure to components with adjuvant effect, such as silicone and vaccines, among others. Adjuvants increase innate immune responses by mimicking evolutionarily conserved molecules and binding to Toll-like receptors. Additionally, they lead to maturation and activation of recruited dendritic cells and activate the inflammasome system. Thus, adjuvants increase the local reaction to antigens, and are associated with secretion of cytokines and chemokines. Siliconosis, the Gulf War syndrome, the macrophagic-myofasciitis syndrome and post-vaccination phenomena were linked with previous exposure to adjuvants.
Recently, some cancer immunotherapies, therapies that directly or indirectly target any component of the immune system that are involved in the anti-cancer immune response, are described as new triggers of autoimmunity. Specifically, inmune checkpoint inhibitors (ICIs) which work on CTLA 4 or PD1 pathways for example, are approved for the treatment of various cancer types because they have a universal effect on immune responses that is not dependent on individual cancer-specific antigens. They have a lot of immune-related adverse events (irAEs) that result from excessive immune response against normal organs and tissues because of the suppression of normal regulatory mechanisms. Potential mechanisms of toxicity to immune checkpoint blockades include autoreactive T-and-B-cell development, molecular mimicry, epitope spread, creating an inflamatory cytokine profile, direct cytotoxicity and amplification for preexisting inflammatory or autoinmune pathologies. They are not mutually exclusive, and different mechanisms likely exist for different immune-related toxicities. These irAEs include over 70 different pathologies, including some organ-specific diseases, such as myocarditis, neumonitis, enteritis or thyroiditis, and systemic diseases including rheumatoid arthritis, spondyloarthropathy, lupus, Sjögren syndrome, and psoriasis, among others. Its frequency is 20 to 90 % of patients who receive ICI. The time of onset, course and severity are unpredictable.

CLINICAL IMPLICATIONS OF DEFECTS IN THE JAK/STAT SIGNALING PATHWAY

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Activation and homeostasis of the immune response are finely regulated by various molecular signaling pathways. Among these, the pathway involving *Janus kinase/signal transducer and activator of transcription (JAK/STAT*) plays a fundamental role in cellular functions of different tissues. Schematically, the following levels of action can be considered: - Initially, cytokines (interleukins, interferons), growth factors and hormones act as ligands binding to their respective receptors. - Receptors are expressed on the membrane of various target cells (hematopoietic, immune, adipose). - Signaling from these receptors to the cell nucleus mediated by various JAK/STAT molecules. Each ligand-receptor pair will use certain members of the JAK/STAT families. Both, phosphorylation and molecular dimerization, are processes intimately involved in this process. - Finally, STATs molecules enter to the nucleus, bind specific gene sequences and modify gene transcription. Through this signaling pathway, functions such as hematopoiesis, immune activation, inflammation, adipogenesis, etc. are regulated. In recent years, gene defects in several members of the JAK/STAT families have been identified as responsible for various diseases in humans. From the cellular point of view, the identified pathogenic variants can be located at germinal level or at somatic level, leading to a situation of loss of function (LOF) or gain of function (GOF). As a consequence, the processes globally presented are linked to malignancies, autoimmunity, inflammation, atopy and/or immunodeficiency. The classification of the International Immunology Societies (IUIS) includes a vast majority of these entities. Some examples: • Defects in *Jak1* autosomal recessive LOF: Susceptibility to Mycobacterial infections. • Defects in *Jak1* autosomal dominant GOF: Immune dysregulation (autoimmunity, inflammation) and hypereosinophilia. • Defects in somatic *Jak2*: Similar picture Systemic lupus erythematosus (SLE), Myeloproliferation, Thrombocytopenia, Polycythemia vera. • Defects in *Jak3* autosomal dominant GOF: Lymphoproliferation, Hypogammaglobulinemia, Interstitial lung disease. • Defect in *Stat1* autosomal dominant GOF: Infections, multiple autoimmunity, inflammation, lymphoproliferation, atopy, malignancy, aneurysms. • Defect in Stat2 autosomal recessive LOF: AicardiGoutieres syndrome, Lymphohistiocytosis. • Defect in *Socs1*: Autoimmunity (similar to SLE, cytopenias), inflammation (similar to Psoriasis), lymphoproliferation, lymphoma, atopy. New advance in massive DNA sequencing methodologies have made it possible, not only to address the diagnosis with certainty, but also to decide on precise therapeutic (Jaks inhibitory molecules) conducts in various medical specialties (Clinical immunology, Rheumatology, Gastroenterology, Dermatology, etc.).

AUTOANTIBODIES IN RHEUMATIC DISEASES: COMMON, RARE & MORE

Carolina Gorlino

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Autoantibodies in rheumatic diseases are vital biomarkers for diagnosing, predicting clinical characteristics, determining prognosis, and guiding treatment decisions. Recent evidence suggests that autoantibodies not only associate with these conditions but actively participate in their pathological processes. This knowledge is essential for appreciating their clinical utility as biomarkers. Our research primarily focused on autoantibodies associated with rheumatoid arthritis and connective tissue diseases, where significant progress has been made in understanding their biological effects and clinical applications. Rheumatoid arthritis (RA) is a chronic systemic inflammatory disease primarily affecting synovial joints, often accompanied by non-joint-related symptoms. Notably, rheumatoid factor (RF) and anti-citrullinated protein antibody (ACPA) are prominent autoantibodies in RA, providing valuable clinical insights. We found that the presence of ACPAs in synovial fluid (SF) correlated with neutrophil infiltration, which, in turn, was positively associated with disease severity. Furthermore, SF-ACPA levels induced a pro-inflammatory profile, leading to elevated cytokine levels, increased neutrophil counts, and more severe clinical manifestations. Serum autoantibodies play a pivotal role in early systemic autoimmune rheumatic disease detection, such as connective tissue diseases. In some cases, they are linked to organrelated complications, like interstitial lung disease (ILD). A newly discovered autoantibody targeting Eukaryotic Initiation Factor 2B (eIF2B) was detected in Systemic Sclerosis (SSc) patients. We recently showed that a small group of patients with SSc exhibit anti-eIF2B and this reactivity was associated with ILD. On another note, serum antimitochondrial antibodies (AMAs) specifically target mitochondrial membrane components and are a hallmark of primary biliary cholangitis. Interestingly, they can also be found in connective tissue diseases, particularly SSc. We confirmed the presence of autoantibodies against pyruvate dehydrogenase complex components in rheumatic patients, regardless of the presence of primary biliary cholangitis or liver dysfunction. Consequently, detecting AMAs in SSc and other rheumatic conditions could serve as a predictor for primary biliary cholangitis. In conclusion, systemic autoimmune rheumatic diseases exemplify scenarios where disease-specific or associated autoantibodies serve multifaceted roles as diagnostic aids, prognostic indicators, and treatment guides, while actively influencing disease progression. Understanding the pathogenic functions of these autoantibodies alongside clinical observations significantly enhances their potential as valuable biomarkers.

DISEASES OF IMMUNE DYSREGULATION: HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS

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CD8+ Cytotoxic T Lymphocytes (CTL) and Natural Killer (NK) cells are cells of the Immune System crucial in immune surveillance against cells infected by viral agents and tumor cells. In recent years there has been crucial progress in the study of complex mechanisms of action of these two cell groups, mainly in the phenomenon of “Cytotoxicity mediated by lytic granules” (dependent on Perforins and Granzymes). The granule-mediated cell death pathway is key in the elimination of viral agents and tumor cells. The formation of the immunological synapse between the immune effector and the target cell induces granule trafficking secretions, the permeabilization of the target cell and its death by apoptosis. The integrity of its effector mechanisms is essential both for the immune response against viral and tumor noxas, as for the maintenance of homeostasis of the immune system. Conditions pathological, lead to a phenomenon of immune dysfunction, with results potentially fatal: hemophagocytic lymphohistiocytosis (HLH). HLH may be inherited, as in the case of primary or familial HLH (FHL), or acquired (secondary HLH). All known genetic mutations responsible for FHL reside in genes that code for proteins in the cytolytic pathway, which is employed by CD8+ T and NK cells to destroy host cells that are compromised by infection. In the absence of a robust cytolytic response, antigen stimulation persists and drives cytokine production. The result is a dramatic presentation in affected patients with fevers, rashes, multi-organ dysfunction, cytopenias, and coagulopathy due to cytokine storm. The diagnosis of HLH is made when a patient is found to have a molecular diagnosis consistent with HLH or fulfills 5 of 8 diagnostic criteria: 1) fever, 2) splenomegaly, 3) cytopenias, 4) elevated triglycerides/decreased fibrinogen, 5) hemophagocytosis, 6) decreased NK cell function, 7) increased ferritin, and 8) increased soluble IL-2 receptor levels. Historically, FHL was diagnosed almost exclusively in infancy and thought to be fatal without chemotherapy and hematopoietic stem cell transplantation (HSCT). In contrast to FHL, secondary HLH is an acquired condition, typically triggered by a viral infection. Patients with secondary HLH tend to be older and have less severe disease that is often managed with chemotherapy but not necessarily with HSCT.

SYMPOSIUM 5 - *Saturday, November 11, 16-18 h*
TRANSLATIONAL IMMUNOLOGY

Chairs: Pablo Baldi – Cecilia Rodríguez Galán

TMEM176B INHIBITION REPROGRAMS CD8$^+$ T CELL EXHAUSTION VIA TH17 COOPERATION

Marcelo Hill

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DEVELOPMENT OF ARVAC CG, A SUBUNIT VACCINE AGAINST SARS-COV-2

Karina Pasquevich

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ARVAC CG is a variant adapted recombinant RBD-based COVID-19 vaccine, adjuvanted with aluminum hydroxide. This vaccine was developed from inception to clinical application in Argentina, with research contributions from the National University of San Martin (UNSAM), the National Scientific and Technical Research Council (CONICET) and the Pablo Cassará Foundation. In 2022, a phase 1 clinical trial was conducted to assess the safety and immunogenicity of two doses of the Gamma variant vaccine. Subsequently, in 2023, a phase 2/3 clinical trial was carried out to evaluate the immunogenicity of three distinct versions of the vaccine: the Gamma Variant RBD-based ARVAC-CG vaccine, the Omicron Variant RBD-based ARVAC-CG vaccine, and the Bivalent Gamma/Omicron RBD-based ARVAC-CG vaccine. This study involved adult volunteers who had previously received vaccinations against the SARS-CoV-2 virus.

Results of these clinical trials indicated that ARVAC CG exhibits a satisfactory safety profile, a robust and broad booster response of neutralizing antibodies against different SARS-CoV-2 variants and a booster effect on T cell immunity in individuals previously immunized with different COVID-19 vaccine platforms.

IMMUNE CHARACTERISTICS IN PANCREATIC CANCER AND THEIR IMPACT FOR CLINICAL OUTCOMES

Eduardo Chuluyan[1], Nicolás Fraunhoffer[1,2], Carla Remolins[1], Gustavo Kohan[3], Kevin Matamoros[1], Diego Guerrieri[1], Olivier Blond[1], Analía Abuelafia[2], Nelson Dusetti[2], Juan Iovanna[2,4]

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Pancreatic ductal adenocarcinoma (PDAC) is one of the malignant neoplasms with the worst prognosis with fewer than 7% of patients surviving past 5 years following diagnosis. Some of the various factors that contribute to this are the lack of appropriate diagnostic and prognostic biomarkers as well as a poor understanding of the dynamic interaction between the immune system and cancer cells within the tumor microenvironment. The new immune checkpoint Inhibitors that revolutionized cancer treatment by boosting immune responses have not been effective in PDAC. However, in a recent small trial in phase I, a combination of a personalized mRNA vaccine, an immune checkpoint inhibitor and chemotherapy after surgery, induced an effective immune response in half of the treated PDAC patients. An essential question is why half of the patients did not respond favorably to the vaccine despite the similarities in their clinical features? We believe it is imperative to study the patient´s immunological characteristics as well as the immune factors produced by the tumor in the interest of personalizing cases better and achieve an accurate stratification. This knowledge could also be useful to find and apply the most effective therapies. Herein, we will discuss factors that lead to diverse mechanisms of immune evasion, in addition to some of the immunologic traits that might be used to classify patients, in order to offer the most-effective therapeutic target for each case.

EXPLORING HIGH-DIMENSIONAL IMMUNOLOGICAL LANDSCAPES: INSIGHTS FROM CHECKPOINT INHIBITORS AND SARS-COV-2 VACCINE COMBINATIONS.

Nicolás Gonzalo Núñez[1,6], Ekaterina Friebel[1], Fiamma Berner[2], Jonas Schmid[1], Laura Power[1], Mitchell P Levesque[3], Lukas Flatz[2,4], Mariana Maccioni[5], Burkard Becher[1].

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The perpetual threats of cancers such as melanoma and non-small cell lung cancer (NSCLC), coupled with the evolving challenges of the COVID-19 pandemic, underscore the importance of expanding our understanding of the immune system for therapeutic advancements. This presentation navigates the diverse domains of immune checkpoint inhibitors (ICIs) and SARS-CoV-2 vaccines, exploring the dynamic and intersecting immunological landscapes they forge.

Firstly, we explore the role of ICIs in cancer treatment, notably melanoma and non-small cell lung cancer (NSCLC). Through combining a multi-omics approach, we reveal early indicators of immune-related adverse events (irAEs) following ICIs' administration, enabling more nuanced risk-benefit assessments, and contributing to a patient-tailored approach.

Subsequently, we analyze the breadth of immune responses elicited by multiple SARS-CoV-2 vaccines, demonstrating that the vaccine type and order of their administration remarkably shape immune signature profiles. Furthermore, we identify specific combinations that result in stronger defenses, thus broadening the possibilities for effective immunization strategies.

In conclusion, this integrative study provides compelling evidence of how various immune modulation strategies can direct us towards truly personalized medicine. From optimizing cancer therapies with ICIs to tailoring COVID-19 vaccines, our investigations highlight the immense potential and inherent complexity of the human immune response. This research illuminates new paths to overcome global health challenges, thus opening up broad possibilities for the future of immunology.

Hypersensitivity and allergies

Thursday, November 9, 14-15:30 h

Chair: Romina Fernández Brando

1 (70) A POLYMERIC NANOPARTICLE PLATFORM WITH TH1-DEPENDENT ADJUVANT PROPERTIES FOR IMMUNOMODULATION OF FOOD ALLERGY

Gastón P. Rizzo[1], Camila Chavero[1], Daiana Bianchi[1], Micaela Quereda[1], Evangelina Dupuy[1], Eugenia Apuzzo[2], Santiago E. Herrera[2], Maximiliano L. Agazzi[2], Omar Azzaroni[2],Alberto Fossati[1], Guillermo H. Docena[1], Paola L. Smaldini[1].

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Nanotechnology is increasingly assuming a significant role in the advancement of vaccine development. This technology can create diverse nanoparticles (Np) with adjustable composition, size, shape, and surface characteristics. Emerging advances in nanotechnology significantly impacted on biomedicine, vaccinology and health research. In this study, we analyzed polyallylamine (PAH) nanoparticles as a vehicle for antigen protection, delivery carrier, and adjuvant in formulating a mucosal therapeutic vaccine for food allergies.

We studied the intracellular inflammasome activation on murine bone marrow-derived dendritic cells (BMDC) using Np and different inhibitors. Additionally, we stimulated bone marrow-derived macrophages (BMDM) from null mice for NLRP3, Caspase-1, Caspase-1/11, or gasdermin D (GSDM D) to identify the pathway involved. The secretion of IL-1β was used as a readout of cell activation. Np´s integrity and carrier capacity throughout the gastrointestinal tract and the adjuvant properties were assessed in a mouse model of IgE-dependent food allergy driven by cholera toxin and cow´s milk protein (CMP) as a food allergen. Sensitized mice were then treated by gavage of β-lactoglobulin encapsulated in Np. Clinical score and immunological parameters were assessed for evaluation of allergy suppression.

We found that Np were up-taken by phagocytic cells and upon cell activation, IL-1β was secreted. Inhibitors of the inflammasome pathway abrogated the cytokine release ($p<0.01$), and using null mice, we confirmed that cell activation is dependent on caspase 1 and NLRP3. *In vivo* tracking experiments showed that Np were stable during the gastrointestinal passage, protected the immunogen and reached the critical immune organs to promote dendritic cell activation with increased CD80 an CD86 expression. Our findings on the food allergy mouse model showed that the β-lactoglobulin-containing vaccine exerted a Th1-dependent immunity that reversed the mucosal allergic reaction. The clinical score, specific IgG1, IL-5 and IL-13 were suppressed.

In conclusion, we found that Np activated antigen-presenting cells in an NLRP3- and caspase-1-dependent manner, promoting the secretion of IL-1β. In addition, Np could be mucosally administered for immune activation and we showed that an intragastric Np-based vaccine reversed experimental food allergy. Our findings may pave the way for developing a mucosal immunotherapy to treat this inflammatory disorder.

2 (34) *Lacticaseibacillus casei* CRL75 MODULATES HUMORAL IMMUNE RESPONSE IN FOOD ALLERGY. PREVENTION OF THE DEVELOPMENT OF ASSOCIATED SYMPTOMS.

Jonathan Emiliano Laiño[1]*, María Hortensia Zelaya[2], María Pía Taranto[1]

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Food allergies constitute a public health problem of great importance as they can lead, in some cases, to anaphylactic shock. About 3% of adults and 3-8% of children suffer some type of this reactivity. In general, food allergy is not exclusive to one type of food, but rather many of them have cross-reactions with other foods and/or pollen. The beneficial effect of some lactic acid bacteria (LAB) on consumer health is widely reported, as well as the ability to release and/or produce specific compounds (nutraceuticals) with positive outcome on the human or animal host. Many exopolysaccharides (EPS) produced by beneficial LAB are powerful immunomodulatory molecules. Previously, it was demonstrated that *Lacticaseibacillus (L.) casei* CRL75 has a potent immunostimulatory effect and produces functional EPS. **Objective:** to evaluate the ability of *L. casei* CRL75 to prevent the development of symptoms associated to food allergies by regulating the humoral immune response and related cytokines. **Materials and methods:** The immunomodulatory capacity of *L. casei* CRL75 was evaluated *in vivo* by an OVA-induced intestinal allergy mouse model. Six week-old BALB/c mice were divided into 3 groups: a) Control (C): non-sensitized animals received a conventional balanced diet and water *ad libitum*; b) OVA: animals sensitized to OVA by bi-weekly intraperitoneal injection; c) *L. casei* CRL75 (CRL75): OVA-sensitized animals supplemented with *L. casei* CRL75 in drinking water for 7 days prior to OVA challenge. Two weeks after second injection, intestinal allergy was induced by feeding egg white diet for 7 days. Samples were obtained to evaluate: a) cytokines: IL-4, IL-10, TNF-α; b) OVA-specific antibodies (OD450nm): IgE, IgG, IgG1 and IgG2a; c) bacterial translocation to liver and spleen. **Results:** Supplementation with *L. casei* CRL75 induced the production of TNF-α, and IL-10, with a significant decrease in IL-4 levels (pg/ml, C: 71.43±18.1, OVA: 941±94.77; CRL75: 364.74±76.64, $p<0.0001$). Besides, *L. casei* CRL75 induced significant decrease of OVA-specific IgE levels (OD450nm, C: 0.064±0.017; OVA: 0.717±0.085; CRL75: 0.449±0.036, $p<0.001$), with an increase in OVA- specific IgG1 and IgG2a compared to OVA group. On the other hand, no bacterial translocation was detected in any experimental group. **Conclusion:** Results showed that *L. casei* CRL75 is able to regulate immune response in an OVA-induced intestinal allergy. *L. casei* CRL75 led to the development of a Th-1 response, decreasing the Th-2 response, with reduced levels of OVA-specific IgE and IL-4. Thus, *L. casei* CRL75 constitutes a promising option for the design of functional foods with beneficial effects on food allergies.

3 (84) UPPER AND LOWER AIRWAY INFLAMMATION IN A POLLEN-INDUCED MURINE MODEL OF ALLERGIC RHINITIS

Marcelo Javier Galvez, Ileana Lencinas, Gisela Giorgi, María Carla Crescitelli, Adriana Martínez, María Gabriela Murray, María Belén Rauschemberger, María Inés Prat

INBIOSUR (Instituto de Ciencias Biológicas y Biomédicas del Sur), Departamento de Biología, Bioquímica y Farmacia, CONICET-Universidad Nacional del Sur, Bahía Blanca, Buenos Aires, Argentina

More than 80 % of asthmatics have rhinitis and 10-40 % of individuals with rhinitis also have asthma. Allergic rhinitis (AR) and asthma are frequently associated and often share therapeutics. Guidelines by World Health Organization emphasize the importance of links between the upper and lower airways and recognize both entities as a global airways disease. In our region, Russian thistle (*Salsola kali*) is a common weed causing pollinosis and previously, our group has used its pollen extract to generate a murine *S.kali*-specific AR acute model. The aim of this study was to establish whether AR may contribute to the inflammatory response of lower airways in a prolonged exposure model. BALB/c mice (n = 5/group) were administrated every three days for two weeks with PBS or *S. kali* pollen extract through i.p. route. Then, mice were challenged by nasal instillation of PBS or *S. kali* pollen respectively weekly for 6 weeks. After sacrifice, the sera and bronchoalveolar lavage (BAL) were obtained. Total serum IgE and *S. kali*-specific IgE, IL-4 and IL-5 levels from splenocytes culture were measured by ELISA. The noses and lungs were fixed and paraffin embedded for histological analysis (H&E). After nasal challenge, frequency of sneezing in sensitized group were higher than in control ($p<0,05$). IgE values were increased three times in sensitized mice compared to control group ($p<0,01$). Moreover, mice in the sensitized group had significantly higher levels (26 times) of *S. kali*-specific IgE than the control ($p<0,001$). IL-4 and IL-5 values in sensitized group (31,7 ± 4,5 pg/ml and 31,4 ± 4,8 pg/ml respectively) were higher than control group ($p<0,01$). Significant increased number of total inflammatory cells were observed in BAL from exposed mice compared to control group ($p<0,05$) including eosinophils which represented up to 90 % of the cells in this group compared to the 20 % observed in non-exposed animals. Histological studies showed high inflammatory cells recruitment and macrophage-like cells infiltration in the lung connective tissue of mice sensitized group with chronic exposition, unlike our previous studies of acute exposure that showed minor changes at the lung level. In nasal mucosa, inflammatory cells infiltration was moderated in mice of sensitized group respect to the control group. Our results confirm an upper and lower airway inflammation associated to an IgE-mediated hypersensitivity reaction induced by *S. kali* pollen proteins. Moreover, chronicity of aeroallergen exposure exacerbates inflammatory response in lower airways as a result of local nasal challenge induced. Regardless of the need for further investigation to clarify the mechanisms under these interactions, this model could be helpful for new therapeutic strategies development against AR and also asthma.

Anti-infectious immunity
Thursday, November 9, 14-15:30 h
Chairs: Ignacio Cebrián - Cinthia Stempin - Griselda Moreno - Javier Eliçabe

4 (196) BIOFILM AND INFLAMMATION. STUDY OF THE ANTIMICROBIALAND INMUNOMODULATORY CAPACITY OF LACTIPLANTIBACILLUS PLANTARUMON ON *STAPHYLOCOCCUS AUREUS* STRAINS ISOLATED FROM DIABETIC FOOT ULCERS

Mirta Rachid[1], Cecilia Werenitsky[2], Nicolás Argañaraz Aybar[1], Gonzalo Domínguez Paredes[1], Gustavo Calvo[3], Fernanda Buzzola[3], Sebastián Nisoria[4]
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Objectives: The objetives of this study were to (i) study the antimicrobial properties of *Lactiplantibacillus plantarum* (Lp) and its supernatant (SLp) on the virulence factors of methicillin-resistant *Staphylococcus aureus* (MRSA) strains isolated from diabetic foot ulcers and (ii) to investigate the effect of Lp and its supernatant on the microbicity activity of polymorphonuclearcells (PMNs) infected with MRSA. **Material and methods:** Diabetic patients with ulcers were treated with a topical application of Lp. This study was approved by the Hospital Ethics Committee. Biopsies were taken at day 0, 10 and 30 post-treatment. Standard bacteriology techniques were applied for the isolation and identification of microorganisms that infect wound. MRSA strains were identified from biopsies obtained before, during and post-treatment. Antibiotic susceptibility was determined accordingto CLSI international standards. Quantitative real-time PCR was utilized to determine genes expression that code for adhesión virulence factors (fnbA,sdrC, eap, emp, cna, clfA).The inhibition capacity of Lp and its supernatant (SLp) on the biofilms of MRSA strains was studied with the O´Toole and Kolter technique and viability was also determined with MTT assay. Antimicrobial susceptibility profiles of MRSA and the growth inhibition capacity of Lp and SLp were determined using the disk-diffusion method. PMNs wereisolated from the circulanting blood of the patients. PMNs were stimulated with Lp and culture supernatant to perform the following tests: phagocytosis byflowcytometry, NETosis by fluorometry and microbicidal activity. **Results:** An increase in the expression of the eap, fnbA and clfA genes of the MRSA strains obtained from the biopsies of day 0 and 15 compared to the biopsies of day 30 was observed. In this study all MRSA strains isolates were identified as biofilmsproducers. Lp and SLp inhibit the MRSA biofilm after 18 h of incubation. Significant differences were observed between the MRSA biofilm without treatment and the biofilm treated with Lp (100% vs 35%<0.005) and SLp (100% vs 40% <0.005). Increased phagocytic activity of PMNs was observed in healthypatients only stimulated with Lp (230 ifm vs 402 ifmp <0.001). The same was observed for diabetics patients PMNs (210 ifm vs 460 p <0.001). The PMNs of healthy patients showed greater induction of NETosis (4000 RFU) vs PMNs of diabetics patients (2000 RFU). A significant increase in NETosis of PMNs of diabetics patients stimulated withLp was observed (6000 RFU). A decrease in UFC/ml was observed in the PMNs of diabetic patients stimulated with Lpcompared with those no stimulated ($2x10^6$ vs $6x10^6$ p <0.01). **Conclusions:** Lp and its supernatant inhibit the growth and biofilm de MRSA strains isolated fromdiabetic foot ulcers. Lp stimulates the

process of phagocytosis, netosis and microbicial activity of PMNs from diabetic patients and favors the control of MRSAstrains infection.

5 (119) DIFFERENCES BETWEEN POTENTIAL PERSISTENCE NICHES OF *BORDETELLA PERTUSSIS*

Carlos Manuel Baroli[1], Kristin Surmann[2], Juan Pablo Gorgojo[1], Martina Debandi[1], Hugo Alberto Valdez[1], Christian Hentschker[2], Uwe Völker[2], Maria Eugenia Rodriguez[1]

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Bordetella pertussis (*Bp*) is a strictly human pathogen and the etiological agent of whooping cough, a reemerging respiratory disease. Current vaccines fail to prevent host colonization and previous findings indicated that *Bp* establishes intracellular niches of persistence within the host, which might have implications for the current epidemiological scenario. Results from our group showed that *Bp* adapts its phenotype and persists in phagosomes with early endosomal characteristics inside human macrophages, by modulating the cellular bactericidal response through pertussis toxin (PT) and adenylate cyclase toxin (CyaA). We recently showed that *Bp* is also able to remain alive inside polarized respiratory epithelial cells in compartments with late endosomal characteristics, in which it has access to extracellular nutrients. Comparative proteomics of intracellular bacteria isolated from macrophages or epithelial cells, together with *in vitro* assays using mutant strains lacking the expression of selected bacterial factors evidenced the differences between these two potential niches of persistence, and helped to explain the higher intracellular survival rate observed in the epithelial cells. Briefly, our results showed that, unlike in macrophages, neither PT nor CyaA are needed to establish an intracellular niche in epithelial cells. The absence of either of these toxins did not impact bacterial intracellular trafficking or survival, as addressed through confocal studies and polymyxin B protection assays, respectively. The proteomic investigation of *Bp* residing inside the epithelial cells revealed the lack of activation of a bacterial stress response and essential metabolic proteins previously identified as crucial for *Bp* survival within these immune cells. *In vitro* experiments with specific mutant strains corroborated the lack of relevance of these factors for intracellular survival within respiratory cells. The changes induced at protein level in bacteria residing in the epithelial cells were mainly related to proteins involved in metabolic processes, and indicated a deceleration of intracellular bacterial metabolism, which is consistent with a persistent phenotype. Collectively, these findings stress the capacity of the pathogen to adapt and survive in different intracellular environments. They, additionally, highlight that airway epithelial cells potentially offer a more favorable environment for *Bp* survival as compared to immune cells. Given the substantial abundance of these cells within the human host, these results identify these cells as potential key determinants in the pathogen's persistence.

6 (66) A PEPTIDE DERIVED FROM THE G GLYCOPROTEIN OF THE RESPIRATORY SYNCYTIAL VIRUS DECREASES NEUTROPHIL FUNCTIONALITY

Federico Birnberg-Weiss[1], Juan Gutman[2], Joselyn Castro[1], José R. Pittaluga[1], Sebastián Esperante[2], Verónica I. Landoni[1], Damián Alvarez-Paggi[2], Gabriela C. Fernández[1].

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Respiratory syncytial virus (RSV) is by far the most frequent cause of bronchiolitis and viral pneumonia in infants and young children worldwide. Two surface RSV glycoproteins, F and G, are key to the biology of infection. Although G has been studied mostly as a possible vaccine candidate against RSV, less is known about its immune modulatory properties, partly ascribed to complex formation with CX3C.R1. Neutrophils (PMN) are highly recruited to the lungs during RSV infection. Moreover, it has been reported that some of the antimicrobial mechanisms of PMN show anti-viral properties. Therefore, our objective was to assess if a peptide corresponding to the receptor-binding site derived from G was able to modulate PMN functional responses. We used purified PMN from healthy donors and measured by flow cytometry their activation (CD11b up-regulation or reactive oxygen species (ROS) generation) after incubation for 30 min with the G peptide (1 μM) alone or together with an activating stimulus (LPS 0.5 ng/ml for CD11b measurement or fMLP 10^{-7} M for ROS generation). We found that the G peptide by itself decreased CD11b expression and completely abolished up-regulation of CD11b caused by LPS (MFI±SEM of CD11b: Basal: 323±37; G: 189±26*; LPS: 455±42*; LPS+G: 208±23*#, *p<0.05 vs basal, #p<0.05 vs. LPS; n=6). The decreased CD11b expression observed by G was not cause by cell death as incorporation of 7-AAD at 1 or 4 h after G incubation was minimal compared to a death control (PMA 100 nM) (% 7-AAD$^+$ PMN: 1 h: Basal=1.5±0.2; G=3.6±0.3; PMA=58.0±12.9*; 4 h: Basal=3.6±1.1; G=3.8±1.4; PMA=70.7±9.0*; *p<0.05 vs, basal; n=2). When we measured ROS generation, we found that the G peptide was able to reduce the percentage of ROS producing PMN triggered by fMLP (% ROS$^+$ PMN±SEM: Basal: 5.6±1.0; G: 3.5±1.1; fMLP: 73.3±12.6*; fMLP+G: 13.3±5.4#, *p<0.05 vs basal, #p<0.05 vs. fMLP; n=6). In conclusion, the G peptide exhibits suppressive properties of PMN functions, and this could represent a potential evasion strategy in RSV infection.

7 (102) A QUANTITATIVE PROTEOMIC STUDY IDENTIFIES DECTIN-1 ACTIVATION BY *Candida albicans* OR CURDLAN AS RELEVANT IN TYPE-I INTERFERON PATHWAY INDUCTION IN EPITHELIAL CELLS OF THE FEMALE GENITAL TRACT.

Emilse Rodriguez[1], Sofía Angiolini[1], Paula Icely[1], María Luisa Hernandez[2], CinthiaStempin[1], Concepción Gil García[2] and Claudia E. Sotomayor[1].

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Vulvovaginal candidiasis (VVC) is primarily caused by *Candida albicans (Ca)*, impacting over 75% of healthy women at least once in their lifetime. Despite extensive knowledge about host predisposing factors, many mechanisms and immune mediators involved in the *Ca*-Epithelial Cell (EC) interaction are unclear. In particular, the role of type I interferons (IFN-I) during fungal infections is not well understood. The activation of the Dectin1 receptor by *Ca* β-glucans and the subsequent induction of IFNβ production in dendritic cells have been documented during systemic candidiasis, but its exact role in EC of the female genital tract (FGT) remains unknown. **Objective:** To study the biological processes and cellular pathways differentially regulated in EC of the FGT after interaction with *Ca* and the Dectin1 agonist, Curdlan, through a label-free quantitative proteomic study. **Methods:** We performed an *in vitro* model of VVC using the human EC line, HeLa. A quantitative proteomic approach using Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) was performed to study the changes in the abundance of EC proteins after interaction with *Ca* SC5314 (5:1 *Ca*:cell ratio) or Curdlan (100 µg/mL) during 4h. Unstimulated EC were considered as control. Four biological replicates of each condition were analyzed. The bioinformatics analysis of differentially regulated proteins (DRPs) was carried outusing Gene Ontology and REVIGO for GO terms enrichment analysis and Reactome for pathways analysis. **Results:** A total of 216 DRPs upon *Ca* interaction were observed in EC (95 upregulated and 121 downregulated). GO term enrichment analysis showed that the more abundant proteins were mainly involved in nucleocytoplasmic transport, regulation of metabolism, and immune response. Pathway enrichment analysis of proteins upregulated after *Ca* stimulation confirmed “Antiviral mechanism by IFN-stimulated genes” (p=9.9e-7)as the most relevant pathway in response to *Ca*. Furthermore, other relevant pathways were closely related to IFN-I signaling mechanisms such us “ISG15 antiviral mechanisms” (p=5.8e-6) and “Interferon signaling” (p=3.5e-3). On the other hand, 429 DRPs were identified in EC stimulated with the β-glucan Curdlancompared to control (252 upregulated and 177 downregulated). GO term analysisshowed that the most abundant proteins were involved in biological processes related to nucleocytoplasmic transport, metabolic process, viral process, and gene expression. Interestingly, the pathway “Antiviral mechanisms by IFN- stimulated genes” (p=2.6E-5) was among the 10 most relevant pathways as in EC interacting with *Ca*. **Conclusion:** This study provides significant evidence about the activation of the IFN-I pathway in EC of the FGT in response to *Ca* infection. Our results also suggest that the regulation of this pathway could involve the activation of the Dectin1 receptor by *Ca* β-glucans. Taken together, quantitative proteomics offered new insights into fungal-host interaction during VVC.

8 (154) ACTIVATION OF PBMCS AND PMNS BY STAPHYLOCOCCAL ENTEROTOXINS N AND U

Daniela Maria Redolfi, Sofia Noli Truant, Maria Belen Sarratea, Laura Valeria Iannantuono López, Maricef Vergara Rubio, Emilio Luis Malchiodi, Marisa Mariel Fernández.
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Bacterial superantigens (SAgs) are enterotoxins usually produced by *S. aureus* that activate the immune system in an exacerbated manner, triggering a massive cytokine release, which could lead to toxic shock syndrome, among other affections.

This study aims to analyze the effects of Staphylococcal enterotoxin N (SEN) and U (SEU) on diverse cell populations representing both the innate and adaptive immune systems. For this propose, SEN and SEU were cloned and expressed as recombinant proteins in *E. coli*, and then purified using Ni++/NTA column followed by size exclusion chromatography. Subsequently, peripheral blood mononuclear cells (PBMCs) from donors who had not been previously exposed to SAgs were incubated with different concentrations of SEN and SEU (0.0001-10 µg/ml) for 48 h. Proliferation was evaluated by [methyl-3H] thymidine incorporation, and supernatants were collected to quantify cytokines (IL-6, IL-12, IL-10, TNF-α, and IFN-□) by ELISA. In addition, polymorphonuclear cells (PMNs), mainly neutrophils, were stimulated with 1 µM of SEN and SEU for 4 or 24 h depending on the assay. NET release was evaluated by indirect immunofluorescence (IFI), IL-6 and TNF-α production was measured in supernatants using ELISA. Hoechst staining and propidium iodide (PI) were used to assess the proportion of living and dead cells while MMPs activity was studied by zymography. Statistical analysis involved t-tests, one-way and two-way ANOVA, followed by post-hoc tests.

Results showed that PBMCs exhibited significant proliferation with SEN and SEU at concentrations above 0.001 µg/mL and 0.1 µg/mL respectively, compared tothe control ($p < 0.05$). Moreover, both SAgs induced a significant release of IL-6,IL-12, IL-10,TNF-α, and IFN-□ at different concentrations compared to untreated cells ($p < 0.05$).Otherwise, PMNs incubated with both SAgs for 4 h showed a significant production of IL-6 and TNF-α compared to basal levels ($p < 0.05$). Thus, NETosis was observed by confocal microscopy in PMNs exposed to SEN for 4 h and a significant increase in cell death compared to the control was found in PMNs incubated with this SAg for 24 h ($p < 0.05$). Additionally, both SAgs significantly enhanced MMP-9 activity at 24 h ($p < 0.05$).

In conclusion, these findings show that SEN and SEU activate immune cells, as PBMCs, promoting their proliferation along with the release of pro-inflammatory cytokines as IFN-γ, TNF-α, IL-12, IL-6, and the anti-inflammatory cytokine IL-10. Among others cellular targets, they display activity on innate immune cells like neutrophils, leading to the release of IL-6 and TNF-α and an increment of MMP-9 activity. Here we provide the first evidence of NETosis induction by SEN, which correlates with the observed increase in cell death within this population. These effects highlight the complexity of the response elicited by these toxins in different populations of the immune system, favoring the success of the bacteria infection at different levels.

9 (35) ADJUVANT TREATMENT WITH LACTIPLANTIBACILLUS PLANTARUM IN CHRONIC VENOUS ULCERA. BASES FOR THE PRODUCTION OF DERMATOLOGICAL DRESSINGS

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Venous leg ulcers (VLU) are caused by venous insufficiency, impaired venous blood flow brought about by venous hypertension. Among people aged 65 years and older, the annual prevalence of VLUs is 1.7**%** and it has been called the 'Silent Epidemic'. Since 1989, in San Miguel de Tucumán city, Argentina, our multidisciplinary research group has investigated the properties of culture of *Lactiplantibacillus plantarum* in wound healing applied topically as adjuvant to the standard wound care consisting of surgical debridement, systemic antibiotic, and routine bandage. **Patients** with CVUs lesions that did not heal after applying the standard treatment for 6 months were enrolled. The treatment sessions took place daily until 6 months. The ulcers size was measurement monthly. **Treatment:** *L. plantarum* ATCC 10241, grown in MRS broth, was spread on a gauze pad and applied to the lesion. **Soft tissue biopsies** (4 mm) were taken after wound debridement for microbiological analyses. **Heparinized venous blood samples** were obtained from patients with CVU, from normal individuals and diabetic patients. Neutrophils was isolated by dextran T-500 and Ficoll-Hypaque gradient centrifugation, suspended in RPMI 1640-HEPES Medium, seeded into 96-well black plates and infected with pathogens bacteria to induce NET formation. For the study of *L. plantarum* effect on this Netosis, neutrophils were preincubated with *L. plantarum*. Then, SYTOX green was added and NET formation was registered in a spectrofluorometer FLx800. Ethical considerations were taken into account. **Dressings:** To enhance the therapeutic effect of *L plantarum*, we are developing its incorporation of supernatant of *L. plantarum* culture **(SNLP)** into biocompatible polymer dressings. SNLP was loaded into Calcium Alginate spheres and Polycaprolactone electrospun nanofiber.

Results and conclusions: At months 6 of treatments, the percentage of healed wounds was 57% for wounds with initial area larger than 20 cm^2 and 60% for those smaller than 20 cm^2. Percentage of standard treatment was 30%.

The most frequently isolated bacteria were *Staphylococcus aureus* (57%) *Pseudomonas aeruginosa* (25%), SAMR (7%), *Enterococcus faecalis* (14%). Four of 5 patients who are cured have a pathogen-induced NETosis greater than or similar to that of the normal individual. The 3 patients who do not cure and the diabetics have NETosis lower than that of the normal. Spontaneous NETosis is lower than or equal to that induced (#$p<0.05$). SAMR-induced Netosis is lower than *S. aureus*-induced ($p<0.05$).

The presence of *L. plantarum* decreases the MRSA-induced Netosis in the 5 cured patients. Electrospun nanofibers showed smooth and uniform surfaces. The nanofibers did not encapsulate SNLP in the electrospinning process but can absorb and release it in a short time. Calcium Alginate spheres release molecules of different PM in a rather controlled fashion and they can encapsulate SLp. We are designing a dressing that contains the SLp with both polymers.

10 (110) ANTIBODY DETECTION AGAINST KUNITZ-TYPE RECOMBINANT PROTEINS IN EXPERIMENTALLY INFECTED SHEEP WITH FASCIOLA HEPATICA USING ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA)

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Fasciolosis is a parasitic disease considered as emerging and neglected by the WHO. Sheep are highly susceptible to this disease showing a decreased productivity due to increased mortality and reduced quality of their products such as wool and meat. To effectively control this disease, reliable and early diagnosis is essential for making decisions regarding the application of antiparasitics and/or the removal of affected animals.

The current diagnosis of *F. hepatica* (Fh) in sheep relies on the detection of parasite eggs in feces, a method that becomes reliable from week 10 post-infection. Consequently, there is a need for earlier diagnostic tools based on immune response. However, the obtaining of antigens for antibody detection has proven to be difficult and expensive.

Kunitz-type (KT) protease inhibitors are low molecular weight proteins with serine protease inhibitor activity. They have recently been described to be involved in the regulation of major parasite and/or host-secreted cathepsin L-like cysteine proteases.

The aim of this work was to compare the performance of recombinant proteins (rFhKT) belonging to the family of FhKT inhibitors (FhKT1.1, FhKT1.3 andFhKT4) produced as fusion proteins with a synthetic (sFhKT) in a ELISA test forthe diagnosis of *F. hepatica* in sheep. We evaluated the antibodies levels using a number of samples collected from experimentally *F. hepatica*-infected sheep six weeks after infection. Among the proteins used, FhKT1.1 showed the most promising diagnostic antigen, exhibiting high precision and low cross-reactivity, thus holding potential for standardized production.

The precision of the diagnostic assay using FhKT was evaluated by determining the sensitivity, specificity, predictive values, likelihood ratio (LR) and *kappa* index. The specificity and sensitivity parameters calculated by the Wilson-Brown method showed thatthe best combination for both parameters is obtained using FhKT1.1(Sensibility (Se)0.83 and Specificity (Sp) 0.92, followed by sFhKT, FhKT4 and FhKT1.3.

The kappa index was used to estimate the concordance between the results obtained by coproparasitological diagnosis (gold standard) and ELISA using sFhKT and rFhKTs. The highest concordance (*Kappa* index value= 0.91) was obtained for FhKT1.1, followed by sFhKT, FhKT4, and FhKT1.3.

The results of our study demonstrated that the application of FhKT1.1 is a valuable tool for early-stage diagnosis of *F. hepatica* in sheep. Such an early diagnosis can aid in implementing timely interventions and effectively managing the disease in sheep populations.

11 (33) BLOOD IMMUNE CELLS SUBSETS IN CHILDREN WITH POST ADENOVIRUS BRONCHIOLITIS OBLITERANS AND FREQUENT RESPIRATORY TRACT INFECTIONS

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Post Adenovirus infection Bronchiolitis Obliterans is a pathologic entity characterized by airways fibrosis. In the worldwide are relatively rare but in Latin America appears significantly elevated. These patients present variable course and outcomes, being one of the long-term complications the frequent respiratory tract infections. Unfortunately, the development of immunology or its long-term consequences of post infection bronchiolitis obliterans PIBO by Adenovirus have not been studied in depth. This work aimed to evaluate blood immune cells subsets in children attending to the neumonology service with a history of PIBO by Adenovirus during the first year of life and frequent respiratory tract infections. Five patients with PIBO (8-12 years, 2 males, 3 females) that attended service for respiratory infection symptoms were selected for the study. Blood samples were taken at time of consultation (t1) and after 21-23 days (t2) for flow cytometric studies. Total leucocytes, monocytes and granulocytes counts, and $CD45^+Annexin^+$, $CD45^+HLA\text{-}DR^+$, $CD16^+CD56^+$ NK, $CD19^+$ B cells as well as $CD3^+CD4^{hi}$, $CD3^+CD8^+$, $CD3^+CD25^+$, and $CD3^+HLA\text{-}DR^+$ T cells were evaluated at t1 and t2. All the patients presented symptoms of respiratory infections at t1. Four children had no symptoms at t2 and patient 1 (P1), despite presenting clinical improvement, still had symptoms. At t1, all the patients had significantly higher ($p<0.05$) levels of blood $CD45^+Annexin^+$ cells, granulocytes, $CD45^+$ $HLA\text{-}DR^{hi}$ monocytes and $CD19^+$ B cells than non-infected controls. While three patients showed significantly higher ($p<0.05$) levels of $CD16^+CD56^+$ NK cells as well as the cytokine producing $CD16^+CD56^{hi}$ and the cytotoxic $CD16^+CD56^{lo}$ NK cells than non-infected controls, the remaining children presented values below the normal range. Significantly reduced levels of $CD3^+$ T cells and activated $CD3^+HLA\text{-}DR^+$ T cells were detected in blood of PIBO patients at t1 compared to non-infected controls. When t1 and t2 were compared, four of the patients showed a tendency towards normalization for all populations of immune cells studied, except for $CD3^+CD8^+$ T cells and $CD19^+$ B cells that reached values lower than uninfected controls. Of note, in P1 the levels of $CD45^+$ $HLA\text{-}DR^{hi}$ monocytes, $CD3^+CD4^{hi}$, $CD3^+CD25^+$, and $CD3^+HLA\text{-}DR^+$ T cells increased from t1 to t2. The results suggest that children with PIBO have a predisposition to the development of exacerbated inflammatory responses against respiratory virus characterized by the increases and activation of granulocyte and monocytes, together with qualitative and quantitative alterations of NK, T and B cells. In virus-infected PIBO patients, the clinical improvement would related to the tendency of the increased immune cells to return to normal values. Further characterization of immune responses in PIBO children should consider multicentric studies, in order to include a significant number of patients.

12 (33) CD39 EXPRESSION BY REGULATORY T CELLS MEDIATES CD8+ T CELL IMMUNITY SUPPRESSION DURING ACUTE TRYPANOSOMA CRUZI INFECTION

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We previously reported that after *Trypanosoma cruzi* (Tc) infection, Foxp3+ regulatory T cells (Tregs) undergo a marked reduction in frequency during the acute phase of the infection, albeit they become activated and acquire a specialization program consistent with suppression of type 1 effector responses. This natural contraction of Tregs was critical to allow the emergence of protective anti-parasite CD8+ T cell immunity. Herein, we aimed at gaining mechanistic insight into how Tregs impact on effector immune response in the context of Tc infection. For this, we studied the expression of Tregs activation and suppressive markers after Tc infection by flow cytometry. We found that while the frequency of CD25+ and CTLA-4+ Tregs showed no differences between infected and non-infected mice at day (d) 7 post-infection (pi), there was a significant increase in the proportion of CD39+ Tregs in the spleen of Tc-infected animals compared to controls, and importantly, of Tregs expressing high levels of CD39. We then performed Tregs depletion followed by adoptive transfer experiments to deeply evaluate if the expression of CD39 by Tregs is necessary for suppressing the Tc-specific CD8+ T cell response. For this, DEREG mice were infected with Tc and injected with diphtheria toxin (DT) to eliminate Tregs or PBS as control at d 5 and 6pi. The following day, a group of DT-treated mice were adoptively transferred with Tregs differentiated *in vitro* (iTregs) from CD4+ T cells of non-infected WT or CD39 KO animals. Notably, depleted animals that were injected with WT iTreg cells reversed the effect of DT treatment, as demonstrated by a reduction in the parasite-specific CD8+ T cell response to levels similar to those of PBS-injected controls. In contrast, injection of CD39 KO iTregs in depleted mice resulted in a robust Tc-specific CD8+ T cell response comparable to or higher than DT-treated animals, and notably higher than Treg-depleted mice that received WT iTreg cells, in both the spleen and liver. Interestingly, the relative and absolute numbersof short-lived effector (SLEC) Tc-specific CD8+ T cells significantly decreased when Treg-depleted animals received WT iTregs, but not when they weretransferred with CD39 KO iTregs. Importantly, Tc load in the spleen and liver wassignificantly reduced when Treg-depleted mice were transferred with CD39 KO iTregs compared to mice that received WT iTregs. These results highlight the involvement of CD39-expressing Tregs in modulating the magnitude of the effector Tc-specific CD8+ T cell response and parasite control during the acute phase of infection. This regulatory function was not compensated by other suppressive molecules expressed in Tregs during acute Tc infection.

13 (122) CHARACTERIZATION OF MYELOID-DERIVED SUPPRESSOR CELLS IN A MODEL OF CHRONIC INFECTION WITH *MESOCESTOIDES VOGAE*

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Myeloid-derived suppressor cells (MDSCs) are a heterogeneous population of immature cells that are activated in various pathological conditions and are characterised by their ability to suppress the immune response. These cells express Gr-1 and CD11b molecules and can be classified in the mouse as polymorphonuclear MDSCs (PMN-MDSCs) and monocytic MDSCs (Mo-MDSCs) based on their morphology and differential expression of Ly6G and Ly6C markers. The suppressive activity of MDSCs is mainly associated with the metabolism of L-arginine, a substrate of the enzymes inducible nitric oxide synthase (iNOS) and arginase-1 (Arg-1). *Mesocestoides vogae* is a parasite related to zoonotic cestodes that usually infects mice but occasionally causes infections in humans. Following oral or intraperitoneal infection in mice, *M. vogae* migrates to the liver and then to the peritoneal cavity, where it proliferates and establishes a chronic infection. In the early stages, these cestodes induce a Th1-type immune response, which is gradually replaced by a Th2-type response, favoring their survival in the host. In the present work, we set out to investigate the characteristics of MDSCs in chronic *M. vogae* infection. First, we examined the accumulation of MDSCs in the spleen and liver of mice 35 days after infection by flow cytometry. We observed that infected mice had an increase in the number of these cells in the spleen ($p<0.001$) and in the frequency and number in the liver ($p<0.05$ and $p<0.01$, respectively) compared to uninfected mice. In addition, a predominance of the Mo-MDSC subpopulation in the spleen and PMN-MDSCs in the liver was observed ($p<0.01$ and $p<0.01$, respectively). Furthermore, we observed that PMN-MDSCs of infected mice showed an increase in the expression of CD101 and Siglec-F ($p<0.05$ and $p<0.01$, respectively). In addition, to investigate the suppressive activity of MDSCs from the liver of infected mice, we perform a co-culture with Th1 or Th2 cells stimulated with anti-CD3/antiCD28. T cell proliferation was analysed by flow cytometry. We observed that MDSCs from infected mice have the ability to selectively suppress Th2 cell proliferation ($p<0.01$). This was reversed by supplementing the culture with the inhibitor of arginase nor-NOHA ($p<0.01$). In conclusion, our results suggest that MDSCs accumulate in the spleen and liver during chronic *M. vogae* infection. These cells are able to regulate the Th2 response of the liver through an Arg-1-dependent mechanism, as part of the systemic immunosuppressive response that would favor the survival of *M. vogae* in the host.

14 (77) DOES SARS-COV-2 INDUCE PROTECTIVE TRAINED IMMUNITY OR TOLERANCE FOR M. TUBERCULOSIS?

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Innate immunity is crucial for defense against pathogens, rapidly mounting a protective but non-specific response. Innate immune cells such as monocytes, macrophages, and NK cells possess the capability to acquire immunological memory, termed trained immunity. It involves reshaping metabolic and epigenetic processes as a response to an antigen, enabling the host to enhance proinflammatory gene expression and mount a secondary functional response against different pathogens. However, depending on the characteristics of the first stimulus, the secondary response could also repress cellular effector functions, which is known as immunological tolerance.

This work is placed in the context of the COVID-19-tuberculosis (TB) co-pandemic. We aimed to investigate how prior infection with SARS-CoV-2 impacts macrophage phenotype, function and metabolic pathways and how this subsequently would intensify or repress the response against *Mycobacterium tuberculosis (Mtb)*.

To accomplish this, a novel trained immunity *in vitro* model was developed, using the THP-1 cell line. Macrophages were trained with active SARS-CoV-2 or exposed to a heat-inactivated virus, emulating an infection or vaccination situation, respectively. Untrained cells were used as control. After 6 days, these macrophages were challenged with *Mtb* antigens or LPS (as control of pro-inflammatory response) for 24 hours. Then, the phenotype of the macrophages was analyzed by studying the expression of different M1(HLA-DR/CD86/CD64) or M2 (CD163/CD209) markers using flow cytometry, and ELISA to evaluate cytokine production. Results showed that exposure to inactivated virus exclusively promoted a macrophage *Mtb*-specific response with a predominant M1 phenotype. Conversely, the training with active virus did not show differences with the control group but induced a robust proinflammatory response in macrophages with higher interleukin IL-6 production, which might contribute to the physiopathology of TB. Furthermore, the metabolic profile of trained macrophages was assessed by measuring glucose consumption and lactate production. Macrophages trained with inactivated SARS-CoV-2 exhibited a glycolytic profile, indicated by increased glucose consumption upon *Mtb* infection. However, this elevated glucose consumption did not result in higher extracellular lactate production.

These results suggest that SARS-CoV-2 infection or vaccination may induce differential changes on macrophages. In turn, the above-mentioned changes would modulate macrophage function and metabolism, training these cells to ultimately shape the specific immune response against *Mtb*.

15 (26) DUAL MODULATION OF LYMPHOCYTE ACTIVATION BYMICROBIAL RNA

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In order to survive inside the host, *Brucella abortus* (*Ba*) must trigger different strategies to evade the robust adaptive T cell response it elicits. We have recently demonstrated that *Ba* RNA (a PAMP related to pathogens' viability or *vita*-PAMP) activates lymphocytes at early time points but, later on it could contribute to circumvent this activation. More specifically, *Ba* RNA increases the expression of the early activation marker CD69 on peripheral blood mononuclear cells (PBMCs) independently of plate-bound anti-CD3 stimulation at 24 h. However, *Ba* RNA decreases the expression of the senescence marker CD28 only in anti-CD3 pre-stimulated PBMCs at 4 days. The aim of this study was to deepen this dual modulation of lymphocyte activation. For this, we first evaluated the effect of *Ba* RNA on the secretion of IL-2 and IFN-γ. For this, unstimulated or anti-CD3 pre-stimulated PBMCs were treated with *Ba* RNA (1, 5 and 10 µg/ml) for 24 h and 4 days. After these times, supernatants were collected and the secretion of IL-2 and IFN-γ was quantified by sandwich ELISA. *Ba* RNA increased IL-2 and IFN-γ secretion ($p<0.05$) both in unstimulated or anti-CD3 pre-stimulated cells, at 24 h and 4 days. Next, we evaluated the effect of *Ba* RNA on the lymphocyte proliferation. This was evaluated by two methodologies: MTT cell proliferation assay and flow cytometry. For the first one, unstimulated or anti-CD3 pre-stimulated PBMCs were treated with *Ba* RNA for 24, 48, 72 and 96 h. After these times, the proliferation was evaluated by MTT colorimetric assay. *Ba* RNA did not modify the proliferation in unstimulated cells at any time evaluated. However, *Ba* RNA decreased the proliferation in anti-CD3 pre-stimulated cells at 48, 72 and 96 h. For the second methodology, unstimulated or anti-CD3 pre-stimulated PBMCs were treated with *Ba* RNA for 24 and 96 h. Then, the number of cells in the gate of lymphocytes was evaluated by flow cytometry. *Ba* RNA did not modify the number of cells in unstimulated cells, neither at 24 h nor at 96 h. However, *Ba* RNA decreased the number of cells in the gate of lymphocytes in anti-CD3 pre-stimulated cells at 96 h ($p<0.05$). Finally, we evaluated whether the observed phenomena could be extended to RNA of other microorganisms. For this, we purified RNA from *Escherichia coli* (*Ec*) and *Klebsiella pneumonia* (*Kp*) and used them to treat unstimulated or anti-CD3 pre-stimulated PBMCs. The RNA from *Ec* and *Kp* increased CD69 expression and IL-2 and IFN-γ secretion ($p<0.05$) at early time points. However, later on both RNA decreased CD28 expression and lymphocyte proliferation ($p<0.05$). These results indicate that these phenomena are not restricted to *Ba* RNA. Overall, our results show that the RNA could be a *vita*-PAMP employed by microorganisms to differentially modulate lymphocytes, activating them at early time points but avoiding this activation and favoring the establishment of chronic infections later on.

16 (207) EFFECT OF IL-1β ON THE AUTOPHAGY PROCESS INMONOCYTES FROM TUBERCULOSIS PATIENTS

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INTRODUCTION: Autophagy, an essential process that maintains cell homeostasis, is also an important protective mechanism against *Mycobacterium tuberculosis* (*Mtb)* infection and other intracellular pathogens. It has been demonstrated that the induction of autophagy suppressed intracellular survival of *Mtb*. Interleukin 1β (IL-1β) is a key mediator of the inflammatory response, essential for the host-response and resistance to pathogens. We previously reported that the cytokines IFNγ and IL-17A augmented autophagy in *Mtb*-infected monocytes from tuberculosis (TB) patients, according to their immunological status of the human host. Here we investigated the role of IL-1β on the autophagy process in two groups of TB patients. Our ultimate goal is to elucidate if this cytokine could play a role in a host directed therapy.

METHODS: TB patients were classified as low and high responders (LR and HR, respectively) according to their immune responses to sonicated *Mtb* (*Mtb*-Ag). Control individuals were healthy donors (HD) from the community. Monocytes were obtained from heparinized peripheral blood and cultured (2×10^6 cells/ml) with an *Mtb* lysate (*Mtb*-Ag, 10μg/ml) ± IL-1β (10ng/ml). To evaluate the effect ofIL-1β on bacterial growth, monocytes were infected with H37Rv *Mtb* strain (MOI:10). After 2h of infection, the medium was replaced and cells were cultured ± IL-1β (10 ng/ml) for 96h. Cells were then washed and lysed to assay mycobacterial colony-forming units (CFU). Flow cytometry was used to evaluate autophagy levels by determining LC3 (the main marker of the autophagy process). P-values<0,05 were considered significantly different.

RESULTS: When we analyzed the effect of IL-1β on monocytes, we observed that *Mtb*-Ag significantly increased $CD14^+LC3^+$ cells in HD and HR TB patients ($p<0.001$) but IL-1β stimulation markedly decreased $CD14^+LC3^+$ cells in HD ($p<0.05$). However, when we added Bafilomycin A1 (BafA1) together with IL-1β and *Mtb*-Ag, the percentage of $CD14^+LC3^+$ cells in HD was higher than when BafA1 was not used ($p<0.05$), indicating that the autophagy flux was increased. The increase of LC3 was confirmed by western blot. In order to determine the effect of IL-1β on monocytes, we treated H37Rv *Mtb* infected-monocytes with IL-1β. Cells stimulated with IL-1β displayed lower CFUs as compared to cells infected without IL-1β.

CONCLUSIONS: Our present findings suggest a differential autophagy response of human monocytes upon stimulation with IL-1β which might indicate that this cytokine might be critical in new potential host directed therapies (HDT) for TB.

KEY WORDS: tuberculosis, autophagy, il-1β, therapy.

17 (186) EFFECTS OF TYPE II DIABETES MELLITUS ON THE IMMUNE RESPONSE TO *Trypanosoma cruzi* EXPERIMENTAL INFECTION

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Introduction and Objectives: Given the increased susceptibility to infections associated with type 2 diabetes mellitus (T2DM), the purpose of the present work was to determine the effects of T2DM on a mouse model of *Trypanosoma (T.) cruzi* infection.

Materials and Methods: To this aim, 8-week-old C57BL/6 mice were fed a medium-fat diet (17% FAT) combined with 20% fructose in drinking water for 20 weeks. At week 8, a single dose of the diabetogenic drug streptozotocin (100 mg/kg) was injected (i.p.) to establish diabetes (D+T). In this group, the glycemia significantly increased compared to those who had not received either the diet or the drug (p=0,01). Diabetic (D+T_i) and non-diabetic (N_i) mice were infected with 1000 *T. cruzi* trypomastigotes (i.p.).

Results: We found that D+T animals had higher plasma IL-10 concentrations than nondiabetic controls (p= 0,02). At 14 days post-infection, the D+T_i group showed greater parasitemia (p=0,03) and plasmatic concentrations of IL-6 (p=0,01), as well as inflammatory cytokines such as IL-1α (p=0,01), TNF-α (p=0,01), IL-12p70 (P=0,03), and the chemokines MCP-1 (p=0,01) or GM-CSF (p=0,02) than the N_i group. In addition, D+T_i had a higher frequency of circulating $CD11b^+Ly6C^{hi}$ cells (p= 0.03) and a lower frequency of $Ly6C^{lo}$ monocytes (p=0,01) than N_i. Notably, the $Ly6C^{lo}$ cells exhibited higher IL-10 expression (p=0,02) in D+T_i than N_i. Moreover, the D+T_i group showed a lower frequency of circulating granzyme B^+ (p= 0,01) and $perforin^+$ (p= 0,01) CD8 T-cells. In the spleen, the frequency of granzymes B^+ CD8 T-cells (p= 0,02) and the frequency and total number of effector memory T cells ($CD44^+CD49d^+$) ($P_{\%}$=0,01; $P_{\#}$= 0,01) were lower in the group D+T_i than in the N_i. In agreement, the production of TNF-α by CD8 T-cells was lower in D+T_i than in N_i (P_{MFI}= 0,03; $P_{\%}$=0,01). Surprisingly, D+T_i animals showed a lower frequency of macrophages ($CD45^+CD11b^+F4/80^+$) in VAT than N_i mice (p=0,01), and the M1-like population ($CD86^+$) showed a lower frequency of TNF-α expression (p=0,03).

Conclusion: Our results suggest a blunted immune response to *T. cruzi* infection in a novel murine T2DM model. In particular, the observed imbalance in inflammatory cytokines and reduced CD8+ T-cell effector molecules highlight the impaired immunocompetence at several immune compartments to deal with infections in T2DM.

18 (43) EVALUATION OF THE EARLY IMMUNE RESPONSE TO *STREPTOCOCCUS PNEUMONIAE* PULMONARY INFECTION IN THE Eμ-TCL1 ADOPTIVE TRANSFER MOUSE MODEL OF CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)

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CLL is the most frequent leukemia in adults characterized by the accumulation of clonal B cells in peripheral blood (PB) and lymphoid organs. Infections are one of the main causes of death in CLL, even at early stages of the disease before any anti-leukemic treatment is needed. Bacterial pneumonia, involving *Streptococcus pneumoniae (Spn),* is a frequent serious infection among untreated patients. By using a murine adoptive transfer model of CLL (AT-TCL1) we aimed to study the susceptibility to *Spn* pulmonary infection in the context of untreated CLL and to characterize innate immune parameters involved in the early response to the bacteria.

To obtain AT-TCL1 mice, female C57BL/6 mice were intraperitoneally injected with 20 x 10^6 leukemic cells ($CD5^+CD19^+$) from spleens of full leukemic Eμ-TCL1 mice. Control (CT) mice were injected with PBS. After 4 weeks, leukemization was confirmed by flow cytometry (FC) (>60% of $CD5^+$ $CD19^+$ cells in PB). Mice were intranasally infected with 2 x 10^6 colony forming-units of *Spn* serotype 3 (clinical isolate) and were either daily controlled for 11 days (survival experiments) or euthanized 24 hs after infection to obtain the bronchoalveolar lavage fluid (BALF). Bacterial load by serial dilution in agar plates, cell number and phenotype by FC, IL-1β, TNF-α and CXCL1 by ELISA and total protein, Lactate Dehydrogenase (LDH) and myeloperoxidase (MPO) by colorimetric assays, were determinate in the BALFs. Neutrophil phagocytic capacity was evaluated *ex vivo* by FC. GraphPad Prism was used for statistical analysis.

After *Spn* infection, we found an increased mortality rate of AT-TCL1 mice compared to CT mice (n=4/group, 3 experiments, $p<0.05$). The BALF analysis 24 hs after infection showed a higher bacterial burden in AT-TCL1 mice than in CT mice (n=12, $p<0.05$), suggesting an impairment in bacterial growth control-mechanisms. Because natural anti-*Spn* IgM provides innate protection against *Spn* infection, we evaluated its plasma levels in non-infected mice by ELISA and found lower titers in leukemic mice (n=4, $p<0.05$). We also observed higher levels of inflammatory parameters in BALF of infected AT-TCL1 mice such as total protein, TNF-α and IL-1β (n=12, $p<0.05$). No difference in LDH were found (n=12), suggesting similar levels of lung damage. Moreover, we found similar neutrophil count in the BALF of AT-TCL1 and CT mice (n=12) and a similar *ex vivo* phagocytic capacity of CFSE-*Spn* by bone marrow neutrophils (n=2). Remarkably, leukemic mice showed lower levels of MPO in BALF which is critical in the oxidative bacteria killing by neutrophils (n=12, $p<0.05$).

Our results show that CLL predisposes to an impaired control of bacterial growth and to a higher mortality rate in a model of *Spn* pulmonary infection. In addition, we found that leukemic mice have defects in early immune mechanisms that might contribute to the increased susceptibility to *Spn* such as a decrease on natural IgM anti-*Spn* and lower levels of MPO on infected lungs.

19 (188) EXPLORATION OF OXIDATIVE STRESS LEVELS AND T-CELL PROFILES IN PATIENTS WITH BOTH CHRONIC CHAGAS DISEASE AND TYPE 2 DIABETES MELLITUS

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Chagas disease (ChD) and type-2 Diabetes mellitus (DM) are both chronic, progressive and often comorbid diseases. However, little is known about their reciprocal influences and the immune patterns of individuals presenting DM comorbidity-associated with chronic ChD and how its influences each other. For this reason, we decided to explore the level of cellular oxidative stress and different parameters of T-cell populations among chronic *T. cruzi* infected patients with and without DM. The study population consisted of 1) Individuals with both pathologies (ChD+DM), 2) patients with chronic infection by *Trypanosoma cruzi* (ChD), 3) individuals with DM, and 4) healthy age- and sex-matched volunteers (Co). ChD patients with distinct clinical forms were included (with and without chronic myocarditis). None of these patients received antiparasitic treatment or had other concomitant pathological disorder (n=6-10/group; age range: 45-65 years). For this, blood samples were obtained in fasting and peripheral blood mononuclear cells (PBMCs) were isolated using Ficoll-Paque gradient. All studies were made by flow cytometry. Cellular oxidative stress of PBMCs was determined by evaluation of reactive oxygen species (ROS) and nitric oxide (NO) using fluorescent probes. We also evaluated phenotypic and functional parameters among T-cells, including Tregs (CD4+CD25+CD127-), effector/memory (CCR7/CD45RA), Th1 (IFN**γ**+) and CD8+ cytotoxic cells (IFN**γ**+/CD107a+). The production of ROS and NO tend to be more pronounced in DM than in ChD patients, (independently DM comorbidity or cardiac involvement), but without statistical significance. A trend towards enhanced CD4+IFNγ+ production was observed in ChD+DM and DM patients compared to ChD and Co [mean±SEM, (%) Co=16±1.5; ChD=19.2±2.0; ChD+DM=19.4±2.1; DM=21.7±1.9]. Treg frequency was slightly enhanced in ChD and ChD+DM compared to Co and DM individuals (in both cases $p<0.05$). CD8+ cytotoxicity was more evident in ChD+DM compared with ChD and DM patients (mean±SEM, (%) ChD=3.2±0.3; ChD+DM=7.5±2.4; DM=5.4±1.0). No differences were observed in the frequencies of CD4+ naïve (TN, CCR7+CD45RA+) or effector memory T-cells re-expressing CD45RA (TEMRA, CCR7−CD45RA+), while T effector memory (TEM, CCR7-CD45RA-) showed slightly expansion among clinical groups compared to Co. Contrary, the frequency of CD8+TEM tend to decreased in ChD+DM and DM compared to Co ($p<0.05$), while CD8+TEMRA T-cells were more expanded in DM and ChD+DM (i.e.: mean±SEM, (%) Co=28.7±2.0*; ChD=35.1±2.1; ChD+DM=41.4±2.6; DM=51.0±4.2, *$p<0.05$ vs rest).

Our findings suggest a certain degree of synergy between ChD and DM in establishing a pro-inflammatory profile, while not revealing differences between

ChD patients with varying levels of myocardial involvement. Future studies with larger patient cohorts may yield more robust insights into how DM comorbidity influences ChD progression and vice versa.

20 (89) FOLLICULAR CYTOTOXIC CD8+ T CELLS INFLUENCE B CELL DIFFERENTIATION IN *Trypanosoma cruzi*-INFECTED MICE

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Chagas disease, caused by the parasite *Trypanosoma cruzi*, is a chronic infection that elicits an early plasmablast (PB) response and a delayed Germinal Center (GC) reaction during the acute phase of infection. In *T. cruzi* infected-C57BL/6 mice, we identified a subset of $CD8^+$T cell in spleen and inguinal lymph nodes called follicular cytotoxic $CD8^+$T cells (Tfc, CD8+CXCR5+PD-1+) whose peak of response was at 18 days post-infection (dpi), as PB response did. Tfc shared gene signature with CD4+ follicular helper T cells (Tfh), conventional providers of B cell help, by expressing ICOS, CD40L and Bcl6. Moreover, near 13% of Tfc and 7% of Non-Tfc (CD8+CXCR5-PD-1-) were specific for the *T. cruzi*-immunodominant peptide TSKB20. Immunofluorescence of spleen sections from *T. cruzi* infected mice revealed CD8+T cells inside B cell follicles (FO) in close contact with PNA+GC B cells, extrafollicular PB and parasites.

The aim of our study was to evaluate the role of splenic Tfc from *T. cruzi* infected-C57BL/6 mice on B cell response.

For that, we performed *in vitro* co-culture assays by using different protocols of B cell stimulation. B cells isolated from uninfected mice were activated using CpG-ODN plus anti-CD40, or anti-IgM plus anti-CD40. Then, these cells were co-cultured with sorted Tfc or Non-Tfc cells or medium during 20 h in a 2:1 ratio (2CD8+:1Bcell). Additionally, same experiments were performed co-culturing Tfc or Non-Tfc cells with naïve B cells from infected mice. All conditions had aCD3/CD28 o TSKB20 stimulation. We observed by flow cytometry, a higher frequency of B220+CD138+Blimp-1+ and B220+IgD- cells in the co-cultures of activated B with Tfc cells versus with Non-Tfc cells o B cells cultured alone ($p<0.05$). By a multiplex bead-based assay, co-cultures also significantly showed the highest levels of IgG2c and IgG2b in the supernatants of B cells cultured with Tfc, indicating that Tfc favored B cell differentiation into antibody-secreting cells. Non-Tfc did not increase any immunoglobulin isotype in comparison to B cells alone. *In vivo* experiments were performed by transferring splenic Tfc and Non-Tfc cells from 19 dpi-CD45.1 mice to CD45.2 mice at 10 dpi. In contrast to mice that received Non-Tfc cells or PBS injections, Tfc-transferred mice showed a significant increase in the number of splenic total B cells, GC (B220+Bcl6+Fas+) cells, and IgM+IgD+ B cells, accompanied by elevated levels of IgG2b, IgM and IgA in sera and displayed the lowest parasitemia ($p<0.05$). Different B cell subsets were also evaluated in infected-CD8 KO mice in which numbers of splenic GC B cells and PB were decreased while the frequency of Tfh and T follicular regulatory were increased with respect to control infected mice.

To summarize, we identified follicular $CD8^+$ T cells in *T. cruzi*-infected mice with a potential role in the control of parasitemia of infected mice, and in the humoral response by favoring the differentiation of B cells into antibody-secreting cells.

21 (152) HIV AND GP120-INDUCED LOSS OF LIPID DROPLETS IN HEPATIC STELLATECELLS CONTRIBUTES TO A PROFIBROTIC PROFILE

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Liver fibrosis involves the excessive accumulation of extracellular matrix proteins in response to chronic diseases. HIV infection accelerates the rate of liver fibrosis progression in patients co-infected with HCV or HBV, compared to those who are only mono-infected. The earliest event in the progression of liver fibrosis is the activation of hepatic stellate cells (HSC), which leads to an increase in the synthesis of extracellular matrix proteins. Quiescent HSC store vitamin A within perinuclear droplets. The initial stage of HSC activation involves the depletion of these lipid droplets, followed by an increase in α-smooth muscle actin (SMA) and the secretion of collagen in a mechanism that involves TGF-β and IL-6.

CCR5 was implicated in the activation of hepatic stellate cells. Given that HIV-R5 tropic strains bind to CCR5 via their gp120 glycoprotein, the goal of this study was to assess the impact of HIV and its gp120 glycoprotein on HSC activation using the LX-2 cell line. CCR5 and CXCR4, in conjunction with CD4, play a crucial role in the adsorption and entry of HIV into cells. We found that 15.1 ± 0.85% of LX-2 cells expressed the CCR5 receptor, while 27.65 ± 0.82% of cells expressed CD4. Negligible levels of CXCR4 were detected.

Experiments were conducted to determine whether HIV-R5 and gp120 induce loss of lipid droplets, the expression of IL-6, α-SMA, TGF-β, and cellular proliferation. To this end, LX-2 cells were infected with HIV-R5 (AD8 strain, MOI 0.5 pg (p24)) or stimulated with gp120 (500 ng/ml). HIV infection induced the loss of lipid droplets (Microscopy, Bodipy 493/503). The gp120 from HIV-R5 mimicked the changes associated with lipid droplets loss induced by the infection. This effect was reversed when experiments were performed in the presence of the CCR5 antagonist TAK-779. This process involved an increase in PPAR-α (**$p < 0.01$) and an augmentation of lysosomal acid lipase (LAL) expression (**$p < 0.01$) (RT-qPCR).

In concordance, HIV infection and gp120 induced an increase in the colocalization of lipid droplets with lysosomes (Microscopy, ****$p < 0.0001$) strongly suggesting that lipophagy could play a role in this process. HIV infection induced IL-6 secretion (ELISA), but was unable to induce the expression of TGF-β (RT-qPCR) and α-smooth muscle actin (α-SMA) (RT-qPCR and immunofluorescence); as well as collagen deposition (Microscopy, Sirius red staining and immunofluorescence). IL-6 could promote HSC survival and proliferation, which correlates with the extent of fibrosis. However, HIV infection was unable to induce cell proliferation (violet proliferation dye, cytometry).

The present study provides evidence that HSC exposed to HIV R5 or the gp120 glycoprotein induce the loss of lipid droplets, a critical step in initiating HSC activation. This sets in motion a series of interactions that may contribute to accelerating the progression of hepatic fibrosis observed in coinfected individuals.

22 (166) HIV MODULATES ADIPOCYTE DIFFERENTIATION VIA A MECHANISM DEPENDING ON VIRAL TROPISM

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Mesenchymal stem cells (MSC) can differentiate into adipocytes and osteoblasts, a process that can be altered in pathological conditions, such as HIV infection. The aim was to determine whether HIV can infect MSCs and modulate their differentiation into adipocytes.

To determine the susceptibility of MSC to HIV infection, we evaluated the expression of the CD4 receptor and the CXCR4 and CCR5 co-receptors by flow cytometry. CCR5, along with its natural ligands CCL3, CCL4, and CCL5, induces the recruitment and differentiation of adipocytes. CXCL12 is the ligand of CXCR4 and increases the sensitivity of adipocytes to insulin. Therefore, we decided to investigate the expression of CCR5, CXCR4, and CD4 at various time points during the differentiation process (1,3, and 7 days). Infections were performed with HIV-X4 (NL43) and –R5 (AD8) at a MOI of 0.5 pg (p24). To assess whether HIV could modulate the differentiation of MSCs into adipocytes, cells were infected in the presence of differentiation medium (0.5mM 3-Isobutyl-1-methylxanthine; 10ug/ml insulin; 0.01 uM dexamethasone) for 10 days. Lipid droplet formation was assessed by Bodipy 493/503 staining (Microscopy). The expression of master transcription factors implicated in adipogenesis (PPARγ, C/EBPβ, and C/EBPα) were evaluated using RT-qPCR.

We demonstrated that MSCs express CD4 (7.63 ± 0.94) and exhibit low levels of CXCR4 (6.7 ± 0.43) and CCR5 (10.5 ± 0.56). After 7 days, we observed that HIV modulated the differentiation of MSCs into adipocytes, with variations between the two viral tropisms: HIV-R5 stimulated adipocyte differentiation, leading to the development of larger lipid droplets ($p<0.0001$), while HIV-X4 induced the formation of smaller adipocytes with perinuclear lipid droplets.

At 10 days of differentiation, both HIV tropisms resulted in larger lipid droplets compared to uninfected controls. Our results indicated that CCR5 expression remained consistent throughout the differentiation process, while CXCR4 expression increased from day 7 of differentiation. This increase in CXCR4 expression could potentially account for the differences observed between the two viral tropisms. Both tropisms were capable of upregulating adipocyte differentiation markers such as PPARγ ($p<0.05$), C/EBPβ ($p<0.05$), and C/EBPα ($p<0.05$) throughout the differentiation process.

Collectively, our results indicate that HIV is capable of modulate the differentiation of these cells into adipocytes, with observed differences between the studied viral tropisms.

23 (164) *IN VITRO,* SARS-COV-2-EXPOSED MACROPHAGES DEPICT ENHANCED OSTEOCLAST DIFFERENTIATION

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Background: The complications attributed to SARS-CoV-2 within the musculoskeletal system are progressively rising, even in asymptomatic patients. Certain studies have linked these complications to mature bone-resorbing osteoclasts (OC), and it is possible that their precursors (macrophages) are influenced by the virus creating a proinflammatory-environment that enhances their differentiation. The aim of this study was to analyze the effect of macrophage infection by SARS-CoV-2 and their subsequent differentiation into OC.

Methods. Macrophages as OC precursors were obtained in cell culture from human monocytes isolated from buffy coats and differentiated with M-CSF (30 ng/mL) for 6 days (MDM). Then, by adding RANKL (50 ng/mL) for 9 days mature OC were obtained. At 3 days of MDM differentiation, SARS-CoV-2 infections with Wuhan (Wh) and Omicron (BA.5) variants were performed using a MOI=0.1. Multinucleated and tartrate-resistant acid phosphatase (TRAP) positive cells with ≥3 nuclei were considered mature osteoclasts. Bone resorption activity was measured by light microscopy on bovine cortical bone slices. Using flow cytometry in cells detached, cell death (annexin-V/7-AAD) and mitochondrial ROS production (MitoSOX) were measured. SARS-CoV-2 replication was assessed at 3, 6, 9, and 12 dpi by measuring viral load in supernatants by RT-qPCR, and by measuring intracellular SARS-CoV-2 nucleoprotein (N)-expressing cells (flow cytometry). Quantitative RT-PCR (qPCR) was used to detect RANK expression (as one of the key genes of osteoclast differentiation).

Results: SARS-CoV-2 exposure of macrophages markedly increased ($p<0.05$) the OC number in response to both viral variants (200x; Ctrl:24.5±2.1; Wh:38.5±2.1; BA.5:38±1.4). Furthermore, viral-exposed cultures (Wh:23.3±3.3; BA.5:25.5±1.7) exhibited significantly greater osteoclast activity ($p<0.05$), as evidenced by examinations of the resorbed bone area, compared to non-infected controls (Ctrl:13.5±1.6). Viral RNA was measured in supernatants even at 12 dpi (Wh:$6.4x10^3$±$2.8x10^3$; BA.5:$2x10^4$±$2.3x10^3$ copies/mL) with a maximum at 3 dpi (Wh:$2.2x10^6$±$7.3x10^5$; BA.5: $6.1x10^6$±$1.9x10^6$ copies/mL), without altering neither cell viability (Wh:2.5±0.9 vs Ctrl:2.9±0.2) or mtROS production (Wh:2.0±0.5 vs Ctrl:3.7±0.8). However, the viral capsid (N) expression has not been detected at 3 dpi by FACS. A significantly higher level of RANK mRNA was measured at 3 dpi in infected MDM than in non-infected controls (fold-change 11.2±12.3).

Conclusions: The exposure of OC precursors to SARS-CoV-2 increases both their differentiation and reabsorbing bone capability, irrespective of the viral variant. Such modulation is independent of viral replication in MDM that does not support the early stages, including the production of newly synthesized viral RNA and protein with a non-productive infection. The expression of pivotal osteoclastogenesis-related genes appears to be upregulated after SARS-CoV-2/OC precursors interaction.

24 (2) INOSITOL HEXAKISPHOSPHATE (IP6) PRODUCTION IS MEDIATED BY YopP AND N-GLYCANS OF MACROPHAGES PARTICIPATE IN THE NITRIC OXIDE REGULATION DURING *YERSINIA ENTEROCOLITICA* INFECTION

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Yersinia enterocolitica (Ye) is a pathogenic bacterium that cause intestinal infections. Different Yops (*Yersinia* outer proteins) are main virulent factors of Ye. YopP is activated by the host factor inositol hexakisphosphate (IP6) and regulates nitric oxide (NO) production during Ye infection. Recently, it has been found that the glycosylation of murine macrophage surface receptors plays a pivotal role in the interaction with pathogens. Here, we investigated the IP6 production and the participation of glycans on NO regulation by YopP during Ye infection. To explore whether YopP is activated by IP6 binding, we measured IP6 at different times post infection (15, 30, 45, 60 min) in lysates of murine peritoneal macrophages (MΦ) infected with Ye wild-type (Ye wt) or with YopP-deficient (Ye Δ*yopP*). We observed that the infection with Ye wt stimulates IP6 production at 30 min post infection ($p<0.01$) with a significant decrease at 45 minutes ($p<0.001$), probably due to its consumption by YopP. No modification in IP6 levels were detected in Ye Δ*yopP* infected MΦ. Then, MΦ were N-deglycosylated by treatment with PNGase F and then, they were infected with Ye wt or with Ye Δ*yopP*. We determined that N-deglycosylation did not impact on the viability, phagocytosis, microbicide capacity and metabolic activity of MΦ. However, it modifies some surface receptors expression such as LPS receptors ($p<0.05$), CD11b and CD115 ($p<0.01$). Then, we demonstrated that YopP displays a dual role in controlling NO production, inhibiting NO production under normal MΦ ($p<0.05$) but increasing NO production by N-deglycosylated MΦ ($p<0.05$). Therefore, to investigate a possible extracellular lectin-like role of YopP, an *in vitro* infection experiment was performed using lactose. When we added lactose, the inhibition of NO was blocked ($p<0.05$). In addition, we demonstrated by western blot that YopP is secreted in the supernatants (SN) of MΦ infected 1 h with Ye wt. Furthermore, the extracellular binding of YopP to MΦ and its glycan dependence were analyzed by immunofluorescence and flow cytometry. The binding of YopP to the surface of MΦ was higher when it was incubated with SN of Ye wt-infected macrophages compared to the binding of Ye purified Yops (*$p<0.0001$*) but decreased when MΦ was treated with SN from Ye wt-infected deglycosylated macrophages ($p<0.001$). Our findings suggest the IP6 induction by YopP and an important role of surface N-glycans on the NO downregulation by YopP during Ye infection. Further studies are required to identify the specific surface receptor involved in these YopP effects.

25 (76) INTERLEUKIN 6 (IL-6) AND TYPE I INTERFERON (INF) SIGNALING ARE NECESSARY TO INDUCE NEURONAL DEATH BY *BRUCELLA ABORTUS*-ACTIVATED MICROGLIA

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Neurobrucellosis is an inflammatory disease caused by *Brucella* spp infection of the central nervous system. We have previously demonstrated that *B. abortus*-infected microglia induces neuronal death through primary phagocytosis of live neurons. This particular mechanism of cellular death involves the activation of microglia and requires 2 conditions to take place: the secretion of nitric oxide and the increase in microglial phagocytic capacity. Since IL-6 and type I IFN have been implicated in the modulation of diverse neuropathological diseases; and considering that activated microglia are capable of secrete various pro-inflammatory mediators, we aimed to investigate the role of these cytokines on primary phagocytosis. Neurons/microglia co-cultures from BALB/c mice were infected or not with *B. abortus* for 48 h, in the presence of IL-6, IFN-β or IFNAR (type I interferon receptor) neutralizing antibodies or isotype control. After that, neuronal density in co-cultures was evaluated by fluorescence microscopy. Neutralization of IL-6 ($p<0.005$), IFN-β ($p<0.05$) and IFNAR ($p<0.05$) completely inhibited neuronal loss caused by *B. abortus*-infected microglia, indicating their relevance in this phenomenon. To evaluate in which of the two requirements each cytokine is involved, we measured nitric oxide secretion by the colorimetric Griess reaction and microglial phagocytic activity by a phagocytosis assay with negatively-charged fluorescent beads in the presence of anti-IL-6 or anti-IFN-β neutralizing antibodies. Neutralization of IL-6 significantly ($p<0.05$) decrease phagocytic activity (both the number of phagocytic microglia and the number of beads taken per microglia), but it did not affect secretion of nitric oxide ($p>0.05$) by *B. abortus*-activated microglia. Conversely, neutralization of IFN-β did not modified phagocytic activity ($p>0.05$) of *B. abortus*-activated microglia, but significantly decrease nitric oxide secretion ($p<0.05$). Altogether these results indicate that both IL-6 and IFN-β signaling pathways are necessary to induce phagocytosis of viable neurons by *B. abortus*-infected microglia. Moreover, our results demonstrate that these signaling pathways have independent effects on microglia functions and highlight their key role in this mechanism of neuronal death caused by *B. abortus*-infected microglia.

26 (183) METFORMIN TREATMENT IN MACROPHAGES COULD HAVE AN EFFECT ON T CELL ACTIVATION AGAINST T. CRUZI INFECTION.

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Macrophages (Mo) play a key role in initial control of *T. cruzi* replication mainly through iNOS expression and ROS production. However, exacerbated ROS and iNOS production could lead to T cell suppression and tissue damage. This can be modulated by various intracellular signals. AMPK is a cellular energy sensor responding to low ATP levels and can be activated by Metformin (MF). MF is a diabetes drug that can modulate several pathways switching Mo activation. We have previously reported that pretreatment of bone marrow derived macrophages (BMDM) with MF prevents intracellular parasite replication and modulates the expression of costimulatory and inhibitory molecules in peritoneal and spleen macrophages of *T.cruzi* infected mice. To investigate the relevance of AMPK in Peritoneal Mo (PEMs) during *T.cruzi* infection, we determine the frequency of p-AMPK+ in Large PM (LPM) and Small PM (SPM) by Flow Cytometry (FC) from Balb/c mice infected with 500 trypomastigotes (Tp) at different time points. We observed a significant decrease ($p<0.01$) of p-AMPK+ cells during the acute phase of infection that restored to normal levels in the chronic phase. As we show before, *ex vivo* treatment of PEMs from infected mice with MF (1 mM) decreased iNOS expression and NO production compared with PBS treatment. To confirm these results, we performed an *in vivo* MF-treatment (100 mg/kg/day from day 5 to 18 pi) on infected and control mice and we observed a significant decrease of iNOS+ cells in LPM and SPM subsets and in spleen Mo assessed by FC. We also found less Foxp3+ CD4 T cells in peritoneal lavage from infected and MF-treated mice, compared with the PBS-treated group ($p<0.01$). To investigate the role of Mo on T cell proliferation and Treg differentiation, we first co-cultured *ex vivo* treated PEMs from infected or control mice with total splenocytes of control animals previously stimulated with PMA/Ionomycin. We found a reduction in T cell proliferation by FC of stimulated CD4 and CD8 T cells in presence of infected PEMs compared to non-infected PEMs. This reduction was significantly reverted when PEMs from infected cells were treated with MF ($p<0.05$). We also found an increase in IFN-γ concentration by ELISA in the supernatant of these conditions, but we found no differences on Foxp3+ frequencies in this model. To study the role of Mo in MF treatment, we did an adoptive transfer of BMDM treated with MF (M-MF), PBS (M-PBS) or vehicle (PBS) to the peritoneum of infected mice at 14 dpi. Four days later, we obtained peritoneal lavage, spleen and inguinal lymph nodes cells from these groups and non-infected (control) mice and evaluated the frequency of Tregs by FC. The M-MF transfer reversed the increase in Treg frequency produced by the M-PBS transfer and it is correlated with Tp blood levels since mice transferred with M-MF had less parasitaemia than M-PBS transferred. These results suggest that MF could modulate Mo response and its potential role in T cell activation.

27 (21) MODULATION OF MEGAKARYO/THROMBOPOIESIS BY THE *VITA*-PAMP *BRUCELLA ABORTUS* RNA

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Thrombocytopenia, or the reduction in the number of circulating platelets, is one of the more frequent hematologic abnormalities associated with *Brucella* infection. However, the reasons for this have not yet been studied. In previous studies, we have shown that platelets are capable of establishing complexes with macrophages and neutrophils, which may contribute to the observed thrombocytopenia. Moreover, *B. abortus* (*Ba*) can be detected in bone marrow cultures of brucellosis patients. Platelets are formed and released by precursor cells found in the bone marrow, called Megakaryocytes (MKs). This leads us to propose that *Brucella* could directly affect MK generation (megakaryopoiesis) and/or platelet biogenesis (thrombopoiesis), contributing to the observed thrombocytopenia. On the other hand, we have recently demonstrated that *Ba* RNA (a PAMP related to pathogens' viability or *vita*-PAMP) is involved in macrophage immune-modulation. Therefore, the aim of this study was to evaluate the effect of *Ba* RNA on megakaryopoiesis and platelet biogenesis. For megakaryopoiesis, hematopoietic progenitor cells purified from human umbilical cord blood ($CD34^+$) were differentiated to MKs with thrombopoietin (TPO) in the presence of different doses of *Ba* RNA (1, 5 and 10 μg/ml) or Heat-Killed *Ba* (HKBA) (2 x 10^5 bacteria/μl). After 11 days of culture, we determined cell expansion, the expression of the MK differentiation marker CD41 and the MK maturation marker CD42b by flow cytometry. Our results demonstrated that *Ba* RNA decreased significantly the number of total cells in a dose dependent manner ($p<0.05$, n=4). In addition, the highest dose of *Ba* RNA significantly reduced the commitment to MK lineage ($CD41^+/CD42b^-$ cells, $p<0.05$, n=4) and MK maturation ($CD41^+/CD42b^+$ cells, $p<0.05$). Due to the reduction in cell proliferation and MK commitment, *Ba* RNA diminished the total number of MKs and mature MKs ($p<0.05$). HKBA could also significantly decrease the number of total cells, and the number of total and mature MKs ($p<0.05$), indicating that megakaryopoiesis was inhibited by *Ba* RNA and HKBA. Next, we evaluated the effect of *Ba* RNA and HKBA on thrombopoiesis. For this, $CD34^+$ cells were differentiated to MKs with TPO in the presence of different doses of *Ba* RNA or HKBA. We stimulated one set of cells on day 0 and another set on day 11. On day 16, platelet production was evaluated in the two sets. Our results show a tendency in reduction of platelet production in both sets. However, this tendency was more evident in the cells stimulated on day 11. Altogether, these results suggest that *Ba* RNA and HKBA impair megakaryopoiesis, contributing to the thrombocytopenia observed in chronic brucellosis.

28 (6) NEUTRALIZING ANTIBODIES IN THE INTESTINAL MUCOSA ARE ESSENTIAL TO CONTROL SHIGA TOXIN- PRODUCING *Escherichia coli* (STEC) GASTROINTESTINAL INFECTION IN MICE

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The humoral response in the intestine against pathogens such as STEC is fundamental to define the evolution to self-limited forms or systemic complications. We previously demonstrated that B cell stimulation and the early antibody production are fundamental in protection against STEC infections in BALB/c mice (BALB). The objective of this work is to characterize the mechanism by which local and/or systemic specific anti-STEC antibodies (aSTECAb) mediate protection against systemic complications and death after STEC infection in BALB.

To achieve this, BALB were gastrointestinally infected with a non-lethal dose of STEC ($1x10^{10}$ CFU/mouse) at weaning; to increase the antibody titer, they were challenged two more times with STEC ($5x10^{10}$ CFU/mouse) at 10-day intervals. Mice were bled 7 days after the 1st and 2nd infection to determine plasma aSTECAb titer. Seven days after the 3rd infection, mice were euthanized, and plasma and feces collected to determine the aSTECAb titer reached by ELISA. To characterize the functionality of these antibodies, in vitro opsonization assays were carried out by incubating feces (1/2 dilution) or plasma (1/10 dilution) with STEC ($1x10^{7}$ UFC) for 2 h and subsequent analysis by flow cytometry using an anti-mouse IgA or IgG antibody, respectively, coupled to FITC. Also, the neutralizing capacity of these antibodies was analyzed by bacterial growth inhibition in tryptic soy broth determining the optical density at 600 nm (OD600) every 20 min for 18 h, and motility in soft agar by measuring diffusion halos at 48 h (10^{3} UFC with 1/10 diluted plasma for growth and motility inhibition. Only plasma was selected for these assays as it is a sterile sample). The statistical analyzes used were ANOVA, Student's parametric t test or non-parametric t test, as appropriate.

IgG aSTECAb titer in plasma was 2048 and IgA aSTECAb titer in feces was 1024 after the 3rd infection (n=4 mice) (levels of aSTECAb measured as OD492 in plasma at 1/2048 dilution: 0.427±0.17/ 0.07±0.11 infected vs control mice, $p<0.05$; in feces at 1/1024 dilution: 0.711±0.34/ 0.06±0.26 infected vs control mice, $p<0.05$). aSTECAb present in plasma and feces were able to opsonize STEC (IgG-coated bacteria 96.0%±5.3/ 9.2%±2.4 infected vs control plasma, $p<0.0001$; IgA-coated bacteria 98.5%±0.6/ 5.94%±0.6 infected vs control feces, $p<0.0001$. n=3). Specificity controls were carried out with commensal *E. coli* and no cross-reaction was observed. Similarly, aSTECAb were able to inhibit bacterial growth (at 600 min: OD600= 0.698±0.01/ 0.964±0.09 infected vs control plasma, $p<0.001$, n=2) and motility (at 48 h: 44.2 mm±10.3/ 75.4 mm±2.75 infected vs control plasma, $p<0.001$, n=4).

We concluded that aSTECAb through their opsonizing and neutralizing capacity would interfere with adhesion to the intestinal epithelium, and as a consequence of this, in limiting STEC pathogenicity and in defining the outcome of a gastrointestinal infection with this pathogen.

29 (87) NOVEL ENZYME LINKED IMMUNOSORBENT ASSAY TO DETECT ANTI-RABIES VIRUS ANTIBODIES IN SERUM SAMPLES DERIVED FROM VACCINATED ANIMALS

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Rabies is a zoonotic disease, which means it can be transmitted from animals to humans. Rabies in bulls is a rare but potentially serious disease. Therefore, any case of rabies in animals, including bulls, is a cause for concern and should be managed seriously to prevent the spread of the disease to other animals and people. There is no test to monitor the effectiveness of the anti-rabies vaccination in our Country. For this reason, the aim of this work was to develop a novel immunoassay to detect antibodies to rabies employing a rabies antigen produced in baculovirus expression system.

Materials and methods: The ectodomain of the rabies virus glycoprotein G (GE) was synthesized using the baculovirus expression system in Sf9 insect cells. The protein was recovered from the cell lysates and purified in one step by metal ion affinity chromatography.

Thirty-two serum samples of bulls vaccinated against rabies and 30 sera from non-vaccinated bulls kindly donated by ProinVet Innovations company were used for the development of the immunoassay. An indirect ELISA was performed, based on the interaction of anti-rabies virus antibodies present in samples with purified GE immobilized in the solid phase. Bound antibodies were detected with anti-bovine IgG antibodies conjugated to peroxidase. The formation of immunocomplexes was revealed by the addition of TMB, the reaction was stopped with H_2SO_4 and the oxidized product was measured using a spectrophotometer. The data obtained for each sample was analyzed with the GraphPad Software and the results were expressed as specific absorbance / OD (OD = the mean of each sample minus the mean of the blank control). The cut-off value of the assay was set at OD = 0.706.

Results: Recombinant glycoprotein G from rabies virus was efficiently expressed in Sf9 insect cells. After chromatography purification the GE was obtained with a yield of 95% and a purity of 87%.

When testing the serum samples using the developed ELISA 19 out of the 32 samples derived from vaccinated animals were positive (sensitivity = 59%) and 29 out of the 30 samples from non-vaccinated bulls were negative (specificity = 97%) when a cut-off value of 0.706 OD was established. The area under the ROC curve was 0.945, demonstrating the strong ability of the assay to differentiate between the two groups under study.

Conclusion: Having a sensitive and specific detection method for rabies antibodies in bull sera such as the ELISA described herein, would offer several significant advantages: i) disease control by rapidly identifying if bulls have been exposed to the rabies virus, ii) public health concern by preventing disease transmission to individuals working with these animals, iii) vaccination programs and evaluation of the effectiveness of the anti-rabies vaccination, iv) animal breeding selection, and v) epidemiological monitoring to better understand the

prevalence and distribution of rabies in bull populations which has an important economic impact.

30 (85) PARTICIPATION OF NUCLEAR RECEPTORS NR4A IN THE IMMUNE RESPONSE DURING TUBERCULOSIS.

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Nuclear receptors (NRs) are a superfamily of ligand-dependent transcription factors that bind to numerous kinds of molecules, mostly hydrophobics, and with an important role in the immune endocrine regulation of homeostasis and pathophysiology. Within the NRs, NR4As orphan receptors have emerged as important regulators of immune cell polarization in immune response (IR) and inflammation, particularly affecting NF-κB signaling. In macrophages (Mf), NR4A receptors modulate NF-κB activity in a dynamic manner, either repressing or enhancing target gene expression. In another hand, Tuberculosis (TB), a major infectious disease caused by *Mycobacterium tuberculosis* (MTB), infects alveolar Mf and, hence, promotes a cellular IR, which becomes harmful when prolonged over time, being central to the immunopathology of TB. This work aimed to evaluate the participation of NR4As in the Mf response when they are exposed to MTB antigens. In this regard, we differentiated cells from the THP-1 cell line into inflammatory and anti-inflammatory Mf (M1 and M2 respectively) and exposed them to MTB irradiated (MTBi) at different times (1, 3, 6, and 24 H). Then, we assessed the mRNA expression of CD80, NR4A1-3, NFKB1, NFKBIA/B, IL-1β, IL-6, IL-10, HIF1α and Caspase 3. M1 cells showed an important increase in CD80, NR4A2 and 3, IL1β, HIF1α, and NFKB1, as well as its inhibitors A and B, particularly after 6 H of treatment, with its expression being even higher, and IL-10 mRNA augmented, provided MTBi was added to these cultures. Regarding M2 cells, only NR4A1 and IL-10 were notably expressed before MTBi treatment. However, after 1 H of mycobacteria exposure, all analyzed genes were expressed, particularly the inflammatory ones. On the other hand, MTBi-treated Mf showed an increase in NR4A1 and 2, IL-1β, IL-10, NFKB1, NFKBIA and NFKBIB expression. These results suggest that MTBi induces a clearly augmented expression in pro-inflammatory genes, together with IL-10 mRNA transcripts. The coexistence of inflammatory and anti-inflammatory mediators, along with an increase in NFκB inhibitors may imply an attempt to regulate the strong inflammatory response elicited by the pathogen presence, wherein NR4As are likely to play a regulatory role in this regard.

31 (178) PREINFECTION OF CACO-2 CELLS WITH ENTEROAGGREGATIVE *Escherichia coli* INCREASES ADHESION OF ENTEROHEMORRHAGIC *Escherichia coli*.

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Escherichia coli enterohemorrhagic (EHEC) O157:H7 causes bloody diarrhea and hemolytic uremic syndrome (HUS) in children below 5 years of age. Shiga toxin production and bacterial adhesion to intestinal cells through type three secretion system are the major pathogenic factors. In recent years, an increased association of HUS pediatric cases with Enteroagreggative *E. coli* (EAEC) strains co-infection was reported by the National Reference Laboratory, ANLIS-Malbrán. The objective of this work was to evaluate if the infection of Caco-2 cells with EAEC increases the adhesion and the pathogenic potential of an EHEC strain. Caco-2 cells were infected for 3 h with EAEC (10^5 or 10^4 CFU/ml) and EHEC (10^6 cfu/ml) [ratios 10:1 and 100:1 (EHEC:EAEC)]. We tested two infection protocols where EAEC was added simultaneously with or 1 h before EHEC. We evaluated the percentage of adherence by colony forming units counting in agar plates, the presence of Shiga toxin in culture supernatants by cytotoxicity in Vero cells, and IL1β, IL-8, TGFβ, CCL20 mRNA by qPCR. When EAEC and EHEC were added simultaneously in Caco-2 cells a decreased adherence percentage of EHEC was observed compared to control (EHEC) (mean % ± SD: 10^6 EHEC:28.27 ±11.62; 10^6 EHEC:10^5 EAEC: 10.46± 5.5 10^6 EHEC:10^4 EAEC 25±13; p≤0.05, Kruskal-Wallis). Otherwise, when EAEC was added 1 h before EHEC a significantly increased percentage of EHEC adhesion was evidenced (mean % ± SD: 10^6 EHEC:29.67 ±12.13; 10^6 EHEC:10^5 EAEC:75.31 ±26.82 10^6 EHEC:10^4 EAEC 171.5±101.1; p≤0.05, Kruskal-Wallis). In addition, preliminary results showed an increased IL1β mRNA level when Caco-2 cells were pre-infected with EAEC (mean Expression Units ± SD: 10^6 EHEC: 0.10±0.01; 10^6 EHEC:10^4 EAEC: 1.50 ±0.02; p≤0.05, t-test). We did not observe differences on Stx production in all the conditions evaluated.

In conclusion, the pre-infection of Caco-2 cells with EAEC increases the percentage of EHEC adhesion as well as the production of IL1β. An increased production of proinflammatory cytokines in the intestine could contribute to the passage of Stx to the systemic compartment. These observations may be related with an increased HUS outcome in children, however more studies should be conducted.

32 (179) PRELIMINARY EVALUATION OF A MURINE MODEL TO DETERMINE THE IMPACT OF NEUTROPHIL INTERLEUKIN-1 BETA (IL-1β) IN HEMOLYTIC UREMIC SYNDROME DEVELOPMENT

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Neutrophils are recruited to the gut upon infection with the enteric pathogen Shiga toxin-producing *Escherichia coli* (STEC). There, they can deploy their microbicidal battery to fight against the pathogen. However, they could also contribute to the extensive tissue damage that accompanies STEC infections and that impacts the development of Hemolytic Uremic Syndrome (HUS), a systemic disease. In Argentina, HUS is endemic showing high incidence in children under five years. There is still no treatment to prevent the progression of the disease or an effective vaccine. We previously found that human neutrophils secrete IL-1β upon challenge with STEC (O157:H7) by a pathway that involves neutrophil serine proteases (NSP) and caspase-1 activity. We here conducted preliminary studies to determine if a murine model would be appropriate to investigate both the role of neutrophil IL-1β in HUS development and the capacity of inhibitors of this secretion to halt the progression to this syndrome. With this aim, we isolated peritoneal cells from either BALB/c or C57BL6 mice after 6-h intraperitoneal injection of Thioglycolate 6% and determined the percentage of granulocytes in these samples. Then, we challenged ex vivo those cell samples containing ~58%-73% of granulocytes with STEC (O157:H7) at a multiplicity of infection (MOI) of 0.5, either in the presence or absence of a PAN-serine protease inhibitor (AEBSF 0.1M) or a caspase-1/4 inhibitor (VX-756 50 μM). After 3 h, we evaluated IL-1β concentrations in culture supernatants by ELISA. STEC induced a significant increase of IL-1β in culture supernatants over the basal levels both in BALB/c ($p<0.001$) and C57BL6 ($p<0.05$) cell samples. Furthermore, AEBSF and VX-765 significantly inhibited IL-1β secretion induced by STEC (BALB/c AEBSF n=5; $p<0.001$; C57BL6 AEBSF, n=3, $p< 0.05$; BALB/c VX-765; n=5; $p<0.05$; C57BL6 VX-765 n=1). These results suggested that IL-1β secretion by mouse neutrophils can be negatively modulated by the same inhibitors that had demonstrated to markedly reduce human neutrophil IL-1β secretion, even when other cell types were also present in the samples of the inflamed peritoneal cavity. Then, with the aim to evaluate plasmatic IL-1β levels upon infection with STEC, mice (17 to 19 days of age; 6 to 10 g of body weight) were withheld from feeding for 4 h immediately after weaning, and then gavaged with STEC ($1x10^{10}$-3.5×10^{10} UFC), and 4.5 hours later blood samples were obtained and the plasmatic IL-1β levels were determined by ELISA. We found a trend towards an increased level of plasmatic IL-1β in infected animals (n=8). However, we obtained a high value of standard deviation, suggesting the necessity to evaluate other time points after infection. Altogether, our findings suggest that a mouse model of HUS might be helpful in evaluating the efficacy of the treatment with NSP inhibitors to limit the inflammatory response that accompanies intestinal infections by STEC and consequently HUS outcome.

33 (142) PRONOSTIC APPROACH IN COVID-19 PATIENTS THROUGH PLASMA PEPTIDOME ANALYSIS USING MALDI-TOF MS TECHNOLOGY

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The COVID-19 disease presents a dynamic nature characterized by distinct and well-defined pathophysiological phases. As the disease progresses, an exacerbated inflammatory response accompanied by marked lymphopenia and dysfunctional immune response that could lead to the sepsis progression and dead. Nevertheless, clinically, accurately determining the patient's stage or identifying those individuals at advanced risk of progressing to more severe conditions remains challenging. Technologies such as proteomics and predictive models based on artificial intelligence have raised significant expectations in the study of various pathologies. Our aim was to investigate the usefulness of plasma peptidome fingerprints as a tool for severity stratification of patients with COVID-19 using MALDI-TOF MS technology. For this, we conducted an observational and analytical prospective cohort study in which the analysis unit consisted of the peptidome biomolecular fingerprints in plasma, along with clinical information collected from patients admitted to the intensive Care Unit (ICU). We followed up 37 patients admitted to the ICU of the Hospital de Clinicas "José de San Martín" -UBA during the August-September 2020 period. Plasma samples (n = 87) and clinical information were collected during their ICU stay. Patients were categorized into survivors (S) (n=12) and non-survivors (NS) (n=19). A control group (C) (n=6) consisted of ICU patients without COVID-19. Multivariate analyses and machine learning algorithms were applied to discriminate between the classification stages in patients. Clinical and biochemical parameters were also evaluated. Neutrophil-Lymphocyte Ratio (NLR), which serves as a predictor of mortality in COVID-19 ICU patients (NLR (median (RIQ): S=7.5 (4.5-9.7); NS=16.6 (10.4-31.6)*#; C=4.8 (2.6-7.1), $p<0.05$), along with serum creatinine and urea levels, exhibited significant distinctions between S and NS on admission at day 1 ICU (urea (mg/dl) (mean±SD): S=40.4±9.1; NS=68.6±34*#; C=35. 3±18, $p<0.05$), indicating compromised renal function. The appearance of complications such as septic shock were significantly more frequent in non-survivors than survivors (S=1/12(8.3%); NS=8/19(42.1%)*; C=0/6 (0%) ;$p<0.05$ Chi-square test).The 797 mass spectra database of COVID-19 patients was processed and utilized to apply binary discriminant analysis (BDA) as a classification method. Through this analysis, it was possible to discriminate survivors from non-survivors based on a matrix of spectral characteristics and additional information from clinical biomarkers. These findings underscore the potential of plasma peptidome fingerprints obtained through MALDI-TOF MS as a prognostic assessment tool, enabling the development of predictive models for patient stratification based on disease severity.

34 (79) REDUCTION OF PEYER´S PATCHES IN ACUTE *TRYPANOSOMA CRUZI* INFECTION

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Chagas disease, also known as American trypanosomiasis, is a tropical parasitic disease caused by *Trypanosoma cruzi*. The acute phase of experimental *T. cruzi* infection results in splenomegaly and expansion in lymph nodes, polyclonal B cell activation, hypergammaglobulinemia and unspecific antibodies (Abs) in sera. Parasite-specific Abs are also present in infected mice and have been described as an important mechanism to parasitemia control. The contribution of the different B cells subsets to this infection are diverse and not completely understood.

In this work, we evaluated B cell response in gut during *T. cruzi* infection since intestine is the tissue with the highest number of B cells. For that, 8-12 weeks old C57BL/6 mice were intraperitoneally injected with 2500 trypomastigotes of *T. cruzi* Tulahuén strain or with PBS (control mice). At different days post infection (dpi) small intestine, Peyer's patches (PP) and mesenteric lymph node (MLN) were obtained. By macroscope evaluation we observed a decrease in the size of PP and MLN at the time of highest parasitemia (18 dpi). PP decrease was transient since at 82 dpi they recovered the normal size. By immunofluorescence (IF), at 18 dpi, we observed a decrease in B and $CD4^+$ and $CD8^+$T cells in PP but infiltrating T cells in the muscle layer of small intestine. Accordingly, by FACS, we observed that PP had a marked decrease in the number of T and B cells, being the greatest reduction in B cell population. Moreover, TUNEL assay performed on PP and MLN at different dpi showed negative result in PP obtained at 10 dpi, while positive signal, indicating apoptosis, was observed inside the follicles and medullary zone of the MLN. In MLN from infected mice, $CD169^+$ metallophilic macrophages disappeared from the subcapsular sinus and clustered around the B cell follicles, and follicles were highly disorganized containing high frequency of $CD4^+$ T cells. The findings suggest that *T. cruzi* infection impacts on gut immune response, leading to changes in B and T cell populations of PP and MLN. In summary, absence of apoptosis and the reduction in lymphocytes number indicate that *T. cruzi* infection is affecting the cellular traffic in PP. In addition, the high tissue disorganization of MLN from infected mice suggest that *T. cruzi* affects the generation of productive gut immune response.

35 (44) RNA FROM *ESCHERICHIA COLI* ENHANCE INFLAMMATORY RESPONSE IN A PNEUMONIA MURINE MODEL

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Escherichia coli (ECO) is a Gram-negative bacteria member of the *Enterobacteriaceae* family, a well-known microorganism capable of causing multiple diseases. In particular, ECO is highly responsible for causing ventilator-associated pneumonias. Prokaryotic RNA has been previously established as an immune modulator. Our previous work demonstrated that depending on the bacterial species, RNA could act as both an inductor or an attenuator of inflammatory responses.

In particular for the RNA from ECO (RNA_{ECO}), an inflammatory response was induced *in vitro* on human endothelial, lung epithelial cells and isolated neutrophils (PMN). For all these cell types, upregulation of activation proteins on their surface and secretion of chemotactic and pro-inflammatory cytokines have been shown after stimulation with RNA_{ECO}. Also PMN elimination of ECO was enhanced in the presence of RNA_{ECO}.

The aim of this study was to determine the effects of RNA_{ECO} *in vivo* in the context of a lung infection using a murine model.

For experimentation, 3 month-old female BALB/c mice were treated (intranasal, i.n) with PBS (Control) or RNA_{ECO} (10 µg), and bacterial infection was induced by administration of live ECO i.n. After 4 h post-infection bronchoalveolar lavage (BAL) and lung cells were obtained, and the number of PMN ($GR1^+$-$CD11b^+$ cells), secretion of inflammatory chemokines as well as bacterial clearance were determined. The number of migrated PMN was analyzed by flow cytometry, pro-inflammatory cytokines were measured using ELISA kits and bacterial clearance was determined counting the number of Colony Forming Units (CFU) of bacteria.

Our results showed that RNA_{ECO} induced an increase in PMN in BAL and lung compared to control mice ($p \leq 0.05$). In line with this result, we observed an increase in the secretion of pro-inflammatory cytokines such as TNF-α and IL-6 in BAL ($p \leq 0.05$).

Finally, we wondered whether this pro-inflammatory response in the lung elicited by RNA_{ECO} could affect bacterial clearance. The number of CFU in lung and BAL from mice treated with RNA_{ECO} were significantly reduced compared to mice challenged with ECO ($p \leq 0.05$).

In conclusion, these results revealed that RNA_{ECO} is able to induce pro-inflammatory responses in the lungs that favors bacterial clearance during pneumonia.

Further and detailed studies will allow us to determine whether the use of bacterial RNA could be implemented as a therapeutic strategy to improve bacterial clearance.

36 (187) SEMEN EXTRACELLULAR VESICLES CONTAIN IFITM3 AND INHIBIT ZIKV INFECTION ON DENDRITIC CELLS

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Recently, sexual transmission of Zika virus (ZIKV) has been reported for the first time, changing our view of arbovirus-host interaction. Infectious virus persists in semen six month safter symptoms onset at higher viral loads. Extracellular vesicles are membrane-enclosed structures released by cells that mediate intercellular communication and are present in all biological fluids; seminal plasma contains trillions of extracellular vesicles. It has been demonstrated that interferon induced transmembrane proteins 1 and 3 (IFITM1, IFITM3) inhibit ZIKV infection. Knowing that semen is not merely a carrier for sexually transmitted diseases, our aim was to evaluate the role of semen extracellular vesicles (SEV) on ZIKV infection of dendritic cells (DCs).

SEV were purified from healthy donors using size exclusion chromatography, and then analyzed by western blot, electron microscopy, NTA and proteomics. Monocytes were purified from peripheral blood and differentiated to DCs with GM-CSF and IL4 for 5 days. ZIKV infection was measured by UFP, qPCR, flow cytometry, and epifluorescence microscopy. Phenotype was analyzed by flow cytometry and the cytokine production was measured by ELISA.

Presence of CD9, CD63, CD81, HSP70 and Alix were measured in purified SEV by western blot. NTA analysis showed that SEV purified samples contained between 2*10^11and 2*10^12 vesicles/ml and that size distribution was homogenous between samples (n=12). Proteomic analysis of SEV showed that vesicles carried IFITM1 and IFITM3, which was confirmed by western blot.

Initially, DCs (2*10^5) were infected with ZIKV (MOI=0.1) in the presence or absence of SEV (200ug/ml) for 72hs. We first analyzed DCs phenotype and measured cytokine production. Neither SEV nor ZIKV altered IL1β, IL6, IL10 or IL12 production (n=5-8). Expression of CD83, HLA-DR, CD86, DC-SIGN or CD40 was not modified by SEV nor ZIKV. Purified SEV inhibited ZIKV infection ($p<0.001$, n=10). When SEV was added to the culture 1h after infection there was a reduction on the inhibition. The binding of ZIKV to cells was not affected by SEV (n=9). Interestingly, the infection of DCs in the presence of SEV increased the expression of IFN-β mRNA ($p<0.05$, n=7), OAS, MX1 and IRF7 ($p<0.05$, n=5). However, inhibiting the interferon response using an IFNa blocking antibody didn't revert the antiviral effect of SEV (n=3). To evaluate if IFITM proteins could explain the inhibition observed we preincubated with blocking antibodies. The blockade of IFITM-3, but not IFITM-1 or IFITM-2, produced a partial decrease in the inhibition exerted by SEV.

SEV inhibit ZIKV infection on DCs. SEV contain IFITM3, which partially explains the inhibition observed. Further studies need to be performed to identify other mechanisms involved.

37 (189) STUDY OF THE MECHANISM INVOLVED IN THE PROTECTIVE EFFECT OF *Lactiplantibacillus plantarum* CIDCA 83114 ON SHIGA TOXIN (Stx)-PRODUCING *Escherichia coli* (STEC) PATHOGENICITY *IN VITRO*

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Shiga toxin (Stx)-producing *Escherichia coli* (STEC) O157:H7 is a foodborne pathogen, which can lead to the life-threatening Hemolytic Uremic Syndrome (HUS). There is no treatment available in order to reduce HUS outcome up to date. We previously showed that the probiotic strain *Lactiplantibacillus plantarum* CIDCA 83114 (CIDCA 83114) completely inhibits STEC growth *in vitro* when co-culturing both bacterial strains for 24h in ratios 1:1, 1:10 and 1:100 (STEC: CIDCA 83114). Besides, CIDCA 83114 reduces STEC adhesion to Caco-2 cells by an exclusion mechanism. In the present work we aim to delve into the mechanism of CIDCA 83114 effect on the pathogenic potential of a clinically isolated STEC strain *in vitro*. We tested EHEC growth at 6 h of co-culture with CIDCA 83114 in ratios 1:1, 1:10 and 1:100 (STEC: CIDCA 83114). We quantified lactate concentration by a colorimetric assay and pH values in all the EHEC:CIDCA 83114 ratios tested at 6 and 24 h. We also used conditioned media obtained by culturing CIDCA 83114 and then reproduced the lactate concentrations and pH values to that of the co-culture treatments. We also quantified TGF_{β} and IL-8 mRNA levels by RT-qPCR in Caco-2 cells after infection with EHEC and CIDCA 83114 in ratios 1:1 and 1:100 (STEC: CIDCA 83114).

We observed EHEC growth inhibition when co-cultured with CIDCA 83114 at 6 h in a 1:100 ratio ($p<0.01$, Bonferroni test). In addition, significantly increased lactate concentration in the 1:10 and 1:100 ratios after 6 h of co-culture ($p<0.001$, ANOVA) and in all evaluated ratios at 24 h of co-culture ($p<0.001$, ANOVA) were obtained. Furthermore, we observed significantly reduced pH values in the 1:10 and 1:100 co-cultured ratios after 6 h ($p<0.001$, ANOVA) and in all evaluated ratios at 24 h of co-culture ($p<0.001$, ANOVA). When EHEC cultures were carried out with conditioned media we observed EHEC growth inhibition only with unneutralized supernatant media in all lactate concentrations tested ($p<0.0001$, Kruskal-wallis).

Besides, we observed a significant reduction in IL8 and TGF_{β} mRNA levels in epithelial cells cultured with CIDCA 83114 1 hour before STEC addition (mean expression units ±SD: IL8= EHEC: 1.9±0.1; CIDCA 83114: 0.6±0.2; 1 EHEC: 100 CIDCA 83114: 0.10±0.03; TGF_{β}: EHEC: 5.1±1.1; CIDCA 83114: 1.1±0.6; 1 EHEC:100 CIDCA 83114: 1.29±0.31 (ANOVA $p<0.01$).

In conclusion, STEC growth inhibition by CIDCA 83114 is exerted, at least in part, by the low pH values observed in co-cultured media, since we only observed EHEC growth inhibition with unneutralized conditioned media regardless of the lactate concentration. In addition, the reduced production of IL8 and TGF_{β} by Caco-2 cells co-infected with 1 EHEC: 100 CIDCA 83114 may be related to a protective effect in epithelial cells. However, more studies are needed in order to better characterize the inflammatory response in these conditions.

38 (148) TH17 PROFILE IN CHRONIC MUCOCUTANEOUS CANDIDIASIS PEDIATRIC PATIENTS IN MENDOZA PROVINCE.

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Chronic mucocutaneous candidiasis (CMC) encompasses a range of genetically diverse disorders characterized by persistent or recurrent infections commonly caused by *Candida albicans*. Th17 lymphocytes produce IL-17A which is relevant component for antifungal immunity. Conversely, Th1 cells mainly yield IFNγ contributing to cell-mediated immunity. Genetic polymorphisms linked to defects in Th17 differentiation, proliferation or subsequent IL-17 receptors signaling can increase susceptibility to CMC.

Our objective was to contribute to CMC diagnosis in pediatric population by evaluating the Th17 cells profile.

An observational, cross-sectional, analytical, prospective study was conducted on 9 patients with CMC from 1 to 17 years of age. As control, 22 individuals of the same age range were included. Peripheral blood mononuclear cells were isolated from both study groups; primary cultures were stimulated with PMA/ionomycin and the frequency ofIL-17A and IFNγ-producing CD4+ T cells was evaluated by Flow Cytometry. In addition, cytokines concentration in both, the culture supernatantand serum of patients were assessed by ELISA.

The percentage of IL-17A producing CD4+ T cells was significantly reduced in CMC`s patients compared to controls ($p<0.05$), evidencing a decrease in the frequency of Th17 cells in individuals with the pathology. When the Th1 profile was evaluated, INFγ-producing CD4+ T cells were increased in CMC´s patients compared to controls ($p<0.05$). The decrease was not related to the diagnostic condition, symptoms recurrence, or the clinical manifestation of CMC. Despite of the limited number of patients studied, CMC of different etiologies were present in this group, as Hyper IgE syndrome (HIES) and STAT1-GOF mutations. A decrease in the frequency of Th17 cells was observed in all subgroups, independent of the molecular mutation. Examination of the Th1/Th17 ratio showed an imbalance, characterized by a clear rise (3.57 fold) among CMC patients. This underlines the importance of the equilibrium between Th17 and Th1 responses in antifungal defense. No correlation was observed between the frequency of Th17 cells with the concentration of IL-17A.

Flow cytometry provides an efficient method to identify deficiencies in Th17 cells, providing valuable information on the patient's immune phenotype, the severity of their dysfunction and the progression of the disease.In Argentina there are few laboratories with the possibility of carrying out this type of study, which determines the great relevance of the development of sensitive methodologies, so the implementation of this diagnostic methodology in a hospital of reference

in Mendoza constitutes a significant contribution for the early identification of these patients and their early treatment. An increase in the number of patients, possibly allows us to corroborate the observed trends.

39 (50) THE DOWN-MODULATION OF IFN-γ-INDUCED MHC-I EXPRESSION BY *BRUCELLA ABORTUS* RNA IN CALU-6, HMEC AND A-549 CELLS SHARES FEATURES WITH MONOCYTES/MACROPHAGES'

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Brucella abortus (*Ba*) is an intracellular pathogen capable of surviving inside macrophages. Since the disease is presented in multiple forms, many different cells are susceptible to be infected by *Ba*. We previously demonstrated that *Ba* RNA is a *vita*-PAMP involved in the immune evasion mediated by this pathogen. One of the mechanisms displayed by *Ba* is the down-modulation of MHC-I on monocytes/macrophages when Th1 response is being held, *i.e*., in the presence of IFN-γ. Moreover, MHC-I total expression is not altered, instead these proteins are retained within the Golgi Apparatus (GA). More recently, we demonstrated that *Ba* RNA diminishes the IFN-γ-induced MHC-I surface expression in other cells able to be infected with *Ba.* However, we do not know if this phenomenon is due to the retention of MHC-I within the GA by *Ba* RNA, as occurs in human macrophages, their preferential niche. To evaluate this, we stimulated the human bronchial epithelium cell line (Calu-6), the human alveolar epithelium cell line (A-549) and the endothelial microvasculature cell line (HMEC) with 10 µg/ml of *Ba* RNA in the presence of IFN-γ. After 48 h, MHC-I expression and GA marker GM130 were detected by confocal microscopy. We observed that *Ba* RNA induces colocalization of MHC-I and GM130 in Calu-6 and HMEC cells. However, no colocalization was detected in A-549 cells. Then, we evaluated the effect of *Ba* RNA on the secretion of IL-8, IL-6 and MCP-1. For this, Calu-6, A-549 and HMEC cells were stimulated with *Ba* RNA (1, 5 and 10 µg/ml) in the presence of IFN-γ for 48 h. Afterwards, supernatants were collected and the secretion of IL-8, IL-6 and MCP-1 was quantified by sandwich ELISA. We did not observe any changes in MCP-1 in *Ba* RNA-treated cells. Conversely to what we expected, Calu-6, HMEC and A-549-*Ba* RNA-treated cells had higher IL-8 and IL-6 levels compared to those from untreated cells ($p<0.05$). In addition, our previous results indicate that *Ba* RNA inhibits the IFN-γ-induced MHC-I surface expression on human monocytes/macrophages by a TLR8-dependent mechanism and through the Epidermal Growth Factor Receptor (EGFR) pathway. In order to extend this finding Calu-6, A-549 and HMEC cells were stimulated with 10 µg/ml of *Ba* RNA in the presence of IFN-γ for 48 h. TLR-8 expression was confirmed by flow cytometry in all cell lines. Next, cells were stimulated with 10 µg/ml of *Ba* RNA in the presence of an EGFR ligand-blocking antibody (Cetuximab). Neutralization of the EGFR partially reversed the inhibition of MHC-I surface expression mediated by *Ba* RNA in HMEC and A-549 cells. Overall, these results show that the down-modulation of MHC-I expression by *Ba* RNA in different cells susceptible to be infected by *Ba* would allow the bacteria to persist successfully within the host, remaining unnoticed and evading $CD8^+$ T cell surveillance.

40 (103) THE MANNOSE RECEPTOR IS A KEY PLAYER IN THE NON-BACTERICIDAL INNATE INTERACTION BETWEEN B. PERTUSSIS AND MACROPHAGES.

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Our previous work revealed that macrophage (MØ) polarization into alternatively activated cells (M2) enhances B. pertussis (Bp) intracellular survival. CR3 has been historically acknowledged as the main target of Bp on these immune cells. Moreover, previous studies suggested that the non-bactericidal macrophage phagocytosis of Bp critically depends on the bacteria-MØ interaction through CR3. In this study, we aimed at evaluating the relative contribution of the mannose receptor (CD206, MR), highly expressed in M2, in the non-bactericidal Bp interaction with macrophages. To this end, primary human monocytes were differentiated into M2 phenotype in the presence of M-CSF+IL-4. We used blocking antibodies against MR and CR3 to selectively study the bacterial interaction of each receptor. M2 macrophages were incubated or not with 10 µg/ml anti-MR or 10 µg/ml anti-CR3 antibody prior to the addition of Bp at an MOI of 100. After 30 min at 37°C, bacterial binding and phagocytosis were quantified by confocal microscopy with double staining to discriminate intracellular and surface attached bacteria. Bacterial trafficking and intracellular survival were evaluated by confocal microscopy and Polymyxin B protection assays, respectively. The use of blocking antibodies against MR resulted in a significant drop in Bp attachment to and phagocytosis by macrophages. We observed a 66 % ($p < 0.05$) decrease of bacterial attachment and a 90% ($p < 0.05$) decrease of bacterial phagocytosis. Surprisingly, in the presence anti-CR3 blocking antibody only the number of bacteria attached per macrophage was affected but not bacterial phagocytosis. We observed a 70 % ($p < 0.05$) decrease of bacterial attachment to macrophage surfaces but no changes in the percentage of phagocytosed bacteria. These findings challenge the previous assumption that the non-bactericidal interaction between Bp and macrophages relied predominantly on CR3, proposing instead receptor cooperation and highlighting that while CR3 serves as a docking molecule for Bp, it is the PAMP receptor, MR, that plays a pivotal role in facilitating Bp phagocytosis and subsequent survival within macrophages.

41 (212) THE ROLE OF SOLUBLE LEISHMANIA PROTEINS AND THE INDUCTION OF INFLAMMATORY RESPONSES IN AMERICAN TEGUMENTARY LEISHMANIASIS

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American tegumentary leishmaniasis (ATL) displays two main clinical forms: cutaneous (CL) and mucosal (ML). ML is more resistant to treatment and displays a more severe and longer evolution. Since both forms are caused by the same *Leishmania* species, the inflammatory response of the host may be an important factor determining the evolution of the disease.

The aims of this work are to assess the specificity of soluble *Leishmania* proteins (SLP)for CL and ML patients in the *in vitro* induction of IFNγ. We also pretend to evaluate if cytokine induction varies depending on the *Leishmania* spp. used, pointed also to the relation of the induction to the clinical form of the disease and analyzing if ATL patients are capable to maintain longtime specific responses.

Methods: We prepared a *Leishmania* lysate (SLP) from *L. (V.) braziliensis* (MHOM/BR/75/M2903) and *L. (L.) amazonensis* (MHOM/VE/84/MEL) massive cultures of promastigotes in the exponential phase of growth. Then we cultured freshly isolated PBMCs from the studied groups ($1x10^6$ cells/ml; 37°C, 5% CO_2) in 24 wells plates in presence or absence of SLP mix composed by equal concentrations (20 µg/ml) of *L. (V.) braziliensisand L. (L.) amazonensis*. Additionally PBMCs were stimulated with each strain separately. We collected culture supernatants after 7 days to assess the levels of IFNγ by ELISA. Studied groups: CL (n=9), ML (n=11), HS (healthy subjects, n=14) and DD (patients with differential diagnosis as vasculitis, psoriasis, n=16).

Results: We determined that SLP specifically induced the production of IFNγ of CL (2360±596) and ML patients (3475±1717), with scarce concentrations obtained from the control groups (*p< 0,0001*). The addition of both species separately showed that *L. (V.) braziliensis* (3487±785) was responsible for higher IFNγ amounts than *L. (L.) amazonensis* (1344±641) (*p=0,0093*) analyzing CL cases, but this effect was not observed in ML, with similar concentrations of the cytokine recorded for both strains (1867±1258; 1299±1056 respectively). Next, we performed the follow up of 4 CL patients with good response to therapy during 1, 3 and 6-10 months post-treatment obtaining similar levels of IFNγ in each studied point.

Conclusions: The specific induction of IFNγ produced by SLP *in vitro* on PBMCs from ATLpatients could be a useful tool to accompany classical methods in difficult diagnosis cases. *L. (V.) braziliensis*is responsible for the majority (>90%) of the infections found in the Northwest of Argentina, but we reported here than *L. (V.) braziliensis* proteins only induced increased concentration of IFNγ in CL patients. Possibly, T cell exhaustion produced in the chronic ML form could account for less capacity to mount stronger responses against the causative agent. In this regard, the results obtained during CL follow up indicate that at least 6-10 months after healing of the lesions, strong inflammatory specific responses are still maintained.

*Results expressed as Media±SE(pg/ml)

42 (117) THE SECRETORY-IGA IN SALIVA OF COVID19-CONVALESCENT PATIENTS IS ASSOCIATED WITH LACK OF REINFECTION FOR AT LEAST ONE YEAR. VACCINE DEVELOPMENT PERSPECTIVES.

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Approved vaccines against SARS-CoV-2 in Argentina are administrated intramuscularly, triggering a systemic immune response but not effectively engaging the mucosal immune response. Given that SARS-CoV-2 enters through the respiratory mucosa, we wonder whether or not, the immune response in the mucosa, specifically the presence of secretory IgA (sIgA) in the respiratory tract may have a protective effect on viral infection. To assess this question, we developed an ELISA to measure the level of IgA against the receptor-binding-domain of the SARS-CoV-2-Spike protein in saliva, assuming that there is an acceptable correlation with the level of neutralizing sIgA. We recruit 35 convalescent patients and 10 controlindividuals that have never been positive for SARS-CoV-2 tests and have remainedCOVID19-symptoms-free since the beginning of the Pandemic. All of them hadreceived at least 2 doses of anti-SARS-CoV-2 vaccines at the time of samples collection. Ours findings revealed that 14% (5/35) of the first group were negative for sIgA and surprisingly, 50% (5/10) of the control group had sIgA in saliva. Kaplan Meier analysis showed that the risk of infection or reinfection is significantly lower in patients with sIgA in saliva ($p=0.0318$). These results suggested that individuals with mucosal immune response, indicated by the presence of sIgA in saliva, have a reduces susceptibility to SARS-CoV-2 infection.
Based on our findings, it appears that a vaccine designed to stimulate the Mucosa-Associated-Immune System may be the way to eradication of SARS-CoV-2.

43 (175) THE VALUE OF HEMOGLOBIN TO RED BLOOD CELL DISTRIBUTION WIDTH RATIO (HB/RDW), NEUTROPHIL-TO-LYMPHOCYTE RATIO (NLR) AND PLATELET-TO-LYMPHOCYTE RATIO (PLR) AND THEIR ASSOCIATION WITH IMMUNOENDOCRINE PARAMETERS FOR A BETTER APPRAISAL OF PULMONARY TUBERCULOSIS

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Pulmonary tuberculosis (TB), caused by *Mycobacterium tuberculosis* is accompanied by an immunoendocrine imbalance, likely to impact the hematopoietic system involving myeloid and lymphoid cells, as well as plasma components. Our study aimed to evaluate in newly diagnosed TB patients the value of Hb/RDW, NLR, monocyte-to-lymphocyte ratio (MLR), and PLR in terms of disease severity (classified by X-Ray as Mild, Moderate –Mod- and Severe –Sev-). The relationship of such parameters with systemic levels of adrenal hormones (cortisol -Cort-, dehydroepiandrosterone –DHEA-, pg/ml) along with pro- and anti-inflammatory cytokines (IL-6, IFN-γ and IL-10, pg/ml) was also assessed. A retrospective observational study was conducted on 47 TB patients (Aged, 18 -75 years; 34 males and 13 females) and 49 matched healthy controls (HCo); all HIV (-). As reported earlier, DHEA plasma levels decreased according to TB severity ($p<0.05$), in the presence of increased values of plasma Cortisol, IL-6, IFN-γ, and IL-10. White blood cells/µl, RDW%, PLR, NLR, and MLR values appeared significantly higher in TB than in HCo ($p<0,0001$), whereas red blood cells/µl, Hb, Hb/RDW values were decreased in this patient group ($p<0.01$ vs. HCo). RDW, NLR, and PLR correlated negatively with plasma DHEA levels ($p< 0,003$) while Hb/RDW was positively and inversely associated with such steroid and IL-6 ($p< 0,004$ and $p<0,01$, respectively). At the same time, the inflammatory biomarkers NLR, PLR, and MLR showed a positive relationship with IL-6 and IFN-γ ($p< 0,01$). Concerning pulmonary TB severity, PLR and Hb/RDW from Mild, Mod, and Sev TB patients differed significantly from HCo ($p< 0,05$), whereas MLR and NLR remained only different among Mod and Sev TB patients ($p< 0,01$, vs. HCo). When comparing among patient groups only Hb/RDW attained statistical significance, particularly in severe cases whose values situated below the ones recorded in moderate and mild patients. In addition to an improved appraisal of disease status, assessment from these routine laboratory surrogates sounds useful for a better TB diagnosis, which in the case of Hb/RDW, appears as a novel and potential tool for TB severity at the time of its recognition.

44 (133) TOWARDS DISSECTION OF IMMUNOGENIC AND IMMUNOMODULATORY PROPERTIES OF RSV SURFACE GLYCOPROTEIN

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Respiratory Syncytial Virus (RSV) is the most prevalent cause of bronchiolitis and viral pneumonia in children, making it a significant global health concern. Despite not demonstrating antigenic variation, RSV causes recurrent infections throughout patients' lives. This phenomenon might be linked to the immunomodulatory effects that some of RSV's proteins exert. RSV encompasses three envelope proteins, including the attachment glycoprotein (G), which exhibits multiple striking features: it's evolutionary origin is still unknown; efforts to obtain it's molecular structure have been sterile (since it is highly disordered and glycosylated) and it encompasses an alternative initiation codon that results in a truncated secreted version, a property that has not been observed in related viruses. In addition, G is physiologically relevant due to its immunogenicity: G epitopes (localized in the central conserved region of the glycoprotein) account for a significant part of the neutralizing antibodies after exposition to RSV. This central conserved region also exerts immunomodulatory effects, mainly via interaction with the fractalkine receptor CX3C.R1. We have set out to study this interaction, focusing on the fifty non glycosylated residues of the central conserved region of G (Gpep). We pursued a bioinformatic approach, modeling the complex between Gpep and CX3C.R1 and identified key residues for the interaction. This model led us to the design of point mutations, aiming to disrupt this interaction while simultaneously conserving the native structure of the peptide, thus maintaining its immunogenicity. Apart from this, we recombinantly expressed the Gpep and evaluated its immunomodulatory properties in bone marrow derived dendritic cells (BMDCs) and splenic dendritic cells (DC), both obtained from C57BL/6J mice. BMDCs and DCs were incubated with Gpep in concentrations from 250 nM to 1 uM, in presence or absence of LPS 0.5 ng/ml. The activation markers CD80 and CD86 were measured, obtaining interesting results: the Gpep exerts an immunosuppressive effect in DCs, since it inhibits its activation in response to LPS in a dose-dependent manner (significance determined by ANOVA test, p values <0.05 were considered significant). In conclusion, a novel interaction within the central region of the G protein was identified, raising profound questions about the implications of this domain in the immune modulating effects of RSV.

45 (149) FIBROBLAST ACTIVATION PROTEIN IS OVEREXPRESSED IN INFLAMED INTESTINAL MUCOSA AND MAY BE MODULATED BY MIR-21

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Fibroblast activation protein (FAP) is a transmembrane endopeptidase present in cancer associated fibroblasts, which contributes to extracellular matrix remodeling and mesenchymal cell activation, favoring tumoral growth. FAP overexpression has been reported in lung and breast cancer, under microRNA-21 (miR-21) regulation. Nevertheless, FAP role is not completely described in colorectal cancer (CRC). We previously showed miR-21 overexpression in inflammatory bowel disease (IBD) patients' mucosa and intestinal fibroblasts. IBD is a predisposing condition for CRC development; we aim to investigate FAP expression in the colonic mucosa of patients with chronic intestinal inflammation. Colonic biopsies and surgical samples were taken from macroscopically inflamed and uninflamed mucosa from patients with IBD, CRC, polyps, or healthy controls (HC). Mucosal pieces were fixed and staining for FAP and α-SMA was performed by indirect immunofluorescence (IF). Total RNA was extracted from mucosal samples, and retro-transcribed. qPCR was performed on cDNA using specific primers for miR-21, *fap*, *α-sma* and *HPRT*. Relative expression was normalized to U6 for miRNA or to HPRT for the rest, and data were analyzed using the comparative threshold method ($2^{-\Delta Ct}$). Fibroblast primary cultures were established after isolating lamina propria cells by mechanical and enzymatic digestion of biopsies or tissue sections. Cells were cultured in DMEM Glutamax 20% FBS with antibiotics. Fibroblast culture supernatants were collected and ultracentrifuged at 100.000xg for 2 h for exosome enrichment, which were visualized by atomic force microscopy. Mi-R21 expression was evaluated by qPCR in cells and exosome fractions. *In vitro* induction of FAP was evaluated on fibroblasts by IF, after stimulation with TGF-β (1 and 10 ng/ml) or exosomal fraccion for 24 h. Images were acquired with Leica SP5 confocal microscope. FAP and α-SMA merge images were analyzed with Image J and QuPath softwares.

We observed an overexpression of miR-21 and its target genes in the intestinal mucosa and fibroblasts from IBD and CRC patients, compared to HC ($p<0,05$). IF staining of FAP and α-SMA in colonic biopsies showed higher expression of these proteins in the stroma of inflamed tissue compared with HC ($p<0,05$), and both signals colocalized in inflamed areas. Incubation of fibroblasts with TGF-β

10 ng/ml or miR-21 rich exosomal fractions induced FAP expression, 30% and 91% FAP+ cells, respectively *vs.* 3% FAP+ cells in unstimulated conditions.
We conclude that miRNA-21 overexpression in stromal cells from chronic inflamed mucosa may participate in FAP activation pathways, implying the remodeling signature and increasing the risk of CRC development in IBD patients.

46 (36) ANNEXIN A1 EXPRESSION IN THE SMALL INTESTINE. ANALYSIS IN A MURINE MODEL OF ENTEROPATHY AND CELIAC DISEASE PATIENTS

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Objective: Annexin A1 (ANXA1) participates in the control of immune response, apoptosis, cell differentiation and modulation of the inflammatory response, and has been implicated in a variety of chronic inflammatory diseases. Although, the anti-inflammatory role of ANXA1 was demonstrated in colitis model of dextran sodium sulphate, and its expression has been described in the small intestine, there are no studies on its functional role in this tissue. ANXA1 is found in different cell types, including T lymphocytes and neutrophils expressing myeloperoxidase (MPO).

In previous work, we showed the activation of inflammatory markers and apoptosis in small intestine mucosa of Celiac Disease (CD) patients. CD is a highly prevalent chronic enteropathy driven by dietary gluten in genetically susceptible individuals.

This study aimed to assess the expression of ANXA1 in the small intestine in a murine model of enteropathy and small intestinal samples of CD patients.

Methods: 8 weeks old C57BL/6 wild type mice were treated by intragastric administration of 20µg/mouse of p31-43 gliadin peptide, following a protocol already established by our group and approved by CICUAL. Small intestines were collected at 16h after treatment. Human duodenal biopsies were collected during the procedure for CD diagnosis, following Ethic approved protocols. Small intestine samples were used for confocal immunofluorescence microscopy (IFI) studies and western blot (WB) analysis.

Results: Increased numbers of dead cells were detected by TUNEL reaction in both duodenum of untreated CD patients and in proximal small intestine of mice orally challenged with p31-43 ($p<0,002$). WB analysis showed significantly increased expression of ANXA1 in small intestine of mice treated with p31-43 ($p<0.05$); similar result was observed by IFI ($p<0.01$). In human duodenal tissue, WB results were no statistically different when control and untreated CD samples were compared, however IFI analysis showed an increase in ANXA1 expression in *lamina propria* of small intestine of CD patients ($p<0.05$). Interestingly, some $ANXA1^+$ cells were $CD3^+$ T cells while others co-expressed MPO (neutrophils), in fact cells $ANXA1^+MOP^+$ showed a significantly increased in small intestine of untreated CD samples ($p<0,05$).

Conclusion: In this study we assessed a model for acute enteropathy (murine model of enteropathy) and chronic inflamed tissue (duodenal samples from CD patients). In both conditions, we found that ANXA1 is differentially upregulated suggesting that it may play a role in regulating immune responses during inflammation or tissue repairing.

47 (139) ANTIMICROBIAL AND PROTECTIVE EFFECT OF ALOE VERA GEL IN *CLOSTRIDIOIDES DIFFICILE* INFECTION

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Clostridioides difficile (*C. difficile*), is a Gram-positive bacillus, anaerobic, spore-forming, that constitutes the major cause of hospital-acquired diarrhea, often in association with previous antibiotic use. *C. difficile* infection (CDI) symptoms can range from low/medium diarrhea to severe diarrhea with toxic megacolon, sepsis and even death. CDI treatment, in Argentina, is based on oral administration of metronidazole or vancomycin. The emergence and spread of *C. difficile* isolates resistant to multiple antibiotics, especially the hypervirulent ribotype 027 strains, are becoming an increasing problem for CDI treatment.

Aloe Vera (AV) has been used in traditional herbal medicine as an immunomodulatory agent inducing anti-inflammatory effects. Moreover, AV gel exhibits antimicrobial properties. The inner leaf gel contains active compounds with prebiotic activity on beneficial microbiota, while others compounds inhibit the growth of pathogenic bacteria making it an alternative therapy for intestinal disbiosis.

We evaluate the AV effect on *C. difficile* growth, and its impact in combination with vancomycin and metronidazole. The antibacterial activity of AV was determined by broth microdilution assays using the hypervirulent and toxigenic *C. difficile* (027/BI/NAP1) strain. The bacteria were cultured in anaerobiosis for 48h, seeded in triplicates in 96 microwell plates in the presence of AV/antibiotics. After 48h, *C. difficile* growth was determined in a microplate reader at 600nm. Two varieties of AV were used, *Aloe barbadensis Miller* and *Aloe saponaria*, in final concentrations of 1, 5, 10 and 20%. The minimal inhibitory concentration (MIC) for metronidazole and vancomycin was calculated and the AV gel was used in combination with 0.25, 0.5, 1 and 2µg/ml of each antibiotic. The results indicated that AV gel inhibits *C. difficile* growth ($p<0.01$) and significantly increases the vancomycin effect ($p<0.01$).

On the other hand, we also evaluate the effect of AV as a protector agent of the intestinal epithelia. To this end, we used human intestinal epithelial cells line Caco2,a model widely employed to assess the intestinal permeability. Caco2 monolayers were prepared in 24 well culture plates. Differentiated cells (late post-confluence, 15 days of culture) were threated during 24h with toxigenic *C. difficile* (027/BI/NAP1) strain at $2.25x10^6$UFC/ml and/or TcdA/TcdB toxins obtained from *C. difficile* (027/BI/NAP1) culture supernatant. By optical microscopy, we observed the AV has a protective effect on the monolayer's integrity treated with *C. difficile* (027/BI/NAP1) strain or TcdA/TcdB toxins.

This first evidence positions AV as a potential promising combination therapy against *C. difficile*, reducing the antibiotics concentration treatment and the detrimental consequences on the beneficial microbiota, protecting the epithelial barrier integrity. More studies are underway to strengthen this hypothesis.

48 (30) ASSOCIATION BETWEEN SEGMENTED FILAMENTOUS BACTERIA (SFB) COLONIZATION AND TNFR1 DEFICIENCY IN THE IMMUNE RESPONSE OF GUT-JOINT AXIS

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Segmented filamentous bacteria (SFB) are potent microbial stimuli of the gut mucosal immune system, including IgA and IL-17 production. In the ileum of mice, SFB appear shortly after weaning, and then quickly decreases. TNF receptor 1 deficient (*TNFR1*$^{-/-}$) mice develop reactive arthritis (ReA) after oral infection with *Yersinia enterocolitica* (Ye) serotype O:3. The aim was to analyze at normal steady state whether TNFR1 deficiency impacts on joint and gut immune response during and after SFB colonization. First, SFB presence was analyzed by Gram staining in the ileum lavage of male 21 to 34 day-old (weaning time) and 8 to12 week-old (adult) C57BL/6 wild-type (WT) and *TNFR1*$^{-/-}$ mice. Then, IgA and IL-17 levels were determined in the intestinal lumen by ELISA, and the number of IgA-producing cells in lamina propria by immunofluorescence. Finally, lymphocytes, neutrophils, macrophages and CX3CR1 macrophages were evaluated in mesenteric lymph nodes (MLN) and regional lymph nodes of the joint (RLN) by flow cytometry. High number of SFB was found in both WT and *TNFR1*$^{-/-}$ mice around weaning time. Although almost no IgA positive-cells were detected in lamina propria in 21 to 34 day-old WT and *TNFR1*$^{-/-}$ mice, IgA levels in luminal intestinal were increased at weaning time, suggesting similar passively acquired maternal IgA. However, a significant decrease of neutrophils and macrophages was detected in RLN of 21 to 34 day-old *TNFR1*$^{-/-}$ compared with WT mice ($p<0.01$). Furthermore, significant decrease in CX3CR1 macrophages in MLN ($p<0.0001$) as well as in RLN of 21 to 34 day-old *TNFR1*$^{-/-}$ mice was detected when compared with WT mice ($p<0.01$). Higher luminal IL-17 levels were found in adults compared with peers at weaning time ($p<0.05$ for WT and $p<0.001$ for *TNFR1*$^{-/-}$). Besides, adult *TNFR1*$^{-/-}$ mice showed significant increases of total T lymphocytes ($p<0.001$), CD4$^+$ T lymphocytes ($p<0.01$) and CD8$^+$ T lymphocytes ($p<0.01$) in RLN. Our results indicate that under normal steady state and during SFB colonization, TNFR1 deficiency impacts on immune cell population of draining lymph nodes of the joint-gut axis. Further work will be needed to determine whether the decreased CX3CR1$^+$ macrophage migration from the lumen to MLN in *TNFR1*$^{-/-}$ mice associates with a reduced transport of commensal bacterial antigens, which conducts to an inappropriate adaptive immune response in RLN in adult mice.

49 (198) BACTERIAL FECAL MICROBIOTA OF FOOD SENSITIZED CHILDREN WITH JUVENILE POLYPS PRESENTS A DIFFERENT COMPOSITION SIGNATURE COMPARED TO THAT OF NON-ALLERGIC HEALTHY INDIVIDUALS

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Food allergy is characterized by an immune reaction to foods. We have previously characterized that 90% of children with juvenile colorectal polyp (JCP) from La Plata Children's Hospital are sensitized to food allergens, JCP showed type 2 inflammation and are active sites of IgE synthesis. It has been reported low levels of gut microbiota diversity in food allergic children, marked by predominance of Firmicutes over Bacteroidetes phylum. Nevertheless, there are no reports regarding microbiota composition associated to JCP. The aim of this work was to characterize bacterial populations in feces and colonic tissue of allergen sensitized children with JCP in comparison to that of healthy children.
Stool samples from food-sensitized children (FSC) with JCP (n=11) and non-allergic children (non-FAC) (controls, n=25) were collected. JCP (n=18) and surrounding tissue biopsies (B) (n=7) were obtained by colonoscopy for microbiota analysis. Microbial DNA was extracted using the QIAmp PowerFecal and AIamp Stool DNA kits, respectively. 16S rRNA V3-V4 hypervariable regions were amplified and Illumina sequencing was performed. Sequences were analyzed through Qiime2 software. Amplicon Sequence Variants (ASV) abundance and representative sequences were generated. The Shannon index was used for evaluation of alpha diversity, while Weighted Unifrac was used for beta diversity. The resulting distances were visualized using Principal Coordinate Analysis (PCoA). Finally, a taxonomic analysis was applied where the representative sequences had been assigned taxonomic labels, giving potential identities of the microbial species represented by the ASVs.
Whereas alpha diversity showed no significant differences in fecal (p=0.73) or tissue (p=0.89) samples (Kruskal-Wallis), we found beta diversity differences between the fetal microbiota of FSC and non-FAC (p=0.001, PERMANOVA). JCP and B tissues showed no significant differences (p=0.732). PCoA highlighted distinct fecal and tissue sample clustering, while fecal samples could be discerned between FSC and non-FAC samples. The taxonomic analysis of the bacterial communities in samples from FSC and non-FAC showed similar profiles to those reported, with a reduction of Bacteroidetes (Bacteroides) and increase in Firmicutes (Lactobacilleae) phila associated to feces from FSC. Also, we detected the presence of *Bifidobacterium bifidum* in B but not in JCP, while polyps present an increase in Firmicutes and Clostridium spp.
In conclusion, we characterized the fecal and tissue-associated microbiota in children with JCP associated to food allergy and in non-allergic children. This is the first description of microbiota composition of food allergic patients carried out in Argentina and we mainly found food allergy-associated bacterial species in JCP tissues. These findings may pave the way for a more comprehensive

understanding of the association between colorectal polyp development and allergy to food antigens.

50 (38) COMPARATIVE ASSESSMENT OF AZITHROMYCIN INDUCED DYSBIOSIS IN MALE AND FEMALE C57BL7 AND FOXP3-GFP MICE.

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The intestinal microbiota is a complex community of microorganisms that plays a fundamental role in the immune competence of the host. Exposure to antibiotics is one of the most common causes of disruption to this community, known as dysbiosis, and is associated with both short-term and long-term diseases. We are studying the effects of the acute administration of Azithromycin (AZM), a widely prescribed antibiotic in our environment, on the regulatory circuits of intestinal immunity. Our objective is to characterize the dysbiosis in male and female C57BL6 and Foxp3-GFP mice after 5 days of AZM administration (50 mg/kg/day) in the drinking water, by measuring bacterial density and the activation of the TLR4 and TLR5 NF-kB pathway. Fecal samples were collected daily or on days D0 (before AZM), D3, and D7 (2 days after AZM administration ended). We used the DNA intercalator SyBR Green, count beads, and flow cytometry to determine bacterial density, and the HEK BLUE TLR4 and TLR5 reporters cells lines and a colorimetric assay to measure TLR4 and TLR5 activity. We observed a decrease in bacterial density as early as 24 h after AZM intake in both strains and sexes, which was significant on D3 in female Foxp3-GFP mice ($p<0.01$) and in male WT mice ($p<0.01$). On D7, females from both strains receiving AZM maintained a 50% reduction in bacterial density. The highest TLR4 activity was observed in untreated female WT mouse samples ($p<0.001$), which experienced the greatest loss of activity on D7 (>90%). In male Foxp3-GFP mice, the decrease in TLR4 activity was also significant on D7. The most pronounced effects of AZM treatment were observed on D3 in both strains and sexes, affecting microbial density and TLR4/NF-kB activity. Upon discontinuation of the antibiotic, recovery was observed in males, while more lasting residual effects were evident in females from both mouse strains. Understanding the disruption differences between sexes is crucial for improving antibiotic management. Providing evidence of the magnitude of these changes will enable better control over antibiotic dispensation and a deeper understanding of the effects of dysbiosis on immune competence.

51 (112) EXPRESSION OF TYPE III INTERFERONS AND ITS RELATIONSHIP WITH TYPE I AND II INTERFERONS IN THE DUODENAL MUCOSA OF CELIAC PATIENTS.

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Objective: Celiac Disease (CD) is a chronic inflammatory disease caused by an abnormal T cell-mediated immune response of genetical susceptible individuals to dietary gluten.

Interferons (IFNs) are divided into three families (type I, type II, and type III) on the basis of sequence homology. While type I and II IFNs have been associated with CD, information about type III IFNs is limited. The aim of this work was to evaluate the expression of type III interferons (IFNL1 and IFNL2/3) in healthy human small intestine and samples from CD patients, and its relationship with both type I and II IFNs.

Material-Methods: Duodenal biopsies were collected from untreated CD patients (CD) and non-celiac (NC) individuals and analyzed by RT-qPCR and fluorescent microscopy. Ethics committees from Health Institutions approved this protocol.

HT-29 cells (model for intestinal epithelial cells) were cultured in 24 wells plate for 72 or 24 h after incubation with 50 ng/ml IFNγ and 1000 U/ml IFNβ. Samples from cell culture were used for RT-qPCR analysis.

Results: Since type I and II IFNs have been associated with CD, we decided to analyse their transcripts levels as well as some of their target genes in duodenal samples. A significant upregulation of IFNβ1 (p-value < 0.05), IFNγ (p-value < 0.05), MX1 (p-value < 0.05), and IRF1 (p-value < 0.05), but not IRF8, was found in duodenum of untreated CD patients compared with control population. Expression of type III IFNs was also increased, IFNL1 (p-value < 0.05) and IFNL2/3 (p-value < 0.05) in duodenal samples of CD patients. Interestingly, IFNL1 and IFNβ1 mRNA levels show a significant positive correlation (p-value <0.05). Furthermore, by immunofluorescent analysis in duodenal sections, type III IFNs were detected in both the epithelial and *lamina propria* compartments of the mucosal samples of CD patients. Particularly, IFNL was found increased (p-value < 0.05) in the leukocytes ($CD45^+$ cells) or epithelial cells ($CD45^-$ cells) of CD patients.

In vitro studies using HT29 cells incubated with type I and II IFNs for 24 or 72 hours showed that the expression of IFNL1 as well as IFNL2/3 was upregulated (p-value < 0.05) and (p-value < 0.001), respectively.

Conclusion: Duodenal mucosae from untreated CD has an increased differential expression of cytokines of the three known IFN families. Epithelial and *lamina propria* cells expressed Type III IFNs. These findings suggest a role for type III IFNs in CD pathogenesis.

52 (32) FOLLOW-UP OF COMPARATIVE STUDY ON IMMUNE BARRIER DEVELOPMENT IN MAMMAL AND AVIAN MODELS: ASSESSING AGE, SECTION, AND DIET EFFECTS IN MICE AND QUAIL ENTEROIDS

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Microbiota colonization initiates the development of the bacterial community in the digestive tract. In mammals, this process begins at birth and is subsequently influenced by lactation. In contrast, avian species start colonization through environmental microorganisms upon hatching. We have conducted a comparative study involving mammals and birds. Previously, we presented results on Japanese quail (*Coturnix coturnix*), evaluating various ages and diets (starter or adult food). Mucin levels displayed significant Age x Section interactions, while bursa cellularity was influenced by diet. Here, we examined C57BL/6 mice at 14, 21, 28, and 35 days old, divided into weaning and mixed-fed (mother-housed) groups on day 21. We investigated the effects of Age, Intestinal Section (Proximal vs. Distal), and Diet (Weaning vs. Mixed) on bacterial density, mucin production, and goblet cell frequency. Age influenced bacterial density/mg of feces in mother-housed animals ($p \leq 0.05$), with an Age x Diet interaction between 28 and 35 days ($p < 0.05$). Age impacted mucin production in the duodenum ($p < 0.01$) for mother-housed animals, with no effect observed in the large intestine. Goblet cell frequency exhibited Age effects in the duodenum ($p < 0.001$) for mother-housed animals, and an Age x Diet effect at 28 and 35 days ($p < 0.05$). Bacterial density increased from day 14 to 28 in mother-housed animals, and mixed-fed animals experienced an increase from day 28 to 35. Mother-housed animals exhibited higher mucin production in the duodenum on day 14, which remained stable thereafter. Duodenal goblet cell frequency increased from day 14 to 21 in mother-housed animals. Additionally, an in vitro enteroid model was developed to study microbiota-immune system-gut interactions in post-hatching quail. Enteroids obtained from small and large intestine segments of 1-day-old quail (n=4) were co-cultured with bursa cells, utilizing a bifactorial design considering intestinal section (small vs. large) and the co-culture of enteroids and bursa cells. A significant effect of the interaction between co-culture and Section on enteroid size was observed ($p < 0.05$). In cultures without bursa cells, large intestine enteroids displayed a larger total area than those from the small intestine. Furthermore, in co-cultures at a 100:100.000 ratio, small intestine enteroids exhibited a notable tendency to higher size, with the opposite effect observed in large intestine enteroids. To conclude, colonization of the microbiota and its interactions with the immune system and intestinal sections differ among species. In both quail and mice, Age, Intestinal section, and Diet differentially impact bacterial density, mucin production, and goblet cell number. In avian species, enteroids exhibit distinct responses based on intestinal section and co-culture conditions. These findings underscore the intricate nature of microbiota-host interactions across diverse organisms.

53 (41) FUNCTIONAL FOODS CONTAINING PEPTIDES FROM AMARANTH AMELIORATE INTESTINAL INFLAMMATION.

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Amaranth is a pseudocereal with a high content of proteins with good nutritional and health quality (antihypertensive, antioxidant, antithrombotic and antiproliferative and anti-inflammatory effects). Intestinal inflammation is characterized by epithelial disruption, leading to loss of barrier function and the recruitment of immune cells. Recently, it has become apparent that the transporter PepT1 may play an important role in the pathogenesis of such inflammation. In healthy individuals, PepT1 is primarily expressed in the small intestine to transport peptides for metabolic purposes. However, during chronic inflammation PepT1 expression is upregulated in the colon. We aimed to evaluate whether the use of a functional food of amaranth (FF) or an amaranth peptide (AP) modulate intestinal inflammation and PepT1 expression.

To evaluate PepT1 expression under inflammatory conditions *in vitro*, we stimulated the Caco-2 cell line with proinflammatory cytokines (TNF-α or IL-1β plus flagellin), and also evaluated its expression in colon biopsies from inflamed and non-inflamed areas of IBD patients by quantitative PCR. Then, colitis was induced in Balb/c mice with an intrarectal administration of TNBS in ethanol on day 0. Thereafter, mice were orally given every day for a week a formulation containing an AP, FF or FF+AP. As controls, mice received PBS or ETOH. Corporal weight and disease activity index were monitored and on day 7 mice were sacrificed. The colonic inflammatory response was analyzed (weight, length, histology, gene expression by qPCR).

In vitro experiments showed higher PepT1 transcript level with proinflammatory stimuli than controls ($p<0,05$) and we found that inflamed areas of IBD patients showed significant up-regulation of mRNA PepT1 expression compared with non-inflamed areas ($p<0.05$).

Furthermore, we found in mice that only treatment with AP ameliorated the TNBS-induced colitis. We found that AP reversed the weight loss of mice with colitis and mitigated clinical signs. The colon of AP-treated mice showed a decreased histological score, decreased expression and production of proinflammatory cytokines (mRNA FI, Ccl20: 2.1 ± 0.2 vs 4.5 ± 0.3; TNF: 2.3 ± 0.45 vs 2 ± 0.7; and IFN-γ protein levels: 326 ± 57 vs 748 ± 34 pg ml^{-1}, $p<0.01$) than those from untreated TNBS-induced colitis mice. We also observed significantly decreased at transcript level of PepT1 in AP-treated mice compared with TNBS-induced colitis mice (4.3 ± 0.2 vs 2.1 ± 0.3; $p<0.05$). On the other hand, FF treatment failed to modulate the colitis, whereas AP supplementation reduced the intestinal inflammation.

In conclusion, our findings indicated that peptide from Amaranth exerted a mucosal anti-inflammatory effect that suppressed the TNBS mediated intestinal inflammation through the reduction of PepT1 expression. In addition, the FF does not exert an anti-inflammatory effect per se, and it would therefore be necessary to supplement it with the peptide.

54 (16) GENERATION OF HUMAN INTESTINAL ORGANOIDS FROM INTESTINAL INFLAMED TISSUES.

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Intestinal organoids are self-organized three-dimensional structures that partially recapitulate the identity, cellular heterogeneity, and behavior of the original intestinal tissue *in vitro*. Intestinal stem cells are located in crypts and are capable of self-renewal and differentiate into major epithelial lineages. As such, organoids have emerged in recent years as valuable models for studying intestinal development, homeostasis, diseases, and regeneration. In this study, our aim was to obtain and characterize human intestinal organoids using stem cells derived from samples under various pathological inflammatory conditions.

We firstly quantified stem cells in intestinal biopsies (n=23) and surgical specimens (n=6) from inflamed regions and adjacent tissue of adult patients with colorectal cancer (CRC), inflammatory bowel diseases (IBD) (n=19), and healthy controls (n=8). We also analyzed samples from colorectal polyps of pediatric patients sensitized to cow's milk protein (n=7) and biopsies of the surrounding areas (n=2). Epithelial cells were obtained by incubation of samples in HBSS supplemented with EDTA 0,5 mM. Crypts were detached by vigorous shaking. Stem cells were identified and quantified as Lgr5+ cells using flow cytometry. Subsequently, we initiated organoid cultures by isolating intestinal crypts from the aforementioned samples and embedding them in Corning® Matrigel® Matrix with IntestiCult™ Organoid Growth Medium. Organoids were passaged every 7 to 10 days and subjected to analysis via fluorescence microscopy.

Our results revealed a higher frequency of LGR5+ cells in inflamed IBD tissue compared to non-inflamed IBD tissue and healthy controls ($p=0.007$). Conversely, a smaller proportion of LGR5+ stem cells was observed in polyp samples compared to the control biopsies surrounding the polyps ($p=0.04$). We obtained organoids from IBD biopsies, surgical samples of the small intestine, colorectal polyp tissues, and biopsies surrounding the polyps. We verified the expression of the epithelial marker Epcam and the stem cell marker Lgr5 through fluorescence microscopy. Furthermore, we detected proliferating cells using the Ki-67 marker.

In conclusion, we effectively generated human intestinal organoids from healthy and inflammatory samples of adult and pediatric patients. These organoids were characterized based on the expression of distinct markers. Further analyses, encompassing functional studies, RNA expression profiling, and co-culture

experiments are planned to gain deeper insights into the role of epithelial cells in different inflammatory settings.

55 (45) HANTAVIRUS PULMONARY SYNDROME: DISTINCT IMMUNE RESPONSE PATTERN OF LUNGS IN FATAL CASES.

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Background. Hantavirus cardiopulmonary syndrome (HPS) caused by Andes orthohantavirus (ANDV) in South America. Some of them are public health threat due to the high mortality rate and the lack of a specific treatment and preventive vaccines. Interestingly, these viruses are non-lytic, and thereby, the strong antiviral immune response is suspected to contribute to pathogenesis. Hantavirus infections may cause severe and sometime life-threatening lung failure. The pathogenesis is still not entirely clear and elucidation is essential for the development of effective specific treatments. We aimed to investigate the immune response directly in the lung to understand the balance between protective and the harmful immunity in HPS cases with severe disease.

Methods. Here, we present three HPS cases with fatal outcome corresponding to two adults and a child from Argentina. Hantavirus infection was confirmed by the detection of IgM and IgG specific antibodies by ELISA and genomic viral RNA through RT-qPCR in serum and lung samples, respectively. Partial genomic viral RNA was amplified and sequenced for viral characterization. Lung samples were obtained post- mortem and harvested and stored at 80^0C until RNA extraction. Gene expressions of FOXP3, GATA, RORC2, and Tbx21 (factors controlling the differentiation of Th1, Th2, Th17 and Tregs cells, respectively) were analyzed by quantitative real-time PCR (StepOne plus, Applied Biosystems) using TaqMan gene expression assays (Applied Biosystems) and the $2-\Delta\Delta CT$ method. Housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used for normalization.

Findings. Our results revealed that in the 3 patients Th1/Th2 (Tbx21/GATA3) expression was suppressed while FOXP3 (Tregs) was overexpressed, compared to control.

Viral load in blood was not associated with disease severity. The genetic characterization analysis on a 496bp fragment of viral S-segment and 611pb of 9 M-segment revealed three different genotypes in each patient: Orán virus (infant from Nothwestern, Argentina), Andes virus (adult male from Southwestern, Argentina), and Buenos Aires virus (adult female from Central/East Argentina).

Interpretation. The best predictor of a severe disease course in HPS was the FOXP3 related Treg cell response, suggesting that the role of Treg cells in acute human hantaviral infections may be critical. In our study we have focused on the characteristics of individual cases, and appears to be a generis response, as it was found in man, women and child, and even in three infections with different hantaviruses.

Our findings are important due the difficulty to obtain lung samples from HPS cases and biosafety concerns. More studies are necessary to determine the immune cell populations involved of Th1/Th2 inside the lung and to characterize the phenotype of CD4+Treg cell that may be responsible for the immune suppression in fatal HPS cases.

56 (69) INTESTINAL MICROBIOTA MODULATES THE NEUTROPHIL HOMEOSTASIS THROUGH A MECHANISM INVOLVING TNFR1.

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Neutrophils are the first leukocytes to be recruited to an inflammatory site and are capable of eliminating pathogens. These cells are continuously generated in the bone marrow (BM) from hematopoietic stem cells. Cytokines and microbiota-derived components play important roles in the granulopoiesis. The purpose of this work was to investigate the role of TNFR1 in neutrophil homeostasis and the influence of the microbiota. Naïve C57BL/6 wild-type (WT) or TNFR1 knockout (*TNFR1$^{-/-}$)* mice were treated with an antibiotic (ABX) cocktail, consisting of ampicillin, neomycin, metronidazole and vancomycin in drinking water to induce microbiota depletion. In addition, microbiota-depleted WT and *TNFR1$^{-/-}$* mice were infected with *Yersinia enterocolitica* (Ye) serotype O:3. Neutrophil frequency was analyzed by flow cytometry in bone marrow (BM), peripheral blood (PB), spleen and mesenteric lymph nodes (MLN) of naïve and infected mice. Our results show that the absence of TNFR1 does not affect the hematopoiesis and granulopoiesis in particular. However, TNFR1-deficient mice showed a marked increase in leukocytes, particularly in thefrequency and the number of neutrophils in SP compared to WT mice ($p<0.01$ and $p<0.01$, $p<0.01$, respectively). However, a decrease in the frequency and number of neutrophils was detected in the MLN of *TNFR1$^{-/-}$* compared to WT mice ($p<0.01$). While no significant differences were observed when comparing serum IL-17 levels between the two groups, *TNFR1$^{-/-}$* mice showed a tendency towards higher levels of this cytokine. Interestingly, this alteration in the number of leukocytes and in particular of circulating neutrophils in *TNFR1$^{-/-}$* mice was normalized after depletion of the intestinal microbiota with ATB. On the other hand, infection with Ye increased the percentage and number of neutrophils in SP ($p<0.01$ and $p<0.05$, respectively) and MLN of *TNFR1$^{-/-}$* mice ($p<0.01$ and $p<0.01$, respectively) compared to the steady-state counterpart. However, microbiota depletion by ATB treatment in *TNFR1$^{-/-}$* mice prior to Ye infection reduced the number of circulating neutrophils in SP and the percentage of these cells in MLN when compared to infected *TNFR1$^{-/-}$* mice that did not receive ATB treatment ($p<0.05$). These changes were not observed in microbiota-depleted and infected WT mice. Our results suggest that the intestinal microbiota modulates the release of neutrophils from the BM to the SP through a mechanism involving TNFR1. In addition, in the absence of TNFR1 signaling, neutrophil migration across the intestinal mucosa would be impaired. This defective migration of neutrophils to the intestinal mucosa of *TNFR1$^{-/-}$* mice can be overcome in the context of an infection, which strongly dependent the intestinal microbiota.

57 (98) *Lacticaseibacillus rhamnosus* CRL1505 OR ITS CELL WALL ADMINISTERED AS IMMUNE ADJUVANTS IN A BREAST CANCER MODEL UNDER CHEMOTHERAPY.

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Lacticaseibacillus rhamnosus CRL1505 and its cell wall are able to enhance basal myelopoiesis in cyclophosphamide (Cy)-affected bone marrow. The aim of this work was to study the immunomodulatory effect of the inmunobiotic *L. rhamnosus* CRL1505 and its postbiotic (cell wall) on basal myelopoiesis deteriorated by chemotherapy treatment during a tumor process. *In vitro*, 4T1 breast cancer cells were treated with Cy in the presence or absence of CRL1505 postbiotic. Cell viability was evaluated by flow cytometry. *In vivo*, BALB/c mice bearing breast cancer treated with Cy or not, were administered the CRL1505 strain or its cell wall during 20 days. Tumor growth, bone marrow and blood cell counts, lung histology and serum cytokines were evaluated. First, we demonstrated that CRL1505 cell wall does not interfere with Cy toxicity in 4T1 breast cancer cells. In addition, mice treated with Cy plus *L. rhamnosus* CRL1505 showed a significant reduction in tumor size compared to the Cy control. While the CRL1505 strain and its cell wall were effective treatments to reduce leukocyte, neutrophil and Px+ cell counts in peripheral blood, the immunobiotic strain was able to reduce the leukemoid reaction that accompanies the growth of this tumor. In the bone marrow, both oral supplements were able to increase the expression of CXCR4 and TLR2. However, only the immunobiotic strain was able to reduce granulocyte and macrophage progenitors. Finally, the CRL1505 strain and its postbiotic reduced splenomegaly, restored the myeloid/lymphoid ratio in the spleen, and the monocyte population in the liver. Therefore, the oral administration of *L. rhamnosus* CRL1505 or its cell wall exerts an adjuvant effect on chemotherapy treatment with cyclophosphamide in mice bearing breast cancer, observing the best benefits administering the viable bacteria, without producing adverse effects.

58 (172) LOW PH ACTS AS AN ENVIRONMENTAL CUE FOR THE HUMAN TISSUE-RESIDENT MEMORY T CELL DIFFERENTIATION.

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Tissue-resident memory T cells (TRM) provide frontline defense against infectious diseases and contribute to antitumor immunity. However, despite intense study, relatively little is known regarding TRM differentiation in humans. PH is a hallmark of a variety of inflammatory processes in peripheral tissues where TRM are present. Our aim was to evaluate if low pH induces the differentiation of CD8+ T cells in a TRM phenotype.

PBMC were purified from healthy donors and CD8+T cells were obtained using magnetic microbeads. CD8+T cells were activated with CD3/CD28 beads for 48hs to generate “early effectors” and then were cultured an additional 3 days at neutral (7.3) or low pH value (6.5). The expression of CD69 and CD103, characteristic phenotype of TRM, was determined by flow cytometry. The production of IFNγ, TNF and IL-10 was evaluated by flow cytometry and ELISA. The viability was evaluated by annexin/propidium and live/dead viability dye.

We observed that exposure at low pH for 72 h considerably increase the percentage of CD69+/CD103+ CD8+T cells compared to neutral pH (24.8 ± 2.4 vs 4.0 ± 0.7, n=24 $p<0.0001$). Also, the percentage of CD103+ TCD8+ cells was increased (44.0 ± 4.3 vs 16.7 ± 1.8, n=24 $p<0.0001$). Notably, CD4+ T cells did not appreciably express CD103 (n=24). We then evaluated treatments for 4, 8 or 24 h at low pH and then cultured T cells at neutral pH. We observed that 4 h at low pH was sufficient to obtain CD8+ CD103+ T cells (n=3). Low pH treatments did not compromise viability (n=24).

We observed a decrease in the IL-10 production in CD8+T cells exposed to low pH compared with neutral pH (398.7 ± 123.0 vs 531.0 ± 125.1, n=4, p=0.0345), but we didn’t observe any difference in the production of IFNγ and TNF (n=4).

Low pH involves the inhibition of the cellular nutrient sensor mTORC1, however, we found that the mammalian target of rapamycin (mTOR) inhibitor temsirolimus did not promote TRM differentiation (n=3).

Given that low pH is a regulator of cellular metabolism and TRM are enrichment in the HIF-1α signaling pathways, we evaluated the role of HIF-1α. Using roxadustat (HIF-1α stabilizer) and a cobalt (II)-chloride solution (HIF-1α inductor) we didn’t observe any difference at low or neutral pH medium conditions.

Our findings identify a likely previously unreported cue for the TRM differentiation program and can enable facile generation of human TRM phenotype cells in vitro for basic studies and translational applications such as adoptive cellular therapy. Further analysis remains to identify the mechanism that explain how low pH induce the differentiation of CD8+ T cells in a TRM phenotype.

59 (105) NASAL PRIMING WITH A NEW POSTBIOTIC IMPROVES ADAPTATIVE IMMUNE RESPONSE AGAINST *Streptococcus pneumoniae* OF THE UPPER RESPIRATORY ASSOCIATED LYMPHOID TISSUE IN MALNUTRITIONED MICE.

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Lymphoid tissue of the upper respiratory tract is responsible for immunosurveillance of inhaled respiratory pathogens. The hosts immunosuppressed by malnutrition are known to be particularly susceptible to *Streptococcus pneumoniae* (Sp) infections, and especially defects in adaptive immunity have been observed. The nasal priming of malnourished mice with the peptidoglycan (PG) of *Lacticaseibacillus rhamnosus* CRL1505 (Lr) is as effective as viable strain for improving systemic and respiratory immune response against Sp. However, the impact of these treatments on mucosa-associated lymphoid tissue is unknown. In this work, the effect of nasal administration of Lr or PG on the adaptive immune response of nasopharyngeal-associated lymphoid tissue (NALT) cervical lymph nodes (CLN) and axillary lymph nodes (ALN) in malnourished mice under repletion treatments was evaluated. Weaned Swiss mice were malnourished with a protein-free diet (PFD) for 21d. Then, malnourished mice received a balanced conventional diet (BCD) during 7d (BCD group) or BCD with nasal supplementation with Lr (10^8 cells/mouse/d) or PG (8 µg/mouse/d) during the last 2d of treatment (Lr or PG groups, respectively). Malnourished control mice (MNC) received PFD while the well-nourished control group (WNC) consumed BCD. On d8, all groups were infected with Sp (10^7 cells/mouse). T and B cell populations of NALT, CLN and ALN as well as production of specific antibodies were studied on days 10 and 14 post-infection. After infection, malnourished mice showed a significant reduction in the number of total cells and the different cell populations studied in both NALT and ganglia. BCD treatment was not able to normalize these parameters. However, only Lr was able to maintain the number of $CD3^+$, $CD3^+CD4^+$ and $CD3^+CD8^+$ lymphocytes at normal values in NALT. Furthermore, Lr and PG treatments induced an increase in total B cells ($B220^+$ cells), mature B cells ($B220^{high}CD4^{Low}IgM^+$) and immature B cells ($B220^{Low}CD4^{High}IgM^-$) in NALT and ALN. Moreover, the Lr and PG mice had higher levels of bronchoalveolar lavage anti-pneumococcal antibodies (IgA, IgG and IgM) than the others groups. These results highlight the importance of NALT as a target for administration of postbiotics and immunobiotics to enhance respiratory immunity in immunocompromised malnourished hosts. Postbiotics could be an interesting alternative as mucosal adjuvants, especially in immunocompromised hosts where the use of live bacteria could be dangerous.

60 (93) PROBIOTIC SUPPLEMENTATION IN RE-NUTRITIONAL DIETS IMPROVES CYTOKINE PROFILE IN THYMUS AND INTESTINE OF MALNOURISHED MICE.

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Malnutrition affects the intestinal barrier, specially the intestinal epithelial cells (IEC), and also influence the thymus (T) activity, leading to a dysfunction of the immune response that can significantly increase morbidity and mortality from infections.

AIM: to evaluate the effect of nutritional recovery supplemented with different probiotic strain on the microenvironment of T and IEC through the production of cytokines in malnourished mice.

Methods: Balb/c mice were divided into groups according to the different diets and supplementation: A) NC: Normal Control; B) DSN: Malnutrition; C) DSN+A: DSN+ conventional food; C) DSN+L: DSN+Milk; D) DSN+Lp: DSN+ *Lactobacillus Paracasei* 1518; E) DSN+M: DSN+ commercial probiotic MIX; F) DSN+A+L: DSN+ conventional food + Milk; G) DSN+A+Lp: DSN+ conventional food + *Lactobacillus Paracasei* 1518; and H) DSN+A+M: DSN+ conventional food + commercial probiotic MIX .

Samples: T and IEC from mice of all experimental groups were taken and we performed *ex vivo* cultures: IL-6, IFN-γ, TNF-α, IL-12 and IL-10 levels were measured by ELISA. Statistical analyzes were performed by GraphPad Prism software using the ANOVA, with Tukey's correction for multiple comparisons, $p< 0.05$ was considered statistically significant.

Results: in T and IEC of mice from the DSN group, levels of proinflammatory cytokines increased respect to the normal control, while values of IL-10 decreased. Re-nutrition diets in malnourished mice significantly decreased levels of proinflammatory cytokines and increased IL-10 values in T and IEC respect to DSN mice, these results being more significant in DSN mice receiving Lp and M, even reaching values close to normal control.

Conclusion: re-nutrition diets (with A or L) in DSN mice demonstrated improvement in level of proinflammatory cytokines and IL-10 in T microenvironment and in IEC, however supplementation with Lp and M (alone or combined with re-nutrition diets) showed to be significantly more effective in the recovery of the regulatory action of the T cells and the IEC.

These results show that probiotic supplementation could be a valuable tool to help improve the functioning of the immune system in malnourished patients.

61 (54) TARGETED DELIVERY OF GALECTIN-1 VIA *LACTOCOCCUS LACTIS*: A NOVEL THERAPEUTIC STRATEGY FOR INFLAMMATORY BOWEL DISEASES.

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Inflammatory bowel diseases (IBD) are chronic and progressive diseases that severely affect quality of life, with ulcerative colitis and Crohn's disease being the most frequent in adults. IBD arises from a combination of genetic and environmental factors leading to exuberant inflammation and pathologic activation of the immune system. Although introduction of TNF-α-neutralizing monoclonal antibodies revolutionized IBD therapy, a third of patients do not respond to this treatment. Thus, the search for new therapeutics is of great importance in IBD. In this regard, development of genetic engineering has given rise to genetically modified probiotics for delivery of therapeutic mediators. Recently, we reported the dysregulation of galectin-1 (Gal1), an immunomodulatory β-galactoside-binding protein in mucosal tissue of patients with IBD and validated its therapeutic potential in experimental colitis. These results prompted us to investigate a selective delivery method of Gal1 through the oral administration. Particularly, we used *Lactococcus lactis (L. lactis)* genetically engineered to deliver Gal1 as a new therapeutic approach for intestinal inflammation. We generated a novel construct for Gal1 recombinant expression in *L. lactis* producing higher amounts of bioactive human Gal1, as evidenced by Western-blot, ELISA and a glycan binding assay. The anti-inflammatory effect of Gal1-secreting *L. lactis* was first evaluated in the 2,4,6-trinitrobenzenesulfonic acid (TNBS)-induced model of colitis in Gal1-deficient (*Lgals1*$^{-/-}$) mice. Daily intragastric administration of 1 x 10^9 CFU of Gal1-secreting *L. lactis* led to active delivery of Gal1 at the ileum and colon, measured by ELISA of tissue homogenates from *Lgals1*$^{-/-}$ TNBS-treated mice. Remarkably, treatment with Gal1-secreting *L. lactis* significantly decreased weight-loss ($p<0.05$) and macroscopic inflammation score ($p<0.05$). This improvement was accompanied by an increase in the expression of IL-10 ($p<0.05$) and IL-17 ($p<0.05$). Moreover, in WT mice we found a considerable decrease in the expression of IFN-γ ($p<0.05$). Furthermore, supernatants of Gal1-secreting *L. lactis* induced more rapid wound closure in the presence of pro-inflammatory TNF-α ($p<0.05$) *in vitro*, as compared to control *L. lactis* or recombinant Gal1. Thus, targeted delivery of human Gal1 via oral administration of genetically-modified *L. lactis* emerges as a potential therapeutic approach for IBD patients.

Conflict of interest: L.G.M., V.S.B., M.A.T., C.M., K.V.M, and G.A.R are co-inventors of the Patent "Galectin-1 delivery for therapeutic control of intestinal inflammation (Provisional patent US 63/274,287, 01-11-2021).

62 (57) URINE METABOLITES PROFILE CHARACTERIZE PATIENTS WITH INFLAMMATORY BOWEL DISEASE.

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Introduction: Inflammatory Bowel Disease (IBD), which comprises Crohn's Disease (CD) and Ulcerative Colitis (UC), is a disorder in which complex interactions between genetic, environmental, and microbial factors trigger alterations in the immune responses of the intestinal mucosa that result in chronic intestinal inflammation. On the other hand, nutrient catabolism from the diet carried out by the microbiota results in the formation of metabolites that also impact immune responses on the intestinal mucosa. Considering that IBD patients have altered microbiota compared to healthy individuals and that the urinary metabolic profile of an individual is the result of a combination of genetic, dietary, and microbial components, we propose the hypothesis that the urinary metabolic profile distinguishes people with IBD from healthy individuals.
Objective: To perform a metabolomic analysis of urine samples from healthy individuals and patients with IBD.
Methods: In this study, 27 patients were divided into control (n=10), UC (n=10), and CD (n=7) groups. The activity of IBD in patients was clinically estimated using the Mayo score (UC) or the Harvey-Bradshaw index (CD). Metabolites in urine were measured using gas chromatography-mass spectrometry (GC-MS). Metabolomic analysis and machine learning were performed using the MetaboAnalyst software. Samples were normalized by sum without data transformation and with autoscaling. PLS-DA was used for multivariate analysis, and clustering separation was performed using hierarchical clustering. To identify and interpret patterns of metabolite concentration changes in a biologically meaningful way we perform Over Representation Analysis (ORA). The statistical analysis included Shapiro-Wilk test for data normality, one-way ANOVA with post-hoc analysis using the Tukey method for group comparison, and Pearson correlation for measuring correlation between values.
Results: In total, 62 organic acids were detected. Comparing UC and control subjects revealed 11 significantly altered metabolites (9 increased and 2 decreased). Increased metabolites comprised lactic acid (p=0.0015), pyroglutamic acid (p=0.0147), isocitric acid (p=0.0325), and phosphoric acid (p=0.0091), whereas comparing CD and control subjects revealed only 2 significantly altered metabolites, glycolic acid (p=0.0214), and lactic acid (p=0.0111). Interestingly, the value of lactic acid varies with the stage of UC, being more concentrated in patients in remission. The most affected metabolic pathways are related to energy metabolism and oxidative stress, including the warburg effect (p=0.0016), gluconeogenesis (p=0.0106), pyruvate metabolism (p=0.0197) and glutathione metabolism (p=0.0039).

Conclusion: We found that the urinary metabolic profile of IBD patients differs from the control group, particularly for UC patients. These metabolites could be used as indicators to improve the diagnosis, monitoring, and treatment of patients with IBD.

63 (161) ALLELE FREQUENCY AND MOLECULAR RELATIONSHIP OF MICA AND MICB IN BRAZILIAN HEALTHY INDIVIDUALS.

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Abstract. The MICA and MICB molecules are constitutively expressed in the membrane of endothelial cells, epithelial cells, fibroblasts and monocytes, and in any other cells in stressful situations, such as tumor or infected cells, as they activate Natural Killer (NK) cells by interacting with NKG2D receptors. These molecules can also be released in a soluble isoform and, by interacting with the same receptor, internalize it, thus decreasing NK activity in the immune system. These molecules are encoded by the *MICA* and *MICB* genes, located on chromosome 6, in the highly polymorphic MHC region. Some polymorphisms have already been described for the *MICA* gene, such as MICA-A5.1, in which, through the insertion of a guanine in the reading frame, there is a premature stop codon and the cytoplasmic tail is not translated, thus these proteins associate with a GPI anchor and can be released in soluble isoform. One of the alleles already described with this polymorphism is MICA*008. *MICB*, on the other hand, has fewer polymorphisms and most do not confer functional changes to the protein. Therefore, the aim of the study was to identify *MICA* and *MICB* alleles in samples from healthy Brazilian individuals and relate them to the immunological functionality of the MICA and MICB molecules. To this end, DNA was extracted from 60 healthy individuals to be amplified by specific primers for the MICA and MICB gene regions for Next Generation Sequencing (NGS) and by bioinformatics tools using HLA-mapper for allele calling. We also amplified 34 SNPs along the genome to find the ancestry of the samples analyzed. Then, plasma and monocyte cultures supernatants were harvested for MICA-B measurement by ELISA. The highest *MICA* allele frequency in our samples was for MICA*008, with 48.36%, followed by MICA*009 with 26.66% and MICA* with 23.33%. For *MICB*, the most frequent alleles were MICB*005 with 55.17%, MICB*002 with 19.83% and MICB*004 with 18.10%. Regarding ancestry, we found majority European ancestry (>80%) in all the samples. The plasma level of soluble MICA samples was significantly higher than membrane samples (89,96 ±13,7 vs 38,34 ±3,9 ng/ml). However, the monocyte culture supernatant showed no difference. In addition, the profile of the samples that release the molecule in a soluble manner is lower in relation to the other group. All MICB results were lower than 0.5ng/ml. Thus, the Brazilian samples analyzed in this study may help to increase public data, as well as indicate the production profile of these molecules related to the allele, favoring that test methods for transplantation and tumor immunotherapies may be more adequate to the patterns of Brazilian miscegenation.

64 (156) CARD9 SIGNALING IS NOT INVOLVED IN THE ANTIFUNGAL INNATE RESPONSE TO EXPERIMENTAL DERMATOPHYTOSIS.

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CARD9, a caspase recruitment domain-containing signaling protein, plays an essential role in downstream signaling and gene activation induced by C-type lectin receptors in response to fungal glycans, contributing significantly to the antifungal immunity. Patients with autosomal recessive CARD9 deficiency suffer uncommon forms of invasive fungal diseases, such as *Candida albicans* encephalitis, extrapulmonary aspergillosis, phaeohyphomycosis, and deep dermatophytosis. Nevertheless, the precise CARD9-mediated mechanisms involved in cutaneous antifungal defenses remain poorly explored. In our laboratory, we have previously developed an experimental model of dermatophytosis in C57BL/6 that recapitulate human infection, characterized by fungal invasion and neutrophil recruitment in the epidermis and an IL-17A-mediated immune response.

In this study, we aimed to investigate the *in vivo* relevance of CARD9 expression during *Nannizzia gypsea* experimental dermatophytosis.

C57BL/6 (WT) and CARD9$^{-/-}$ (CARD9 KO) mice were epicutaneously infected in the back with a *Nannizzia gypsea* suspension (OD 1.0 at 450 nm) or treated with PBS (uninfected controls). At 3-, 6/7- and 20-days post-infection (dpi), back skin sections were incubated with Trypsin/EDTA (2 h,37°C) and epidermal cell suspensions were obtained to analyze *Card9* gene expression (RT-PCR), IL-17A-producing cell populations (FACS), chemokine and cytokine production (ELISA) and fungal burden (CFU/gr skin).

The RT-PCR analysis of sorted epidermal cells from *N. gypsea*- infected WT mice (3 and 6 dpi) revealed an exclusive detection of *Card9* RNA expression within myeloid cell populations (CD45+CD11b+ epidermal cells).

Interestingly, CARD9KO mice exhibited a lower fungal burden after 3 dpi compared to WT mice, (1657 ± 1167 vs. 4306 ± 2968 CFU/gr skin, respectively, $p<0.001$), but there were no significant differences after 7 dpi and the dermatophyte infection was cleared after 20 dpi. Furthermore, CARD9 KO and WT mice had comparable frequencies of IL-17A-producing CD45+ epidermal cells at 7 dpi as well as similar levels of CXCL1, CXCL2, and TNF production by PMA-stimulated epidermal cells.

Collectively, our findings indicate that CARD9 signaling does not participate in fungal sensing in this experimental model of dermatophyte epicutaneous infection. To gain deeper insights, further investigations are necessary to explore whether susceptibility to deep dermatophytosis in CARD9 deficient hosts is contingent on the site of fungal inoculation, such as intradermal or subcutaneous infection routes.

65 (190) CHARACTERIZATION OF CELLULAR AND MOLECULAR FACTORS INVOLVED IN MUCOSAL INFLAMMATION IN EOSINOPHILIC GASTROINTESTINAL DISEASES.

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Eosinophilic Gastrointestinal Disorders (EoGD) are chronic and immune-mediated conditions marked by gastrointestinal (GI) symptoms and eosinophilic inflammatory infiltration of the GI tract. Food allergens triggered both Food Allergies and Eosinophilic Esophagitis (EoE) and their prevalence is increasing globally. Eosinophils are recruited to lamina propria due to a local type 2 inflammation dominated by IL-4, IL-5, IL-13, and eotaxins. In addition, persistent inflammation leads to fibrosis and tissue remodeling. Nevertheless, the underlying mechanisms are poorly understood. We previously showed that juvenile polyps from food allergic patients are infiltrated by eosinophils and fibroblasts, and high levels of type 2 cytokines and CCL26 were detected. In this study, we aimed to characterize the cellular and molecular interplays involving human intestinal eosinophils and fibroblasts.

Isolated eosinophils and fibroblasts from colonic polyps of food allergic patients were primarily cultured. Fibroblasts were stimulated *in vitro* with rh-IL-13 (10ng/ml), rh-IL-9 (10ng/ml) and rh-TGF-β (10ng/ml), and vimentin and IL-8 secretion were evaluated by immunofluorescence and ELISA, respectively. Eosinophils enriched by cell sorting were exposed to IL-5 (10ng/ml) and cell viability was measured by trypan blue exclusion. Esophagus biopsies from adult patients with EoE were *ex vivo* stimulated with IL-13 (10ng/ml). Secreted CCL26, IL-33, IL-25 and TSLP were assessed by ELISA. Inflammatory parameters (α-SMA, CCL26, IL-33, TSLP) were evaluated in esophagus biopsies by immunofluorescence. Comparison between groups was made using Student's t-test.

We found an eosinophil-dominant cell infiltration in polyps (1,55+/-2,79% of live cells) with IgE^+ cells (35,41+/-20,36% of the total cells). Sorted $Lin^-Siglec\text{-}8^+$ eosinophils (35% of yield) showed a higher viability when the culture medium was supplemented with rh-IL-5. Polyp fibroblast primary cultures expressed vimentin and secreted IL-8 under different Th2 stimuli (IL-13: 645+/-4,9; IL-9: 629,2+/-104 *vs.* medium: 482,3+/- 45 pg/ml). Eosinophil-conditioned media also triggered IL-8 secretion by fibroblasts (172,4 *vs.* medium: 57,6 pg/ml). Esophageal biopsies from adult EoE patients showed expression of α-SMA in lamina propria, while CCL26 and TSLP were detected in the epithelium. *Ex vivo* stimulation of esophageal biopsies with IL-13 induced TSLP (124,3+/-35,7 *vs*. medium: 45,26+/-9,3 pg/ml), but no CCL26, IL-25 or IL-33 secretion.

In conclusion, we observed eosinophilic infiltration and activated fibroblasts in the inflamed mucosa of food allergic patients, particularly within polyps and the esophageal mucosa of EoE patients. To study cell responses and interactions, we optimized eosinophil and fibroblast isolation and culture conditions from GI

human tissues. Our findings showed that the type 2 cytokines may activate EoGD fibroblasts.

66 (114) *Corynebacterium pseudodiphtheriticum* MODULATES TLR3-TRIGGERED INNATE IMMUNE RESPONSE IN BRONCHIAL EPITHELIAL CELLS

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Nasally administered *Corynebacterium pseudodiphteriticum* 090104 enhances the resistance of infant and adult mice to the challenge of the respiratory syncytial virus. In order to advance in the knowledge of the mechanisms involved in the beneficial effects of the 090104 strain, this work aimed to evaluate the effect of *C. pseudodiphteriticum* on the immune response of bronchial epithelial cells (BECs) triggered by the activation of TLR3. For this purpose, porcine BECs (1.8 10^4 cells) were stimulated with *C. pseudodiphteriticum* 090104 (Cp) (10^7 cells) for 48 h, and then challenged with poly(I:C) for 24h. Samples of mRNA were taken before (0h) and 3, 6, 12 and 24 h after TLR3 activation, for the evaluation of type I and III interferons (IFNs), antiviral factors, inflammatory and regulatory cytokines as well as pattern recognition receptors and negative regulators of the TLR signaling. BECs non-stimulated with Cp and challenged with poly(I:C) were used as controls.

The stimulation of BECs with Cp significantly increased the expression of *IFN-β*, *α*, *λ1*, and *λ3*, along with *Mx1*, *Mx2*, *OAS1*, *OAS2*, and *OASL* ($p<0.05$) and the receptors *MDA-5* and *RIG-I* ($p<0.05$). In addition, enhanced expression of *IL-6*, *IL-8*, *MCP-1*, and *TNF-α* ($p<0.05$) as well as *IL-10*, *IL-27*, and *TGF-β* were observed in BECs treated with Cp when compared to controls. Cp also increased the negative regulators *A20*, *BCL-3*, *IRAK M*, *MKP-1* and *SIGIRR* ($p<0.05$).

Subsequently, the effect of Cp on the response of BECs with poly(I:C) was examined. TLR3 activation in BECs significantly augmented the expressionlevels of all the immune factors evaluated. Of note, BECs treated with Cp beforethe challenge with poly(I:C) had levels of *IFN-β*, *IFN-λ1*, and antiviral factors thatwere higher than controls. In addition, Cp induced a decrease in the levels of *IL-8*, *MCP-1*, *IL-12* and *TNF-α* in comparison to control cells. A notable increase in the expression of *IL-10* was also evident in BECs treated with Cp ($p<0,05$). The analysis of the TLR negative regulators revealed decreased expression of *A20* and *MKP-1*, along with increased expression of *BCL-3* in BECs treated with Cp, in comparison to controls.

The transcriptional profiling performed in this work allows the obtention of a global overview of the expression patterns of immune genes involved in the response of BECs cells to poly(I:C) stimulation. This study also demonstrated that *C. pseudodiphteriticum* 090104 differently modulate gene expression in poly(I:C)-challenged BECs inducing changes that could help to explain the protective activities against viral infections observed in animal models. These results provided clues for the better understanding the mechanism underlying host–respiratory commensal bacteria interaction.

67 (72) CYTOKINES DEPENDENCE OF MURINE AND HUMAN TVM CELLS INTHEIR DEVELOPMENT AND FUNCTIONALITY

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Memory-like cells are a subset of $CD8^+$ T cells that present a memory phenotype ($CD44^{hi}$ $CD122^{hi}$ $Eomes^{hi}$ $CD49d^{lo}$) without previous antigen encounter. They are a heterogeneous group of cells compose of innate $CD8^+$ T cells that arise in the thymus (T_{IM}) and are exported to secondary lymphoid organs (SLO) or, could develop directly in SLO where are named as virtual $CD8^+$ T cells (T_{VM}). T_{VM}/T_{IM} cells produce large amount of IFNγ after IL12 and IL18 stimulation and play a protective role during the early phase of bacteria, viral and parasite infections. We have previously reported that thymi from *Trypanosoma cruzi* (*Tc*)-infected mice are highly enriched on T_{IM} cells.

It is known that T_{IM} require IL-4, IL-15 and type 1 interferon (IFN-I) for thymic development, but the role of these cytokines in the development andmaintenance of T_{VM} cells in periphery remains unclear. Using IL4KO and IFNAR KO mice, we evaluated the phenotype of this population by flow cytometry and the capacity to produce IFNγ after IL12 and IL18 stimulation. We observed that in the absence of IL4 and IFN-I there is a decrease in the expression of Eomes, a transcription factor important for their functionality (<0,05). Moreover, in the absence of IFN-I and IL-4, at day 2 post-infection, T_{VM} cells show a decrease in IFNγ production (<0.05), suggesting that these cytokines are important not only for the phenotype but also for their functionality of T_{VM} cells.

An analogous population to murine T_{VM} cells was described in human peripheral blood. This population is characterized by mutually exclusive expression of NKG2A or KIR on the $CD8^+$ T cell population. $CD8^+KIR^+$ cells have a more TEMRA phenotype with high expression of the transcription factor Helios than $CD8^+NKG2A^+$ cells (<0,05). Through the analysis of a large number of consensus markers, we evaluated by spectral flow cytometry the response of both populations to IL15, IL4 and IFN-I *in vitro* exposure and we observed that they present a differential expression pattern of CD57, CD27, CD56, CD16 (<0,05). Moreover, we observed that IL4 induces a higher proliferation on $CD8^+NKG2A^+$ cells than $CD8^+KIR^+$ cells (<0,05), IL15 induce proliferation on $CD8^+NKG2A^+$ and $CD8^+KIR^+$ cells whereas IFN-I has a negative effect on the proliferative capacity of both population (<0,05).

Since human T_{VM} cells have been reported to be present in umbilical cord blood it could be inferred that they can acquire their innate characteristics in the thymus. We evaluated by spectral flow cytometry the presence of T_{VM} in human thymi and observed the presence of a $CD8^+$ $TCR\alpha\beta^+$ $Eomes^+$ population with expression of a phenotypic pattern similar to $CD8^+KIR^+$ T cells present in peripheral blood. There results will contribute to the understanding of T_{VM} cells role both in human and mice in steady-state and after cytokine stimuli helping to comprehend their involvement in different pathological scenarios.

68 (67) CYTOSKELETON DYSREGULATION: A MECHANISM OFNEUTROPHIL EVASION BY KLEBSIELLA PNEUMONIAE ST258?

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The cytoskeleton plays a key role in different neutrophil (PMN) functions, such as migration, phagocytosis, the assembly of NADPH oxidase and ROS production, degranulation and NETs formation. We have previously demonstrated that *Klebsiella pneumoniae* ST258 (Kp) is capable of inhibiting neutrophil microbicidal responses, such as ROS production, azurophilic granule mobilization, and NETs release. To understand the mechanism behind this phenomenon, our aim was to assess the impact of Kp on the PMN cytoskeleton.

To evaluate actin cytoskeleton dynamics, we used phalloidin to perform kinetics studies on isolated human PMN by flow cytometry, in the presence of Kp or *Escherichia coli* (Eco), a potent inducer of PMN responses. While Kp did not show differences compared to untreated neutrophils, Eco induced a sustained gradual increase in the levels of actin polymerization from one hour post-incubation ($p<0,05$).

We then investigated whether Kp was capable of affecting cytoskeleton dynamics in response to a positive stimulus, measuring cytoskeletal-dependent functions. For this, we pre-incubated PMN for 1 h with Kp in a ratio PMN:Kp 1:10, and evaluated actin polymerization, chemotaxis, and ROS production in response to fMLP (10^{-7} M), a known positive stimulus for these responses.

After pre-incubation with Kp, actin polymerization in response to fMLP, assessed by flow cytometry, was significantly lower with respect to non-pretreated PMN ($p<0,05$).

We also observed a decrease in chemotaxis towards fMLP, using a Boyden chamber ($p<0,05$), and a decrease in fMLP-induced ROS production, measured by flow cytometry ($p<0,05$).

In conclusion, Kp affected actin polymerization as well as cytoskeleton dependent functions. Therefore, Kp-mediated evasion of PMN responses could be related to mechanisms targeting cytoskeleton dynamics.

69 **(160)** DIFFERENT BIOGEOGRAPHIC REGIONS POINT TO DISTINCT *MICA* GENE DELETION FREQUENCY

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The complete deletion of major histocompatibility complex class I-chain related A (MICA), known as MICA*del, may be an important point of weakness for the immune response, since this gene encodes a NKG2D receptor-ligand, highly polymorphic and responsible for activating an immune response during intracellular infections, neoplasic cells development and after allograft transplantation. However, data on the deletion frequencies in different populations are limited. To evaluate the distribution of the MICA*del in different biogeographic regions, including a large sample of admixed individuals from Brazil we use a specific bioinformatic pipeline and hla-mapper software to correct the alignments, we addressed MICA*del and others *MICA* and *MICB* null alleles in eight different populations of biogeographical regions. Different deletion patterns were found: MICA deletion complete, called MICA*del and the partial MICA deletion, which involves the promoter region, exon 1 and intron 1, called MICA*partial. MICA*del was detected in all biogeographical regions, except Oceania. It's more frequent in the Asian population. Interestingly, America was the second region where MICA*del were most frequent, specially in samples from Mexican, Peruvians and Native Americans individuals from Brazil (Surui). MICA*partial was detected only in America and the Middle East, with low frequency. Most alleles with MICA deletion were in LD with B*48:01 and MICB*009:01N in Asia and America. Therefore, the presence of different patterns of deletion suggests independent deletion events. Moreover, we found two samples with MICA deletion in both chromosomes and one of these samples did not have functional MICB, which may indicate that another mechanism compensates for the lack of functional MIC.

70 (169) EFFECTS OF PURINERGIC-RECEPTOR ANTAGONISM ON NEUTROPHIL INFLAMMATORY RESPONSE INDUCED BY CALCIUM PYROPHOSPHATE DIHYDRATE CRYSTALS

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Deposition of calcium pyrophosphate dihydrate (CPP) crystals in joints and soft tissues is the cause of acute or chronic inflammatory responses known as Calcium Pyrophosphate Crystal Deposition Disease (CPPD). CPP crystals arise extracellularly and their formation requires sufficient extracellular PPi, which is derived from local production by the breakdown of ATP. Purinergic P2Y receptors represent a main class of receptors by which neutrophils respond to extracellular nucleotides and the drug suramin is widely used as a general inhibitor of P2Y receptors. We have previously showed that the presence of CPP crystals in synovial fluid from CPPD patients induced in neutrophils extracellular DNA exposure as well as an up-regulation of CD66b expression and reactive oxygen species (ROS) generation. The aim of this study was to shed light on possible roles of purinergic signaling on neutrophil pro-inflammatory response induced by CPP crystals. Purified human neutrophils, isolated from healthy subjects, were treated with suramin (30 μM) and then incubated with CPP crystals (CPP). Degranulation (measured by the expression of CD66b), ROS production (determined by DHR-123 assay) and NET formation (detected by Sytox Green fluorescence) was studied by flow cytometry in basal neutrophils (basal), neutrophils stimulated with CPP crystals (control) or in neutrophils treated with suramin 1 h before CPP crystal-stimulation (suramin treatment). Results are expressed as mean ± SEM and *p* value is the result of one-way ANOVA (Kruskal-Wallis test) followed by Dunn's post-hoc test. We found that suramin exhibited a partial inhibitory effect on extracellular DNA exposure in neutrophils which were stimulated with CPP crystals (%SYTOX-Green positive cells, control (43 ± 5%) vs suramin treatment (20 ± 8%); p=0.05; n=9). Moreover, treatment of human neutrophils in vitro with suramin inhibited the respiratory burst induced by CPP crystals (% control (112 ± 3%) vs % suramin treatment (88 ± 7%); p=0.02; n=10). No effects were observed in CD66b expression (Gm control (144 ± 16) vs Gm suramin treatment (136 ± 8); p=0.8; n=5). In conclusion, we showed that purinergic receptor inhibitor suramin seems to reduce the pro-inflammatory action of CPP crystals on neutrophils. Further studies are needed to determine which purinergic receptors are involved in this process.

71 (150) EVALUATION OF GENE EXPRESSION PROFILE AND PRO-INFLAMMATORY MOLECULES PRODUCTION IN MICROGLIAL CELLS AFTER TLR2 STIMULATION.

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Toll-like receptors (TLRs) are a family of pattern-recognition receptors in the innate immune system. Exogenous and endogenous TLR ligands activate microglia that trigger inflammatory reactions in CNS.

Studies using a mouse experimental brain abscess model have revealed a complex role for TLRs in the disease pathogenesis.

These studies showed that TLR2 participates in the innate immune response, during the acute stage of brain abscess formation induced by *Staphylococcus aureus* influencing the adaptive immune response.

Therefore, identification of potentially neurotoxic molecules released by TLR stimulation, may provide clues about neuropathological mechanisms involved in these diseases. Thus, here we evaluated gene expression profiles and proinflammatory molecules production in murine microglial cells after TLR2 stimulation.

RNA-seq public datasets from mouse microglial cells and/or macrophages stimulated with TLR2 ligands, were obtained from NCBI Gene Expression Omnibus (GEO) repository and analyzed using integrated Differential Expression and Pathway (iDEP) analysis tool.

Murine BV2 microglial cells were cultured in the presence or the absence of autophagy inhibitors (3-MA) or a general phosphatidyl-inositol-3 kinase (PI3K) inhibitor (LY294002) and then stimulated with Pam3CSK4 or LPS (control) at different timepoints. After treatment, the cells were processed to evaluate: (1) cytokine production by ELISA and (2) nitric oxide (NO) production by Griess reaction. All experiments were performed 3 times and $p < 0.05$ was considered statistically significant.

Preliminary exploration of the RNA-seq data showed enhanced pro-inflammatory gene expression in microglial cells stimulated with TLR2 agonists, compared to the control groups.

On the other hand, we confirmed that activation of microglial cells with Pam3CSK4 induced increased production of TNFα and NO ($p < 0.001$).

Interestingly, treatment of microglial cells with autophagy or a general PI3K inhibitors, prevented the increased production of NO ($p < 0.001$). Additional experiments are currently performed to evaluate the effects of these inhibitors on TNFα production.

Both, RNA-seq datasets analysis and cell culture experiments suggest that TLR2 stimulation of microglial cells modulates pro-inflammatory gene expression and cell responses that may involve the participation of PI3K activation.

72 **(163)** EVALUATION OF THE ROLE OF INTERLEUKIN-10 IN THE PATHOGENESIS TRIGGERED BY STX2

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Hemolytic Uremic Syndrome (HUS), characterized by hemolytic anemia, thrombocytopenia, and renal failure, is caused by Shiga toxin (Stx)-producing E. coli (STEC). Previously we demonstrated that IL-10 deficient (IL-10 -/- KO) Balb/c mice are protected against a Stx2 dose which is lethal for wild-type (WT) mice, in a model of intravenous (I.V) inoculation. The aim of this study was to further analyze the role of IL-10 in HUS, using a different Stx2 inoculation model, referred to as the split-dose model. WT and IL-10 -/- Balb/c mice (6-8 weeks) were injected with a lethal dose of Stx2 divided into four doses, over four days. Mice were bled at days 3, 4, and 5. Percentages of PMN were higher in IL-10 -/- mice compared to WT at days 3 (WT: 13 (9-17); KO: 47 (43-54)*; n=8; $p<0.05$) and 4 (WT: 18 (13-24); KO: 35 (33-37); n=8; $p<0.05$). On the other hand, circulating free DNA (cf-DNA, µg/mL), a parameter of NETosis (inflammatory process), was lower in KO mice compared to WT mice at days 3 (WT: 1.24 (0.72-2.9); KO: 0.33 (0.21-0.96); n=5; $p<0.05$) and 4 (WT: 0.6 (0.47-1.13); KO: 0.30 (0.21-0.34); n=5; $p<0.05$). Interestingly, the protection for KO mice was lost, as 100% of them died between days 5 and 6, while 90% of WT mice survived until day 7. However, urea (mg/dL), a parameter of kidney damage, was significantly higher in WT compared to KO at days 4 (WT: 93 (87-118); KO: 59 (51-81); n=5; $p<0.05$) and 5 (WT: 256 (195-267); KO: 164 (150-186); n=5; *$p<0.05$). Our next step is to evaluate the effect of IL-10 overexpression by *in vivo* transfection in both HUS models (one lethal dose and split-dose). As a preliminary assay, IL-10 -/- mice were administered with 1.8 mL of saline alone (control) or with 0.7 µg of plasmid containing the murine IL-10 gene, through the hydrodynamic injection technique. After 72 h, they were bled to confirm IL-10 expression and inoculated with 5 µg of LPS (I.P.). Control mice reached high levels of TNFα (µg/mL) whereas the plasmid group did not show detectable levels (Control: 9.5 (8.02-10.5); Plasmid: undetectable). Control group died 24 h after LPS, and the plasmid group survived, validating the biological activity of plasmid-derived IL-10. In this study, we evaluated the role of IL-10 in an alternative model of systemic Stx2 inoculation, revealing a distinct function for this cytokine. KO mice exhibited reduced levels of cf-DNA and elevated PMN levels, suggesting a decrease in NETosis. These results imply that IL-10 might not only impact PMN recruitment but also influence their functionality. Moreover, these mice lost the protection reported with a single dose but reached mortality with less severe kidney damage than WT mice, indicated by urea levels. Therefore, the absence of IL-10 appears to influence the response to Stx2 when administered in small doses over 4 days. It is possible that another factor contributes to the heightened susceptibility observed in KO mice. Further experiments are essential to explain this phenomenon.

73 **(184)** EXPLORING THE ROLE OF hBD1, *Candida albicans* AND EPITHELIAL CELLS IN AN *IN VITRO* MODEL OF VULVOVAGINAL CANDIDIASIS

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Vulvovaginal Candidiasis (VVC), mainly caused by *Candida albicans* (Ca), affects reproductive-age women. Around 75% face acute episodes (AVVC), while 9% endure recurrent ones (RVVC), significantly impacting their quality of life. Therefore, a need for effective antifungal therapies. The vaginal epithelium's role in innate immunity is crucial, producing antimicrobial peptides (AMPs) like Beta defensins (BDs), which possess microbicidal, chemotactic, and immunomodulatory properties. Among these, Human Beta Defensin 1 (hBD1) is a major AMP in the non-inflamed vaginal mucosa. However, BDs role in AVVC and RVVC is limited; our team reported reduced hBD1 levels in vagina epithelial cells from RVVC patients. Our objective is to evaluate the interactions between hBD1, Ca and epithelial cells (EC) in an *in vitro* model of VVC. The virulence of Ca strains, including clinical strains (RVVC=20, AVVC=20) and reference strains (SC5314 and ATCC 36801), was evaluated by characterizing virulence factors (VF) such as morphogenesis, adherence to epithelial cells (%AFGT), biofilm formation (BFC) and hydrolytic enzyme activity (lipases-LIP, aspartyl proteinases-SAP). An *in vitro* model was implemented using HeLa cells infected with Ca strains in a 5:1 ratio. After 4 hours, hBD1 mRNA regulation was assessed by qPCR. Besides, we also studied the effect of different concentrations of recombinant hBD1 (rhBD1) (0.78, 1.56 and 3.12 µg/ml) on fungus and *in vitro* model of VVC. We assessed cell viability (LDH release), inhibition of fungal growth (MTT assay) and candidicidal activity (UFC). As controls, fluconazole (FLU) and amphotericin B (AMB) were included. The result indicates that Ca SC5314 was the most virulent strain with higher SAP, LIP ($p<0.01$), and BFC ($p<0.001$) compared with clinical strains and ATCC 36801. This strain inhibited the transcription of hBD1 mRNA in EC after 4h of culture, meanwhile, Ca SC5314 heat killed were unable to produce the same effect ($p<0.05$), showing the relevance of fungal VF in the modulation of AMPs. When the direct effect of rhBD1 on fungus was evaluated after 4h of treatment, the AMPs were able to reduce the fungal growth between 34-39%; for FLU and AMB the values were 69% and 55% respectively. rhBD1 also showed candidicidal capacity. On the other hand, the addition of different doses of rhBD1 on EC doesn't modify the cell viability. In the Ca-EC system, rhBD1 showed dose-related variability in the candidicidal activity compared with antifungal drugs. Interestingly, in the *in vitro* model, the candidicidal activity of rhBD1 was higher than observed on the fungus alone. These findings provide information for addressing unresolved aspects of VVC and exploring therapeutic strategies. The impact of rhBD1 and detailed VF characterization in Ca strains contribute to fungal infection knowledge and could elucidate mechanisms of resistance or susceptibility.

74 (153) EXTRACELLULAR VESICLES RELEASED FROM HUMAN NEUTROPHILS BIND SHIGA TOXIN, THE AGENT RESPONSIBLE FOR HAEMOLYTIC UREMIC SYNDROME.

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Infection with Shiga toxin (Stx) producing *Escherichia coli* (STEC) can cause from self-limited diarrhea to a systemic life-threatening condition called Haemolytic Uremic Syndrome (HUS). The STEC colonizes the intestine where it secretes the Stx, its main virulence factor, which can translocate to the bloodstream and reach target organs. In HUS patients, Stx is not found free in circulation but is detected associated with blood cells and extracellular vesicles (EV). Furthermore, neutrophil (PMN) count and blood EV amounts are increased during HUS acute phase. We have previously described that human PMNs upon stimulation with Stx2 release EV that bear functional Stx (EVstx). The aim of this study was to further characterize the release of EV by PMNs and their interaction with Stx. Human PMNs ($1x10^7$) were treated with purified Stx2 (100 ng/ml, EVstx) or vehicle (EVc). After incubation for 1 or 4 h, samples were spun down, cellular debris was removed, and EV were isolated by differential centrifugations. The capacity of the EV to carry functional Stx was evaluated by a cytotoxicity assay employing the Vero cell line. We determined that the release of EV by PMNs was not modulated by Stx2 stimulation, as we found similar protein concentrations (n=5) and equivalent levels of CD63 (n=10) in samples of EVc and EVstx by western blot. Moreover, both EV samples expressed equal amounts of myeloperoxidase (MPO) and elastase measured by western blot (n=3) and a comparable enzymatic activity of both proteases when analysed by spectrophotometry (n=7). Thus, we then investigated if EVc could bind Stx once released from PMNs. To this end, we isolated EVc and incubated them for 1 h without or with Stx2 (100 ng/ml, EVc+Stx). After a washing step, EVc+Stx, EVstx and EVc were seeded onto Vero cells at different dilutions to evaluate their cytotoxic capacity. EVc+Stx and EVstx significantly reduced cell viability (n=7, $p<0.05$) compared to EVc. Treatment of EVc+Stx with Proteinase K (50 µg/ml, 20', 37°C) did not modulate their cytotoxicity, indicating that the Stx once in contact with the EV turned inaccessible to enzymatic degradation (n=4, $p<0.05$). Altogether these results suggest that EV from human PMNs can bind Stx in the extracellular milieu once released from the cell, keeping it protected from the action of extracellular proteases. Whether during HUS, EV liberated from PMNs already bear Stx or bind it once in the circulation after being released, remains to be investigated.

75 (51) FUNCTIONAL PARAMETERS OF NEUTROPHILS RELATED TO INITIAL STEPS OF NEUTROPHIL-*PROVIDENCIA* SPP. RECOGNITION **Joselyn E. Castro[1], Federico Birnberg-Weiss[1], Jose R. Pittaluga[1], Agustina Serafino[1],Sonia Gómez[2], Verónica I. Landoni[1], Gabriela C. Fernández[1].**
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Antimicrobial resistance is a significant problem for the treatment of infectious diseases worldwide. *Providencia* spp. are Gram-negative bacteria that belong to the Enterobacteriaceae family. Infections caused by species of *Providencia stuartii* (Ps) and *Providencia rettgeri* (Pr) are mainly of the urinary tract, followed by pneumonia, meningitis, endocarditis, wound infections and septicemias. In recent years, the relevance of *Providencia* spp. infections is growing since they have intrinsic antimicrobial resistance, which makes treatment of these multidrug-resistant (MDR) strains challenging.
The innate immune response against these pathogens has not been previously studied. In our first study we reported some striking differences in human neutrophils (PMN) responses against *Providencia* spp. compared to E. coli ATCC (Eco, positive control). In this sense, we observed that Pr poorly induces the respiratory burst in PMN compared to Ps and Eco. Additionally none of the strains, Ps or Pr, were able to induce Neutrophil extracellular traps (NETs) compared to Eco. In concordance with these results, we found that bacterial killing by PMN resulted less effective for Pr.
In this work, our aim was to deepen the study of the interaction between human PMN and multi-resistant strains of Ps and Pr, focusing on early events of PMN activation upon bacterial challenge. For this purpose, we exposed isolated human PMN (n=6) to a clinical isolate of Ps and Pr and used Eco as a positive control in a PMN:bacteria 1:10 ratio. First, we evaluated PMN chemotaxis for 30 min towards bacteria using a Boyden chamber, and found that all strains were chemotactic for PMN compared to basal ($p<0.05$), but migration was similar between Ps, Pr, and Eco. CD11b up-regulation was determined by flow cytometry after 30 min of incubation with the different bacteria. Both Ps and Pr up-regulated CD11b expression compared to basal levels ($p<0.05$), but this increase was similar to that observed for Eco. In addition, increased IL-8 secretion, measured by an ELISA kit, was increased with both Pr and Ps compared to basal ($p<0.05$). Finally, phagocytosis of bacteria after 1 h was determined using Transmission electron microscopy (TEM). PMN were able to phagocyte Pr and Ps, and no differences in the percentage of PMN that phagocyte bacteria was observed compared to Eco.
In conclusion, in contrast to our previous results that demonstrate that *Providencia* spp. were unable to trigger NETosis, they are capable of stimulating early functions of PMN, indicating that PMN are able to recognize and become activated upon bacterial encounter.

76 (124) GLIOBLASTOMA CELL LINE-DERIVED EXTRACELLULAR VESICLESMODULATE γδ T CELL ACTIVATION.

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Tumoral cells are able to modulate their microenvironment for their own benefit by different means such as ligand expression, soluble factors secretion and the release of extracellular vesicles (EVs), among them, medium/large EVs (100-2000 nm). EVs can interact with target cells by membrane receptors, by being internalized or by their fusion with plasma membranes. In consequence, proteins, lipids and nucleic acids carried by EVs can be transferred to other cells present in the tumor microenvironment, among them, immune cells, modulating their functions. γδ T cells are non-conventional T lymphocytes involved in innate responses, that recognize malignant cells and can trigger their apoptosis. Based on the anti-tumoral capacity of γδ T cells, in the last years immunotherapy protocols has been proposed to employ γδ T lymphocytes to treat pathologies like Glioblastoma multiforme (GBM). GBM is the most aggressive malignant cerebral tumor in adults and has a median survival of less than a year after diagnosis. In this work, we aimed to analyze the interaction and effect of GBM cell line-derived EVs on γδ T cells. For that purpose, peripheral blood γδ T lymphocytes were purified by using an anti-TCR γδ MicroBead isolation kit and EVs were obtained from U251 cell line conditioned medium by differential centrifugation. Activated γδ T cells, with specific agonist HMBPP, were incubated overnight with EVs. After incubation, we analyzed the activation state of the γδ T lymphocytes measuring the expression of CD69 by flow cytometry and the release of IFN-γ by ELISA. Moreover, EVs were stained with the lipophilic fluorescent dye PKH26 and incubated with γδ T cells. Then, cells were analyzed by confocal microscopy. The studies showed that there was a physical interaction between γδ T cells and U251 EVs, and such interaction was greater when the T cells were pre-activated with HMBPP ($p<0.0001$). They also indicated that U251 EVs downregulated CD69 expression ($p<0.01$), and elicited a decrease trend in IFN-γ production (n= 4). Our findings suggest that the GBM-derived EVs obtained from the cell line U251 can diminish γδ T cells activation state.

77 (143) HOMING EXPRESSION MOLECULES RANK, CXCR4 AND AXL ARE MODIFIED BY INDOMETHACIN TREATMENT IN LANGERHAN CELL HISTIOCYTOSIS AND IN VITRO DENDRITIC CELLS

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Langerhans cells histiocytosis (LCH) is a rare disease with an abnormal accumulation of pathological Langerhans cells (LC) in almost any tissue. When the bone is compromised, Indomethacin, a non-selective COX inhibitor (NSAID) is an effective treatment. Nevertheless, the specific mechanism of action involved in this therapeutic benefit is not defined. We hypothesized that Indomethacin restrain the homing and migration of pathological LC and/or their precursors to the bone. We analyzed the effect of indomethacin on the expression of homing receptors CCR1, CXCR4, AXL and RANK in monocytes, *in-vitro* differentiated dendritic cells (DC) and inflammatory Langerhans like “LC-like” cells. Moreover, to determine if this effect is extended to another NSAID, we compared it with Ibuprofen. Finally, we have also measured the expression of AXL, RANK and CXCR4 in monocytes obtained from LCH patient’s vs controls and before and after indomethacin treatment.

$CD14^+$ monocytes from Peripheral Blood Mononuclear cells of healthy volunteers were used as precursor cells, and conventional DC (GM-CSF + IL-4) and inflammatory Langerhans like cells “LC-like” (IL-4 + GM-CSF + TNFα + TGFβ + dexamethasone) were *in-vitro* differentiated from monocytes. The treatment was performed with 100uM of Indomethacin or Ibuprofen or vehicle. qPCR and flow cytometry assays were performed to evaluate their effect on gene expression and proteins, respectively.

Indomethacin significantly reduced RNA expression of RANK (N=6, P=0.015), AXL (N=6, P=0.031) and CCR1 (N=8, P= 0.007) in conventional DC. AXL was reduced in both conventional DC and “LC-like” cells confirmed by qPCR and flow cytometry. Treatment of monocytes with Indomethacin also reduced the expression of AXL (N=9, P=0.004) and CXCR4 (N=6, P=0.031), measured by flow cytometry, meanwhile, when monocytes were treated with Indomethacin, RNA expression of RANK was upregulated (N=9, P=0.003). Interestingly, when the cells were treated with Ibuprofen, conventional DC showed a trend reduction in the RNA expression of CCR1 (N=4, P=0.062) and RANK (N=4, P=0.062). Monocyte treatment with ibuprofen does not modify AXL expression, but significantly reduces CXCR4 levels measured by flow cytometry (N=6, P=0.031). Interestingly, preliminary results with AXL (N=6), RANK (N=5) and CXCR4 (N=6) showed a decrease in their expression in monocytes from patients with bone LCH under indomethacin treatment.

Indomethacin affects homing molecules on *in-vitro* differentiated DC, “LC-like” cells, and their precursors, with some specific differences when compared with Ibuprofen. Importantly, patients with bone compromise and under Indomethacin treatment also showed a reduction of these molecules in monocytes suggesting a possible mechanism involved in its therapeutic benefit.

78 (42) HOMOLOGOUS DESENSITIZATION TO PROSTAGLANDIN E2 IN HUMAN MONOCYTES IS MEDIATED BY INDUCTION OF PHOSPHODIESTERASE 4B EXPRESSION

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Prostaglandin E2 (PGE2) is a well-known lipid mediator with inflammatory and anti-inflammatory properties. While PGE2 is recognized for inhibiting LPS-induced activation of monocytes, our previous work has demonstrated that long-term exposure of monocytes to PGE2 increased their ability to produce LPS-induced TNF. We found that increased TNF production was explained by disruption of the negative feedback elicited by LPS-induced endogenous PGE2. This PGE2-mediated abrogation of PGE2 signaling is known as homologous desensitization.

In cell line models, PGE2 has been shown to trigger desensitization either by downregulating specific receptors EP2 and EP4, or by inducing phosphodiesterases (PDE), which terminate PGE2 signal by hydrolyzing second messenger cyclic AMP. In this work we aim to study the mechanism leading to homologous desensitization to PGE2 in human monocytes.

Monocytes were purified from peripheral blood and desensitized by culturing for 15 hours in the presence of low concentrations of PGE2 (10^{-8} M). Control monocytes were cultured for 15 hours without treatment. Spontaneous apoptosis was measured by annexin V staining and was found equivalent in both conditions. To test desensitization, cultured monocytes were stimulated with LPS (25 ng/ml), in the presence, or not, of a second pulse of PGE2 (10^{-7} M). Then, TNF was evaluated by intracytoplasmic staining flow cytometry.

As expected, LPS-induced TNF expression in monocytes pre-treated with PGE2 for 15 hours was not inhibited by a second pulse of PGE2 ($n=7$, $p<0.01$). Washing of pre-treatment PGE2 at 30 or 60 min before the second pulse did not reverse desensitization, implying that receptors were not transiently internalized. In fact, EP2 surface levels studied by flow cytometry scarcely decreased in PGE2-pretreated monocytes compared to controls (mean fluorescent intensity 868 vs 825, $n=11$, $p<0,05$). Furthermore, pre-treatment with PGE2 did not change EP2 and EP4 gene expression as measured by qPCR, suggesting receptors are not downregulated at the transcriptional level either.

On the other hand, pre-treatment with PGE2 increased expression of PDE4B, the main PDE isoform expressed in monocytes. PDE4B expression was induced as early as 1 h after exposure to PGE2 ($n=4$, $p<0,05$), and further sustained for at least 15 hours ($n=8$, $p<0,01$). Conversely, the transcription of the other PDE isoforms expressed in monocytes (PDE3A and PDE3B) was not induced by PGE2 ($n=7$). In agreement, desensitization was abrogated by addition of PDE4 inhibitor rolipram (10 □M, $n=8$, $p<0,01$), but it was not altered by addition of PDE3 inhibitor cilostazol (10 □M, $n=3$).

These results indicated that homologous desensitization to PGE2 in monocytes is associated to increased PDE4B expression. Our findings might be relevant to modulate monocyte activation in diseases characterized by chronically elevated levels of PGE2.

79 (63) IMMUNOMODULATION OF RAW 264.7 MACROPHAGES BY *BACILLUS* LIPOPEPTIDE EXTRACTS UPON LPS CHALLENGE AND EVALUATION OF THEIR ANTIMICROBIAL ACTIVITY AGAINST SWINEENTERIC PATHOGENS

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Weaning is a stressful period in swine production where susceptibility to pathogens such as enterotoxigenic *Escherichia coli* (ETEC) and *Salmonella* Typhimurium causing post-weaning diarrhea (PWD). The aim of the present study was to explore the potential of *Bacillus* lipopeptide extracts (LPE) naturally containing surfactin (SF) — a lipopeptide with anti-inflammatory and antimicrobial capacity, among other biological activities — to mitigate PWD by assessing their immunomodulatory and antimicrobial effects. LPE were obtained from *Bacillus* spp. isolated from bovine feces and soil and their SF concentration quantified by RP-HPLC. LPE's effect on RAW 264.7 murine macrophages viability was assessed by an MTT-based assay. Cytokines (TNF-α; IFN-Y; IL-10) and NO production after LPS challenge was measured in RAW 264.7 cells pre-treated with LPE and untreated control cells. Effect of LPE on macrophages phagocytic and microbicidal capacity was also assessed pre-treating RAW 264.7 cells with extracts containing 0.01 to 60 µg/ml SF. Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of LPEs against ETEC and *Salmonella* Typhimurium were calculated. LPE from *Bacillus* spp. isolates MFF 2.2 and TC12, containing 40 and 60 µg/ml of SF showed a significant (p<0.05) ability to induce IL-10 production in unstimulated (≤587 pg/ml) and LPS-stimulated (≤155.20 pg/ml) cells. Furthermore, these LPE reduced significantly (p<0.05) NO production to levels comparable to unstimulated cells (0.80 ± 0.05 to 1.19 ±0.02) and IFN-γ to undetectable levels upon LPS stimulation, indicating an anti-inflammatory effect. However, TNF-α levels were over 1000 pg/ml in all treatments. LPE of TC12 with SF concentrations from 60 to 0.1 µg/ml did not compromise macrophage viability (V% >90%), while MFF 2.2 showed cytotoxic effects only in the highest concentrations. LPE of both MFF 2.2 and TC 12 did not affect phagocytic activity, or bactericidal capacity of RAW 264.7 cells, and exhibited a bacteriostatic activity that reduced *Salmonella* Typhimurium and ETEC growth (TC 12 MIC: 29.08 µg/ml for ETEC and 3.64 µg/ml for *S.* Typhimuriun; MFF 2.2 MIC: 13.77 µg/ml for ETEC and 0.86 µg/ml for *S.* Typhimuriun). In conclusion, LPE from native *Bacillus* spp. showed immunomodulatory and antimicrobial effects *in vitro*.These effects suggest administration of LPEs could potentially improve gut health in piglets during weaning by reducing inflammation and inhibiting pathogen growth, causing a positive impact on the animal's overall health and reducing the need of antibiotics as growth promoters.

80 (115) INNATE IMMUNE RESPONSES TRIGGERED BY HYPERMUCOVISCOUS CARBAPENEM-RESISTANT *Klebsiella pneumoniae* ST25 STRAINS IN BRONCHIAL EPITHELIAL CELLS AND ALVEOLAR MACROPHAGES

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In recent years, an increase in the prevalence of hypermucoviscous carbapenem-resistant *Klebsiella pneumoniae* (Kp) of the sequence type (ST) 25 was detected in hospitalized patients in Northwest Argentina. Our previous studies have shown that Kp carbapenemase-2 (KPC-2)-producing ST25 strains are endemic in hospitals of Tucuman and are associated with respiratory and systemic infections. Furthermore, studies in mice showed that Kp ST25 strains have different degrees of virulence when infecting the respiratory tract. In this work, the innate immune responses triggered by two *K. pneumoniae* ST25 strains (LABACER 01 and LABACER 27) in bronchial epithelial cells (BECs) and alveolar macrophages (AMs) were evaluated.

For this purpose, porcine BECs (1.8 10^4 cells) or AMs (10^5 cells) were challenged with LABACER 01 or LABACER 27 (10^7 cells). Samples of mRNA were taken before (0h) and 3, 6, 12, and 24 h after Kp challenge, for the evaluation of inflammatory and regulatory cytokines as well as pattern recognition receptors and negative regulators of the TLR signaling. BECs and AMs challenged with LPS were used for comparisons.

The stimulation of BECs with LABACER 01 or LABACER27 significantly increased the expression of the negative regulators *A20, BCL3, IRAK M, and SIGIRR* when compared to the LPS-treated cells ($p<0.05$). In addition, enhanced expression of *IL-6*, *IL-8*, *MCP-1*, and *TNF-α* ($p<0.05$) as well as *TGF-β* were observed in BECs treated with both Kp ST25 strains when compared to controls. LABACER 01 also increased the expression of *IL-10* and the negative regulator *TOLLIP* while LABACER 27 enhanced the expression of *IL-17* and *IFN-γ* ($p<0.05$).

Only slight impacts were noted among the AMs exposed to the two *K. pneumoniae* ST25 strains when compared to controls. LABACER 01 enhanced the expression of *A20* and LABACER 27 induced the expression of *IFN-γ, TNF-α* and *IL-10* compared to the LPS control ($p<0.05$). However, neither of the two strains studied proved as effective as LPS in inducing the expression of the remaining negative regulators and cytokines assessed in this investigation.

Studies on the cellular and molecular interactions of multiresistant *K. pneumoniae* ST25 with the host are of fundamental importance to assess the association of their virulence factors with the intensity of the inflammatory response. In this sense, this work explored the ability of two ST25 strain to trigger inflammatory responses in two important cell types involved in the defences of the respiratory tract, expanding the knowledge of the immunobiology of the emerging ST25 clone in Argentina.

81 (155) INNATE LYMPHOID CELLS CONFER PROTECTION IN A MURINE MODEL OF DERMATOPHYTOSIS

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Dermatophytosis is a cutaneous fungal infection caused by filamentous keratinophilic fungi. Despite its prevalence of 25% worldwide, the skin-specific immune response against dermatophytes has been scarcely investigated. Previously, using an experimental model of dermatophytosis in C57BL/6 mice, we demonstrated that IL-17RA-dependent signaling is crucial to control the superficial infection caused by the human pathogens *Microsporum canis and Nannizzia gypsea*. Furthermore, we demonstrated that, after *N. gypsea epicutaneous* infection, IL-17A-producing cells in the skin were mainly αβ T and γδ T cells and the skin resident lymphocytes were sufficient to prevent fungal overgrowth.The aim of this study was to further characterize the *in vivo* role of lymphoid cell populations in the protective immunity to *Nannizzia gypsea experimental dermatophytosis.*

Wild type (WT), mice deficient in mature T and B cells ($Rag1^{-/-}$, $Rag2^{-/-}$) and mice deficient in lymphoid cells ($Rag2^{-/-}Il2rg^{-/-}$ C57BL/6 mice, Rag2γC KO) were epicutaneously infected in the back with a fungal suspension of *N. gypsea* (DO=1.00 at 450 nm). At 3-, 7- or 20-days post-infection (dpi), mice were euthanized, and the back skin was removed and trypsinized (2h, 37°C) to obtain an epidermal cell suspension used for fungal burden quantification (CFU/gr skin), cytokine production (ELISA) and FACS analysis of IL-17A-producing or RORγt-expressing skin cell populations.

Results showed that *N. gypsea*-infected $Rag1^{-/-}$, $Rag2^{-/-}$ and WT mice have similar fungal burden at the different days post-infection. In addition, WT and $Rag2^{-/-}$ mice completely cleared the infection by 20 dpi. Unsupervised tSNE and supervised FACS analyses revealed that infected $Rag1^{-/-}$ mice maintained a lymphoid skin population characterized as innate lymphoid cells ($CD45^+$ $CD90^+$ $CD3^-$ $CD11b^-$ $CD11c^-$ $Ly6G^-$ $Ly6C^-$ $CD19^-$), which expressed high levels of the transcription factor RORγt suggesting an ILC3 phenotype. Interestingly, despite $Rag1^{-/-}$ mice controlled the dermatophytosis and harbored $ROR\gamma t^+$ ILC cells, these mice showed a significant decrease in the frequency of skin IL-17A-producing cells when compared to WT mice ($P<0.01$).

To study the role of ILC, we infected Rag2γC KO mice which lack ILC and mature T and B cells. Fungal burden quantification showed that these mice were significantly more susceptible to infection compared to WT or $Rag1^{-/-}$ and $Rag2^{-/-}$ mice (CFU/gr skin at 6-7 dpi, $P<0.0001$) and histopathology showed intense skin inflammation and abundant PAS+ hyphae in the epidermis without fungal invasion to deeper skin layers. Furthermore, *N. gypsea* was still detected in the skin of $Rag2^{-/-}Il2rg^{-/-}$ mice at 20 dpi.

Taken together, we demonstrate that ILC play a central role in skin protection against *N. gypsea* superficial infection in the absence of T-cell mediated immunity. These data suggest that T cell deficiency is not enough to establish a susceptible phenotype in this model of dermatophytosis.

82 **(48)** LOW pH ENHANCES NEUTROPHIL TROGOCYTOSIS

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Trogocytosis is a biological process by which cells acquire membrane fragments from donor cells through a contact-dependent process, allowing the acquisition of receptors that can modulate the function of receiving cells. Trogocytosis has been shown to be involved in different processes including cell-cell communication, antigen-presentation, immune regulation, and anti-tumor immunity. However, the mechanisms involved in this process remain poorly characterized. We here analyzed whether low pH values (pH 6.5), similar to those found in inflammatory processes, could modulate neutrophil-mediated trogocytosis. Neutrophils were isolated from peripheral blood from healthy donors using conventional methods. Jurkat (a T cell line) and Ramos (a B cell line) were used as donor cells, and were previously coated or not with anti-CD3 or anti-CD20 IgG antibodies, respectively. Afterward, neutrophils were co-cultured at 37°C for 2 hours with either Jurkat or Ramos cells using a cell-cell ratio of 1:1, at pH 7.3 or pH 6.5. In a first set of experiments, Jurkat cells were stained with the lipophilic dye PKH-26, which labels the cell membrane, and the transference of PKH-26 to neutrophils was evaluated by flow cytometry. No transference of PKH was observed when using unopsonized Jurkat cells (not shown). By contrast, we found that 49 ± 12 % and 96 ± 14% of neutrophils were PKH-26 positive at pH 7.3 and pH 6.5, respectively (n=3, $p<0.01$ pH 7.3 vs pH 6.5) when anti-CD3-coated Jurkat cells were used as donor cells. No phagocytosis was observed under these experimental conditions (not shown). In a second set of experiments, we used unlabeled Ramos cells, and the transference of CD19 and HLA-DR, from Ramos cells to neutrophils, and of CD11b from neutrophils to Ramos cells was evaluated. No transference was observed when using unopsonized Ramos cells. By contrast, when using anti-CD20 IgG-coated Ramos cells we observed a bidirectional transference of cell receptors that was significantly increased at low pH (pH 6.5). Neutrophils co-cultured with opsonized Ramos cells acquired the surface expression of CD19 and HLA-DR when cultured at pH 7.3 (5 and 9%, respectively) and pH 6.5 (20 and 24%, respectively). Moreover, we found in this context that Ramos cells also acquired the expression of the highly expressed neutrophil integrin CD11b (7% at pH 7.3 vs 24% at pH 6.5) (values of a representative experiment are shown, n=3, $p<0.01$ pH 7.3 vs pH 6.5). Our observations show that neutrophil trogocytosis is induced by antibodies directed to cell surface molecules expressed by donor cells and that the process of transference is enhanced at low pH values.

83 (127) *LSP1*$^{-/-}$ DENDRITIC CELLS EXHIBIT IMPAIRED SOLUBLE ANTIGEN UPTAKE AND DELAYED PARTICULATE ANTIGEN DEGRADATION KINETICS.

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Leukocyte-specific protein 1 (LSP1) is a 52kDa cytoplasmic F-actin binding phosphoprotein expressed within human, murine leukocytes, and endothelial cells. It serves as a crucial regulator of actin cytoskeleton remodeling and potentially plays a role in antigen processing within endomembrane compartments of dendritic cells (DCs). Previous findings from our research highlighted that *Lsp1*$^{-/-}$ DCs exhibit impaired antigen presentation to CD4+ T cells in comparison to DCs from wild type (WT) mice, for both soluble and particulate antigens. Specifically, in the case of soluble antigens, we observed diminished uptake in *Lsp1*$^{-/-}$ DCs as opposed to their WT counterparts. As a result, we investigated whether this impaired antigen presentation in *Lsp1*$^{-/-}$ DCs stems from alterations in antigen uptake or processing.

To address this, we derived DCs in vitro from bone marrow precursors with Flt3-L. For assessing uptake of particulate antigens, DCs were co-cultured for 1 hour with Yellow-Green fluorescent microspheres. Subsequently, cells underwent two PBS washes, followed by staining with distinct antibodies and subsequent flow cytometry analysis. Intriguingly, *Lsp1*$^{-/-}$ DCs displayed statistically non-significant differences compared to *Lsp1*$^{+/+}$ DCs in this regard. To evaluate degradation of soluble antigens, DCs were incubated with OVA-AF 647 (a fluorochrome unaffected by pH changes) and OVA-FITC for 30 minutes. Afterward, cells were washed with PBS and were placed in RPMI media and were analyzed by flow cytometry at 1, 2, 3, and 4-hour intervals. Despite the previously noted reduced antigen uptake in *Lsp1*$^{-/-}$ DCs compared to their *Lsp1*$^{+/+}$ counterparts, these cells surprisingly exhibited consistent degradation kinetics. Contrastingly, in exploring degradation of particulate antigens, DCs were co-cultured with OVA-bound microspheres for an hour. Following two PBS washes, cells were cultured for 1, 2, 3, and 4-hour periods. After these intervals, DCs were fixed, permeabilized, and subjected to anti-OVA staining to gauge intact protein or incomplete proteolysis. Strikingly, at the 1-hour mark, *Lsp1*$^{-/-}$ DCs demonstrated diminished OVA degradation ($p<0.05$) compared to their *Lsp1*$^{+/+}$ counterparts, though both groups reached similar values at the 2-hour mark. These trends persisted at 3 and 4-hour intervals.

These findings suggest that the impaired antigen presentation in *Lsp1*$^{-/-}$ DCs may, in part, result from impaired uptake of soluble antigens rather than alterations in degradation kinetics. Conversely, the capture of particulate antigens by *Lsp1*$^{-/-}$ DCs appears unaffected, with differences instead noted in degradation kinetics. This discrepancy could arise from distinctions in the intracellular compartments participating in the uptake and degradation of soluble versus particulate antigens.

84 (64) *MINTHOSTACHYS VERTICILLATA* ESSENTIAL OIL MODULATES INNATE IMMUNE RESPONSE IN BOVINE EPITHELIAL CELLS CHALLENGED WITH *STAPHYLOCOCCUS AUREUS* VÍA NFκB

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In a previous study we demonstrated the ability of essential oil (EO) of *Minthostachys verticillata*, an Argentinian plant, to modulate the IL-1β and IL-6 synthesis in bovine epithelial cells (MAC-T), in the presence or absence of an inactivated *Staphylococcus aureus* strain (iSa), isolated from cows with mastitis. In cells pre-treated with EO and then challenged with iSa, we observed a decrease in both cytokines' levels between 24 and 48 h post-challenge. Therefore, the aim of this work was to evaluate the effect of EO on the synthesis of anti-inflammatory cytokines as well as to determine the relative expression of NFκ-B transcription factor and TLR2 receptor in MAC-T cells, in the presence or absence of iSa. The cells were treated with EO (25, 50 and 100 µg/mL), iSa ($5x10^6$ CFU/mL) and pretreated with EO and then challenged with iSa, at different times (2, 6, 24 and 48 h). Cells without stimulation were used as control. IL-4 levels were quantified in the cell supernatant by sandwich ELISA. The mRNA relative expression of NFκ-B and TLR2 was measured by qPCR. As expected, in cells stimulated with iSa, IL-4 values were similar to those found in control cells. In cells stimulated with EO at three concentrations, the IL-4 values were significantly higher than those observed in control cells at 2, 6 and 24 h ($p<0.05$, $p<0.01$ and $p<0.001$, respectively) and were similar to those found in control cells at 48 h. In cells pre-treated with EO and then challenged with iSa, IL-4 values were variable and dependent on dose and time. The pre-treatment with EO (100 µg/ml) induced the highest IL-4 levels at 24 and 48 h. As expected, NFκB mRNA expression levels were significantly increased in cells stimulated with iSa compared to control cells ($p<0.001$) at 6 h. Interestingly, in cells stimulated with EO (100 µg/ml) a significant increase in NFκB mRNA expression levels was also observed compared to control cells ($p<0.001$) at 6 h, although these expression levels were lower compared to iSa-stimulated cells ($p<0.001$). These results demonstrated that after 24 h, the pre-treatment of MAC-T cells with EO stimulate the production of IL-4 down-regulating the production of IL-1β and IL-6 induced by iSa. These results are related to down-regulation of NFκB mRNA expression by the pre-treatment of MAC-T cells with EO. In conclusion, *M. verticillata* EO, modulated the innate immune response activated by *S. aureus* in bovine cells, decreasing pro-inflammatory cytokine levels and increasing anti-inflammatory cytokine levels vía down-regulation of NFκB transcription factor. These results serve as a basis to continue studying this natural product as a potential immunomodulator with future application in the control of bovine mastitis.

85 (86) NOVEL, SENSITIVE, *IN VITRO* RADIOLABELING ASSAYS ALLOW THE MONITORING OF CYTOSOLIC VIMENTIN PROTEOFORMS BY SDS-PAGE IN NON-INFECTED, MYCOBACTERIAL-INFECTED AND TLR2-LIGATED THP-1 CELLS. POSSIBLE VIMENTIN ROLES IN MONOCYTE TO MACROPHAGE DIFFERENTIATION, INFECTION AND INFLAMMATION.

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Discovering low-abundant macrophage proteins/proteoforms, altered in level/PTMs during intracellular bacterial infection and innate immune responses, needs sensitive proteome screening tools in electrophoretic gels, but outperforming dyes. AIMS: to search for, in human THP1 macrophage-like cells, cytosolic proteins reproducibly altered in a time-dependent and sustained manner, at days 1-4 post-infection with mycobacteria (live or killed). METHODS: The cytosolic fraction was obtained and used in novel, post-cell harvest, cell-free, *in vitro* radiolabeling (IVR) assays, allowing the covalent labeling of cytosolic proteomes with P-32. Labeled proteomes were separated in 1D/2D gels to detect bands/spots with altered labeling, normalizing them against total stained and total labeled proteomes. Proteins of interest were identified by MS and characterized. Bibliometric and bioinformatic studies were initiated to interpret findings in terms of PTMs, protein-protein interactions and possible roles of altered proteins and to plan how IVR might help future studies. RESULTS: in all 12 time-course infection experiments, cytosolic vimentin (VIM) was upregulated by infection in a time-dependent manner. In 3 monocytic- to-macrophage differentiation experiments (PMA-treated, non-infected), the VIM IVR increased during 4 days. We identified cytosolic kinases allowing detection of VIM with cleaved forms. Metabolic labeling in cell culture detected VIM profiles different to IVR. In WB, different antibodies and sera against other proteins often did bind non-specifically to VIM. So, to monitor minor cleavage/expression changes in VIM, IVR was more sensitive, quantitative and robust than WB. The literature indicated that VIM: **a)** is emerging as a multifunctional protein located in the perinuclear area, cytosol, endosomes, viral factories, cell surface, extracellular space and blood; **b)** has roles in auto-/xeno-/aggre-phagy, apoptosis, scaffolding of signaling complexes and in binding to DNA, RNA, phospholipids, O-GlcNAc, Rab7a, p62, HDAC6, MTOC, NFκB, NOD2, NLRP3, ERK; **c)** is a modulator of infectious, immune, autoimmune, inflammatory, cell stress, and fibrotic responses and is a target of toxins from many bacteria; **d)** has roles other than the cytoskeletal/mechanical by using different PTMs and by assembling as 1-, 2- and 4-mers, cages, and filamentous networks; **e)** Surface VIM binds many bacteria and viruses including SARS-Cov-2; **f)** Non-specific WB signals might depend on VIM-Fc and/or on citrullinated VIM-Ab interactions; **g)** VIM can be cleaved in cells. CONCLUSIONS: IVR helped detecting dynamic changes in cytosolic VIM levels, complementing WB. IVR would help to study VIM functional diversity, to correlate VIM alterations with those in binding partners, and to study VIM as biomarker or drug-target in cell infection and/or differentiation. Dissecting pro-infection and infection-restricting VIM roles will improve our knowledge of host–pathogen interaction complexity.

86 (81) PLASMA EXTRACELLULAR VESICLES AS PROANGIOGENIC AND WOUND HEALING AGENTS

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Chronic inflammation alters the physiological state of tissues, being the underlying cause of the pathogenesis of a group of diseases with increasing prevalence. We recently demonstrated that plasma extracellular vesicles (pEVs) from healthy donors regulate inflammation in macrophages exposed to PAMPs. Herein we investigated the pro-angiogenic and pro-resolving functions of macrophages exposed to pEVs and PAMPs. pEVs were obtained by size exclusion chromatography, followed by centrifugation at 30,000 g for 90 min. The identity and purity of pEVs was performed by western blot, electron microscopy and NTA. pEVs were used to stimulate primary macrophages derived from blood monocytes, which were simultaneously stimulated with LPS (5ng/ml) or Resiquimod (R, 2ug/ml) as models of bacterial or viral PAMPs respectively, for 24h. Modulation of the expression of genes related to angiogenesis/tissue repair was analyzed by quantitative PCR. The secretion of proteins involved in tissue repair/angiogenesis was evaluated in the supernatant of macrophages exposed to the different stimuli by ELISA or dot blot. Finally, the ability of macrophage supernatants, exposed to the different stimuli, to promote tube formation in HUVEC and to modulate activation surface markers in these cells was analyzed. Macrophages exposed to R+pEVs simulatenously exhibited increased expression of the resolution-associated genes VEGF ($p<0.05$), CD300e ($p<0.001$), CD93 ($p<0.05$), RGS2($p<0.01$) and TIMP-1 ($p<0.05$). Also, pEVs induced HB-EGF ($p<0.05$) and SERP-E1($p<0.01$) expression, while decreased gene expression of SERP-F1 ($p<0.001$). Moreover, we detected an augmented VEGF release ($p<0.001$) on R+pEV-treated macrophages and an increased PF4 and THBS-1 secretion on pEV and R+pEV-treated cells. Finally, we observed a promotion of tubulogenesis ($p<0.01$) and a reduced expression of ICAM, V-CAM, VE-CADH in HUVEC stimulated with R+pEVs/pEVs supernatants. Our results support the hypothesis that pEVs induce the expression of proangiogenic factors in macrophages, having also the ability to promote tissue repair, even in the absence of proinflammatory factors as co-stimuli. These findings support the therapeutic use of pEVs in the resolution of chronic wounds.

87 (22) PLATELET RICH PLASMA TEMPERS THE INFLAMMATORY SKEWING OF MACROPHAGES IN THE CONTEXT OF CHRONIC HEMOPHILIC SYNOVITIS

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INTRODUCTION & AIMS: Intra-articular bleeding is a common feature of patients with hemophilia, contributing to the development of chronic hemophilic synovitis (CHS). The increased presence of monocytes/macrophages with hemosiderin deposits in the sinovia is one of the main contributors in the maintenance of inflammation and promotion of M1 profile. We aim to evaluate the modulating effect of platelet rich plasma (PRP) on the phenotype and skewing of inflammatory macrophages in the presence of synovial fluid from CHS patients.

METHODS: Monocytes CD14+ and PRP were obtained from peripheral blood of healthy donors and differentiated into non-polarized (M0) and polarized with IFNy plus LPS (M1), IL4 (M2a), dexamethasone (M2c) or synovial fluid (SF) alone or plus PRP after 7 days of culture. The cellularity of SF after Giemsa dye was evaluated by optical microscopy and the expression of polarizing markers were assessed by flow cytometry and qPCR.

RESULTS: The macrophages from the SF of patients with CHS, before PRP treatment, were characterized as large cells containing cytoplasmic hemosiderin deposits stained in dark violet-blue as well as by high percentage of cells expressing CD64 (78.5±4.2%) and lower levels of CD206 (22.74±8.4%) and CD163 (26.33±15.4%), indicating the M1 profile signature. Furthermore, the *in vitro* monocyte-derived macrophages differentiation in the presence of SF also showed a skewing to M1 pattern. The gene program of SF-macrophages segregates separately from the cytokine-polarized cells, suggesting a specific inflammatory program. The addition of PRP significantly modulate the relative expression of CD64 and CD206 ($*p<0.05$) with a particular gene induction that results in an M2-type profile. Finally, after 1 to 2 weeks of intra-articular injection of PRP in CHS patients, the microscopy analysis showed a recomposition of a normal cellular content of SF in 3 follow up patients, in concordance with the *in vitro* results.

CONCLUSION: Macrophages from SF samples of CHS patients and those differentiated *in vitro* under SF challenge, showed a prototypical M1 signature of surface markers and a unique gene pattern. Moreover, the modulative effect of PRP to a pro resolving-tissue repaired cell type was evidenced both in patients and in monocyte-derived macrophages differentiated with SF, underlying the potential therapeutic action of PRP in CHS.

88 (201) PROTEOME RADIOLABELING ASSAYS REVEAL THAT INFECTION OF THP1 MACROPHAGES WITH DIFFERENT BACTERIA UP-REGULATES A NOVEL CYTOSOLIC VARIANT OF AN ER CHAPERONE, SHOWING A pI-REDUCING PTM OF UNKNOWN STRUCTURE. DEPENDENCY ON TLR2-LIGATION AND MICROTUBULES.

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Finding proteins altered in level/PTMs during bacterial infection in macrophages requires sensitive screening protocols in gels, outperforming dyes. We searched cytosolic proteins altered in a time-dependent, sustained manner, at days 1-4 post-infection (pi). Thus, we optimized cell-free, *in vitro* radiolabeling (IVR) assays of cytosolic proteomes of THP1 cells to covalently label some proteins with P-32. Proteomes were resolved by 1D/2D gels to detect bands/spots with altered IVR. 3-4 experiments were performed per condition. Interesting proteins were identified by MS. Bibliometric and informatic studies were initiated to interpret the findings. RESULTS: After infection with *Mycobacterium avium*, a labeled 78 KDa band, p78, was always upregulated at day 1 pi, equally by live or heat-killed bacteria. Time-course infection experiments revealed that live bacteria sustained p78 longer, with a 2-fold difference with killed, at day 4. So, p78 was sustained by live intracellular bacteria. The p78 spot had pI=3.9 in 2D gels. A stained spot was identified by MS as HSPA5, an ER chaperone (normal pI=5.3). So, p78 was an ultra-acidic, cytosolic, charge variant of HSPA5, but with similar mobility in gels. p78 was upregulated at day 1 also by 2 gram-negative bacteria (live or killed). Thus, p78 was likely a conserved cell response to heat-resistant molecules of different bacteria. Since it did suggest participation of TLRs, we treated cells with pure TLR2 ligands like lipopeptides, LTA, etc., upregulating p78 again. Contrary to the normal HSPA5 precursor, p78 was ER-stress independent. So, p78 might arise from a minor precursor pool after addition of a PTM. p78 was undetectable in WB and IVR outperformed silver and dyes in sensitivity. We improved the IVR to monitor p78 only in 1D gels and identified a kinase labeling p78 by IVR (did not label HSPA5). Taxol treatment revealed that microtubules were involved with p78. LPS as TLR4 ligand did not generate p78. TLR2 negative HeLa cells had no upregulation. The 1.4 pH units shift, without intermediate spots, would imply a single-step polyanionic PTM, incompatible with multisite phosphorylation. Besides, a phosphate-binding dye did not detect p78. So far, the PTM escaped MS identification. We conclude that any candidate PTM should comply these characteristics: a) be poly-anionic and/or neutralize many basic HSPA5 residues, b) generate a reproducible pI reduction, maintaining equal mobility, c) compatibility with the spatiotemporal aspects of the upregulation plus association to microtubules, d) TLR2 ligation dependency, e) ER-stress and ligand-structure independency, f) if more than 1 enzyme is involved, they should be spatiotemporally coordinated. We discuss the literature about candidate PTMs, together with reports of other HSPA5 forms. p78 in THP1 is a reproducible biomarker useful to compare different TLR2 ligands and bacteria and deserves more studies about its roles in innate immune responses and its PTM.

89 (83) PULSE OF PROSTAGLANDIN E2 TRIGGERS GENETIC REPROGRAMMING TO DRIVE HUMAN MONOCYTE-DERIVED MACROPHAGES INTO A PRO-RESOLUTION PROFILE

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Prostaglandin E2 (PGE2) is a lipid mediator with recognized inflammatory properties but, paradoxically, is also critical for the initiation of the resolution of inflammation. Macrophages play a fundamental role in resolution through cytokine secretion and the removal of apoptotic cells. We have previously shown that PGE2 induces in macrophages a unique pro-resolution profile characterized by increased efferocytosis (EC), increased VEGFa expression and resistance to LPS-mediated TNF and IL-6 production. Our study aims to evaluate the mechanism through which PGE2 induces this profile.

For this purpose, monocytes were purified from buffy coats and were differentiated into macrophages over 7 days by the addition of M-CSF (50 ng/mL). Macrophages were treated at day 5 of culture with PGE2 (1 μM) until day 7. Control macrophages were cultured without PGE2. EC was quantified by flow cytometry measuring incorporation of CFSE-stained apoptotic Jurkat cells. Transcriptional changes were analyzed by RNAseq and by qRT-PCR.

First, we studied PGE2-canonical cAMP-mediated signaling. Treatment with cAMP analog 8-Br-cAMP and with EP4 receptor agonist L-902,688 ($n=5$, $p<0,05$) increased EC, although no effects were observed with EP2 receptor agonist butaprost. Notably, PGE2-mediated effect was abolished by PKA inhibitor H89 ($n=4$, $p<0,01$).

To study the role of cyclooxygenase enzymes, we evaluated EC after treatment with indomethacin (COX1/2 inhibitor) and celecoxib (specific COX2 inhibitor). Interestingly, while indomethacin abrogated PGE2-mediated increase in EC ($n=7$, $p<0,01$) celecoxib did not ($n=10$, 5 to 50 μM). However, other COX1 products such as PGI2, thromboxane A2 (using specific agonist U46619) and 15-d-PGJ2 were not able to recapitulate PGE2-mediated effects.

Next, we found that adding PGE2 only for 6 hours during day 5 of culture replicated the EC increase induced by the 48-hour treatment ($n=8$, $p<0,001$), suggesting that a transient treatment with PGE2 triggers macrophage reprogramming. Indeed, transcriptome analysis by RNAseq of macrophages treated for 6 h with PGE2 showed 130 genes differentially expressed compared to control (not treated macrophages). Gene set enrichment analysis indicated that PGE2-treated macrophages were enriched in genes associated to glycolysis, angiogenesis and EC, most notably apoptotic-cell bridging protein thrombospondin-1 (7.5-fold increase). Genes associated with synthesis of pro-resolution lipids or with oxidative phosphorylation (two pathways previously associated to pro-resolution profiles) were not significantly upregulated. In fact, we confirmed that PGE2-mediated EC was not affected by blockade of PPARγ signaling nor by mitochondrial membrane potential disruption.

Thus, we have shown that exposure to PGE2 during differentiation of macrophages elicits a transcriptional reprogramming associated to increased efferocytosis but not through lipid switch or oxidative phosphorylation.

90 (130) RESPONSES OF γδ T CELLS TO SOLUBLE FACTORS RELEASED BY SHIGA TOXIN -TREATED INTESTINAL EPITHELIAL CELL LINE OR BY *ESCHERICHIA COLI* O157:H7.

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Hemolytic uremic syndrome (HUS) is a serious public health concern that mainly affects children younger than 5 years old. They have a higher risk of developing severe consequences such as acute or chronic renal injury. HUS associated with diarrhea, hemolytic anemia, and thrombocytopenia is a consequence of Shiga toxin (Stx)-producing *Escherichia coli* (STEC) infection. Stx type 2 (Stx2)-producing strains are associated with severe cases of HUS in Argentina, where that disease is endemic. γδ T cells are a specialized subset of T cells, which act as early sensors of cellular stress and infection. They can exert cytotoxicity against infected cells and produce cytokines and chemokines. Previously, we evaluated the response of γδ T cells to stress signals emulating an inflammatory microenvironment that could be developed at the gut epithelial barrier. In this work, we aimed to evaluate the response of human peripheral blood γδ T cells to supernatants obtained from the intestinal epithelial cell line HCT-8 treated or not with Stx2 (0.01 - 1000 ng/ml). As well, we studied the response of γδ T cells to soluble factors released by *Escherichia coli* O157:H7 strain 125/99 which produce Stx2 (STEC 125/99), and from Stx2 non-producing bacteria (STEC125/99deltaStx2), as control. For that purpose, we analyzed in γδ T cells, after 24 h incubation with the supernatants mention above, the CD69 expression by flow cytometry, and cytokine (IFN- γ and TNF-α) production by ELISA. As a result, we observed that the treatment of γδ T cells with HCT-8 supernatants treated with Stx2 did not induce changes in lymphocyte activation compared with non-treated cells (n= 11). In addition, similar CD69 expression and cytokine production were observed when γδ T cells were expose to both *Escherichia coli* supernatants obtained from Stx2-producing bacteria and their controls (n= 7). In conclusion, our results suggest that γδ T cells seem to be not sensitive to the stress signals released by Stx2-stimulated HCT-8 or by STEC.

91 (59) ROLE OF α-GLUCANS FROM THE CAPSULE OF *MYCOBACTERIUM TUBERCULOSIS* IN THE INDUCTION OF THE RESPIRATORY BURST IN IMMUNE SYSTEM CELLS.

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Mycobacterium tuberculosis (Mtb), the bacterium responsible for tuberculosis (TB), has a complex cell envelope composed of lipids and polysaccharides with chemical structures that confer low permeability as a survival strategy. The capsule is the outermost layer weakly attached to the cell wall, composed of three main polysaccharides: α-D-glucan, D-arabinose-D-mannose, and D-mannose. The α-D-glucan is a high molecular weight homopolysaccharide that is stored in the cytoplasm as glycogen and constitutes 80% of the sugars in the capsule. The α-D-glucan consists of α-D-glucose residues connected by α-1,4 linkages to form a linear core, partially substituted at position 6 with additional α-D-Glc residues, with a molecular weight of 100 kDa. These are produced through the cooperation of the enzyme GlgE, which is solely responsible for producing linear chains, and the branching enzyme GlgB. Even minimal variability in the capsule could lead to marked phenotypic differences affecting its immunogenic capacity. Previously, we demonstrated that the most representative clinical isolates from our country exhibited significant differences in their ability to induce reactive oxygen intermediates (ROS), possibly mediated by the direct interaction of capsule α-glucans. *Mtb* lacking glucans (treated with α-amyloglucosidase, *Mtbe*) loses its oxidative capacity. We evaluated the ability to induce ROS by neutrophils from healthy donors stimulated with H37Rv (*Mtb* wt) or variants possessing mutations in genes encoding key enzymes in α-glucan synthesis pathways: treS-glgB and treS-glgE. We used the dihydrorhodamine (DHR) oxidation assay in neutrophil cytoplasm and an indirectly non-glucan-dependent approach (bacteria loaded with DHR). Additionally, apoptosis at 15 hr was measured by Annexin V binding. Results: All strains induced ROS compared to untreated control ($p<0.0001$). However, treS-glgB, treS-glgE, and *Mtbe* induced significantly lower ROS than *Mtb* wt ($p<0.007$; $p<0.005$; and $p<0.02$, respectively). When the respiratory burst was not glucan-dependent, there were no significant differences between the strains studied. Only *Mtb* wt ($p<0.008$) and treS-glgE ($p<0.05$) were capable of inducing apoptosis compared to the control. These findings allow us to advance our understanding of the pathways involved in the recognition of *Mtb* that trigger the induction of the respiratory burst and the pathophysiological consequences associated with *Mtb's* virulence. The host-pathogen interaction would be conditioned by these sugars, not only affecting the phagocytosis of the bacteria but also the signaling cascade involving ROS that play a role in the immune response.

92 (170) SERINE PROTEASES ACTIVITY IS LINKED TO NEUTROPHIL PROINFLAMMATORY CAPACITY AND CELL FATE

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Neutrophils represent the first line of defense against bacteria and fungi. They exert their microbicidal effects by phagocytosis and the release of extracellular traps (NET). These granulocytes can also modulate immune responses by releasing the potent pro-inflammatory cytokine Interleukin-1 beta (IL-1β). We previously determined that in human neutrophils pro-IL-1β is processed to its active isoform mainly by both serine proteases (NSP) leaked from azurophil granules into the cytosol and caspase-1. However, we found that excessive activation of these enzymes is detrimental to IL-1β secretion. Considering that NETosis and pro-IL-1β processing/secretion usually involve the participation of elastase, ROS-derived from NADPH oxidase, and pores made by Gasdermin D, we conjectured that both processes might be mutually exclusive. To test this hypothesis, we stimulated human neutrophils from healthy donors with a wide range of concentrations (2.5-25 ng/ml) of phorbol myristate acetate (PMA), the gold standard NETosis activator, together with or without LPS (150 ng/ml)+ATP (2.5 mM) to promote pro-IL-1β synthesis and inflammasome activation respectively, and consequently caspase-1 activation. We evaluated the levels of secreted IL-1β by ELISA, NSP activation and reactive oxygen species production by flow cytometry, MPO by spectrophotometry, and DNA release to the supernatants by fluorometry. We found that neutrophils released significant levels of IL-1β when stimulated with PMA 2,5 and 5 ng/ml ($p<0.01$; n=4) but the release of this cytokine gradually decreased as the concentration of PMA increased being negligible at 25 ng/ml, a usual NETosis-triggering dose. This reduction at higher PMA concentration was not due to an inhibition of pro-IL-1β synthesis, as the cytokine could be intracellularly found at earlier time points by immunostaining and confocal microscopy (n=2). When neutrophils were stimulated with LPS+ATP+PMA, we detected a similar secretory profile as with PMA, although higher IL-1β levels were detected in this case. By contrast, the MPO (n=4) and DNA (n=5) levels in culture supernatants, evaluated as a readout of NETosis, augmented as PMA increased ($p<0.05$). We also found that intracellular NSP activation gradually increased the higher the concentrations of PMA, reaching statistical significance at concentrations higher than 5 ng/ml. Moreover, Disulfiram, an inhibitor of Gasdermin D pore formation, significantly decreased MPO release induced by different PMA concentrations ($p<0.05$, n=4). These results, together with our previous data, agree with the notion that high levels of intracellular NSP activation contribute to reduce IL-1β secretion by promoting its intracellular degradation, and according to the agonist, also increase NETosis. Altogether, our findings suggest that significant IL-1β secretion and NETosis might be mutually exclusive processes. Further studies are ongoing in our laboratory to confirm this conclusion.

93 (208) STUDY OF THE MECHANISMS TRIGGERED BY A NOVEL AMNIOTIC MEMBRANE DERIVED TREATMENT FOR SKIN INJURIES

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Skin wound repair is a complex process balancing among innate immunity interactions leading either to scar formation or tissue regeneration. The former comprises patching of the wound due to quick extracellular matrix (ECM) deposition mainly by fibroblasts and might lead to a dysfunctional sealing, while the latter is resolved over a longer time lapse and also requires tissue architecture remodeling guided by M2 macrophages. Human amniotic membrane has been used as a biological dressing. We found that the healing of complex skin wounds has been surprisingly improved in patients treated with a novel homogenized, lyophilized and sterilized amniotic membrane patch (hAMpe). We previously found that these derivatives induced a shift in the activation profile of macrophages, from M1 to M2, diminished IL-6 and TNFa secretion, and increased IL-10 and IL-8. We recently found that IL-1b secretion was also downmodulated in a classical way, not involving inflammasome inhibition. These results emphasize the effects of hAM on the macrophage profile contributing to an anti-inflammatory background.In order to further unravel other mechanisms involved in tissue regeneration, we experimentally focused on fibroblasts.

No cytotoxicity was found on the human cell line fibroblasts HT1080 over the evaluated range of hAM preparations, from 50 to 800 ug/ml, with a significant increase in cell viability under 400 ug/ml stimulation (ANOVA+Tuckey test).

We quantified collagen deposition in human fibroblasts from both line and primary culture. Cell cultures were treated with hAMpe soluble components at different total protein concentrations for 24 hours and after removal of culture media and PBS washing, the insoluble collagen was picrosirius red stained, and quantified by staining elution in alkali, which absorbs at 540 nm. Primary fibroblast conditioned with 100 ug/ml hAMpe rehydrates secreted 60% more collagen than the control group, and the HT1080 fibroblasts also increased in 40% its collagen deposition compared to the control group (ANOVA+ Dunnet`s test, λ=0,05).

To study cell migration, we performed the wound assay. HT1080 cells showed wound closure of 4,48% for untreated fibroblasts against 22,53% for 200 ug/ml hAMpe treated condition, being significantly different with $p<0,05$. The assay performed on primary fibroblasts showed similar results with a more significant difference on the percentage of wound closure between the untreated, -3,66%, against the 150 ug/ml hAMpe treated condition, 25,66% ($p<0,005$; Kruskal-Wallis + Dunn´s tests).

Finally, preliminary results in a murine wound skin model also showed that hAMpe boosted collagen deposition. The histological sections showed no differences on the type I/III collagen, 2 weeks after injury.

With all these results we continued characterizing this new and promising amniotic membrane therapeutic device for complex skin wounds. Further research is still needed, especially regarding its role on ECM architecture remodeling.

94 (132) SUPERANTIGENS OF THE *EGC OPERON* SEI, SEO, SEG AND SEM INDUCE HUMAN NEUTROPHILS ACTIVATION PROMOTING DIFFERENTIAL PEPTIDOME PROFILES, MODIFICATION OF MMPS ACTIVITY, CYTOKINES AND NETS RELEASE

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Superantigens (SAgs) are potent immunomodulatory enterotoxins primarily produced by extracellular bacteria, such as *S. aureus*. While extensive research has focused on SAgs activation of T cells, recent studies have unveiled their targeting of innate immune cells. Polymorphonuclear cells (PMNs), mainly neutrophils, are the first line of defense against these pathogens, being a key component of the innate immune system.

Here, we investigated the effect of four natural SAgs of the *egc* operon (SEG, SEI, SEO and SEM) on the neutrophil population, its peptidome, and activation markers.

For this purpose, SEI, SEO, SEG and SEM were cloned and produced as recombinant proteins in *E. coli* and purified by Ni^{++}/NTA column. PMNs were enriched from healthy donors and incubated with 1 µM of each SAg and PMA as a positive control, during 4 and 24 h depending on the assay. To characterize the proteomic profile within the 2 to 20 kDa range, the protein extract of neutrophils challenged with different stimuli was analyzed using MALDI-TOF-MS. NET release was evaluated by IFI, metabolic activity assessed via XTT assay, and viability determined using Hoechst/IP fluorescent microscopy. Supernatants were collected to measure IL-6, IL-12, IL-10, and TNF-α by ELISA. Concurrently, MMPs activity was evaluated by zymography. Results were analyzed by one-way ANOVA with the corresponding post-hoc test.

Stimulation of PMNs with 1 µM of SEI, SEO, SEG, and SEM led to distinct peptidome profiles, with SEM inducing the most unique pattern. Additionally, a noteworthy increase in metabolic activity after 24 hours ($p < 0.05$) was observed, accompanied by augmented cell death, primarily attributed to SEG ($p < 0.005$). In addition, SAgs were found to trigger NET release and enhance MMP-9 activity in both multimeric and monomeric forms ($p < 0.05$). Furthermore, all SAgs induced a higher production of IL-6, IL-12, and TNF-α by PMNs compared to untreated cells ($p < 0.0001$).

In conclusion, these results show that SEI, SEO, SEG, and SEM stimulate NET release from PMNs with the associated production of pro-inflammatory cytokines, IL-6, TNF-α, and IL-12, each exhibiting a unique peptidome profile. Moreover, these toxins induced increased metabolic and MMP activity. Overall, these findings underscore the distinct modulation of PMNs by *egc* SAgs, highlighting their capacity to influence not only adaptive but also innate immune responses.

95 (136) THE GUT-LUNG AXIS *IN VITRO*: EXPLORING *C. DIFFICILE*'S INFLUENCE ON MACROPHAGES' *M. TUBERCULOSIS* UPTAKE

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Over the last years, interest has aroused in the gut-lung axis as a regulator of the immune system homeostasis. Intestinal dysbiosis has been implicated in numerous respiratory infections or in the chronic exacerbation of certain lung diseases such as Tuberculosis. Antibiotic-mediated dysbiosis can lead to *Clostridioides difficile* infection (CDI), an opportunistic potentially lethal pathogen that colonizes large intestine. Since the impact of CDI on immune cells functions against *M. tuberculosis (Mtb)* infection remains unknown, here we evaluate the effect of pre-exposing human macrophages to *C. difficile* (*CD*) on *Mtb* uptake.

To this end, monocytes were obtained from healthy donors' blood after Ficoll-Hypaque gradient and CD14 positive magnetic selection. Monocytes were cultured in the absence of FBS for 2h and then in complete media ON. Afterwards, monocyte-derived macrophages (MΦs) were cultured in the presence or absence of *CD* (NAP1/BI/027 strain) inactivated by heat treatment (*CD*H) for different times (24h, 48h, 5d and 7d). Then, MΦs were stimulated with FITC-coupled *Mtb* (BEI Resources/NR-14819, H37Rv) for 1h. Endocytic levels for *Mtb* were evaluated by flow cytometry and macrophages morphology by microscopy.

Our results show that pre-exposure to *CD*H induce characteristic changes of MΦs' differentiation. Increases in cytoplasmic volumes and granularity were evidenced by enlarged forward and side scatter on flow cytometry. MΦs also exhibited typical endocytic structures (e.g. pseudopods). Moreover, cell viability seems not to be affected by *CD*H pre-exposure in none of the time points evaluated as analyzed by using eFluorT^{M} 780 viability dye. When evaluating *Mtb*-FITC uptake, preliminary data indicate that MΦs differentiated for 5 and 7 days enhanced their endocytic capacity compared to 24-48h-cultured MΦs (30 vs. 60% of FITC^{pos} MΦs). At day 5, MΦs also evidenced the highest median intensity of fluorescence, indicating that not only the percentage of endocytic cells increases, but also the amount of bacteria per MΦ. Interestingly, pre-exposure to *CD*H for 5d induced different *Mtb*-FITC uptake levels when *CD*H was washed before *Mtb* addition or not. While the presence of *CD*H in the cell culture induced *Mtb*-FITC uptake compared to MΦs without *CD*H pre-exposure (controls), the removal of extracellular *CD*H led to reduced uptake capacity. Although we have not studied it yet, these variations could be explained by the activation of macropinocytosis pathways by *CD*H, a mechanism that we have addressed in a previous work.

In conclusion, we observed a modulation of the endocytic range against *M. tuberculosis* elicited by *C. difficile* in human macrophages. These results are the first testing *C. difficile*-*M. tuberculosis*-macrophages crosstalk, exploring how an intestinal pathogen might affect innate immune responses against a respiratory bacterium.

96 (185) THE INVOLVEMENT OF B-CATENIN ACTIVATION IN THYMIC INNATE CD8+ T CELL DEVELOPMENT

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We have demonstrated that during the acute stage of Th1 infectious processes, as *Trypanosoma cruzi* infection, SP CD8 thymocytes alter their differentiation from “conventional” to “innate” lineage due to thymic production of IL-4 and IL15. Innate CD8+ thymocytes (IM CD8+ cells) express a particular phenotype ($CD44^{hi}$, $CD122^{hi}$, $EOMES^{hi}$), produce high levels of IFN-γ and have cytotoxic activity. The Wnt/β-catenin (β-cat) signaling pathway, apart from playing key roles in different cellular processes, assumes a crucial regulatory function in infectious and inflammatory processes. Accordingly, we have reported the activation of β-cat in both spleen cells and macrophages following *in vivo* and *in vitro T. cruzi* infection. Furthermore, the Wnt/β-cat signaling pathway contributes to the differentiation of CD8+ T cells, functional CD8+ memory T cells and positively regulates CD44 expression in different cell types. In addition, the activation of β-cat leads to the production of IL-4 by CD4+ cells, and it has been demonstrated that IL-4 triggers β-cat activation in macrophages. Additionally, transgenic mice expressing β-cat exhibit an increased presence of CD4+ PLZF+ cells within the thymus, which secrete IL-4 and thus facilitate the development of IM CD8+ cells. Based on these observations, we hypothesized that acute *T. cruzi* infection induces the activation of β-cat within the thymus, thereby establishing a suitable microenvironment that promotes the development of IM CD8+ cells, which participate as the first line of defense against this infection. Firstly, we observed the upregulation of β-cat expression in the thymus on day 2 post-infection using Western blot. With the aim of studying whether β-cat activation is involved in the development of these cells in the thymus, an *in vitro* model previously described was used. Thymocytes from normal OT-I mice (targets) were co-cultured with thymocytes obtained from normal mice or mice infected with *T. cruzi* (effectors), in the presence or absence of the β-cat pharmacological activator (BIO) or inhibitor (iCRT14), along with their vehicle MeBIO or DMSO respectively. After 48 h of co-culture, the expression of IM CD8+ cells markers were evaluated in the OVA tetramer+ (OVAt+) population by FACS. Activation of β-cat increased the percentage of cells expressing CD44hi (p<0.001) and induced up-regulation of CD122 in OVAt+ cells co-cultured with either normal or infected targets, while did not induce Eomes expression. Inhibition of β-cat with iCRT14 decreased the percentage of OVAt+ cells expressing CD44+ CD122+ (p<0,001) and the MFI of CD122 expression (p<0,001) in this population. Interestingly, β-cat activation with BIO increased the percentage of cells expressing the IL-4R (CD124, p<0.001) and induced up-regulation of CD124 in OVAt+ cells. Together, these results indicated that β-cat activation promotes innate differentiation in an antigen-independent and cytokine-driven system.

97 (107) THE SECRETION OF HUMAN NEUTROPHIL IL-1β TRIGGERED BY SHIGA TOXIN-PRODUCING *ESCHERICHIA COLI* IS REGULATED BY THE ACTIVITY OF NEUTROPHIL SERINE PROTEASES WHICH IS CONTROLLED BY GASDERMIN D

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Shiga toxin (Stx)-producing *Escherichia coli* (STEC) is an enteric pathogen that causes self-limited gastrointestinal infections and bloody diarrhea, but also a severe systemic condition known as Hemolytic Uremic Syndrome (HUS). Neutrophils are recruited to the intestine upon STEC infection. Previously we determined that STEC stimulated neutrophils to release elevated levels of IL-1β through a mechanism that involved the activation of caspase-1 driven by the NLRP3-inflammasome. Based on the effect of a PAN-neutrophil serine proteases (NSPs) inhibitor, we also found that NSPs' activity is required for IL-1β processing and release. Other studies showed that active Gasdermin D (GSDMD) produced by caspase-1 processing form pores on the membranes of neutrophil azurophil granules that allow the leakage of the NSP elastase (NE) to the cytosol, explaining its capacity to process cytosolic substrates, among them the IL-1β precursor and GSDMD. We also found that neutrophils IL-1β secretion was higher at lower STEC multiplicities of infection (MOI) and it was independent of the capacity of the bacteria to secrete Stx. The aim of this study was to investigate the molecular mechanism that underlies this secretory profile. We first evaluated the effect of Ecotin, another serine protease inhibitor, capable of inhibiting NE, on STEC-induced IL-1β secretion. Ecotin significantly reduced IL-1β secretion in a concentration-dependent manner ($p< 0,0001$) but neither affected IL-8 secretion nor induced neutrophil lytic death (n=4). These findings confirmed the role of NSPs in neutrophil IL-1β secretion induced by STEC. We then evaluated the effect of Disulfiram (DSF), an inhibitor of GSDMD pore formation. DSF significantly reduced the activation of NSPs induced by STEC (n=6, $p< 0,05$) supporting a role of GSDMD pore in NSPs cytosolic leakage and activation. DSF also reduced the total levels (intracellular and extracellular) of mature IL-1β (n=3, $p< 0,05$) and increased the intracellular pro-IL-1β levels (n=5, $p< 0,05$). Also, pre-activation of caspase-1 which would be expected to increase GSDMD cleavage and pore formation, led to a reduction in IL-1β secretion when challenging cells with STEC compared to cells without pre-activated caspase-1 (n=7, $p< 0,05$). None of these treatments modulated neutrophil lytic death. We previously determined that the higher the MOI, the greater was the activation of both caspase-1 and NSPs. These findings together with those of this study, and the ability of NE to cleave IL-1β at numerous sites, suggest that higher STEC MOIs induce more NSPs leakage to the cytosol, which instead of contributing only to IL-1β processing could lead to its degradation. Overall, our findings suggest that NSPs are key regulators of human neutrophil proinflammatory capacity in response to STEC.

98 (46) TRANSCRIPTOMIC PROFILING OF HIGH-SALT STIMULATED HUMAN NEUTROPHILS

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Neutrophils are the first cells recruited into sites of inflammation or infection, where they play a pivotal role in eliminating pathogens through a wide array of microbicidal mechanisms. Once recruited, neutrophil function can be modulated by physicochemical changes in the inflammatory milieu, including the accumulation of sodium. In fact, high salt (NaCl) concentrations are found in a number of tissues under physiological and pathological conditions. We have previously reported that human neutrophils exposed for a short period of time to high salt concentrations display a decreased ability to produce IL-8 and oxygen reactive intermediates (ROS in response to conventional agonists. By contrast, exposure to high salt concentrations for longer periods of time resulted in a strong activation signal, leading to the production of high levels of IL-8, an increased respiratory burst, and a marked synergistic effect on TNF-α production induced by LPS. To gain insight into the mechanisms by which high salt modulates neutrophil phenotype and function, we performed transcriptional profiling by RNAseq. Neutrophils were isolated from peripheral blood of healthy donors by conventional methods and were cultured for 4 hours in culture medium supplemented, or not, with NaCl (50 mM) and/or LPS (100 ng/ml) (n=4). We found that only 75 genes were differentially expressed between untreated (controls) vs LPS-stimulated neutrophils, being NFkB- signaling associated genes the top enriched pathway among upregulated genes (padj<0.01, fold change>4). In contrast, 4812 genes were differentially expressed between untreated vs. high salt-treated neutrophils (padj<0.01, fold change>4), revealing a broader and distinct transcriptional profile compared to the changes induced by LPS. Retinoic acid signaling and cell-cycle associated genes, as well as paraspeckle components (subnuclear bodies involved in the regulation of cell function), were among the top enriched pathways upregulated by exposure to high salt concentrations. Interestingly, the genes that were markedly up-regulated (>200X) in neutrophils by high salt, but not by LPS, included genes whose expression and function in neutrophils have not been previously analyzed such as ICAM-4, CENPU (Centromere Protein U), OSM (Oncostatin M), CYB5D1 (Cytochrome b5 domain containing 1), DDIT3 (DNA damage-inducible transcript 3), among others. Further studies are required to determine the role of these genes in neutrophil function.

99 (193) UP-REGULATED EXPRESSION OF PD-L1 ON CYTOKINE-ACTIVATED NK CELLS INVOLVES JNK, p38 MAPK and NF-κB PATHWAYS.

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Natural Killer (NK) cells are key effectors in the antitumoral and antiviral immune response. However, these cells can also adopt a regulatory role, suppressing T cell responses. We have shown that tumor-experienced human and mouse NK cells express high levels of PD-L1 and are able to inhibit $CD8^+$ T cell priming. Immunosuppressive PD-$L1^+$ NK cells can also emerge upon viral infections or after cytokine stimulation. The **aim** of this work was to study the mechanisms and signaling pathways involved in PD-L1 up-regulation in human NK cells in proinflammatory conditions. To this end, human NK cells from healthy donors were isolated and stimulated with the proinflammatory cytokines IL-12, IL-15 and IL-18 individually or in combination. After 24 hours PD-L1 expression and intracellular IFN-γ were evaluated by flow cytometry. We observed that when added individually, none of the cytokines increased PD-L1 expression or IFN-γ production in NK cells. However, the combination of IL-12+IL-18 significantly increased the frequency of PD-L1 expressing NK cells ($p<0.0001$) and the percentage of IFN-γ-producing NK cells ($p<0.0001$). Of note, nearly all PD-$L1^+$ NK cells produced IFN-γ (98.10% ± 1.26%; $p<0.0001$). Subsequently, monocytes were stimulated overnight with LPS+R848, which induce IL-12 and IL-18 secretion, and the cell-free supernatants were used to stimulate NK cells. Concordantly, activated monocytes induced the up-regulation of PD-L1 ($p<0.001$) and the production of IFN-γ ($p<0.001$) on NK cells. Next, we used pharmacological inhibitors (SP600125, SB202190, BAY11-7082, Rapamycin, U0126, AG490 and Ly294002), to assess the signaling pathways involved in the IL12+IL18-induced response. Our findings indicate that JNK ($p<0.001$), p38 MAPK ($p<0.0001$) and NF-κB ($p<0.0001$) pathways, but not mTOR, MEK1/MEK2, Jak2 or PI3K, are involved in the cytokine-induced PD-L1 expression on NK cells. Furthermore, the JNK ($p<0.05$), p38 MAPK ($p<0.01$), NF-κB ($p<0.01$) and mTOR ($p<0.05$) pathways, but not MEK1/MEK2, Jak2 and PI3K, were implicated in the IL-12+IL-18-induced IFN-γ production. In summary, our results show that cytokine stimulation as well as soluble factors secreted by activated monocytes induce PD-L1 up-regulation and IFN-γ production on NK cells, through partially overlapped pathways. These findings provide the first description of the signaling pathways regulating PD-L1 expression on NK cells in a proinflammatory environment.

Immunometabolism

Friday, November 10, 8-9:30h

Chair: Cristina Motrán – Silvia Di Genaro

100 (78) DIFFERENT METABOLIC PATHWAYS ASSOCIATED WITH TIME-DEPENDENT SEVERITY IN SEPTIC MICE

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Sepsis is a syndrome caused by a dysregulated host response to pathogens and represents the leading cause of death from infection. In murine models of endotoxemia, the mortality rate is largely dependent on the circadian system: Mice inoculated intraperitoneally with high doses of lipopolysaccharide (LPS; 20 mg/kg) at the end of the day show a higher mortality rate (~80%) compared to those inoculated at midnight (~30%), along with a greater inflammatory response and increased hypothermia. To study the mechanisms involved in this daily variation, we conducted a proteomic analysis on serum samples obtained 2 hours after the administration of LPS or vehicle (VEH) at the end of the day, ZT11, or at the middle of the night, ZT19 (ZT0: the time lights are turned on; ZT12: the time lights are turned off). We performed a two-way ANOVA analysis and observed that proteins increased at ZT19 are associated with glucose metabolism, energy utilization, and lipid metabolism ($p < 0,05$ for all proteins). On the other hand, differentially expressed proteins at ZT11 are involved in the inflammatory response, oxidative stress, and cell communication, migration, and adhesion ($p < 0,05$ for all proteins). Due to a higher food intake prior to LPS stimulation in animals inoculated at ZT19 than animals inoculated at ZT11 ($p < 0,05$), we administered glucose intraperitoneally 3 hours before the ZT11 stimulus to evaluate if the difference in prognosis is determined by glucose availability. However, we observed that this manipulation does not change the severity of sepsis (p n.s.). Furthermore, we evaluated blood glucose levels after stimulation at both time points and in the ANOVA test, we observed that animals exhibit hyperglycemia in response to LPS, within 2 hours post-stimulation, only at ZT11 (LPS ZT11 vs LPS ZT19, VEH ZT11 and VEH ZT19 $p < 0,05$). In conclusion, the prognosis of septic mice is not dependent on glucose availability prior to the stimulus. However, the increase in blood glucose in response to LPS at ZT11 could be signaling at the central level and/or promoting the activation of the inflammatory response, leading to greater severity in this condition.

101 (96). EARLY PREGNANCY REPROGRAMS MATERNAL CIRCULATING MONOCYTE METABOLISM TO HIGHER GLUCOSE DEPENDENCY ASSOCIATED TO INCREASED EFFEROCYTOSIS

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Aim: Metabolic reprogramming of macrophages is associated to functional polarization upon environmental stimuli. In normal pregnancy, monocytes recruited to the pregnant uterus differentiate into M2-like decidual macrophages under the control of extravillous trophoblast cells. However, the immunometabolic profiling of human maternal circulating monocytes at early pregnancy and whether the trophoblast has a conditioning role have not been reported so far. We aim to characterize the metabolic pathways active in circulating monocytes from pregnant women at 16-20 weeks in basal and ex vivo LPS-stimulated conditions. Methods and Results: Peripheral blood mononuclear cells (PBMC) were isolated from fertile non-pregnant and 16-20 weeks pregnant women by Ficoll-Paque and stimulated or not with 100 ng/ml LPS from E. coli. CD14+ cell metabolism was immediately analyzed using SCENITHTM by flow cytometry. Briefly, metabolic inhibitors (2-Deoxy-D-glucose, Oligomycin or their combination) were added to PBMC for 15 min. Then puromicyn was added 30 min and CD14 antibody staining was followed by intracellular anti-puromycin staining. Glucose and long chain polyunsaturated fatty acids (LCPUFA) uptake was assessed by flow cytometry with D-glucose fluorescent analog (2-NBDG) or Bodipy FL-C12, respectively. IL-1β and IL-10 secretion was measured by ELISA and efferocytosis of apoptotic autologous neutrophils stained with CFSE was quantified by flow cytometry. Results: Monocytes from pregnant vs. non-pregnant women showed higher glucose dependency ($p<0.05$) and lower fatty acid and amino acid oxidation capacity ($p<0.01$). This metabolic reprogramming was accompanied by higher levels of efferocytosis. LPS-stimulated monocytes showed no changes in glycolytic and mitochondrial dependency in the pregnant group in contrast to the non-pregnant where the characteristic higher glucose uptake and dependency was observed. Interestingly, we observed higher levels of IL-10 secretion by monocytes from pregnant women without changes in IL-1β respect to monocytes from non- pregnant women while LPS stimulation increased IL-1β and IL-10 production in both monocyte groups. Conclusion: Results presented here indicate that early pregnancy reprograms maternal circulating monocytes priming efferocytosis and modulating their response against pro-inflammatory stimuli.

102 (27) METABOLIC PROFILING OF MACROPHAGE SUBSETS IN *TRYPANOSOMA CRUZI* INFECTION: IMPLICATIONS FOR CHAGAS DISEASE PATHOGENESIS AND IMMUNE RESPONSE MODULATION

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Host-parasite coevolution has led to a complex interplay where the immune system often fails to eliminate parasitic infections, such as *Trypanosoma cruzi* (Tc), the causative agent of Chagas Disease (CD). During the acute infection, Tc invades several tissues including the spleen (SPLN) and the adipose tissue (AT), boosting strong inflammatory responses. In these tissues, discrete subsets of macrophages (Ms) are engaged in both the anti-parasitic response and the maintenance of tissue homeostasis. To note, this includes marginal metallophilic Ms (MMMs) from the SPLN, and AT Ms (ATMs). Currently, the emergence of immunometabolism allows us to exploit the interplay between immune effector functions and metabolic pathways. However, information regarding the Ms metabolic fitness during Tc infection is still limited. We hypothesized that Ms exploits specific metabolic pathways like glycolysis (GLY) or lipid metabolism during the tissular invasion of Tc. Hence, we aimed to characterize MMMs and ATMs metabolic programs during the acute phase of infection. We infected C57BL/6 (B6) mice intraperitoneally with 5,000 trypomastigotes (Tulahuen), and the SPLN and AT were processed at 18 days post-infection. Uninfected B6 mice were used as controls. To identify glucose and fatty acid (FA) dependencies of MMMs and ATMs, GLUT1 and CD36 were evaluated by FACS. We found that Tc infection prompted higher frequency (%) of MMMs ($p<0.05$) and ATMs ($p<0.01$) in mice. In addition, both Ms subsets demonstrated higher expression of the GLUT1 glucose transporter ($p<0.01$, and $p<0.0001$, respectively). Notably, the % of Ms expressing the FA transporter CD36 was reduced in the AT ($p<0.05$) but markedly enhanced in the SPLN ($p>0.001$) of the infected group compared to controls. To complement these findings, bone marrow-derived Ms (BMDMs) were infected *in vitro* with Tc, and the metabolic profile was inspected by gene profiling (bulk-RNAseq) and protein expression. With respect to controls, the transcriptional analysis revealed that infected BMDMs up-regulated the expression of GLY-related genes (Hk2, Slc2a1, and Pfkfb3) whereas repressed lipid-metabolic genes (Cd36, Pparg, and Hmgcs1). FACS analysis revealed that Tc infection enhanced GLUT1 ($p<0.0001$), and CD36 ($p<0.01$) protein expression. In addition, we noted elevated L-lactate release in the culture supernatant during Tc infection ($p<0.0001$). In summary, our findings could suggest that Tc infection shapes Ms metabolic fitness towards enhanced GLY, fueling the acute anti-parasitic responses. Moreover, we observed that the expression of CD36 varies based on the specific tissue, underscoring distinct metabolic demands between MMMs and ATMs. In perspective, by identifying immune cell metabolic programs during protective or detrimental responses, we could exploit the cell metabolic machinery to enhance protection or reduce inflammatory damage associated with CD.

103 (7) RESOLVIN D5 PRESENT IN TUBERCULOUS PLEURAL EFFUSIONS PROMOTES A PRO-RESOLVING PROFILE IN MACROPHAGES BY IMPAIRINGHIF1-α IN RESPONSE TO MYCOBACTERIUM TUBERCULOSIS

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Tuberculosis, caused by *Mycobacterium tuberculosis*, remains a major global health problem accounting for millions of deaths annually. Previously, we found that proinflammatory (M1) macrophages exposed to the acellular fraction of pleural effusionsfrom TB patients (TB-PE) displayed a reduced glycolytic activity by targeting the hypoxia- inducible factor (HIF)-1α expression, and ultimately impairing the resistance to infection. Such properties were driven by polyunsaturated fatty acid metabolites within TB-PE fractions. In particular, we found that the abundances of 18-HEPE, 7(R)-Maresin 1, Protectin Dx and Resolvin D5 correlated with the inhibition of M1 macrophages' glycolysis ($p<0.05$). Therefore, in this work we focused on the study of RvD5, exploring its involvement in the regulation of M1 metabolism. For this purpose, human monocyte- derived macrophages were stimulated with LPS/IFN-γ for 24h (M1 profile) in the presence or not of RvD5 at physiological doses found in TB-PE. The metabolic profile was evaluated using the SCENITH method and lactate was measured by enzymatic assays. In addition, IL-1β and IL-10 release was measured by ELISA, and MerTK and HIF-1α expression by FACS. Macrophages were also cocultured with CFSE-labeled apoptotic neutrophils to assess their efferocytic capacity. Finally, macrophages were infected with *M. tuberculosis* and colony forming units were assessed on the third day ofinfection.

We found that RvD5 was able to inhibit glycolysis by decreasing lactate release, glycolyticcapacity and HIF-1α expression when added to M1 macrophages ($p<0.05$). Furthermore, RvD5-treated M1 macrophages showed lower IL-1β secretion and a reduced Mtb clearance capacity ($p<0.05$) as well as elevated IL-10 production, increased MerTK expression and elevated efferocytic activity compared to M1 cells ($p<0.05$). Interestingly, stabilization of HIF-1α obtained after dimethyloxalylglycine treatment restored the alterations in lactate release, microbicidal activities and efferocytosis observed in RvD5- treated M1 macrophages ($p<0.05$). In summary, these results indicate that the omega-3-derived lipid RvD5 is able to reprogram the proinflammatory profile of M1 macrophages towards a more pro-resolving profile and with a reduced ability to clear *M. tuberculosis* by impairing HIF-1α expression. We have yet to elucidate the molecular mechanisms involved in this phenomenon, which could explain the reduced control capacity of pleuralmacrophages in TB.

104 (37) SEXUAL DIMORPHISM IN THE IMMUNOMETABOLIC PROFILE OFHOFBAUER CELLS

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Aims: Hofbauer cells (HB) are the only foetal immune cell population within the stroma of healthy placenta. However, the functional properties of these cells are poorly described. HB are transcriptional and chromosomically similar to yolk sac macrophages. Here we studied the effect of sexual dimorphism in the immunometabolism of human placental HBat term.

Methodology: HB cells were obtained from placental tissue by enzymatic digestion from healthy pregnant women at term along with autologous PBMCs. HB were cultured overnight and the phenotypic profile, efferocytosis, glucose uptake and lipid droplets accumulation were evaluated by flow cytometry and ELISA. We have started with bioinformatic analysis in public database to understand the differential imprinting of placentas with male or female newborns.

Results: HB from female placenta (fHB) present higher production of antiinflammatory markers CD163, CD206 and CD39 accompanied by lesser secretion of IL-1□ than HB from male placentas (mHB) (*P<0.05). Metabolically, fHB incorporate less glucose, secrete less lactate and accumulate more lipid droplets than mHB (P<0.05),consistent with a lower glycolytic activity. fHB present 2.5 times more efferocytosis than mHB (P<0.05), all factors associated with an alternative profile.

Conclusions: These results support a differential immunometabolic regulation in male vs. female HB, with a stronger antiinflammatory profile on fHB.

105 (53) THE INHIBITION OF HIF-1α LIMITS THE MIGRATION OF DENDRITIC CELLS TO LYMPH NODES IN BCG-VACCINATED MICE

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Dendritic cells (DCs) are key players in the host response to the tuberculosis (TB) agent, *Mycobacterium tuberculosis* (Mtb), which has been shown to interfere with DC functions, delaying the onset and development of adaptive immunity. Given our prior work demonstrating the importance of HIF-1α-mediated glycolysis in the migration of Mtb-stimulated DCs into lymph nodes, herein we wondered whether the inhibition of HIF-1α in bone marrow derived-DCs (BMDCs) could have an impact on their migration to lymph nodes, limiting the induction of the adaptive immunity driven by the Bacillus Calmette-Guérin (BCG) vaccine strain.

First, we evaluated the metabolism of BCG-infected BMDCs from C57Bl/c mice at a single cell level using SCENITH technology. This technique revealed a lower reliance on OXPHOS in parallel with an increase in the glycolytic capacity of BCG-infected BMDCs compared to uninfected cells ($p<0,05$). We measured lactate release (the end product of glycolysis) by enzymatic assays in the supernatants of BMDCs infected or not with BCG for 24h. BCG-infected BMDCs released increased levels of lactate in comparison to uninfected BMDCs, and this increase was abolished by using PX-478 (PX), a HIF-1α inhibitor ($p<0,05$). Thus, BCG vaccine strain induces glycolysis in a HIF-1α dependent manner in BMDCs.

To investigate the effects of glycolysis in BMDCs migration, BMDCs were infected with BCG in the presence or absence of PX and labelled with either CTV or CFSE. Next, a mix of both labelled populations was transferred into naïve mice. PX treated-BCG-infected BMDCs were less efficient at reaching the lymph nodes compared to untreated BCG-infected BMDCs ($p<0,05$). Therefore, HIF-1α is required for an efficient migration of BCG-infected BMDCs to lymph nodes.

To determine whether a diminished migration of BMDCs to lymph nodes could limit the induction of the BCG-driven Th1 immune response, we inoculated BCG-infected BMDCs treated or not with PX into naive mice. After 7, 19 or 30 days, spleens were collected and splenocytes were stimulated in-vitro with mycobacterial antigens (purified protein derivative or PPD) for 24h. IFN-g -producing cells were assessed by flow cytometry. On day 19, a higher number of CD3+ CD4+ IFNγ-producing cells were detected in cultures from mice that received BCG-infected BMDCs compared to those treated with PX ($p<0,05$). Nevertheless, at later times, no differences were observed. Therefore, this data indicates that the inhibition of HIF-1α in BCG-infected BMDCs leads to lower numbers of IFNγ-producing CD4+ lymphocytes specific for mycobacterial antigens at earlier time points.

Taken together, our data provide new insights into the involvement of metabolic pathways in DC trafficking to lymph nodes. The development of tools aimed at enhancing the migratory capacity of DCs by promoting their HIF-1α-mediated glycolytic activity may increase the efficacy of TB preventive strategies.

106 (111) THE PRESENCE OF PLASMID pYV IMPACTS ON THE IMMUNOMETABOLIC RESPONSE OF *YERSINIA ENTEROCOLITICA* INFECTION

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Yersinia enterocolitica (Ye) is a zoonotic pathogen that causes bacterial gastrointestinal infections in humans. The virulence plasmid, termed pYV (plasmid of*Yersinia* virulence) encodes a type III secretion system essential for the delivery of anti-host virulence factors collectively known as Yops (*Yersinia* outer proteins). Although the majority of infections are self-limiting, immunocompromised hosts have higher risk of developing significant disease. Immunometabolism is a key mechanismto develop both adaptive and innate immune responses by triggering functional and metabolic reprogramming in immune cells. However, the role of pYV in metabolic reprogramming remains unclear. Here, we examined the relationship between the presence of pYV with the immune and metabolic responses during the course of Ye infection *in vivo* under normal and TNFR1-deficiency conditions. C57BL/6 wild-type (WT) or TNFR1-deficient (KO) mice were orally infected with Ye serotype O:8 plasmid bearing (pYV+), or plasmid cured (PC) WAP strain by gavage. Body weight, food intake, glycemia, cholesterol and serum TNF levels were evaluated during the courseof the infection. Cell recruitment was analyzed by flow cytometry. Ye O:8 pYV+ infected WT and KO mice exhibited significant weight loss ($p<0.05$) and a decrease infood intake ($p<0.001$), compared with noninfected control mice. Ye O:8 PC-infected mice showed no changes in either weight nor food intake. Ye O:8 PC infection induced serum TNF overproduction in KO mice on day 3 after infection ($p<0.001$) but lower TNF levels in WT mice on day 5 ($p<0.05$), compared to noninfected mice. WT mice developed hypoglycemia on day 1 post-infection with Ye O:8 pYV+ ($p<0.05$) while KO mice started hypoglycemia on day 3 ($p<0.001$). Meanwhile, Ye O:8 PC-infected KO mice showed a slight decrease in blood glucose on day 1 post-infection ($p<0.05$) that was followed by hyperglycemia on day 3 ($p<0.001$). Serum cholesterol levels increased in both WT ($p<0.05$) and KO ($p<0.01$) mice after Ye O:8 pYV+ infection. Thenumber of neutrophils, macrophages, lymphocytes, and dendritic cells in the spleen was higher in Ye O:8 pYV+ infected KO mice ($p<0.01$). Our results show that pYV-encoded-virulence factors impact on glucose and cholesterol homeostasis, cytokine production and immune cell recruitment, suggesting an immune-metabolismregulation during Ye infection, and the participation of Yops in this regulation. Furtherexperiments will allow us to elucidate the specific role of Yops on the metabolic reprogramming of immune cells in this infection.

107 (144) UNRAVELING METABOLIC AND MITOCHONDRIAL PATHWAYS IN *T. CRUZI*-SPECIFIC CD8 EFFECTOR T CELLS: EXPLORING POTENTIAL INTERVENTIONS

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*Trypanosoma cruzi*causes Chagas' disease, endemic in Latin America and globally present. The infection involves an acute phase (AP) with high parasitemia that transitions to chronic stage, potentially leading to Chagasic pathology. CD8 T cells are crucial for host resistance, but *T. cruzi*-specificCD8+ T cell (Tc-CD8) immunity develops slowly, with reduced breadth and may acquire dysfunctional features. Mitochondria plays a key role in CD8 T cell immune response. Mitochondrial membrane potential (MMP) and mitochondrial superoxide (mROS) levels impact in effector function. Our previous results showed an increase in total CD8 T cells with depolarized mitochondria (DM) and mROS during AP of BALB/c infection. Then, the aim of this study was to improve mitochondrial quality on CD8 T cells and explore its impact on infection. To achieve this, Nicotinamide Riboside (NR), a precursor to NAD+ and mitophagy inducer was used. BALB/c mice were infected (I) with 500 trypomastigotes from Tulahuen strain (tp-Tul). Non infected (NI) animals were used as controls. Mice were treated with NR (500 mg/kg/day by oral gavage) or PBS from 5-20 days post infection (dpi) (I-NR or I-PBS). Spleen cells were isolated at 21 dpi. We examined mitochondrial mass (MM), MMP and mROS production by FACS combining MMP-dependent, MMP-independent mitochondrial dyes and MitoSOX respectively. We observed that I-NR mice with lower parasitemia levels exhibit better biochemical parameters in plasma. They also displayed a tendency to decrease in the frequency of effector (E) CD8+ T cells with DM and mROS production. Likewise, I-NR mice exhibited a higher frequency of IL-2-producing E CD8+ T cells. Moreover, we aimed to extend our investigation of mitochondrial metabolism to Tc-CD8 T cells. Then, C57BL/6 mice were inoculated with 5000 tp-Tul. Spleen cells were obtained at 9, 20 and 78 dpi. Tc-CD8 T cell response was measured by FACS using tetramers loaded with the parasite immunodominant peptide Tskb20 in E cells. Moreover, glucose uptake through 2-NBDG probe and expression of the glutamine transporter CD98 was assessed by FACS. We observed that the peak of parasitemia correlates with a higher number of total and E Tc-CD8 cells. These cells showed an increase in MMP at 9 dpi ($p=0,05$) while mROS production and MM were higher at 20 dpi ($p<0,05$; $p<0,0005$) and these parameters decreased at 78 dpi. Besides, glucose uptake and CD98 expression were increased at 20 dpi ($p<0,0005$; $p<0,05$). However, at this point of infection a high proportion of Tc-CD8 T cells lose MMP ($p<0,05$). These Tc-CD8 T cells with DM showed less glucose uptake and reduced CD98 expression ($p<0,0001$; $p<0,05$). Our findings indicate that while Tc-CD8 T cells engage metabolic and mitochondrial programs during infection, further research could shed light on novel pathways that modulate the induction, maintenance, and regulation of CD8 T cell responses to *T. cruzi.* This insight might guide new strategies to improve infection outcomes.

Autoimmunity
Friday, November 10, 14-15:30h
Chair: *Virginia Rivero - Laura Pérez*

108 (52) TOLEROGENIC SIGNALS DELIVERED BY KERATINOCYTES TO LANGERHANS CELLS SUPPRESS SKIN INFLAMMATION THROUGH A GALECTIN-7-DRIVEN CIRCUIT

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Background: Infiltrating and resident immune cells, including keratinocytes (KCs), Langerhans cells (LCs) and T cells orchestrate skin inflammation but also contribute to restore homeostasis in response to external and internal insults. Psoriasis, a chronicinflammatory skin disorder is triggered by an imbalance of these immune cells, promoting polarization toward Th1 and Th17 responses. However, the molecular mechanisms leading to the establishment of tolerogenic circuits remain uncertain.
Objective: To investigate the role of Galectin-7 (Gal7), β-galactoside-binding proteinabundantly expressed in injured skin, in orchestrating skin homeostasis during psoriatic inflammation.
Methods: Mice lacking Gal7 (Lgals7$^{-/-}$), overexpressing Gal7 in KCs (Tg46) or WT were subjected to psoriasis-like dermatitis by daily topical application of 5% imiquimod (IMQ) for 1 week. Skin samples were then processed for FACS, histological, and transcriptomic analyses. CD207-DTR-GFP/Tg46 mice, displaying high Gal7 levels and the ability to eliminate LCs, were subjected to LC depletion followed by IMQ. LCswere obtained by differentiation of CD34^{+} cells isolated from the bone marrow of B6 mice.
Results: Overexpression of Gal7 in KCs in Tg46 mice resulted in milder skin inflammation compared with Lgals7$^{-/-}$ and WT mice with psoriatic-like disease, as evidenced by less epidermal thickening, an increased frequency of regulatory T cells(Treg), a lower proportion of CD8+T cells, and a higher Foxp3/CD8 ratio in skin- draining lymph nodes, spleen and skin compared with WT and Lgals7$^{-/-}$ mice. In selected experiments, after one week of IMQ treatment, WT and Lgals7$^{-/-}$ mice were treated with IMQ and rGal7 cream or control cream every other day. Interestingly, treatment of Lgals7$^{-/-}$ mice with rGal7 improved skin inflammation reflected by decreased skin thickness.
Remarkably, despite overexpression of Gal7, depletion of LCs abrogated the anti-inflammatory effect of this lectin after induction of psoriatic-like disease, as evidencedby increased skin thickness in CD207-DTR-GFP/Tg46 mice which was comparable tothat of WT mice, decreased frequency of Tregs and enhanced CD8^{+}T cells in the skin,dLN, and spleen of LC-depleted mice.
In vitro experiments showed higher binding of Gal7 to the surface of activated vs non-activated LCs and augmented secretion of immunosuppressive cytokines, (IL-10 and IL-27) and increased expansion of the Treg compartment. Similar results were observed in the differentiation of human LCs from monocytes in the presence of Gal7and confirmed by bioinformatic analysis of human psoriasis databases.
Conclusion: During skin inflammation, KCs can reprogram the LCs immunoregulatory activity through glycosylation-dependent, Gal7-driven

mechanisms, thus promoting Treg cell expansion and resolution of psoriatic-like disease. Gal7 topical administrationmay contribute either alone or combined with other anti-inflammatory strategies to mitigate inflammatory skin disorders.

109 (126) CHARACTERIZATION OF CCR8+ REGULATORY T CELLS IN RHEUMATOID ARTHRITIS

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Rheumatoid Arthritis (RA) is a chronic autoimmune inflammatory disease characterized by joint destruction. In RA, immunoregulatory mechanisms mediated by Foxp3+ regulatory T cells may play fundamental roles but are poorly explored. The Treg compartment is heterogeneous, comprising several specialized subpopulations. Among them, CCR8+ Treg cells show enhanced suppression capacity and tissue repair functions that endows them with a detrimental role in cancer. The role of specialized Treg subsetsin RA is unknown. We aimed study the phenotypic and functional characteristics of CCR8+ Treg cells in RA patients determining their protective or pathogenic role.

We studied 53 patients diagnosed with RA according to ACR/EULAR 2010 classification criteria at the Hospital Nacional de Clínicas de Córdoba (24 untreated, 17 with syntheticDMARDs, 12 with biological DMARDs). Thirty healthy individuals age- and sex- matchedwere recruited as controls (HD). RA activity was assessed by the DAS28 index. Frequencies of Treg cells (CD3+CD4+CD25+FoxP3+) and CCR8+ Treg cells was measured by flow cytometry in cryopreserved mononuclear cells isolated from peripheralblood and synovial fluid (SF). Phenotypic characterization of CCR8+ Treg cells includedthe expression of regulatory molecules (CTLA-4, TIGIT, ICOS, CD39, and CD73) and tissue repair profile (GATA-3). Serum levels of CCL1, the CCR8 ligand, were measured by ELISA.

We found that Treg cell frequency was slightly but significantly higher in the PBMC from patients versus controls (RA 4,41 ±1,81 vs HD 3,72 ± 1,30; p 0.046), while the frequencyof the CCR8+ subset was similar between the two groups (RA 6.94 ± 3.77 vs HD 7.14 ±2.99). Phenotypic characterization showed similar expression of CTLA-4, TIGIT, ICOS, CD39, CD73, and GATA-3 between CCR8+ Treg cells of RA and HD. Also serum levelsof CCL1 were similar in RA and HD, and showed no correlation with the frequency of peripheral CCR8+ Treg cells. An initial functional evaluation determined lack of correlation between the percentages of Treg or CCR8+ Treg cells and the expression ofIL-2, TNF, CD107, and CD57 in memory CD4+ and CD8+ T cells in RA and HD. Accordingly, percentages of Treg and CCR8+ Treg cells in RA showed no correlation with DAS28. Of note, CCR8+ Treg cells appeared to be increased in SF, where their frequencyranged between 18.5 and 39.4, (n=4).

Altogether, we showed that, despite exhibiting increased peripheral Treg cells, RApatients present a conserved CCR8+ Treg cell frequency. No correlations were found between Treg cells and CCR8+ Treg cells frequencies and production of immune effectormediators or overall disease scores. The higher occurrence of CCR8+ Treg cells in LS suggests a likely preferential migration and enrichment in tissues. A deepercharacterization of the CCR8+ Treg cell population in RA

patients, including functional and transcriptomic evaluation, would allow us to better delineate the possible function ofthis subset in RA.

110 (90) CHICKEN TYPE II COLLAGEN-INDUCED ARTHRITIS IN MICE.EXPERIMENTAL MODEL FOR AUTOIMMUNE DISEASE STUDY

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Background: Rheumatoid arthritis is a progressive and chronic process characterized by systemic and local inflammation that generates damage, articular destruction, and pain. The aim of this study was to evaluate and characterize the arthritic process induced by purified chicken type II collagen (CII) in mice.

Methods: different conditions of CII isolation and purification protocols were optimized. Once CII was purified and well-characterized, it was used to induce the arthritic process in adult C57BL/6 mice (12 week-old). Mice received an intradermal injection in the tails ofan emulsion of complete Freund adjuvant (CFA) and purified CII (CFA+CII) to develop acollagen-induced arthritis (CIA). Control mice (C) were injected with saline solution. The establishment and progression of arthritis were evaluated for 45 days after injection. Body weight and swelling measurement (paws and ankles) were studied as clinical parameters.At 31 and 45 days post-injection (dpi), samples were withdrawn to evaluate total and differential leukocyte counts, neutrophil myeloperoxidase activity, platelet counts, IgG andsubtypes (IgG1 and IgG2a) in peripheral blood, and histological slides analysis from paws.

Results: chicken CII was successfully purified and characterized by sodium dodecyl sulphate-polyacrylamide (SDS-PAGE) gel electrophoresis, ultraviolet absorption, Fourier Transformed-InfraRed (FT-IR) spectroscopy, and circular dichroism. In mice, CII+CFA induced significant swelling in paws (31 dpi, right paw, C=2.18 mm, CIA=2.98, p=0.0014)and ankles (31 dpi, right ankle, C=2.89 mm, CIA=4.27, p<0.0001), without significant bodyweight alterations. Arthritic process was characterized by local articular changes with inflammation demonstrated by hematoxilin-eosin histological study, significant decrease of total leukocyte and lymphocyte counts in peripheral blood, but increased neutrophils. In addition, myeloperoxidase activity in blood neutrophils was significantly decreased. No changes in platelet counts were observed in arthritic mice respect to control.

Conclusions: purified and well-characterized chicken CII induced arthritis in C57BL/6 mice demonstrated by evaluated parameters. Taking into account that autoimmune arthritis experimental mouse models are widely used because of their similar characteristics to those observed in arthritic patients, setting up a CIA mouse model wouldbe a useful tool for evaluation of involved mechanisms in this autoimmune disease.

111 (17) DESMOGLEIN-4 DEFICIENCY PROMOTES ANTI-DNA AUTOANTIBODIES IN RESPONSE TO IMIQUIMOD

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Desmogleins (Dsg) are transmembrane proteins involved in cell-cell junction. Keratinized epithelia, such as skin, expresses several forms of Dsg. Dsg4 deficiency is associated with hair loss in humans, mice, and rats. Recently, we have reported that topical administration of imiquimod (IMQ) to Dsg4 deficient rats exacerbates skin inflammation. Unfortunately, the role of Dsg4 in the induction of IgG humoral immunity has not been addressed. Moreover, the role of certain Dsg, such as Dsg4, in modulatinglymphatic drainage in response to local inflammation has yet to be studied. Lastly, whether Dsg4 deficiency may favor autoimmune disease is entirely unknown. Our work aimed to determine whether Dsg4 deficiency affects the induction of an antigen-specific immune response and anti-DNA autoantibodies development in response to imiquimod. For this purpose, Dsg4 deficient Oncins France Colony A hairless/hairless (Dsg4 null) and wild-type Sprague-Dawley (SD) rats were inoculated intradermally with OVA or PBS, and imiquimod cream was subsequently administered topically for three consecutive days in dorsal skin. Two weeks later, serum samples were obtained to determine OVA-specific IgG levels by ELISA assays. Additionally, Dsg4 null and SDrats were chronically administered thrice weekly for twelve weeks to evaluate the induction of anti-DNA antibodies. Surprisingly, Dsg4 null rats showed lower OVA- specific IgG and IgG2a levels than the SD group. By contrast, Dsg4 null rats displayed higher IgG natural antibodies against the dinitrophenyl group but similar natural IgM anti-LPS levels compared to control SD rats. When we evaluated brachial lymph node expansion after topical administration of IMQ we observed that Dsg4 null rats showed increased brachial lymph node weight compared to SD rats. Additionally, anti-DNA antibodies from Dsg4 null rats treated with IMQ chronically exhibited significantly higher levels than SD-treated rats. By contrast, spleens size and brachial lymph nodes from chronically treated Dsg4 null and SD rats were enlarged similarly. These results suggestthat Dsg4 may contribute to the induction of a specific humoral immune response. Several mechanisms may be involved in the altered Ig response of Dsg4 null rats, including alterations in lymphatic drainage, keratinocyte-derived cytokines, fine crosstalkbetween keratinocytes and dendritic cells, and germinal center reaction. Additionally, the higher induction of anti-DNA autoantibodies suggests that Dsg4 deficiency unleashes an autoimmune susceptible condition. Although further investigations are necessary, our results suggest a novel role for Dsg4 in supporting humoral immunity and immune tolerance under inflammatory conditions.

112 (91) EVALUATION OF SEROTONYLATION INHIBITION IN A MURINE MODEL OFREACTIVE ARTHRITIS

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Reactive arthritis (ReA) prone mice have higher basal serotonin (5-HT) serum levels, as we have previously observed. Serotonin can regulate innate and adaptive immune functions; accordingly, selective serotonin receptor inhibitors (SSRIs) such as fluoxetine that modify 5-HT availability also have an anti-inflammatory effect. On this line, fluoxetinereduces the severity of ReA in our murine model. This outcome could be related to reduced 5-HT availability and transglutaminase-2 (TG2)-dependent pos-traduction modifications known as serotonylation. Nevertheless, the mechanism involved is still unknown. Thus, in this work, we aim to evaluate the impact of serotonylation blockade in the development of reactive arthritis (ReA). In our study model, mice deficient in TNF receptor 1 (TNFR1 KO) develop ReA as a sequela after infection with *Yersinia enterocolitica* (Ye). Thus, TNFR1 KO mice were orally infected with Ye O:3 (1-5x10^8 bacteria/mouse) and divided into four groups. Group 1 received water (control) in the drinking bottle, Group 2 received fluoxetine, Group 3 cysteamine (TG2 inhibitor) and Group 4 received the combined treatment (fluoxetine and cysteamine) administrated in the drinking water. The mice that survived the infection developed ReA. The incidence and clinical score were periodically recorded, considering the inflammation of the four legs. On day 21, the mice were euthanized, and flow cytometry was performed from blood and draining lymph node samples. DCs, macrophages, neutrophils and T cell infiltrates were evaluated in the samples. Finally, one-way ANOVA and linear regression was used to determine statistically significant differences. Our results indicate that chronic inhibitionof TG2 with cysteamine and the serotonin reduction by fluoxetine attenuates ReA severitysimilarly in TNFR1 KO mice. In addition, the combined treatment not only reduces the clinical score but also delays the onset of ReA and reduces the incidence (p=0.0004). Importantly, at day 21 post-infection, a reduction in neutrophils and DCs infiltrate was observed in the joint draining lymph nodes of treated mice compared with control mice (p=0.027). Besides, a significant positive correlation was detected between neutrophil infiltration and clinical score (p=0.026, r=0,29). On the other hand, a negative correlation between T cell infiltration and ReA clinical score was also observed (p=0.032, r=0.33). To conclude, the inhibition of serotonylation by reduction of serotonin levels and TG2 activity attenuates the ReA severity in the murine model, decreases the neutrophils and DCs migration and enhances the T cells infiltration of lymph nodes. Our results suggest that the increment in serotonylation events under inflammatory contexts could be involved in the establishment and maintenance of a chronic condition. On the same line, SSRIs andTG2 inhibitors could be proposed as a therapy to reduce ReA severity.

113 (94) NOVEL IMMUNOASSAY BASED IN FLOW CYTOMETRY FOR THE SIMULTANEOUS AND DISCRIMINATIVE DETECTION OF THE MAIN AUTOIMMUNEMARKERS IN DIABETES MELLITUS

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Type 1 Diabetes Mellitus (T1DM) is characterized by the presence of autoantibodies against different beta cells structures: insulin or proinsulin (IAA / PAA), the 65 kDa isoform of glutamic acid decarboxylase (GADA), the protein tyrosine phosphatase-related IA-2 molecule (IA-2A) and the 8 isoforms of the Zn transporter (ZnT8A). Its detection is a useful tool for the diagnosis of T1DM, enabling the timely applications of appropriate treatments. The reference method to detect these autoantibodies is the radioligand binding assay (RBA) a radiometric method, highly sensitive and specific, butexpensive, with environmental impact and limited to specialized laboratories. The aim ofthis work was to develop an immunoassay based on flow cytometry for the combined and discriminative detection of GADA, IA-2A and ZnT8A.

Materials and methods: 30 serum samples of pediatric patients, recently diagnosed withT1DM, and 41serafrom normal control individuals were assayed. A double bridge model was employed incubating the samples with a mixture of polystyrene microspheres of 4,5 and 7 □m each one adsorbed with a recombinant autoantigen -Trx-GAD, Trx-IA-2 orTrx-ZnT8, respectively- and the corresponding biotinilated proteins. After an over-nightincubation at 4 °C in an end-over-end shaker, the immunocomplexes formed weredetected using Streptavidin-Ficoeritrin, and then acquired in a flow cytometer. The dataobtained for each sample was analyzed with the Cyflogic software and the results wereexpressed as Standard Deviation scores. All the samples were also evaluated by RBA. Results: Among the 30 patients evaluated, GADA positivity was observed in 22 (73%),IA-2A in 28 (93%), and ZnT8A in 24 (80%). In comparison to the RBA, the alternativemethod demonstrated analytical sensitivities of 88% for GADA (22/25 RBA-positivecases), 92% for IA-2A (23/25 RBA-positive cases), and 78% for ZnT8A (22/28 RBA-positive cases). The specificity was 93% for GADA and ZnT8A, and 90% for IA-2A. Thearea under the ROC curves were 0.869 for GADA, 0.930 for IA-2A, and 0.844 forZnT8A. These values indicate that the method presents exceptional capacity todistinguish between the two groups under study. Moreover, the novel method was ableto detect at least one autoimmune marker in all samples examined, achieving an overall sensitivity of 100%. In comparison, the cumulative sensitivity of the three markers individually assayed using RBA was 97%.

Conclusions: The developed method described herein is a useful tool for the simultaneous and discriminative detection in a single analytical act of the different T1DM markers. This method represents an operative advantage over the determination of each marker individually by RBA, reducing costs, time and being environmentally friendly. Additionally, with this approach it is possible to combine the detection ofautoantibodies present in other related autoimmune diseases, such as celiac disease orthyroid autoimmune disease.

114 (101) TLR-7 ACTIVATION INDUCES LUPUS-LIKE PHENOTYPE IN C57BL/6 MICE: A STUDY ON THE IMMUNOLOGICAL CONSEQUENCES OF CHRONIC IMIQUIMOD EXPOSURE

Fernando Nicolas Ferreyra[1], María Sol Martínez[1], Yair Aron Chocobar Torres[1], Carolina Olivera[1], Daniela Andrea Paira[1], Rubén Darío Motrich[1], Virginia Elena Rivero[1]

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The critical role of Toll-like receptors (TLR) in the pathogenesis of Systemic Lupus Erythematosus (SLE) has been the subject of diverse investigations over the past decade.Multiple studies have elucidated that abnormalities in TLR signaling play a pivotal role in the pathogenesis and exacerbation of SLE. Recently, the involvement of TLR-7 in the etiopathogenesis of SLE has gained significance. Since TLR7-signaling strongly induces the production of interferon alpha, which is able to enhance innate and adaptive immune response, we applied topically imiquimod to C57BL/6 mice over an 8-week period and after that analyze different parameters indicative of lupus disease induction. Anti-DNA and anti- histone antibodies were tested by ELISA and splenic lymphocyte populations were evaluated by flow cytometry technique. Additionally, leukocyte infiltration, histological changes and renal functionality were evaluated.

Imiquimod treated mice exhibited splenomegaly and a significant increase in spleen cellularity ($p< 0.0003$) when compared to vehicle control group. Analysis of spleen T and B cell populations showed significant changes between imiquimod and vehicle treated mice. Indeed, higher levels of CD3+CD4+, CD3+CD8+ activated T cells expressing CD69marker was observed in Imiquimod treated mice ($p< 0.009$). Regarding B cells, a significant increment in B220+CD69+ cell levels was observed ($p<0.03$). In addition, higher proportions of B220+IL-10+ regulatoryB cells ($p< 0,0002$), B220+CD11c+Tbet+ age-associated B cells ($p>0,0009$); and CD19+ CD138+ CMHCII+ plasma cells ($p> 0,005$) were observed, reflecting a substantial activation of the adaptive immune system in imiquimod treated mice. Imiquimod treatment also induced the production of antibodies specifically associated with SLE. Indeed, elevated levels of IgG against double-stranded DNA and IgG against histones were detected in serum samples at 4 weeks and continued to increase at 8 weeks of treatment ($p<0.02$). Significant changes were also observed at renal level between vehicle and Imiquimod treated mice, with significant increments in CD45+ ($p<0.002$), CD3+CD4+ ($p<0.003$), CD3+CD8+ ($p<0.003$) and B220+ ($p<0,03$) cells infiltrating the organ. In addition, higher proportions of age-associated B cells were detected in Imiquimod treated ($p<0.008$). When serum urea and creatinine levels were measured, we found a statistically significant increment in Imiquimod treated mice suggesting the onset ofrenal damage.

Taken together our results provide a clear overview of the immunological repercussions of the chronic TLR-7 activation and not only validates a murine model of SLE induced bya TLR-7 agonist but also underscores the immunological disturbances and the onset of renal injury in C57BL/6 mice. This research broadens our understanding of TLR-7's role in SLE and paves the way for future studies.

Primary immunodeficiencies
Friday, November 10, 14-15:30h
Chair: Laura Pérez

115 (202) INBORN ERRORS OF IMMUNITY: DISCOVERY OF NOVEL VARIANTSAND GENES INVOLVED IN THEIR DEVELOPMENT

Lorenzo Erra[1], Franco G. Brunello[1], Daiana Flores[1], AINCA[2], Marcelo Martí[1], M. Belén Almejun[1] *[1]Departamento de Fisiología, Biología Molecular y Celular (DFBMC) e Instituto de Química Biológica (DQB-IQUIBICEN), FCEN, UBA, CONICET, Buenos Aires, Argentina. [2]Asociación de InmunologíaClínica Argentina.*

Patients with Inborn Errors of Immunity (IEI) have monogenic defects that lead to immune system dysregulation phenotypes. Advances in molecular genetics and the use of next-generation sequencing have led to the identification of an increasing number of IEI associated genes.
The overall objective of this study is to improve the genetic diagnosis of patients with IEI through comprehensive analysis of exome data performed by our laboratory. Molecular diagnosis is complex because the same clinical phenotype can be caused by mutations in different genes. Additionally, different pathogenic variants in the samelocus can cause different forms of IEI.
By the end of 2022, in collaboration with AINCA, we conducted massive sequencing on 100 index cases of Argentine patients with IEI and neurological manifestations fromdifferent healthcare centers across the country. Bioinformatic analysis of the exome data was performed (fastQ processing, variant prioritization, and association with clinical phenotype). Following ACMG classification standards, this analysis revealed: 18.0% of cases had potentially pathogenic variants in the genes BCL11B, RUNX1, TNFRSF13B, AICDA, while 40.7% had variants of uncertain significance. Within this 40.7%, there are previously reported genes (MAGT1, MSH6, POLE, INO80, TBX1, MST1, RELA, LRBA, RHOG, JAK3, STAT3, CARMIL2, NLRP1), as well as new candidates.
We evaluated the coding exonic regions and the exon-intron junctions of genes linkedto, or indirectly affect, the immune system function. We also analyzed the copy numbervariations (CNVs). Our results showed that 77% of the identified variants are located in the coding region while 23% in regulatory regions, CNVs or genes related to epigenetic regulation.
This work highlights the importance of a comprehensive analysis of candidate variants directly impacting the percentage of variants found in this cohort compared to the literature for IEI. These results remark the value of collaborative work betweenresearchers and clinical physicians to carry out the NGS analyses. Our findings furthersupport the heterogeneity in monogenic defects of the immune system, emphasizing the non-redundant and fundamental functions of individual genes and proteins in the development and function of host defense.

General Clinical Immunology
Friday, November 10, 14-15:30h
Chair: Laura Pérez

116 (137) RISK EXPOSED: EXAMINATION OF *C. DIFFICILE* INFECTION RISK FACTORS IN THE NOBA REGION

Nicolás D. Moriconi[1], Angela M. Barbero[1,2], Sabina Palma[1,2], Gabriel Erbiti[3,4], Carlos Altamiranda[4], Martina Calvo Zarlenga[4], María G. Balbi[5], Mónica Machain[5], María Guadalupe Martínez[5], Jorgelina Suárez[5], Rodrigo E. Hernandéz Del Pino[1,2], Virginia Pasquinelli[1,2]
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Clostridioides difficile infection (CDI) is the leading cause of antibiotic-associated diarrhoea. CDI represents a nosocomial infection ranging from an asymptomatic carrier state or mild diarrhoea to the severity of pseudomembranous colitis and ultimately death. The administration of antibiotics is a widely recognized CDI risk factor, attributable to disruption of the commensal microbiome. Advanced age and coexisting conditions such as diabetes, chronic kidney disease, and cardiopulmonary diseases have also been linked to CDI due to the need for hospitalization they entail.Besides, the suppression of gastric acid may facilitate the persistence of *C. difficile* spores, potentially amplifying the risk of CDI in patients using proton pump inhibitors.Since the information on CDI in Argentina remains both scarce and heterogeneous, inthis study we aim to characterize risk factors and demographic patterns linked to CDIin hospitalized patients. A cohort of 200 patients with gastrointestinal symptoms and diarrhoea from the Northwestern Region of Buenos Aires (NOBA) were evaluated. Thepresence of *C. difficile* within stool samples was ascertained by an algorithm that includes 3 tests (EIA, PCR, and toxigenic culture), accompanied by an exhaustive analysis of the patients' medical records. Clinical and demographic characteristics were evaluated employing the Mann-Whitney test or the exact Fisher's test, as appropriate for each variable. Additionally, a multivariate logistic regression was performed to identify independent risk factors. The obtained results were contrasted against findings of other 39 studies through a meta-analysis. All statistical analyses were conducted using RStudio, considering the CDI negative patients as the control group.

CDI positive patients showed an increased number of total leukocytes, neutrophils, monocytes, lymphocytes, basophiles, and platelets, suggesting that these immune cells count as predictors of CDI risk ($p<0.05$). The meta-analysis demonstrated that advanced age, antibiotics or proton pump inhibitors use in the previous 3 months, leukocyte and platelet counts, along with the presence of comorbidities, emerged as significant CDI risk factors ($p<0.05$).

The implications of our findings allowed for a comprehensive clinic-demographic characterization of patients within Sanitary Region III. This information could be valuable for epidemiological decision-making regarding CDI surveillance, facilitating the implementation of robust prevention and control measures. Moreover, in light of the emerging status of antibiotic resistance as one of the major threats to global healthas stated by the WHO, our study could contribute to the monitoring of antibiotic- resistant infections.

117 (195) *TRYPANOSOMA CRUZI* INFECTION AS AN UNDERLYING FACTOR CATALYZING THE ONSET OF HEMODYNAMIC PERTURBATIONS AND CARDIOVASCULAR DISEASES

Ximena Volpini[1,2], Juan Nahuel Quiroz[1,2], Melisa Rocío Herrera[1,2], María Florencia Hellrriegel[1,2], María Belén Brugo[1,2], Melina Musri[3], Laura Fozzatti[1,2], Cinthia Stempin[1,2], Ana Rosa Perez[4], Claudia Cristina Motrán[1,2].

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According to the World Health Organization, cardiovascular diseases (CVD) are the leading global cause of death. Chagas disease (CD), caused by *Trypanosoma cruzi* (TC) infection, is considered the primary cause of death due to infectious cardiomyopathy. Historically, seropositive patients without discernible symptoms of cardiomyopathy have been grouped under the "indeterminate" classification. This categorization has perpetuated the lack of care of ~70% of CD patients. By the measurement of the carotid-femoral pulse wave velocity (cf-PWV), recent research has unveiled that indeterminate patients manifest increased aortic stiffness (IAS), theprimary risk indicator of developing CVD. To contribute to the current extent of knowledge of TC infection as a factor in triggering CVD and hemodynamic disorders, we have evaluated “residual” hemodynamic and metabolic risk factors in infected miceand CD-patients, respectively.

To study hemodynamic risk factors associated with vessel alterations, Balb/c mice were infected with TC (Tulahuen strain), and the cell populations of thoracic, abdominal, and brachiocephalic aortic segments were analyzed by FACS at differentdays post-infection (dpi), using uninfected mice as controls. In the acute phase of infection (18 dpi), TC induced increased frequency (%) of CD45+ CD3+ ($p<0.0001$) and CD45+ CD11b+ ($p<0.0001$) cells. During both acute and chronic (60 dpi) phases,the infected group exhibited a significant increase of %CD45- αSMA+ population withhigh expression of αSMA+ ($p<0.001$). Interestingly, high α-SMA expression, an IAS-associated mechanism, was also found in CD45+ cells; particularly in CD45+ CD11b+population during the acute phase ($p<0.0001$), and correlating with the % of CD45+ CD11b+ in the chronic ($p<0.01$).

Finally, we focused towards residual metabolic risk factors linked to aortic stiffness. In this regard, we explored the plasma metabolic profile of CD patients using LC-MS. We compared the metabolic profiles between healthy donors and three patient cohorts representing different degrees of CD severity (indeterminate, moderate- cardiomyopathy, or severe-cardiomyopathy). Thus, we identified IAS-associated metabolites that could be potential candidates for differential biomarkers indicative of the cardiovascular and cardiac risk degree in patients with the infection by TC. Taken together, our results support evidence that TC infection may act as an underlying factor catalyzing the onset of hemodynamic perturbations and CVD. Considering that cf-PWV is the gold standard determination of IAS but currently it isonly used in research, our findings promote the importance of contemplating the evaluation of some residual metabolites associated with cardiovascular risk in all TC-infected patients. This would be critical to promote the opportunity to preventcardiomyopathy, but also hemodynamic alterations, and CVD among individualsaffected by TC infection, particularly those categorized as “indeterminate”.

Reproductive Immunology
Friday, November 10, 14-15:30h
Chair: Rubén Motrich

118 (120) BRUCELLA INFECTION AFFECTS FUNCTIONALITY OF DECIDUA STROMAL CELLS AND TROPHOBLASTS

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The ability of trophoblasts to migrate, form tubes and invade the decidua is essential for a successful pregnancy. These processes are modulated by cytokines produced by decidual cells. *Brucella* infections are associated with reproductive complications in humans and animals. We evaluated if *Brucella* can impair functions of decidual cells and trophoblasts that are relevant for a successful pregnancy. The effect of *Brucella* infection on trophoblast migration was evaluated with the *scratch test* using the human trophoblastic cell line Swan71. Wound closure was significantly diminished in trophoblasts infected with *B. abortus* (Multiplicity of Infection 50: 45%; MI 500: 36% vs. 83% in uninfected controls), and similar results were observed with *Brucella suis and Brucella melitensis* infections. Human endometrial stromal cells (THESC) were infected with different *Brucella* species (*B. abortus, B. suis, or B. melitensis*) before or after decidualization with medroxyprogesterone and cAMP, and conditioned media (CM) were used to stimulate Swan71 cells during the *scratch test*. Woundclosure was reduced in Swan71 cells treated with CM from *B. abortus*-infected decidualized THESC as compared to uninfected CM (20% vs. 86%). Similarly, wound closure was inhibited by CM obtained from THESC cells infected before decidualization (8.5% vs. 72.8%). Comparable results were observed with the other strains. Inflammatory signals are known to affect or impact trophoblast functions. Trophoblast migration (wound closure) improved when CM from *B. abortus*-infected THESC at different MI was treated with neutralizing antibodies (Ab) against CXCL8 or CCL2 chemokines before addition to the *scratch test.* Incubation with CM plus anti-IL-8 antibody increased wound closure by 59 and 45 % for MI 50 and 500 respectively vs. CM untreated, while, anti-CCL2 antibody treatment increased 55% and 52% for MI 50 and 500 respectively. Moreover, trophoblast *tube formation assay* was more successful in uninfected controls (NI) being reduced in the presence of infected THESC CM, as revealed by the number of master junctions (NI: 88; MI 50: 33; MI 500: 19), master segments (NI:152; MI 50: 28; MI 500: 46.5 and meshes (NI:56; MOI 50: 16; MOI 50: 20; MOI 500:13.5). In addition, when THESC were infected, the attachment area of trophoblast spheroids (invasion) was affected (NI:0.98; MOI 50: 0.71; MOI 250: 0.53; MOI 500: 0.47). Overall, the normal function of trophoblasts and decidual cells, and cross-talk between them, can be affected by *Brucella* infection.

119 (8) CHRONIC EXPOSURE TO GLYPHOSATE BASED-HERBICIDES IMPAIRES SEMEN QUALITY

Yair Aron Chocobar Torres[1], María Sol Martinez[1], Fernando Nicolás Ferreyra[1], Daniela Andrea Paira[1], Andrea Tissera[2], Rosa Molina[2], Virginia Elena Rivero[1], Rubén Darío Motrich[1].

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In the last two decades, more than 90% of the cultivable land is dedicated to the production of herbicide-resistant genetically modified crops, which has significantly increased the production and use of glyphosate-based herbicides (GBH). Human studies assessing detrimental effects of GHB on reproductive health, especially in men, are scarce.

The aim of this work was to study the impact of chronic exposure to GBH in men seeking care for infertility analyzing its impact on sperm quality and semen inflammation.

We performed a retrospective analysis (2016-2022) of sperm quality parameters in a cohort of 242 reproductive-aged male patients who claimed to be exposed to GBH and in 155 healthy control individuals. The samples were collected by masturbation after 48-72 hours of sexual abstinence and semen analysis was performed according to the WHO manual. On the other hand, a prospective study assessing sperm quality in 19 patients who were under the same conditions and 11 healthy control individuals was carried out to analyze the composition of pro- and anti-inflammatory cytokines in seminal plasma. IL-4, IL-2, IP-10, IL-1B, TNF-α, MCP-1, IL-17a, IL-6, IL-8, TGF-β, IL- 10, IFN-□ and IL-12 p70 concentrationsin seminal plasma were analyzed by Legend Plex. Data were analyzed using the non-parametric Mann-Whitney test.

Patients chronically exposed to GBH showed significantly reduced sperm counts per mL and impaired sperm motility and morphology ($p<0,05$). In addition, semen counts of peroxidase-positive leukocytes were significantly increased in patients exposed to GBH respect to controls individuals ($p<0,05$). Remarkably, significantly higher levels of IL-8 were found in semen from patients exposed to GBH with respect to control individuals ($p<0,05$).

Our results indicate that chronic exposure to GBH induce changes in the composition of seminal plasma and decrease sperm quality thus impairing male fertility potential.

120 (15) DEFICIENT LACTATION BY SPONTANEOUS CENTRAL HYPOPROLACTINEMIA IN OFA RATS ALTERS LYMPHOID ORGANS AND INCREASES NATURAL ANTIBODIES IN THE OFFSPRING

María Belén Sánchez[1,2], María Cecilia Michel[1,3], Luciana Belén Viruel[2], Claudio Rodríguez-Camejo[4], Flavia Judith Neira[1,3], Elisa Olivia Pietrobon[1,5], Marta Soaje[1,6], Ana Hernández[4], Susana Ruth Valdez[1,7], Juan Pablo Mackern-Oberti[1,6].

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Prolactin is a lactogenic hormone that plays a vital role in developing the mammary epithelium and synthesizing milk for proper breastfeeding. Maternal milk is an excellent source of bioactive components, immune factors, hormones, and nutrients vital for the offspring's development. Our laboratory has worked for many years with an experimental model of deficient breastfeeding in rats associated with low prolactin levels (hypoprolactinemia or hypoP) during early lactation, known as Oncins France Colony A (OFA). Our work aimed to evaluate the influence of hypoP on milk quality and bioactive components and whether this affects offspring's developmental and immunological parameters in early lactation. To this end, 12-week-old Sprague Dawley (SD- control group n=7) and OFA (hypoP group n=12) rats were mated and euthanized on day 2 of lactation (L2). Serum, spleen, milk, and mammary glands from dams and offspring serum, and several organs were obtained for further analysis. Prolactin serum levels were analyzed by RIA. Total IgG and IgG2a in maternal serum and milk and offspring serum were analyzed by ELISA. Moreover, natural IgG anti-DNP (dinitrophenols) was evaluated in offspring and L2 dam serum. Milk nutritional components like total proteins, reducing sugars (lactose), caloric value, and fat content were measured. Also, histological analysis of the mammary gland was performed, and offspring developmental parameters were evaluated on L2. Our results show that OFA rats had lower prolactin levels than SD rats ($p<0,001$). OFA pups had lower weight on days 1 and 2 ($p<0,0001$), less weight gain ($p<0,05$), length ($p<0,05$), and diminished thymus weight, splenic weight, and gastric weight ($p<0,01$) without differences in head circumference. Concerning the mammary gland, the mammary adipose tissue was significantly lower in the OFA group than in the SD-control group ($p<0,05$). Furthermore, we observed a diminished epithelial elongation in the whole mount mammary gland analysis ($p<0,05$). We found that OFA milk displayed a reduced caloric value and fat concentration ($p<0,01$) with higher total IgG and natural IgG anti-DNP in mothers' serum and IgG2a in offspring serum. Moreover, IgG2a and IgG anti-DNP were higher in OFA offspring serum. In conclusion, our data suggest that hypoP affects the development of the offspring's lymphoid organs and mammary glands. Additionally, our results indicate that humoral immunity may be maintained in early deficient lactation. HypoP may alter dam and offspring's energy intake and humoral immune factors.

121 (194) DYSREGULATION OF THE INFLAMMATORY RESPONSE IN OVARY ASSOCIATED TO ADVANCED REPRODUCTIVE AGE

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The concept of 'inflammaging' represents the inflammation associated to aging, which is systemic, chronic and linked to the increase of pro-inflammatory mediators. Even if inflammatory mediators contribute to ovarian physiology, dysregulation of the inflammatory response can affect ovarian reserve and oocyte quality. Our aim is to investigate the mechanisms underlying the exacerbated inflammatory response in ovarian microenvironment associated to advanced reproductive age. Since, oocytes develop in the context of ovarian follicles, we obtained follicular fluid (FF) from women with indication of assisted fertilization treatment and we investigated ovarian immune cell profile associated with clinical parameters.

First, we confirmed a negative correlation between the number of oocytes and age. In our population (n=70), we identified two subpopulations: women with >10 oocytes (corresponding to the youngest group, from 21-29y) and <10 (corresponding to the aged group, 35-42y). Then, we recovered mononuclear cells from de FF by Ficoll-Hypaque gradient and we focused on macrophages and lymphocytes profiles by immune stained analyzed by FACS. We observed that the frequency of FF-macrophages from aged women show a significant increase in IL-1β production in comparison with the younger group ($p<0,05$). Moreover, the production of IL-1β also correlates negatively with the number of oocytes recovered in a meiosis II phase. Focusing on macrophage characterization, we evaluated their activation and we observed that FF from aged women displayed an increased frequency of $CD68^+$ macrophages in comparison with the observed in young women.

Since CD68 is a scavenger receptor involved in the transport of lipids inside macrophages, and the induction of foamy cells associated with aged ovaries; we evaluated lipid accumulation in FF-macrophages. We determined lipid droplets using BODIPY 493/503 and flow cytometry. Interestingly, we found an increase of lipid droplets in FF-macrophages from aged women in comparison with the younger group. Getting insight into the mechanism involved in foamy generation, CD68 shuttles between the cytoplasm and the plasmatic membrane to incorporate lipids. Therefore, we discriminated surface and intracellular CD68 detection, and we found a higher intracellular CD68 expression in aged macrophages, correlating with lipid droplet accumulation, in comparison with young macrophages. Finally, we focus on a novel Treg subpopulation, $CD3^+CD4^-CD8^-$ (DNT), that is emerging as effector cells capable of mediating immune tolerance in the female reproductive system. We found that FF from aged women displayed a reduced frequency of DNT lymphocytes ($p<0,05$), in comparison with FF from younger women.

Based on these results, we conclude that women of advanced reproductive age show a dysregulated immune microenvironment that may contribute to a chronic inflammation and alter oocyte quality

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122 (9) ENDOMETRIAL DECIDUALIZATION ENHANCED BY ANGIOTENSIN IITREATMENT MODULATES TROPHOBLAST CELL MIGRATION

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Angiotensin (Ang) II is a proinflammatory and vasoactive peptide that has been demonstrated by our group to enhance endometrial decidualization, increasing FOXO1 expression and nuclear translocation and stimulating prolactin secretion. Pharmacological blockage of the 'classical' Ang II signaling axis, through use of Angiotensin Converting Enzyme inhibitors or Ang II type 1- receptor (AT1R) blockers, leads to severe pregnancy complications. Here we aimed to investigate how the action of Ang II on decidual stromal cells can affect the communication between these cells and trophoblast, with a focus on invasion.

Human endometrial stromal cells (T-HESC) were treated for 4 days with 1μM medroxyprogesterone acetate (MPA) and 0.5mM cAMP (DC cocktail) to induce decidualization. T-HESC were pretreated with AT1R antagonist losartan (5μM) before treatment for further 48h with Ang II (125-500nM) in the presence of DC. Culture supernatant was collected after 6 days of treatment; culture media was replaced and after 2 more days conditioned media (CM) were obtained. HTR-8/SVneo (H8) trophoblast cell migration was assessed through an *in vitro* wound healing assay. Reactive oxygen species were scavenged using N-acetylcysteine (NAC). CXCL8 and CCL2 involvement was determined using neutralizing antibodies (NAb) or isotype control antibody. IL-6, VEGF, CXCL8 and CCL2 production and MMP-2 and MMP-9 activity were assessed in T-HESC and H8 culture supernatants by ELISA and gelatin zymography, respectively. Swan-71 blastocyst-like spheroids (BLS) were co-cultured with Ang II-treated DC T-HESC and invasion index was determined after 24h by fluorescence microscopy. Differences were considered statistically significant with $p<0.05$.

Ang II increased VEGF ($p<0.05$), CCL2 ($p=0.051$), IL-6 ($p<0.05$) and CXCL8 ($p<0.001$) secretion, and MMP-2 activity ($p<0.01$) in DC T-HESC (p *vs.* DC). These effects were inhibited by pretreatment of DC T-HESC with losartan. Ang II-treated DC T-HESC CM increased H8 trophoblast migration ($p<0.01$), effect that was inhibited by pretreatment of T-HESC with losartan ($p<0.05$), or pretreatment of T-HESC CM with CXCL8 NAb ($p<0.05$) or with CCL2 NAb ($p<0.05$). Ang II-treated DC T-HESC CM did not modify CXCL8, CCL2 or MMP- 9 production by H8. Pretreatment of H8 with NAC inhibited the migration increaseinduced by Ang II-treated DC T-HESC CM ($p<0.01$). Swan-71 BLS area ($p<0.05$)and invasion index ($p<0.05$) over DC T-HESC was increased by Ang II, effect thatwas inhibited by pretreatment of DC T-HESC with losartan ($p<0.01$).

In conclusion, Ang II modifies the secretome of DC T-HESC, which promotes trophoblast migration and invasion. Ang II/AT1R axis could actively play a role regulating communication between trophoblast and decidual stromal cells during early pregnancy. Further investigation is required to elucidate the molecular mechanism triggered in Swan-71 by Ang II-treated DC T-HESC to increase BLS outgrowth.

123 (173) ENDOPLASMIC RETICULUM STRESS TRANSMITTED BY ENDOMETRIAL STROMAL CELLS DEFINES THE FATE OF DECIDUAL DENDRITIC CELLS TOWARD TOLERANCE OR INFLAMMATION

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During the implantation window, endometrial stromal cells acquire a secretory profile associated with the expansion of its endoplasmic reticulum (ER), suffering a physiological ER stress (ERS) and the consequent unfolded protein response (UPR). The signaling pathways involved in ERS/UPR are related with the onset of a sterile inflammatory response that have a key role in endometrial receptivity and embryo implantation. We previously showed an altered ERS/UPR in endometria of women with reproductive complications. Considering that stromal cells condition maternal monocytes to a tolerogenic dendritic cell (DC) profile and ERS can be cell-to-cell transmitted modulating immune profiles, here we evaluated the impact of ERS/UPR triggered on stromal cells on DCs differentiation and the involvement of ERS-transmission in their conditioning. Thus, human endometrial stromal cell line (HESC) was treated or not with a potent ERS-inducer Thapsigargin (Tg, an ER Ca^{2+} pump inhibitor) for 4h. Conditioned media (CM) were collected after 48h. Then, isolated monocytes from peripheral blood mononuclear cells from healthy women were cultured with rhGM-CSF^{+}rhIL-4 for 5 days in the absence/presence of CM.

Monocyte-derived cultures differentiated with HESC^{+}Tg CM showed a lower frequency of CD1a^{+}CD14^{-} compared with HESC CM ($p<0.01$, Wilcoxon Test). Also, we observed an increased CD86^{high} cell-population accompanied by a higher COX-2 expression. Moreover, we also detected a higher IL-1β secretionin these cultures associated with an increase in AnnexinV^{+}/PI^{+} cell-population ($p<0.05$), indicating that the highly inflammatory response leads a lytic programmed cell death. Then, to confirm the ERS-transmission, first we evaluated the expression of ERS-sensors on DC cultures. Whereas IRE1α and PERK expression was increased, a lower expression of ATF6 was observed on HESC^{+}Tg CM cultures ($p<0.05$). In fact, using an IRE1α inhibitor (STF-083010), we prevented the conditioning to a pro-inflammatory DC profile. When we evaluated the expression of genes downstream the UPR pathways, we noted a higher expression of ATF4 and CHOP ($p<0.05$). Finally, to investigate the impact of the pro-inflammatory DC profile induced by stressed stromal cells on blastocyst implantation ability, we used an *in vitro* model of human trophoblastic spheroids migration. Thus, human trophoblast cells (HTR-8) were cultured on non-adherent plates for 48h to form blastocyst-like spheroids (BLS). The BLS were selected and seeded on adherent plates in the presence or absence of supernatants derived from conditioned DC cultures for 72h. We observed an altered trophoblast migration when BLS were cultured with supernatant derived from dendritic cell cultures conditioned by stressed HESC. These results suggest that ERS might be transmitted from stromal cells to DC and its fine balance is required to maintain the tolerance needed for successful embryo implantation and trophoblast migration.

124 (171) EXTRACELLULAR VESICLES OF PORPHYROMONAS GINGIVALIS IMPAIR TROPHOBLAST CELL INTERACTION WITH IMMUNE CELLS

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Periodontitis is proposed as a risk factor for preterm delivery, fetal growth restriction and preeclampsia with severe consequences for maternal and neonatal health, however the biological mechanisms involved are elusive. Porphyromonas gingivalis (*Pg*), one of the main pathogens in periodontal disease, produces extracellular vesicles referred to as outer membrane vesicles (*Pg*OMV), which serve a relevant function in the interaction with host cells. In the process of placentation, trophoblast cells (Tb) release soluble factors while closely engaging with maternal leukocytes to maintain immune homeostasis. Disruption in this trophoblast-immune interplay has been linked to pregnancy complications.

Objective: The aim of this work is to study the effect of *Pg*OMV on trophoblast-immune interaction.

Methods: Trophoblast cells were exposed to *Pg*OMV stained with PKH67 fluorescent dye at different concentrations and time periods to examine internalization. mRNA expression of inflammatory mediators was evaluated by qPCR. Neutrophils were purified from healthy donors and cultured with conditioned media from Tb (CM) pre-treated or not with *Pg*OMV (*Pg*OMV CM). Migration to CM was evaluated by flow cytometry using transwell assays and Reactive Oxygen Species (ROS) production using DCFH-DA probe. Monocytes were isolated from the peripheral blood of healthy female donors using Ficoll-Paque/Percoll and were cultured with rhGM-CSF+rhIL-4 for 5 days in the absence/presence of CM or *Pg*OMV CM. Additionally, monocytes were differentiated into macrophages with rhM-CSF for 5 days. These macrophages were then cultured for 48 hours with conditioned media from Tb CM or *Pg*OMV CM. The macrophage and dendritic cell profile was assessed by flow cytometry.

Results: *Pg*OMV are internalized by trophoblast cells in a time and dose dependent manner. *Pg*OMV treatment induces a selective modulation of inflammatory mediators with reduced mRNA expression of IL-8, IL-6 accompanied by an increased expression MCP-1 ($p < 0.05$). Trophoblast cells primed with *Pg*OMV enhanced neutrophil chemoattraction ($p < 0.05$) and increased ROS production (CM 15967 ± 2989; *Pg*OMV CM 20068 ± 3982; X±SEM n=9; $p < 0.05$). Enhanced ROS production was associated with an increased mRNA expression of gp91^{phox} p47^{phox} and p67^{phox}, subunits of the NADPH oxidase complex and Glucose-6-phosphate dehydrogenase, the rate-limiting enzyme of the pentose phosphate pathway that fuels NADPH oxidase with NADPH to produce superoxide ($p < 0.05$). *Pg*OMV CM also affected the profile of macrophages and dendritic cells.

Conclusion: Taken together, results support a pathogenic role of *Pg*OMV at early stages of pregnancy and placentation through disrupting trophoblast contribution to immune homeostasis maintenance.

125 (29) HYPERTHYROIDISM ALTERS IMMUNE MILK QUALITY AND OFFSPRING NUTRITION

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Hyperthyroidism (hyperT) alters maternal behavior and disrupts milk production in experimental models. However, it remains unclear if hyperT could change maternal milk's nutritional and immunological composition. We investigated the influence of hyperT on milk quality, bioactive components, offspring development, and immunity in early lactation. For this, 12-week-old *Wistar* rats were injected with 0.25 mg/kg T_4 (hyperT n=20) and vehicle (control n=19), mated with *Wistar* male rats and euthanized on day 2 of lactation (L2) to address this objective. Serum, spleen and milk from mothers and offspring serum were obtained for further analysis. Milk was obtained after ketamine/xylazine sedation and oxytocin stimulation for milking. Total IgG, IgG2a and IgA levels from mothers andoffspring serums and milk were determined by ELISA. Nutritional components: total proteins, reducing sugars (lactose), energy, and fat content were measuredin milk. Moreover, offspring developmental, biochemical, and hormonal parameters were evaluated on L2. To assess whether maternal hyperT may havean immune systemic effect, we analyzed the splenic weight and splenic index inmothers on L2. HyperT dams displayed higher serum levels of T_4 and corticosterone than control mothers. By contrast, T_4 and corticosterone levels were reduced in offspring from hyperT mothers (*p<0,001* and *p<0,01*) without differences in progesterone levels. We found increased splenic weight and index(*p<0,05)* in hyperT mothers compared to control females. Pups from hyperT mothers had lower weight on days 1 and 2 (*p<0,001*), less weight gain, and decreased length, cerebral weight, and head circumference (*p<0,001*) compared to pups from healthy dams. By contrast, pups from both, hyperT and control dams, displayed similar thymus weight. Glucose and albumin serum levels from pups from hyperT mothers were reduced (*p<0,01*) compared with controls. We found that hyperT milk reduced energy and fat concentration (*p<0,01*). Finally, principal component analysis revealed a self-consistent data-set (all hyperT group) that represents mothers with higher loadings factors for IgG and IgG2a (inserum and milk), and higher loadings for IgG levels in the serum of offspring. Theset of results suggests that hyperT may contribute to developmental alterations of the dams and their offspring. In conclusion, hyperT may affect humoral and nutritional milk factors that could impact offspring parameters at crucial early development stages.

126 (121) IMPACT OF *BRUCELLA ABOURTUS* AND *B. MELITENSIS* INFECTION ON THE INNATE IMMUNE RESPONSE AND THE RELEASE OF EXTRACELLULAR VESICLES BY THE PLACENTA

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Placental immunity is crucial for fetal well-being in pregnancy, since invading pathogens can be transmitted from the maternal blood to the fetus through this organ. However, excessive inflammatory responses in the placenta can be detrimental to both the fetus and the pregnant mother. Placental extracellular vesicles (pEV) are generated by trophoblasts and regulate immunological tolerance towards the fetus and the placenta itself.

Brucella infection has been associated with reproductive complications in humans and animals, and inflammation has been shown to be involved. In this study, we investigated the impact of *B. abortus* and *B. melitensis* infection on the innate immune response of the placenta and the production of placental pEV. To do so, we utilized an *ex vivo* infection model of explants from human placenta at term. “Small pEV” were isolated by ultracentrifugation of culture supernatants from infected and uninfected explants at 18 h post-infection (p.i.). Our findings demonstrated that infection with *B. abortus* and *B. melitensis* induces significant increases in CCL2 ($p<0,0001$), CXCL8 ($p<0,0001$), IL-6 ($p<0,0020$) and IL-1β ($p<0.005$), but not of TNF-α or IL-10 ($p<0.97$ and $p<0.44$ respectively), comparedto uninfected explants. Furthermore, total collagenase activity (measured using EnzChek, Invitrogen) increases significantly in infected explants (*B. abortus*: 2.3-fold and *B. melitensis*: 2.4-fold versus non-infected, $p<0.0001$). In agreement with these results, we observed an increase in MMP-2 activity in the supernatants of infected explants, as assessed by zymography. Transmission electron microscopy analysis revealed the presence of spherical double-membrane pEV in the supernatants of placental explants. pEV from *Brucella*-infected or uninfected explants were positive for CD63 and CD81 by fluorescence microscopy. Remarkably, infection with both *Brucella* species reduced the total number of released pEV, specifically affecting the number of $CD63^+$ pEV. Taken together, these results suggest that placental infection with either *B. abortus* or *B. melitensis* triggers an inflammatory immune response and affects the quantity and characteristics of the released pEV.

127 (174) NR4A NUCLEAR ORPHAN RECEPTORS EXPRESSION DIFFER IN IMMUNE CELLS FROM URSA PATIENTS WHEN COMPARING LIVE BIRTH OUTCOMES

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Spontaneous abortion is the most common complication of early pregnancy in women. Recurrent Spontaneous Abortion (RSA) is defined as three consecutive losses within the first 20 weeks of gestation. The majority of these recurrent losses remain unexplained (URSA). The NR4A orphan receptors function as ligand-independent transcription factors that regulate the expression of target genes. These receptors have been implicated in broader functions within the immune system cells. NR4A orphan nuclear receptors may play a role in restraining or increasing the effects of immune system cells in embryo development and implantation. Our goal is to unveil if these orphan receptors integrate the numerous mechanisms of various origins responsible for the maternal tolerance towards the embryo/foetus, through their involvement in immune cell functions. We have previously reported that URSA patients show significant lower mRNA expression levels of NR4A2 and NR4A3 nuclear orphan receptors, when compared to control fertile women. Here we report a new retrospective study analysing the mRNA expression levels in primary and secondary URSA patients, and compared to their results in achieving a successful pregnancy up to two years after consultation, regardless any treatment or strategy they followed. NR4A mRNA expression levels were determined by real-time PCR in peripheral blood mononuclear cells (PBMCs), and women were divided in two groups considering our medical records indicating successful pregnancies (Yes or No live-birth). Statistical analysis was performed using GraphPad Prism 5 software. Groups were compared by Mann-Whitney test. The significance threshold was set at 0,05. When analysing this outcome, URSA women who reversed their condition had, at the time of consultation, significant lower mRNA expression levels of NR4A2 ($p=0.0324$) and NR4A3 ($p=0.0162$) compared to URSA women that did not reverse. Even if NR4A1 expression levels showed a similar tendency, the comparison of the two groups for this nuclear orphan receptor levels did not result statistical significant ($p=0.0957$). We also tested for NR4A expression levels differences between successful pregnancies or not in unexplained infertility patients. In these comparisons, none of the nuclear orphan receptors resulted in differences statistically significant. Our results, obtained from PBMCs, strengthen our hypothesis that members of the NR4A subfamily, particularly NR4A2 and NR4A3, would participate as immune restrictive/promotive factors for embryo/foetus development in primary and secondary URSA patients. These results point the NR4A transcription factor subfamily as being involved in reversible pathways leading to RSA or live birth according to their expression levels and further encourage our efforts to extend the study in endometrial infiltrated immune cells.

128 (135) RELEVANCE OF VIP IN THE MAINTENANCE OF THE IMMUNE OVARIAN HOMEOSTASIS

Lara Castagnola[1], Lucila Gallino[1], Daiana Vota[1], Esteban Grasso[1], María Soledad Gori[1], Fernanda Parborell[2], Claudia Pérez Leirós[1], Vanesa Hauk[1] and Rosanna Ramhorst[1]

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Ovarian aging is associated with inflammation, contributing to the decline in oocyte count and quality. Since the vasoactive intestinal peptide (VIP) is an immunopeptide found in the reproductive tract with anti inflammatory effects inducing a tolerogenic response, we aim to understand VIP contribution to control ovarian immune homeostasis in order to prevent premature aging.

Wild type (WT) mice 3 (young) or 8 (aged) months old and young VIP Knockout (KO) in estrus were used. The ovaries were examined after histological staining, reactive oxygen species (ROS) levels were measured by flow cytometry using DCFH-DA probe and IL-1β secretion was determined by ELISA. Long-term phagocytosis assays (24, 48, 72h) of apoptotic thymocytes were performed to obtain foamy macrophages. Peritoneal WT or VIP KO macrophages were preconditioned with ovarian media from WT or VIP KO mice for 48h, followed by short-term phagocytosis assays (120 min) using apoptotic bodies stained with PKH26 to assess their phagocytic capacity. Phagocytic capacity was measured as the percentage of $PKH26^+$ cells in the macrophage gate. In both assays, after phagocytosis, lipid droplets were determined by flow cytometry using BODIPY 493/503.

We confirmed that during reproductive aging WT females show a smaller number of ovarian follicles, accompanied with altered ovarian histology, in comparison with young WT mice. These parameters were found to be exacerbated in young VIP KO mice, with significantly more atretic follicles and less mature follicles ($p<0,05$), indicating ovarian failure. Moreover, young VIP KO mice displayed a compromised ovarian histology with increased fibrosis deposition (Picrosirius Red staining), accompanied by an inflammatory ovarian microenvironment with increased ROS and IL-1β production ($p<0.05$). In the WT aged ovary, we verified the presence of a unique macrophage population associated with advanced age known as ‘foamy macrophages’. Interestingly, this population was significantly increased in young VIP KO ovary, indicating premature aging. Getting insight into the mechanism involved in foam cell generation, we evaluated long-term phagocytosis in young and aged WT macrophages. Lipid droplets accumulation increased over time in both populations ($p<0.05$), but the formation of foam cells only was detected after 72h of phagocytosis in macrophages from aged mice. Finally, we pre-conditioned young WT or VIP KO macrophages with WT or VIP KO ovarian media for 48h to mimic the ovarian microenvironment. After short-term phagocytosis assays, we observed an enhanced phagocytic ability in VIP KO macrophages preconditioned with WT or VIP KO ovarian media. However, only in VIP KO macrophages conditioned with VIP KO ovarian media we found an increased lipid droplet accumulation, consistent with the formation of foamy macrophages.

These findings highlight the role of VIP in regulating ovarian immune microenvironment, thereby preserving ovarian health and preventing premature aging.

Vaccines
Friday, November 10, 14-15:30h
Chairs: Gabriel Morón - Gabriel Cabrera - Pilar Aoki - José Arias - Juan Silva

129 (68) A NATURAL-BASED NANOADJUVANT ENHANCES HUMORAL AND CELLULAR IMMUNE RESPONSE INCORPORATED INTO AN *INACTIVATED* ENTEROTOXIGENIC *Escherichia coli* (ETEC) VACCINE

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In a previous study, we have demonstrated the adjuvant potential of a Minthostachys verticillata essential oil-based nanoemulsion (NEO), an Argentinian plant, into an inactivated enterotoxigenic Escherichia coli (ETEC) vaccine administered subcutaneously in mice. We observed anti-ETEC IgG levels similar to incomplete Freund's adjuvant (IFA), without a granuloma induction in the area of inoculation. In addition, the binding between NEO and ETEC was demonstrated by SEM. The aim of this study was to evaluate the IgG subclasses, the functionality of antibodies and the cytokines profile induced by NEO incorporated in an experimental inactivated vaccine against ETEC. The NEO was synthetized with 20% v/v of EO, 1% v/v of a surfactant mixture (0.75% Tween 80 and 0.25% Span 60) and 79% v/v of distillated water. Experimental vaccines were prepared as follow: a) formaldehyde-inactivated ETEC at $1x10^9$ UFC/mL and saline solution, b) ETEC with NEO (0.5 mg/mL), c) ETEC with incomplete Freund's adjuvant (IFA) (50%), d) ETEC with EO (0,5 mg/mL) and e) ETEC with surfactant mixture (Tween80/Span60 0,5 mg/mL). Mice were divided into six groups of four animals each and vaccines were administrated subcutaneously on days 1, 14, 28 and 42. One group received only saline solution as a negative control. Animals were kept under controlled temperature, provided *ad libitum* access to food and water and were sacrificed 7 days after the last inoculation. For each group, a pool of sera was obtained and anti-ETEC IgG titers as wells as the IgG1 and IgG2a subclasses were determined through the indirect ELISA method. As in previous study, results showed that IgG titers of the group that received the vaccines formulated with EO as adjuvant were significantly increased compared to group that received ETEC ($p<0.0001$), reaching similar values of those in IFA group. All groups vaccinated showed IgG1 levels higher than control group, being IFA the adjuvant that achieved the highest levels ($p<0.001$). The IgG2a levels in all groups were also higher than control group; however, IFA, ETEC and surfactant mixture-groups induced significant IgG2a levels compared with other groups ($p<0.01$). The functionality of the synthesized antibodies was evaluated by the opsonophagocytic assay performing serum dilutions (1/100, 1/200, 1/500, 1/750 and 1/1000). NEO induced antibodies with opsonophagocytic ability against ETEC similar to IFA up to 1/750 serum dilution. IL-10 and IFN-γ levels were measured in splenocyte culture supernatants from immunized mice. Only the group immunized with NEO as adjuvant showed a

significant increase in IFN-γ compared to the other groups ($p<0.001$) and the group immunized with IFA showed a significant increase in IL-10 compared to theother groups ($p<0.001$). In conclusion, NEO as adjuvant was able to activate humoral and cellular immune response in mice with functional antibodies againstETEC and will continue to be studied for its incorporation into vaccines.

130 (205) A POTENTIAL MUCOSAL VACCINE AGANIST TUBERCULOSIS

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BACKGROUND: *Mycobacterium bovis* BCG is not effective against pulmonary tuberculosis (TB), the most common form of TB in adults and teenagers. Thus, new vaccines conferring protection during the whole life of the individual are required. With the purpose to achieve multistage resistance against *M. tuberculosis* (*Mtb*) infection, we investigated the response of the host to two *Mtb* antigens: the 85A antigen (Ag85A), the protein most expressed in the initial stages of TB infection, and the Rv2626c antigen, a protein expressed under hypoxic conditions. To do this, we analysed the potential of both recombinant proteins or Modified Ankara Virus (MVA) vectors to be used as mucosal vaccines.
METHODS: Balb/c mice were initially immunized by the sublingual (sl) or intranasal routes with Rv2626c and Ag85A proteins together with the Omp19 adjuvant (3 doses every 7 days). After 4 weeks, the animals were challenged with the pathogenic H37Rv *Mtb* strain by intratracheal inoculation. Thirty days post infection, lungs were aseptically removed and *Mtb* CFU counting was performed. Besides, we also assayed other immunization schedule by using two doses of MVA2626c □ Omp19 delivered by sl or intramuscular routes. Ten days after the last immunization, splenocytes were obtained and stimulated with recombinant Rv2626c, $CD4^+$T cell-specific Rv2626c peptides, or $CD8^+$T cell-specific MVA peptides. IFNγ production was measured by ELISA or Flow cytometry. Moreover, a group of experimental mice was challenged with H37Rv *Mtb* strain and afterward, CFUs were determined in lungs.
RESULTS: By determining the CFUs in mice lungs, we observed that the intranasal $Rv2626c^+Ag85A^+$Omp19 vaccine preparation induced higher protection as compared to the same vaccine administered sublingually ($p<0.05$). Regarding the ability of $MVARv2626^+$Omp19 to stimulate the host immune response, cells from mice intramuscularly immunized with the vaccineformulation produced lower levels of IFN-γ after *in vitro* stimulation with Rv2626c Ag as compared to cells from animals immunized by the sl route($p<0.001$). Moreover, the sl route induced an increase of $CD4^+IFN\gamma^+$ and $CD8^+IFN\gamma^+$ cells in response to specific Rv2626c and MVA peptides respectively($p<0.05$).
Moreover, we also compared the use of Omp19 as adjuvant by the sl route. The use of Omp19 increased the percentage of $CD8^+IFN\gamma^+$ cells but decreased the percentage of $CD4^+IFN\gamma^+$ cells. Surprisingly, sl administration of MVARv2626 did not conferred protection against H37Rv *Mtb* challenge but the use of Omp19 as adjuvant decreased significantly the CFUs in mice lungs($p<0.001$).
CONCLUSIONS: Our results show that not all mucosal routes respond induce the same response in the mice host and that the use of Omp19 as an adjuvant could improve the immune response conferred by the vaccine candidate. Together, our findings suggest that our preparations administered by the mucosal route could be potential vaccines against *Mtb* infection.

131 (11) ACELLULAR VACCINES BASED ON OUTER MEMBRANE VESICLES FROM *B. SUIS* STRAINS

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Outer Membrane Vesicles (OMV) from various Gram-negative bacteria have been studied as potential acellular vaccines. We have previously demonstrated that vaccination with *B. suis* wt and Δ*mapB* OMV induced systemic and mucosal specific humoral immune response, and protected against systemic and respiratory acquired brucellosis. In this work we continued characterizing the immune response after OMVwt and OMVΔ*mapB* vaccination and evaluated the protective capacity of this vaccines against intragastric *Brucella* infection.

Female BALB/c mice were immunized intramuscularly (i.m.) with OMVwt (20 µg), OMVΔ*mapB* (20 µg) or saline at 0 and 30 days. One week after last immunization serum samples were obtained and spleens were harvested, processed and the cells obtained were cultured in the presence of both OMV, ConA mitogen or RPMI. After 24h cells were stained with anti-CD3, anti-CD4 and anti-CD8 antibodies for flow cytometry analysis. *In vitro* production of IFN-γ, IL-2, IL-17 and IL-5 was determined after 72h of stimulation. Other batch of vaccinated mice were injected intradermally in opposite footpads with the correspondent OMV or saline to evaluate DTH (48 and 72h). Two weeks after last immunization mice were challenged with *B. suis* through the intragastric route. CFU counts were determined in spleens and mediastinal lymph nodes 20 days after challenge. Antibodies capacity to neutralize *Brucella* infection and to induce opsonophagocytosis were determined in Caco-2 and RAW cells cultures, respectively.

Serum specific antibodies from OMVwt and OMVΔ*mapB* mice promoted *Brucella* phagocytosis by RAW cells ($p<0.01$; $p<0.05$), but only OMVwt sera significantly reduced *B. suis* adherence to Caco-2 cells ($p<0.01$). The CD4/CD8 ratio among the OMVwt immunized group was significantly decreased regarding the control group ($p<0.05$). A reduced CD4/CD8 ratio was also observed for OMVΔ*mapB* immunization group, but without statistical significance. Stimulation of splenocytes with OMVwt or OMVΔ*mapB* did not significantly increase the evaluated cytokines´ secretion when compared to RPMI condition, but splenocytes secreted high levels of IL-2, IL-5 and IFN-γ in response to ConA. Consistent with these results, no DTH reaction was observed in any of the OMV immunized groups. Just OMVwt immunization achieved a reduction of spleen ($p<0.05$) and mediastinal lymph nodes ($p<0.0001$) burden after i.g. infection.

Vaccination with both *B. suis* strains' OMV proved to protect against systemic and respiratory acquired brucellosis, which could be probably related to an increase in the CD8 T cell population. Nevertheless, only OMVwt immunization achieved protection at the gastrointestinal mucosa.

132 (138) ADJUVANT CAPACITY OF OUTER MEMBRANE VACCINE DERIVED FROM BORDETELLA PERTUSSIS

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In order to enhance control over the resurging preventable disease known as pertussis, the development of new strategies is imperative. This objective poses a significant challenge, as the strategy must effectively address the limitations of the current vaccines in use. It is essential that the new strategy is less reactogenic than the cellular-vaccine and, when compared to acellular-vaccines, it must elicit a long-lasting immune response without imposing excessive selective pressure on the circulating bacterial population. We finally achieved these objectives using a vaccine based on outer membrane vesicles derived from Bordetella pertussis (OMVBp), the causative agent of the disease. In this presentation, we provide a summary of the results we obtained in evaluating the OMVBp role as an adjuvant. To accomplish this goal, we used a two-dose murine schedule to assess formulations based on OMVBp combined with 3 heterologous immunogens: HIm1, HIm2, and HIm3 for confidentiality reasons. To adjust the composition, we conducted dose-response assays varying the quantities of OMVBp (low, medium, and high) and the immunogens. Heterologous immunogens formulated without OMVBp were employed as the control group. Humoral response triggered by the tested formulations was evaluated by ELISA and cellular response was examined through splenocytes proliferation assays. These assays revealed that even at the low concentration used, OMVBp acts as an adjuvant. Since this effect was pronounced for medium and high concentrations, we selected the medium concentration to identify the minimum dose that can be employed for each immunogen. In the dose-response assays, we noted that the disparity between OMVBp-based formulations and those comprising solely immunogens became more pronounced as lower concentrations of heterologous proteins were employed (2.5-fold increase, $p<0.0001$). When comparing the adjuvant capacity of OMVBp with that of alum, we observed comparable levels of specific IgG against HIm1 and HIm2 for both adjuvants. Importantly, the OMVBp-based vaccine led to a 1.6-fold increase in IgG-HIm3 ($p<0.0001$) compared to the alum formulation. OMVs derived from *E.coli* were used to determine whether the OMVBp adjuvant capacity could be extended. The obtained results indicated no significant distinctions in the humoral response between these two formulations. Trough the implementation of OMVs derived from *E.coli* we were able to detect that the adjuvant capacity could be extended to other species other than B. pertussis. However, it's important to note that the OMVBp more strongly directed the immune response towards a Th1 profile ($p<0.05$). The IgG2a/IgG1 ratio and IFNγ levels were higher for OMVBp-based vaccine induced than those detected for alum and OMVEc formulations ($p<0.001$). Collectively, these findings underscore the role of OMVBp as an adjuvant and modulator of the immune response, positioning it favorably for its use in combined vaccine formulations.

133 (168) ANTIGENS CO-ENCAPSULATED WITH PROTEASE INHIBITOR (U-Omp19) IN POLYMERIC NANOPARTICLES

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Vaccination continues to be one of the most important tools for the prevention of infectious diseases. The oral pathway of immunization presents many advantages, such as, easy application and development of local and systemic immunity. There are few oral vaccines in the market, most of them are inactivated or attenuated. The development of oral subunit vaccines still faces multiple challenges because of the hostile gastrointestinal environment.

Previously, we have shown that the protease inhibitor U-Omp19 from *Brucella spp*. is an oral vaccine adjuvant that protects co-delivered antigens from proteolysis in the gastrointestinal tract and increases antigen specific adaptive immune responses.

This project seeks to increase the protection of the antigen (OVA), by encapsulating it with U-Omp 19 in polymeric nanoparticles.

The method chosen for the synthesis of the nanoparticles was double emulsion-solvent evaporation (DE-SE). Encapsulation efficiency of each protein was determined by SDS-PAGE and antigen specific ELISAs. Characterization was done using DLS, SEM and z-potential. Cytotoxicity was measured using propidium yodide (IP) and LDH release. Internalization of the antigen was evaluated using flow cytometry and confocal microscopy in HT-29 and Caco-2 cell lines.

Encapsulation efficiency of proteins was between 20-60%. Nanoparticles presented a hydrodynamic diameter between 328 and 744 nm, surface charge between -8,2 mV and -32 mV and a PDI of 0,23-0,29. The particles were not cytotoxic for the cell when the concentration was below 0,5 mg/ml. An increase in the internalization of the encapsulated antigen was observed compared with the non-encapsulated antigen in both cell lines.

In conclusion, we developed polymeric nanoparticles that encapsulates both the antigen and the adjuvant to be used as a potential tool to increase antigen protection that resulted in higher amounts of antigen internalized in cell lines that mimic the intestinal barrier. Together these properties make these antigen-adjuvant containing nanoparticles suitable candidates for potential oral immunization.

134 (165) ANTI-*TRYPANOSOMA CRUZI* PROPHYLACTIC VACCINE DECREASES HEART TISSUE DAMAGE AND IMPROVES CARDIAC FUNCTION IN MICE

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Currently there are no prophylactic vaccines to combat Chagas disease. We previously showed that a nasal vaccine formulated with a N-terminal fragment of Trans-sialidase (TS) combined with c-di-AMP(A), generated specific humoral and cellular immunogenicity and protects against the parasite during acute phase. Given that an effective anti-*T. cruzi* vaccine might avoid or attenuate myocardialdamage, here, we aim to evaluate at myocardial level the prophylactic efficacy during the acute (17 days post-infection–dpi-) and the chronic (110 dpi) phases. For this, BALB/c female mice, were intranasal immunized with the TS+A vaccine(3 doses, one every 2 weeks). As controls, groups of mice were also immunized with saline solution (SS) or TS and A alone. After immunization, animals were orally challenged with 3000 Tulahuen strain/mice. A group of mice not immunized and not infected (NI) was also included. Hearts were removed at the different dpi and longitudinally dissected, afterwards part of tissue was fixed in buffered formalin and later paraffin-embedded and 5-mm tissue sections were stained withboth hematoxylin/eosin (for the evaluation of intensity and magnitude of inflammatory infiltrates), and picrosirius red (for fibrosis evaluation). The rest of tissue was cryopreserved for cytokine expression evaluation by RT-qPCR (IL10,TGF-β, IFN-γ, TNF-α, IL17a). Additionally, chronic functional impairment was determined by electrocardiogram (ECG), and different parameters (corrected QTinterval –QTc-, QRS) were analysed. Acute and chronic myocarditis showed mildinfiltrates in TS+A group, while SS, TS and A groups showed moderated or severe damage (overall,$p<0.05$). Fibrosis was more evident in SS, TS, and A groups, while TS+A mice showed minor intensity (overall,$p<0.05$). Chronic histological findings are clearly associated to an improve heart function, since ECG showed less alterations in QRS duration and QTc in TS+A compared with SS, TS and A, being the values of these parameters similar to observed in NI mice. The proportion of arrhythmias was also less evident in TS+A mice compared with the rest of infected mice. Heart cytokine expression during acutephase showed that TS+A vaccine decreased the expression of TNF-α and TGF-β (in both cases, TS+A vs SS, $p<0.05$). Contrary, IL17a was enhanced in TS+A vaccinated animals compared with SS mice ($p<0.05$), while no differences in IL10and IFN-γ expression were found among groups. During the chronic phase, hearts of TS+A mice also showed a decreased expression of TGF-β (TS+A vs SS, $p<0.05$), and a trend to increase IL17a (mean±SEM, arbitrary units-AU-,TS+A=37±15; SS=18±7, n.s.) and decreased IFN-γ (mean±SEM, AU, TS+A=3±1.9;SS=6±2, n.s.). Moreover, no significant differences were found in TNF-α, while IL10 expression could not be detected. Taken together, these results showed that the

administration of TS+A vaccine decreases the damage at myocardial level in both acute and chronic phase and protects cardiac function.

135 (56) APPLYING MACHINE LEARNING TO EXPLORE CORRELATES OF PROTECTION FOR A VACCINE AGAINST *TRYPANOZOMA CRUZI*

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Introduction: Chagas disease, caused by the protozoan parasite *Trypanosoma cruzi (T. cruzi)*, is a tropical neglected disease for which a vaccine has yet to be developed. We previously described a vaccine candidate composed of a trans-sialidase fragment (TSf), and a cage-like particle adjuvant (ISPA). Correlates of protection (CoPs) are immunological biomarkers which can be used to predict the efficacy of a vaccine. Machine learning algorithms can be used as an important tool to establish CoPs.

Objective: to apply machine learning to search for potential CoPs in the development of a vaccine against *T. cruzi*.

Methods: BALB/c mice that received a protocol of vaccination with TSf-ISPA were included in the study. IgG antibodies anti-TSf were measured by ELISA. Delayed hypersensitivity reaction (DTH) was measured 48 h post-inoculation of 5 ug TSf in the footpad of TSf-ISPA treated and PBS-control inoculated mice. Vaccinated and control mice were challenged intraperitoneally with 1000-2000 *T. cruzi*. (n=20 per study). Parasitemia were measured at day 15 post-infection (p.i.) and survival was recorded until day 40 p.i. Python'sscikit-learn library was used to construct machine learning classification models.

Results: Logistic regression models were generated to assess the use of optical density (OD 450 nm) as a CoP for the survival of vaccinated mice challenged with 1000 or 2000 *T. cruzi*. In all cases, the results were obtained using k-fold methodology. In order to consider not only the death-live difference but also the time of survival, a criterion was developed. A classification label of "1" was assigned to each mouse that died before day 21 p.i. (coincident with the peak of parasitemia), and a label of "0" was assigned to each mouse which died after day 21 p.i. or even did not die. This approach yielded a substantial predictive capability. For instance, confusion matrix analysis returned average values of sensibility of 80,5%, specificity of 86%, and an area under ROC curve of 0,84 for data from mice infected with 1000-2000 parasites.

Regarding DTH as a CoP, when logistic regression was used to analyze data after the challenge with 1000-2000 *T. cruzi*, confusion matrix returned average values of 83% sensitivity, 90,5% specificity, and a value of 0,87 for the area under the ROC curve.

For parasitemia studies, to develop the models, the following criterion was used: if parasitemia value of each mice/mean PBS parasitemia>1, a classification label of "1" was assigned to that mouse. If parasitemia value of each mouse/mean PBS parasitemia <1, a label of "0" was assigned to that mouse. After the analysis, lower predictive capacity was obtained as compared with the use of antibodies as CoPs.

Discussion: Results suggest that machine learning models could be used to analyze potential CoPs of vaccine candidates against *T. cruzi*. Higher number of experimental animals may allow to increase the robustness of the models.

136 (209) CHARACTERIZATION AND IN VITRO EVALUATION OF TRIPLE-TARGETED NANOVESICLES

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The first line of defense against pathogens is mediated by the innate immune system. One of the main activation mechanisms of this system occurs through pattern recognition receptors (PRRs) for structurally conserved microbial motifs. Among the existing PRRs, Toll-like receptors (TLRs) are found, wich are transmembrane proteins located on the extracellular surface and endosomal compartments. TLRs are a highly conserved family among vertebrates, typically expressed in macrophages and dendritic cells.

Imiquimod (IMQ) a nucleoside analogue and polyI:C a synthetic double-stranded RNA are ligands for endosomal TLR 7 and TLR 3, respectively. Both can be used as adjuvants to enhance and optimize the resulting adaptive immune response. However, strategies are required to prevent their escape from the administration site, as IMQ can cause potentially lethal septic shock reactions and contribute to remote organ dysfunction. Meanwhile, high doses of parenterally administered polyI:C can lead to fever and anemia. Therefore, we propose combining polyI:C and IMQ within archaeolipid nanovesicles (nanoARQ). These nanovesicles are recognized by the scavenger Receptor-A1 and allow increased capture by target cells, leading to massive delivery to endosomes. Our goal is to enhance endosomal internalization and achieve synergy in the simultaneous activation of TLR 3 and 7, reducing the required doses and amplifying their adjuvant capacity. Through gradually adding IMQ to a fixed concentration of acridine orange and PolyI:C changes in the fluorescence emission spectrum were observed. Thus determining that IMQ intercalated between the nitrogenous bases of polyI:C, forming what we refer to as a complex (C). Subsequently, the electrostatic combination of archaeolipid nanovesicles with C was developed obtaining nanoARQC. Using dynamic light scattering and phase analysis light scattering measurements we determined that nanoARQC exhibited an average size of 569 ± 172 nm, a PDI of 0.58 ± 0.15, and a Z potential of -11.1 ± 0.9 mV.

In vitro assays were conducted on THP-1 human macrophages by incubating the cells with the nanovesicles using a phospholipid concentration of 100 µg/ml for 24 hours. Cell viability was above 75% in all the used samples. The obtained supernatants were evaluated using enzyme-linked immunosorbent assay (ELISA), revealing that nanoARQC induced a significant release of TNF-α and IL-8 compared to C. Both results were analyzed via one-way ANOVA with $p < 0.0001$. Lastly, nanoARQC were labeled with a fluorescent lipid and cellular capture was determined through fluorescence microscopy.

In conclusion, we successfully mitigated the electrostatic repulsion between polyanions (PolyI:C) and surfaces with high negative Z potential (archaeolipid nanovesicles) by combining PolyI:C and IMQ within a unique nanostructure. This nanostructure is small enough to be endocytosed and capable of inducing a pronounced release of TNF-α and IL-8 in macrophages.

137 (95) CHARACTERIZATION OF A NANOPARTICLE-BASED VACCINE AS A BOOSTER FOR COVID-19

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is an emergent causative agent of a global pandemic. Vaccines development was the most prominent approach to prevent the spread of the virus and COVID-19. However, the emergence of SARS-CoV-2 variants has led to concerns of viral escape from natural- or vaccine-induced immunity. While effective vaccines for severe COVID-19 are currently deployed, we aimed to develop a vaccine platform that could be used as an initial vaccination regime or as a booster.
We characterized polycationic nanoparticles (Np) of poly allylamine as adjuvants in terms of cell internalization and activation, cytotoxicity (IFI, ELISA and flow cytometry) and immunogenicity. Balb/c mice were immunized with two doses at 21 day-interval as initial schedule with Receptor Binding Domain (RBD) in combination with Np, Alum or Alum+CpG as adjuvants or in a boosted scheme, with two doses at 21 day-interval with the *Pfizer®* vaccine followed 28 days after with RBD and Np, Alum or Alum+CpG. Fifteen days after the boost humoral (IgG, IgG1, IgG2a) and cellular (IFNγ-producing CD4 and CD8 T cells) immune responses were evaluated by ELISA and flow cytometry.
We found that Np was internalized by dendritic cells and induced lysosomal destabilization, cell activation, with inflammasome activation followed by IL-1β secretion (200 vs 1 pg/ml compared with medium). We found in vaccinated mice with the initial regime that Np-RBD induced higher levels of serum and bronchoalveolar lavage specific-RBD IgG than mice that received Alum-RBD ($p<0.05$), with predominant production of specific IgG2a ($p<0.05$). Also, Np-RBD vaccinated mice showed a higher frequency of CD4+IFN-γ+ T cell and CD8+IFN-γ+ T cells than the other groups ($p<0,05$). In boosted mice, we found significant higher levels of serum specific-IgG and IFN-γ production compared to the initial schedule, however we did not find differences in the immune responses between the different boosts used.
In conclusion, Np showed adjuvant properties promoting cell activation through the inflammasome pathway and immune response induction at high levels following an initial regime or as a boost. These results are promising for ARGENVAC, a vaccine for COVID-19.

138 (125) CYTOKINES PROFILE INDUCED IN PERIPHERAL BLOOD MONONUCLEAR CELLS OF PIG AFTER STIMULATION IN VITRO WITH DIFFERENT CPG-ODN

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We developed an innovative strategy for formulating vaccine components, which involves the formulation of antigen and CpG-ODN with a nanostructure formed by self-assembly of 6-O-ascorbyl palmitate (Coa-ASC16). Previously, we demonstrated that mice immunized with the OVA/CpG-ODN/Coa-ASC16 nanoformulation elicited antigen-specific antibody and cellular responses superior to those induced by an aqueous solution of OVA and CpG-ODN. CpG-ODNs, due to their chemical structures and immunostimulatory effects, can be categorized into three classes: A, B, and C. Furthermore, the immunostimulatory activity triggered by CpG-ODNs varies among different species. Our goal is to validate the CpG-ODN/Coa-ASC16 adjuvant strategy in the porcine species. To identify which CpG-ODN elicits the best response in pigs, peripheral blood from Large White x Landrace pigs was collected from the jugular vein in vacutainer tubes containing 18 mg of EDTA K2. Peripheral blood mononuclear cells (PBMC) were isolated through FICOLL gradient separation and incubated at 37°C in an environment with 5% CO_2 for 12 hours with four different types of CpG-ODN: 2395, 1826, 1018, and 2007. Medium without CpG-ODN was employed as the control (baseline). The mRNA expression of cytokines and TLR9 was assessed through RT-qPCR. Our findings indicate that CpG-ODN 2395 (a class C CpG-ODN originally used for humans and mice) is capable of inducing a broad spectrum of innate immune response mediators, including IL-6, IL-12p40, TNFα, IFNβ, and IFNγ ($p<0.05$). In second place, CpG-ODN 1018 (a class B CpG-ODN employed in humans in HEPLISAV-B® vaccine and vaccines against SARS-CoV-2) stimulated the production of IFNβ and IFNγ ($p<0.05$) but the levels of these cytokines were lower compared to those induced by CpG-ODN 2395 ($p<0.05$). CpG-ODN 1018 also stimulated the production of TNFα and the level of this cytokine was higher than that observed when PBMC were stimulated with CpG-ODN 2395 ($p<0.05$). Conversely, the other types of class B CpG-ODN evaluated (CpG-ODN 1826, widely used in mouse, and CpG-ODN 2007, utilized in experimental research with chickens, cattle, and pigs) did not elicit PBMC activation under the analyzed conditions. Significantly, PBMC stimulated by CpG-ODN 2359 and 1018 exhibited a significant increase in TLR9 mRNA expression compared to the baseline condition ($p<0.05$). In summary, our study reveals that only CpG-ODN 2359 and 1018 stimulate in vitro the innate immune response in PBMC, albeit with a differential profile of cytokines. Based on these findings, we conclude that CpG-ODN 2395 is the most suitable candidate for formulation with

Coa-ASC16 and should be investigated further in vivo as a vaccine adjuvant in pigs.

139 (158) DC TARGETING AS A STRATEGY FOR INDUCING *TRYPANOSOMA CRUZI* SPECIFIC CD8⁺ T CELL RESPONSES

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In the immune response against *Trypanosoma cruzi*, CD8⁺ T cells are crucial for parasite control and survival during the acute phase of infection. We have previously determined that cross-presentation of antigens (XP) is a relevant mechanism involved in the induction of such responses orchestrated by dendritic cells (DCs). Within the subpopulations of DCs, conventional type 1 DCs (cDC1s), which express the endocytic receptor Clec9A, perform XP most efficiently. Since there are currently no vaccines to prevent or treat Chagas disease, in this work we evaluated a prophylactic vaccination strategy based on antigen targeting of cDC1s in C57BL/6 mice with the aim of inducing CD8⁺ T cell responses. Chemical conjugates of the TsKb20 peptide – immunodominant epitope derived from the *T. cruzi* trans-sialidase protein – with anti-Clec9A monoclonal antibody or an antibody without receptor affinity (isotype control) were obtained to immunize mice intravenously together with poly (I:C) as adjuvant. Non-immunized animals, animals immunized with non-targeted TsKb20 and poly (I:C) or animals that received only the adjuvant were used as additional controls. One week later, the animals were sacrificed and splenocytes were obtained to assess the specific effector response of CD8⁺ T cells by staining with tetramers, analysis of CD25 and CD69 activation markers, and Th1-type cytokine production after *in vitro* restimulation with TsKb20 peptide. Flow cytometric analysis of splenocytes derived from mice that had received TsKb20-conjugated anti-Clec9A showed a population of tetramer-positive CD8⁺ T cells and increased expression of CD25 and CD69 markers in response to incubation with TsKb20 peptide in contrast to the rest of the groups (overall $p<0.05$). In addition, immunization of mice with TsKb20-conjugated anti-Clec9A specifically increased the number of CD8⁺ T cells producing IFN-γ, IL-2, and TNF-α after TsKb20 restimulation when compared to splenocytes derived from animals immunized with the isotype control (overall $p<0.05$). Statistical comparison between the TsKb20-conjugated anti-Clec9A group and the isotype control was performed using the non-parametric Mann Whitney test. In conclusion, this work shows that DC targeting with a parasite epitope is capable of activating specific CD8⁺ T cells and inducing an effector response mediated by the production of cytokines, which are known to be important during the response against *T. cruzi* infection.

140 (74) DEPLETION OF MYELOID-DERIVED SUPPRESSOR CELLS CAUSES DENDRITIC CELLS INCREASES DURING IMMUNIZATION AGAINST *TRYPANOSOMA CRUZI*

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Introduction: Chagas Disease is caused by the protozoan parasite *Trypanosoma cruzi (T. cruzi)*. To date, there is no prophylactic or therapeutic vaccine available. We have reported that a vaccine based on a trans-sialidase fragment (TSf) formulated with a cage-like particle adjuvant (ISPA) protects BALB/c mice against *T. cruzi* infection. We also described that depletion of CD11b+Gr-1+myeloid-derived suppressor cells (MDSCs) with 5-fluorouracil (5FU) during TSf-ISPA-immunization potentiated the protective capacity of the vaccine candidate.

Aim: To study whether MDSC depletion influences spleen and lymph node dendritic cells (DCs) numbers during the immunization phase, and whether MDSCs from immunized mice are able to suppress maturation of DCs in vitro.

Methods: 3 doses of TSf-ISPA were given to BALB/c mice, with a 15-day interval between each dose. 5FU (50mg/Kg) was given one day before and 8 days after each TSf-ISPA inoculation. Spleen and lymph node CD11c+ DCs and conventional type I CD11c+CD8α+dendritic cells (cDCI) were measured by flow cytometry in PBS-inoculated and double 5FU-TSf-ISPA vaccinated mice, at day 2 and 7 post-immunization (p.imm). In addition, Gr-1+ splenocytes were purified by magnetic cell sorting from TSf-ISPA immunized mice (without 5FU) and the capacity to suppress the expression of MHCII and CD80 was assessed in vitro using bone-marrow derived dendritic cells (BMDCs).

Results: The absolute number of CD11c+ DCs and CD11c+CD8α+cDCI significantly increased in TSf-ISPA-immunized mice that were depleted of MDSCs with 5FU. For instance, at day 7 p.imm. mean number of CD11c+ (DCs) splenocytes $x10^6$ ± standard deviation (SD): PBS-treated mice: 3,05±0,4; double 5FU-TSf-ISPA mice: 5,1±0,3 ($p<0,05$). In addition, mean number of CD11c+CD8α+(cDC1) splenocytes $x10^6$±SD: PBS-treated mice: 0,75±0,2; double 5FU-TSf-ISPA mice: 2,2±0,2 ($p<0.05$). In addition, GR-1+ splenocytes purified at day 7 from TSf-ISPA immunized mice suppressed the expression of costimulatory molecules on BMDCs stimulated with LPS. For instance, after 72 h of culture, geometric mean MFI MHCII±SD: BMDCs alone: 129321± 26192; BMDCs + MDSCs 72763±16171, $p<0,05$. Geometric Mean CD80±SD: BMDCs alone: 130110±11183; BMDCs + MDSCs 64081±12710 ($p<0.05$).

Conclusion: Depletion of MDSCs during immunization allows an increase in the absolute number of CD11c+ cells in the spleen and lymph node of double 5FU-TSf-ISPA immunized mice. The fact that purified MDSCs suppressed BMDC maturation suggests that MDSCs may play a role suppressing DC maturation during the immunization process. Results presented here in support the depletion of MDSCs as a target to improve the efficacy of a vaccine against *T. cruzi.*

141 (20) DEVELOPMENT OF A VACCINE CANDIDATE BASED ON VIRUS-LIKE PARTICLES AND AN IMMUNO-STIMULANT ADJUVANT AGAINST ZIKA VIRUS CAPABLE OF INDUCING A STRONG MUCOSAL RESPONSE

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Virus-like particles (VLPs) are a type of subunit vaccines that possess diverse applications in therapeutics, immunization, and diagnostics. On one hand, VLP-based vaccines are being extensively used because of their efficacy, safety and diversity. On the other hand, *Giardia lamblia* variable surface proteins (VSPs) have been shown to act as adjuvants and endure extreme conditions. These protozoan proteins can be used to generate oral vaccines due to their outstanding resistance to proteases and to changes in pH, immunogenicity and absence of toxicity. Previously, using an Influenza viral model, it was shown that surface decorated VLPs with VSPs triggered a successful immune response after oral and subcutaneous immunization with hemagglutinin glycoprotein. Considering the increasing global impact of arboviruses, our goal is to develop a safe and effective vaccine against Zika virus (ZIKV, Flavivirus) using VLP-VSP in conjunction with specific viral antigens. ZIKV infection causes an acute febrile disease including rash, conjunctivitis and arthralgia, and it is also associated with complications such as Guillain-Barré syndrome and complex congenital disorders. So far, there are no available vaccines against ZIKV, which is spread not only by hematophagous mosquitoes but also sexually among humans. In this context, for an optimal Zika vaccine, ensuring safety is of utmost importance, particularly when considering its administration to pregnant women. Additionally, the vaccine should elicit a robust mucosal response to effectively counteract sexual transmission. In this sense, the VLP-VSPs platform fulfills the safety criteria owing to the absence of viral genome and its adaptability for administration through various routes. At the outset, we amplified and inserted the gene encoding the ZIKV-E into a mammalian vector (pEZIKV). We then verified its accurate expression in different cell lines through immunofluorescence observation. For the production of VLPs, we co-transfected HEK cells that constitutively express a specific r *Giardia* VSP (VSP1267) with two plasmids: one encoding the Murine Leukemia Virus capsid, promoting the subsequent self-assembly of the VLPs (pMLV-Gag) and pEZIKV. This approach led to the successful expression of the ZIKV E glycoprotein in VLP-VSP constructs. The proper assembly was confirmed by Western blotting and transmission electron microscopy (TEM), using anti-Zika E antibodies. Finally, we assessed the immune response in Balb/c mice by administering VLP-VSP-EZIKV through both oral and subcutaneous routes. The presence of anti-ZIKV antibodies, confirmed by ELISA, verified the humoral response in both serum and mucosal samples. Additionally, ongoing analysis of the cytokine profile is underway. The data were analyzed by one-way ANOVA followed by Tukey's multiple comparison test. Our

findings demonstrate the capacity to produce targeted antibodies using a secure vaccine that can accommodate various administration protocols.

142 (25) DIFFERENCES IN HUMORAL RESPONSE TO SARS-COV-2 VARIANTS, INCLUDING DELTA AND OMICRON, IN LIVER TRANSPLANT RECIPIENTS AFTER RECEIVING THE THIRD DOSE OF COVID-19 VACCINES

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Solid organ transplant recipients (SOTR), including liver transplant recipients (LTR), often exhibit reduced vaccine responses compared to immunocompetent individuals (IC). While SARS-CoV-2 vaccines have shown significant effectiveness in reducing mortality among the general population, not all individuals produce adequate vaccine responses. Efficacy varies across vaccines, and access to diverse vaccine platforms is unequal across countries, as is the case with Argentina. The emergence of new variants has heightened SARS-CoV-2 transmissibility, severity, and compromised the efficacy of initial monovalent vaccines targeting the original Wuhan strain. Thus, we set out to evaluate the humoral response after a third dose of SARS-CoV-2 vaccines, with a specific focus on their effectiveness against emerging variants. For this purpose, samples from a cohort of 81 LTR and 27 IC were taken between 21 and 90 days after the third vaccine dose. The vaccination schemes that were analyzed in this study included viral vector vaccines (VV) ChAdOx-1 or rAd26/rAd5, inactivated vaccines (IN) BBIP-CorV and mRNA vaccines (mRNA) mRNA-1273 or BNT162b2. Serology for anti-Spike IgG antibodies and neutralizing antibodies against Wuhan, Delta and Omicron variants were evaluated. We found that 73.5% of LTR were responders for anti-Spike IgG, while 100% of IC mounted a measurable response. LTR who responded to the third dose showed significantly lower anti-Spike IgG levels and neutralizing antibodies than IC. Also, we found that there was less neutralization in LTR compared to IC across all variants. Specifically, the neutralization titers in both groups decreased when tested against the Delta variant, and this decline was even more pronounced with the Omicron variant, compared to the Wuhan variant. Furthermore, we identified that the use of high doses of mycophenolate and advanced age were factors that negatively affected the development of anti-Spike IgG antibodies. Regarding vaccine regimes, the regime viral-vector/mRNA/mRNA elicited significantly higher responses in LTR compared to other vaccine schemes. In addition to the recommended and necessary booster doses in this population, strategies that achieve adequate immunization should be evaluated.

143 (31) DOUBLE 5-FLUOROURACIL ADMINISTRATION POTENTIATES A TRANS-SIALIDASE-BASED VACCINE AGAINST *TRYPANOZOMA CRUZI*

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Introduction: *Trypanosoma cruzi* (*T. cruzi*) is the etiological agent of Chagas disease, a tropical neglected illness for which a licensed vaccine is still unavailable. We previously described a vaccine candidate composed of a trans-sialidase fragment (TSf) and a cage-like particle adjuvant (ISPA). In addition, depletion of myeloid-derived suppressor cells (MDSCs) using one or two doses of 5-fluorouracil (5FU) during immunization enhanced the efficacy of the vaccine formulation.

Objective: to characterize the impact of one versus two doses of 5FU on the kinetics of MDSCs during a two-week period following TSf-ISPA immunization, and to compare the efficacy of both protocols against high-dose challenges of *T. cruzi.*

Methods: BALB/c mice were given 3 doses of TSf-ISPA, with a 15-day interval between each dose. For mice receiving the 5FU-TSf-ISPA treatment, a single dose of 5FU (50mg/kg) was administered one day before each immunization. In the case of DOUBLE 5FU-TSf-ISPA treatment, 5FU was given one day before and eight days after each immunization dose. Flow cytometry was used to measure CD11b+GR-1+ MDSCs in splenocytes (suppressive capacity already controlled) on days 2, 7, and 15 following the final immunization dose. Additionally, mice were intraperitoneally infected with Tulahuen *T. cruzi* in varying doses (1500, 2000, 3000). Parasitemia were monitored on different days' post-infection (p.i.), and survival rates were assessed until day 40 (p.i.).

Results: At days 2 and 7 post-immunization (p.imm), both 5FU-TSf-ISPA and DOUBLE 5FU-TSf-ISPA mice exhibited similar percentage and absolute number of CD11b+Gr-1+ MDSCs splenocytes. Conversely, by day 15 p.imm, the DOUBLE 5FU-TSf-ISPA group displayed a reduced percentage and absolute number of CD11b+Gr-1+ MDSCs compared to 5FU-TSf-ISPA mice. Mean percentage ±standard deviation (SD): 5FU-TSf-ISPA 11,6±0.8; DOUBLE 5FU-TSf-ISPA 3,2±1,3 ($p<0.05$). Mean absolute number: 5FU-TSf-ISPA 14,3±1,1; DOUBLE 5FU TSf-ISPA 2,8±1,2 ($p<0.05$). (Mean percentage and absolute number of PBS-treated mice: PBS=4,1±1,1 and 3,26 ±0,5 respectively).

Following *T. cruzi* challenge, the DOUBLE 5FU-TSf-ISPA group exhibited lower parasitemia and higher survival rates than the 5FU-TSf-ISPA mice. For instance, after infection with 2000 Tulahuen parasites (mean parasitemia on day 28 p.i±SD): 5FU-TSf-ISPA 133.4±97.6; DOUBLE 5FU-TSf-ISPA 16.2±15.0 ($p<0.05$). Overall survival: 5FU-TSf-ISPA 22%; DOUBLE 5FU-TSf-ISPA 100% ($p<0.05$).

Conclusion: The second dose of 5FU has a significant impact on the kinetics of MDSCs, leading to reduced percentage and number of this population by day 15 post-immunization. In addition, the protocol based on two doses of 5FU during each TSf-ISPA immunization decreased parasitemia and increased survival after infection with a high parasite dose. Thus, these results support the use of two doses of 5FU to deplete MDSCs and improve the protective capacity of the TSf-ISPA vaccine.

144 (191) EFFECT OF CO-ADMINISTRATION OF LACTOBACILLIS-LAYER PROTEINS ON IMMUNE RESPONSE AGAINST S-LAYER PROTEINS FROM PATHOGENIC *CLOSTRIDIOIDES DIFFICILE*

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The anaerobic, Gram-positive, spore-forming pathogen *Clostridioides difficile* is one of the leading causes of nosocomial antibiotic-associated diarrhoea (AAD) worldwide. Active immunization with surface components or sporulation factors emerges as an alternative to the antibiotic-based therapy. The S-layer is a two-dimensional self-assembled (glyco)-proteinaceous envelope covering the surface of several pathogenic and non-pathogenic bacteria. Previously, we have shown that glycosylated S-layer proteins (SLPs) from different strains of *Lentilactobacillus kefiri* enhance LPS-induced stimulation in murine macrophages. Moreover, it has been reported that *C. difficile* SLPs could act as a Toll-like receptor 4 ligand. Thus, with the aim to find new *C. difficile* antigenic targets and potential adjuvants, we started our study assessing the ability of SLPs derived from *C. difficile* ATCC 43255 (SLP-Cd), and the SLPs of different *L. kefiri* strains (SLP-Lk8335, SLP-Lk8343, SLP-Lk8344, SLP-Lk83111, SLP-Lk83113) to activate murine macrophages *in vitro* both alone and combined. Cultured RAW264.7 cells were treated with individual SLPs or a combination of SLP-Lk + SLP-Cd, and secreted IL-6 after 24h of stimulation was measured by capture ELISA. SLP-Cd did not exert a strong stimulus on macrophage even when were tested at 30 mg/ml. On the other hand, only SLP-Lk8343 and SLP-Lk83111 were able to stimulate IL-6 secretion on RAW264.7 cells at 10 mg/ml ($P< 0,05$). Interestingly, cellular activation was significantly increased ($P<0,05$) after incubation with a combination of SLP-Cd and almost all the SLP-Lk tested (with exception of SLP-Lk8335), in comparison to stimulation with SLP-Cd or SLP-Lk alone. Regarding these results, groups of 5-6 weeks-old BALB/c mice were subcutaneously inoculated with combinations of SLP-Cd and SLP-Lk83111 (3 dose-scheme, every 15 days). Groups receiving only SLP-Cd or phosphate-buffered saline (PBS) were included. Specific anti-SLP-Cd antibodies were detected by indirect ELISA in serum, and production of IFN-g was tested in SLP-Cd-stimulated cells from inguinal lymphoid nodes. Under the experimental conditions tested, no adjuvant effect of any of the SLP-Lk on the specific response against SLP-Cd was observed. Although the *in vitro* results suggested that the combination of both stimuli could improve the capacity of macrophages as antigen-presenting cells, this effect did not lead to an enhancement of the adaptive response against SLP-Cd. However, other immunization schemes can be tested, considering the development of active immunotherapies against *C. difficile* AAD.

145 (176) EVALUATION OF THE POTENTIAL OF OUTER MEMBRANE VESICLES FROM *BRUCELLA OVIS* AS A VACCINE AGAINST BRUCELLOSIS

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Sheep brucellosis is caused by *Brucella ovis* and *B. melitensis*. Control strategies for this disease include periodic diagnosis through serological tests and/or bacteriological cultures, as well as the culling of positive animals. Application of *Brucella melitensis* Rev-1 vaccine is recommended when prevalence is high. However, this attenuated vaccine strain presents significant drawbacks including the development of antibodies that interfere with serodiagnosis, and its virulence in humans, among others. Therefore, it is important to develop new safe and effective vaccines against ovine brucellosis. Outer Membrane Vesicles (OMV) from Gram-negative bacteria have been used in the development of acellular vaccines against numerous infectious diseases. In this study, we assessed the immunogenicity of OMV from *B. ovis* (strain Reo 198) with the aim of evaluating their potential as an acellular vaccine. For this purpose, *B. ovis* was cultured in tryptic soy broth supplemented with 0.5% yeast extract for 72 hours. OMV were isolated from the cell-free culture supernatant through ultracentrifugation. The protein content of the OMV fraction was determined using the bicinchoninic acid method, and the size of the isolated OMV was measured using Dynamic Light Scattering (DLS). DLS analysis demonstrated that OMV have a hydrodynamic diameter of 60 nm. To assess whether the *B. ovis* OMV antigens are recognized by serum antibodies from naturally infected sheep, an indirect ELISA assay was conducted using serum samples from both diseased and healthy animals. In another experiment female BALB/c mice were immunized by intramuscular route with 5 ug of OMVs, 5 ug of OMV plus 10 ug c-di AMP (adjuvant), or saline solution at 0 and 30 days. Sera samples were obtained at 21 and 45 days for the first immunization to determine specific antibody production by indirect ELISA. The levels of anti-OMV IgG in the sera of infected sheep were significantly higher than those of healthy animals ($p<0.05$). Similar results were obtained when sera from naturally infected pigs with *B. suis* were tested. In the mouse model, vaccination with OMV alone or OMV+c-di AMP significantly increased specific IgG titers (Titers: 32000 and 16000, respectively) at 21 days post-first immunization. Taken together, these results demonstrate that *B. ovis* OMV are immunogenic and self-adjuvant, thus potentially constituting a novel vaccine for brucellosis.

146 (61) EVALUATION OF THE SPECIFIC IMMUNE RESPONSE INDUCED BY A RECOMBINANT BCG VACCINE EXPRESSING THE SARS-COV-2 NUCLEOPROTEIN.

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Abstract: SARS-CoV-2 is the causative virus of COVID-19, which is responsible for millions of deaths worldwide. One of the most important antigens among the different variants of this virus is the nucleoprotein, which is immunogenic and conserved. Along this line, our laboratory generated a vaccine against SARS-CoV-2 using the *Mycobacterium bovis* Bacillus Calmette-Guérin (BCG) vaccine as a platform expressing this structural protein. BCG is a vaccine that has been used for more than 100 years in children and the elderly for tuberculosis control. Here, we evaluated the safety and specific immune response induced by a recombinant BCG vaccine expressing the SARS-CoV-2 nucleoprotein (rBCG-N-SARS-CoV-2) in coadministration with recombinant Spike and nucleoprotein and aluminum hydroxide (rProts-A) proteins in a murine model.

Methods**:** BALB/c mice were immunized with $1x10^5$ CFU of rBCG-N-SARS-CoV-2 and recombinant proteins to evaluate safety and immunogenicity parameters. On day 28, post-immunization, a booster with recombinant proteins was performed, and on day 42, post-immunization, animals were euthanized. Lymphocytes were purified and co-cultured with dendritic cells that were stimulated with SARS-CoV-2 nucleoprotein (N), SARS-CoV-2 Spike protein (S), and PPD. After 48 hours, T cell activation markers $CD25^+$, $CD69^+$, and $CD71^+$ were evaluated by flow cytometry. Co-culture supernatants and serum samples were analyzed by ELISA to evaluate cytokines and specific antibodies against SARS-CoV-2 proteins N and S, and surrogate neutralization assays were performed to evaluate the secretion of neutralizing antibodies.

Results**:** Immunization with rBCG-N-SARS-CoV2 vaccine was safe and promoted the activation of antigen-specific neutralizing antibodies and $CD4^+$ and $CD8^+$ T cells recognizing the nucleoprotein and Spike proteins of SARS-CoV2. Additionally, T cell-APC co-culture supernatants showed IFN-γ and IL-2 secretion upon antigenic stimulation.

Conclusions: The BCG vaccine is a promising platform for recombinant antigen expression that can potentially be used to control respiratory pathogens such as SARS-CoV-2. In addition, this vaccine induces a marked immune response characterized by a Th1-type cellular immune response necessary for intracellular pathogen control, secretion of IFN-γ and IL-2 cytokines, and secretion of specific antibodies against the most immunogenic antigens of SARS-CoV-2. We believe that this rBCG-N-SARSCoV-2 vaccine can be an excellent candidate for children to combat SARS-CoV-2. Moreover, our formulation will allow us to efficiently have a vaccine for emerging variants only by changing the proteins of interest.

147 (65) FREE OR NANOENCAPSULATED *Minthostachysverticillata* ESSENTIAL OIL AS CANDIDATES FOR ADJUVANT IN ORAL VACCINES

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In previous studies, we have demonstrated that free and micro or nanoencapsulated essential oil (EO) from *Minthostachysverticillata*, an Argentinian plant, have adjuvant ability enhancing the immune response of different antigens by subcutaneous immunization in mice. In a recent study we reported that oral administration of EO result safe and maintained a tolerogenic environment in the intestinal mucosa of mice. The aim of this study was to evaluate the effect of oral administration of free (EO) and nanoencapsulated (NEO)essential oil on weaned piglets as possible candidates for adjuvants in oral vaccines to prevent post-weaning diarrhea. EO was extracted by hydrodistillation and nanoencapsulation was performed by the high-energy method using Tween 80 and Span 60 as surfactants. EO and NEO were chemically analyzed by gas chromatography-mass spectrometry (GC-MS). The cytotoxic effect of both was evaluated on human colorectal adenocarcinoma (Caco-2) cell line. For *in vivo* assay, thirty-six weaned piglets were randomly distributed in six groups of six animals each group (n=6). Each group received orally EO or NEO for 30 consecutive days. Group 1: Control group (not treated), Group 2: Vehicle control (Tween 80 0.75% v/v, Span 60 0.25% w/v and deionized water 99% v/v). Group 3: EO (10 mg/kg/day). Groups 4-6: NEO (2.5, 5 and 10 mg/kg/day, respectively). Subsequently, histological and hematological parameters, cytokines production, oxidative markers, and $CD4^+/CD8^+$ T cells were evaluated. The GC-MS analysis was similar in both. NEO was more toxic on Caco-2 cells than EO, but the toxicity was acceptable. NEO did not alter the morpho-physiology of digestive organs and decreased malondialdehyde concentrations in liver and kidney ($p<0.05$), resulting safer than EO. No differences were observed in the total leukocyte count of piglets treated orally with EO or NEO, however, NEO groups showed an increase in $CD4^+/CD8^+$ T cells ratio ($p<0.001$). TNF-α and IL-1β levels were not altered by oral administration of EO and NEO, however, NEO induced the highest levels of IL-10 ($p<0.01$). Results showed that NEO administered orally to weaned piglets was safer than EO and maintained a tolerogenic immune response. In future studies, we will evaluate the adjuvant effect of NEO in orally administered vaccines based on microorganisms or subunit of microorganism that cause porcine diarrhea.

148 (99) HUMORAL RESPONSE INDUCED BY A FIFTH DOSE OF AN INACTIVATED-VACCINE AGAINST SARS-COV-2.

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Introduction: Vaccines against SARS-CoV-2 have successfully reduced COVID-19 severity and death. However, while SARS-CoV-2 continues to circulate in the population, the humoral response induced by either vaccines or natural viral exposure has shown to decline over time. In response, booster doses against wild-type (WT) virus and different variants such as Delta and Omicron have been applied, showing positive effects in restoring the neutralizing immunity against SARS-CoV-2.

Aim: In the context of a clinical study, we determined the effect of a third booster (fifth) dose of an inactivated virus vaccineagainst variants of the virus, in the humoral immunity against SARS-CoV-2.

Methods: In the context of the CoronaVar clinical study phase III (NCT05593042), 216 volunteers were recruited at one medical center and randomly selected for analyses. Blood samples were obtained at the time of vaccination (Day 0) and 28 days' post-immunization (Day 28). In 180 subjects, the humoral immunity against ancestral SARS-CoV-2 and variants of concern was evaluated by quantifying total IgG against SARS-CoV-2 by Meso Scale Discovery (MSD) technology. In addition, neutralizing response was assessed by conventional virus neutralization test (cVNT) was determined in 135 subjects and surrogate virus neutralization test (sVNT) was evaluated in a subgroup of 45 subjects. Multiple comparisons were analyzed using ANOVA with the Geisser-Greenhouse correction test followed by a Sidak post-hoc test. The significance level was set at 0.05 for all the analyses.

Results: Our data show a significant increase in the levels of IgG-Anti-SARS-CoV-2 between day 0 and day 28 against ancestral SARS-CoV-2 (2694 vs. 1913; $p<0.0001$), the Delta variant (2389 vs. 1740; $p<0.0001$), and in five different Omicron subvariants (BA.1, BA.2, BA.2.12.1, BA.2.75, BA.4; BA5). The neutralizing response assessed by conventional method (cVNT) was evaluated in 135 of them and showed that vaccination induced a significant increase in neutralization of Omicron BA.1 variant (43.5 vs 63.4; $p=0.0380$). Finally, sVNT analyses evaluated in 45subjects, showed a significant increase between day 0 and day 28 post-immunization in neutralization against the Delta variant (179.6 vs. 151.6; $p=0.0329$), as well as against the Omicron BA.1 (33.5 vs 25.8; $P=0.02$).

Conclusion: Our data show that a third (fifth) booster dose of an inactivated vaccine against SARS-CoV-2 increases the amount of IgG antibodies that recognize different variants of concern of SARS-CoV-2, several of those that are currently circulating among the global population. In addition, we showed that the produced antibodies are capable of neutralizing the Delta variant as well as the Omicron BA.1 variant.

149 (157) IMMUNITY AGAINST PERTUSSIS IN EARLY STAGES OF LIFE

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Pertussis is considered a resurgent respiratory disease that can affect individuals of all ages. Newborns, until they receive their first dose of vaccination, have the highest rates of hospitalization and death. In response to the resurgence scenario, several countries have incorporated a booster dose for pregnant women. With this incorporation, there has been renewed interest in the impact of whole-cell pertussis vaccine (wP) versus acellular vaccine (aP) on disease control, particularly in terms of the best priming approach. To gather evidence on this topic, we analyzed the impact of aP or wP priming on aP vaccination during pregnancy (aPpreg) in mice. We utilized two-mother vaccination schemes (wP-wP-aPpreg and aP-aP-aPpreg) and assessed the immune response in the mothers and their offspring, as well as the protection of the offspring against *Bordetella pertussis* challenge. Offspring from non-immunized mothers were used as the control group. By measuring IgG and IgG isotypes in the pups we detected that the aP-aP-aPpreg schedule triggered a murine antibody response mainly to a Th2-profile, while wP-wP-aPpreg induced a Th1/Th2 mixed profile (IgG2a/IgG1 ratio: 0.34±0.02 vs 1.26±0.06 respectively, $p<0.0001$). The sera avidity from wP primed mother's offspring also turned out to be higher than the offspring from mothers whose regimen includes only aP (Retention rate at NH4SCN 0.375M: 98.19±1.67 % vs 73.92±1.86 % respectively). Both immunization schemes administered to the mothers protected the 21-day-old offspring against pertussis (more than 6,2 orders of magnitude lower than control pups, $p<0.0001$), but the wP-wP-aPpreg vaccination conferred offspring protection in all pregnancies at least up to 20 weeks after receiving the aPpreg-dose. In contrast, the immunity induced by aP-aP-aPpreg began to decline in births that occurred 18 weeks after receiving the aPpreg dose ($p<0.05$). We also evaluated the effect of maternal immunity in 7-day-old pups, as we observed their increased susceptibility to infection. At this age, pups exposed to a bacterial suspension 2 orders of magnitude lower than that used for older pups become colonized at levels comparable to adult animals (6.66±0.78 vs 7.23±0.49 log CFU/lungs respectively). Regarding the impact of maternal immunity on preventing lower airway colonization in these 7-day-old pups, we detected a reduction of over 5.6 orders of magnitude in pups born to immunized mothers, regardless of the received vaccination schedule, compared to those born to non-immune mothers ($p<0.0001$). However, this protection was not evident in the upper airways. Our results support the feasibility of a gestational vaccination strategy to prevent pertussis infection during the earliest stages of life. It's worth noting that in cases of maternal immunity derived from the wP-wP-aPpreg scheme, the transferred immunity provides longer-lasting protection, thereby supporting the use of the wP vaccine in the primary schedule.

150 (140) MOLECULAR MODIFICATION OF A NOVEL PERTUSSIS VACCINE CANDIDATE FOR MORE EFFECTIVE IMMUNE RESPONSE AGAINST BACTERIAL COLONIZATION

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Pertussis is a respiratory disease that affects individuals of all age groups, with infants under 6 months being the most vulnerable, as they can be hospitalized or even die in severe cases. The period from birth until the receipt of the first vaccine dose between 6 and 8 weeks of age is particularly critical. Among vaccine-preventable diseases, pertussis remains one of the least controlled. In recent years, the epidemiological situation has worsened, with resurgence even in countries with high vaccination coverage. To improve the disease control, a novel strategy has emerged: maternal immunization. By immunizing the pregnant women, who can be a source of infection, both she and the newborn are protected. While this approach appears promising, the potential blunting effect of maternal immunity on childhood vaccination requires attention. To explore complementary alternatives, we have proposed neonatal vaccination strategies. Our focus on inducing a protective Th1 immune response against the disease has led us to employ our novel vaccine candidate based on outer membrane vesicles (OMVwt), which we modified to reduce the humoral response while maintaining a mixed Th1/Th2 profile. We utilized OMVs with altered LPS, referred to as OMVLPS*. In vitro assays using whole human blood conducted to assess IL-6 induction levels and in vivo mouse weight gain test allowed us to determine that OMVLPS* exhibits a better safety profile than OMVwt. By these assays we detected that OMVLPS* induced 5176 pg/ml of IL-6, while OMVwt quadrupled that value (22296 pg/ml, $p<0.0001$). Both formulations successfully met the criteria set by international regulatory entities in the weight gain test. ELISA assays aimed at evaluating immunogenic capacity revealed that a two-dose scheme in a murine model with formulations containing OMVLPS* induced lower levels of specific IgG than OMVwt (A490nm: 0.52±0.2 vs 0.92±0.2), as well as for the IgG1 (A490nm: 0.69±0.09 vs 1.8±0.3) and IgG2a (A490nm 0.26±0.03 vs 0.88±0.5) isotypes. Splenocyte restimulation assays showed that levels of secreted IFN-γ, IL-5, and IL-17 did not significantly differ between the two formulations. In 4- week-old mice, formulations containing OMVLPS* demonstrated the ability to reduce bacterial counts in the lower respiratory tract, resulting in a reduction of 2.4 log for OMVLPS* and 2.28 log for OMVwt in comparison to non-immunized mice ($p<0.01$). Encouraged by these results, we expanded our studies to neonatal vaccination models. In this age group, we observed that OMVLPS* significantly reduced lung colonization by 3.74 log in comparison to non-immunized animals, whereas OMVwt did not induce significant levels of protection. Collectively, these results position OMVLPS* as a promising vaccine candidate for protecting neonates against pertussis.

151 (106) MULTIPARAMETRIC FLOW CYTOMETRY ANALYSES REVEAL CHANGES IN THE T CELL RESPONSE INDUCED BY A FIFTH DOSE OF INACTIVATED SARS-COV-2 VACCINES BASED ON THE OMICRON VARIANT OR ON A TRIVALENT VACCINE CONTAINING THE ANCESTRAL, DELTA AND OMICRON VARIANTS.

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Background. The COVID-19 pandemic pushed the rapid development of vaccines to protect the population from severe disease and death caused by SARS-CoV-2 infection. Previous data from our laboratory showed that two doses of CoronaVac®, a SARS-CoV-2 inactivated vaccine, induce a strong cellular response characterized by the activation of Spike (S) specific $CD4^+CD137^+OX40^+$ T cells. In addition, the frequency of activated $CD4^+$ and $CD8^+$ T cells remained stable after the administration of twoboosters of CoronaVac®.

Aim. Due to the emergence of the Omicron subvariants, we evaluated by traditional and multi-parametric flow cytometrythe activation of $CD4^+$ and $CD8^+$ T cells in subjects blindly vaccinated with an inactivated vaccine based on the Omicron BA.1 variant or a trivalent vaccine based on the Wuhan, Delta and Omicron BA.1 variant.

Methods. Peripheral blood mononuclear cells (PBMCs) collected from 6 subjects before (Day 0) and after immunization (Day 28) were stimulated with a megapool (MPs) of peptides derived from the S protein of the Omicron BA.1 and BA.2 variants, the Delta variant and the respective MPs derived from the ancestral SARS-CoV-2 virus (WT). Activated $CD4^+CD137^+OX40^+$ and $CD8^+CD137^+CD69^+$T cells were quantified by traditional flow cytometry. In parallel, barnes-hut distributed Stochastic Neighbor Embedding (bh-SNE) and clustering analyses using the EM algorithm for Gaussian mixtures were performed in stimulated live $CD14^-CD19^-CD3^+$ T cells. Changes in frequencies of T cells before and after vaccination were evaluated by paired t-test. Results. No major changes in the frequency of S specific activated $CD4^+$ and $CD8^+$T cells before and after vaccination were detected. Bh-SNE analyses grouped $CD3^+$ T cells in 24 clusters, where 3 of them showed significant changes in their frequency after vaccination. Cluster 17, identified as activated fully differentiated $CD4^+$memory T cells, increased its frequency after vaccination following stimulation with MP-S derived from WT. Cluster 21, identified as $CD8^+$ central memory T cells, reduced its frequency after vaccination when stimulated with MP-S derived from WT and the BA.2 variants**.** Finally, frequency of cluster 24, classified as effector $CD8^+$memory T cells, decreased after vaccination following stimulation with MP-S derived from WT.

Conclusion. Multi-parametric flow cytometry analysis showed that the T cell response induced by a fifth dose of SARS-CoV-2 vaccine is far more complex than the induction of $CD4^+CD137^+OX40^+$ T cells. Addition of functional markers aimed to evaluate cytokine production, regulatory, follicular and exhausted T cells

will improve our current understanding of the global effects of SARS-CoV-2 boosters over the host cellular immunity. These analyses could also be used to evaluate the effect of vaccination in cellular immunity against other viruses such as Influenza virus, where due to the high vaccination rate and natural viral exposure, studies of this nature can be challenging.

152 (129) NEW VACCINATION STRATEGIES AGAINST THE RESURGENT RESPIRATORY DISEASE NAMED PERTUSSIS

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Pertussis is a preventable respiratory disease that has reemerged in recent years in many countries, including those with high vaccination coverage. The waning immunity induced by the vaccines currently in use (whole cell and acellular vaccines) along with the emergence of *Bordetella pertussis* strains resistant to vaccine-induced immunity, appear to be the main reasons for this resurgence. In the face of this health issue, it's imperative to improve existing vaccination strategies. Our lab has succeeded in developing a new acellular multi-antigen vaccine formulation derived from *B. pertussis*. Preclinical trials have demonstrated that this formulation outperforms both existing vaccines, being safe, immunogenic, and protective against various genotypes of the causative agent. To further advance the positioning of our vaccine candidate for clinical trials, we assessed the potential of utilizing our vaccine prototype in combined formulations and/or heterologous schedules. Two-dose heterologous schedules Vac1-Vac2 and Vac2-Vac1, where Vac2 represents a novel combination were used. Naïve and treated with homologous-vaccination-schedules animals were used as controls. The employed nomenclature is chosen because of confidential reasons. Total IgG levels and specific isotopes were measured by ELISA assays and cellular response was evaluated through splenocyte proliferation assays. We found that for the heterologous schedules and homologous Vac2-Vac2, specific IgG levels were at least 1.6 times higher compared to the homologous Vac1-Vac1 schedule ($p<0.001$). Regarding IFNγ (Th1 marker), treatments including Vac2 as a second dose exhibited the highest levels either in homologous and heterologous schedules ($p<0.05$). Vac1-Vac1 schedule induced the lowest IFNγ levels. As for the IL-5 (Th2 marker), only the homologous Vac1-Vac1 treatment showed a significant difference to naïve infected control group ($p<0.01$). To assess the protective capacity, treated animals were exposed to a sublethal dose of *B. pertussis* with different genotypic backgrounds. All Immunized animals exhibited a significant reduction in bacterial colonization in lungs compared to naive animals ($p<0.05$), regardless of the challenge strain used. When comparing the reduction in bacterial colonization between the homologous Vac1-Vac1 schedule and the heterologous ones, the latter proved to be more effective (0.7 Log difference in CFU counts, $p<0.05$). Regarding the reduction in colonization for *B. pertussis* strains in the upper respiratory tract, the heterologous Vac2-Vac1 schedule and the homologous Vac2-Vac2 treatment achieved at least a 1 log reduction compared to the non-immune control ($p<0.05$). All these results demonstrate that schedules incorporating Vac2 as a second dose have a greater capacity to prevent bacterial colonization, thus positioning this strategy as a means to enhance disease control.

153 (100) QUADRIVALENT INACTIVATED INFLUENZA VIRUS VACCINES INDUCES A CELLULAR RESPONSE CHARACTERIZED BY IFN-γ PRODUCTION IN HEALTHY ADULTS

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Quadrivalent vaccines against Influenza virus have been used since 2013 for people older than six months of age. Quadrivalent vaccines are designed to protect against four different strains of Influenza viruses: two Influenza A viruses and two Influenza B viruses. Cell-mediated immunity plays a vital role against Influenza virus infection in humans, being essential to protect the host against severe disease, especially in older adults. However, less is known about the nature of cell-mediated immunity induced by vaccination. Aim: The objective of this study was to evaluate the cellular response induced by a single dose of two inactivated quadrivalent vaccines against Influenza virus that were blindly administered to healthy adults. Materials and methods: In the present study, we collected blood samples from 38 healthy adults before (Day 0) and 28 days after immunization (Day 28). Peripheral blood mononuclear cells (PBMCs) were isolated and cryopreserved until the experimental protocols were carried out. To evaluate the cell-mediated immunity induced by vaccination, PBMCs were stimulated with specific hemagglutinin (HA) proteins derived from influenza A and B viruses, and activated $CD4^+$ T cells ($CD137^+OX40^+$), $CD8^+$ T cells ($CD137^+CD69^+$) and memory $CD4^+$ and $CD8^+$ T cells, as well as interferon-γ and IL-4 production by total PBMCs were evaluated by flow cytometry and (ELISpot) assay, respectively. A non-parametric paired Wilcoxon test followed by a Dunn's post hoc test were used for multiple comparisons. The significance level was set at 0.05 for all the analyses. Results: Our data show that the majority of analyzed volunteers (31/37) had pre-existing immunity against influenza A and B viruses and no major changes were detected in activated $CD4^+$ T cells or $CD8^+$ T cells following vaccination when compared with prior vaccination. However, ELISpot assay shows that influenza vaccine administration significantly boosted the production of IFN-γ and IL-4 production by PBMCs after specific stimulation with HA proteins derived from influenza A and B viruses. The increased IFN-γ response post- vaccination is important as it modulates the response of $CD8^+$ T cells which mediate viral clearance. Conclusion: Despite volunteers had pre-existing cellular immunity against influenza viruses, our study suggests that inactivated quadrivalent vaccines may improve host response against seasonal Influenza viruses by inducing the production of IFN-γ and IL-4, conferring protection against severe disease in adults.

154 (39) SEXUAL HORMONE EFFECTS UPON EXPERIMENTAL VACCINATION PROCESS AGAINST *T. cruzi*

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Sexual hormones can affect the immune response, and therefore may also influence the efficacy of vaccines. However, most preclinical studies about vaccine immunogenicity and efficacy tend to evaluate only females (F), since they are known to have a better humoral response, assuming that the results apply to the opposite sex. Additionally, there is evidence that male (M) mice are more susceptible to *T. cruzi* infection than F. In this context, our objective was to evaluate the influence of sex and sexual hormones on the humoral and cellular response triggered after administering an experimental vaccine against *T. cruzi*. We worked with both sexes of BALB/c mice (n=5-8/group). Additionally, M were divided into two groups, one of them was gonadectomized -MGx- and another group underwent sham surgery -MSham-. All of them received 3 doses (1 every 15 days) of the following formulations: Trans-sialidase (TS, 10 µg/dose), TS plus c-di-AMP adjuvant (TS+A) or saline solution as vehicle (V). Fifteen days after immunization completion, the levels of specific antibodies against TS (total IgG and IgG2a) were assayed by in-house ELISA. Cellular response against TS was evaluated through a delayed hypersensitivity test by the inoculation of 5 µg of TS on the footpad. The result showed that in both sexes, TS+A induced a significant enhancement of specific total IgG and IgG2a compared to the rest immunized groups ($p<0.05$), despite their levels in M being lower than in F. Also, in both sexes, TS+A triggered an enhanced cellular response against TS ($p<0.05$ vs. rest), being more evident in M than F after 24 and 72 h ($p<0.05$). When compared between MGx and MSham mice that received TS+A, the first group showed significantly higher levels of specific IgG and IgG2a ($p<0.05$). In addition, when compared to the cellular response at 48 h post-challenge, the MGx TS+A mice displayed a higher cellular response than MSham TS+A. The results suggest that sexual dimorphism affects vaccine humoral and cellular immunogenicity, while testosterone affects mainly humoral response in M mice. Therefore, these results suggest that it is mandatory to consider the vaccine response in both sexes when designing the experiment.

155 (10) VACCINE AGAINST *TRYPANOSOMA CRUZI* INFECTION USING THE PARASITE ANTIGEN TcTASV DISPLAYED AT BACULOVIRUS CAPSID

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Chagas' is a neglected disease caused by the eukaryotic kinetoplastid parasite *Trypanosoma cruzi.* Currently there are about 8 million infected people worldwide, most of them at the chronic phase of the disease, which involves cardiac, digestive or neurologic manifestations. There is an urgent need for a vaccine because treatments are only effective at the initial phase of the infection, which is generally underdiagnosed. Selection and combination of antigens, adjuvants, and delivery platforms for vaccine formulations should be designed to trigger mixed humoral and cellular immune responses, considering that *T. cruzi* has a complex life cycle with both intracellular (amastigotes) and bloodstream circulating (trypomastigotes) parasite stages in the vertebrate host. TcTASV is a *T. cruzi* multigene family highly conserved among lineages and without orthologs in other organisms, including trypanosomatids. TcTASV has approximately 40 members that can be classified in subfamilies according to close sequence similarity. TcTASV-A and TcTASV-C are the most abundant subfamilies, present the highest levels of expression and both are in contact with the host immune system *in vivo*. TcTASV-A subfamily is expressed both in trypomastigotes and amastigotes, with an intracellular location. TcTASV-C subfamily is solely expressed in trypomastigotes, located at *T. cruzi* surface, and secreted both freely and contained in extracellular vesicles. Here we report the effectiveness of the vaccination TcTASV protein family, which consisted in a prime with TcTASV-C recombinant protein with aluminum hydroxide and a boost with recombinant baculovirus displaying a TcTASV-A antigen at the capsid. Vaccination stimulated immunological responses with production of lytic antibodies and antigen-specific CD4+ and CD8+ IFNɣ-secreting lymphocytes. After several assays with similar results, an average of 92% of vaccinated animals survived to lethal challenges with *T. cruzi* (RA strain, high virulence) while all control mice died before 30 days post infection. Vaccination also induced a strong decrease of tissue parasitism at the chronic phase, and generated immunological memory that allowed vaccinated and infected animals to control both the reactivation of the infection after immunosuppression as well as a second challenge with *T. cruzi*. Similar results were obtained in another mice strain, and additional trials are ongoing to evaluate the protective capacity against another parasite strain. Interestingly, inoculation with *wild type* baculovirus partially protected mice against *T. cruzi*. In brief, we demonstrated, for the first time, that the combination of the baculovirus platform and the TcTASV family provides effective protection against *Trypanosoma cruz*i, being a promising vaccine for Chagas' disease.

Adaptive immunity
Saturday, November 11, 8-9:30h
Chairs: *Laura Chiapello - Carolina Montes*

156 (5) ATP AFFECTS FOLLICULAR T HELPER CELLS DURING PEDIATRIC RSV INFECTION

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Background: Respiratory syncytial virus (RSV) infection represents a major burden in infants. Follicular T helper cells (Tfh) play a critical role in the generation of protective antibodies. The purinergic receptor P2X7R has been recognized as a signature gene of Tfh cells. The expression and function of P2X7R on Tfh cells from RSV-infected infants have not been analyzed previously.
Aims: 1) To characterize circulating Tfh cells (cTfh) in RSV-children 2) To quantify IL-21 levels in serum, ATP and P2X7R cleaved soluble form (P2X7Rs) levels in serum and nasopharyngeal aspirates (NPA) 3) To analyze the membrane P2X7R expression in cTfh 4) To explore the biologic response of cTfh to ATP (P2X7R agonist) and KN-62 (P2X7R antagonist) 5) To study the correlation with the Clinical Disease Severity Score (CDSS).
Methods: Plasma, NPA and PBMCs from children with non-severe (n=46) and severe RSV infection (n=26) and controls (n=23) were used.
Results: We observed decreased cTfh frequency in non-severe (n=30) and severe RSV-infected infants (n=15) compared with controls (n=23, $p<0.0001$). Severe RSV-children presented the lowest IL-21 serum levels compared to non-severe ($p<0.01$) and controls ($p<0.0001$). Plasma IL-21 levels positively correlated with cTfh frequency ($r=0.31$, $p<0.01$). Severe RSV-children also exhibited higher levels of ATP in plasma and NPA in comparison with non-severe ($p<0.05$) and controls ($p<0.01$). Plasma ATP levels negatively correlated with cTfh frequency ($r=-0.3$, $p<0.05$). Interestingly, severe children showed an increased frequency of P2X7R+cTfh cells related to non-severe ($p<0.05$) and controls ($p<0.01$). ATP serum levels positively correlated with the membrane receptor expression ($r=0.44$, $p<0.05$). Importantly, ATP exposure decreased cTfh proliferative response and *de novo* IL-21 production, while also inducing apoptosis ($p<0.001$, $n=14$). These effects were significantly restored with KN-62. Moreover, the levels of P2X7Rs, due to negative receptor regulation, were higher in non-severe than in severe RSV-infected children ($p<0.001$). Finally, plasma and NPA ATP levels positively correlated with CDSS ($r=0.6$, $p<0.0001$), while IL-21 plasma levels correlated negatively ($r=-0.36$, $p<0.005$).
Conclusions: RSV infection induces up-regulation of P2X7 receptor in cTfh cells modifying their function. Levels of ATP in plasma and NPA could be useful biomarkers of disease severity. A better understanding of the Tfh cells in young infants is critical for vaccine development.

157 (200) CD39: AN ANTIBODY-SECRETING CELL MARKER AND A REGULATOR OF B CELL RESPONSE

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Antibody-secreting cells (ASC) are the only ones able of conferring the organism the ability of protection through immunoglobulin (Ig) release. Antibodies (Abs) are essential components for immunity against pathogens and have different functions depending on its isotype. ASCs can also act beyond Abs production; they have heterogeneous functions extending from dampening the immune system, via regulatory mechanisms, to antigen presentation and cytokine production. Recently was shown that ASCs hydrolyze extracellular ATP (eATP) to AMP through the ectoenzyme CD39 and generate Adenosine (ADO) which inhibits macrophage function. Yet, whether CD39 and ADO control the magnitude or quality of B cell responses remains unknown.

Here, we deeply characterize acute ASC response in *T. cruzi* experimental infection and further investigated the expression and function of CD39 on B cell compartment in a complete range of experimental models. Through FACS and bulk RNAseq we found that short-lived ASCs, known as plasmablasts (PBs), from spleen and lymph nodes of *T. cruzi* infected mice have not only different phenotype and genetic expression but also distinct Ab profile, which could be conditioned by differential distribution on T cell subsets and cytokines in these tissues. Nevertheless, both type of PBs expressed higher levels of CD39 than any other B and T cell populations. Additionally, long-lived plasma cells from chronic *T. cruzi* or Influenza infected mice had elevated CD39 levels.

High expression of CD39 was also observed in ASCs after immunization with red blood cells, infection with *S. aureus, P. falciparum* or Influenza virus, autoimmune models (Sanroque and FASLpr) and from COVID-19 patients. CD39 on PBs from *T. cruzi*-infected mice was functional since they had exonuclease activity, evidenced by a decrease in eATP levels in PBs-culture supernatant in comparison to naïve B cell-culture medium. This activity was partially reversed by ARL, a CD39 inhibitor, and reduced in PB from infected CD39KO mice.

To assess the role of CD39 in B cell responses, we infected CD39KO mice with *T. cruzi.* We found that CD39 was dispensable for PB differentiation, but ASC from *T. cruzi* infected CD39KO mice developed an altered profile and concentration of Ig compared to WT mice. Yet, chimera mice lacking CD39 only on B cell compartment and infected with Influenza exhibited an increased frequency of GCs when compared to controls, suggesting that $CD39^{+}$PBs may limit GC reaction.

Then, we evaluated whether this phenomenon was mediated by ADO. We found that ADO administration significantly impaired GC reaction. These results go in line with increased ADO receptor (Adora2) expression observed by bulk RNAseq on activated B cell.

Our findings highlight CD39 as ASC marker with an important role in shaping the profile and magnitude of Ab response. Also, we unveiled an unknown regulatory axis between PBs and GCs, where early formation of CD39+PBs modulates eATP and regulate the GC expansion.

158 (58) G PROTEIN-COUPLED RECEPTOR KINASE 2 (GRK2) MODULATES LEUKEMIC CELL HOMING AND ACTIVATION IN B-CELL CHRONIC LYMPHOCYTIC LEUKAEMIA (CLL)

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CLL leukemic cell proliferation and resistance to therapy occur within lymphoid tissues, supporting the idea that their presence within this tumour microenvironment contributes to disease progression and relapse. GRK2 plays a central role in B cell homing to lymphoid organs by inducing Sphingosine-1 phosphate receptor-1 (S1PR1) downregulation, which allows lymphocytes to overcome the S1P-mediated retention in blood necessary to enter into lymphoid tissues. In addition, GRK2 has been implicated in signalling pathways related to cancer progression. The role of GRK2 on CLL cell migration, activation and survival has not been studied yet.

Leukemic cells were obtained from CLL patients´ peripheral blood (PB). GRK2 expression was evaluated by western blot and qRT-PCR. Normal B cells from age-matched healthy donors (HD) were used as control. CLL cell activation and viability were evaluated by flow cytometry (FC). *In vitro* migration was evaluated with the transwell cell migration assay. Primary murine leukemic cells were obtained from Eμ-TCL-1 mice. GRK2-deficient murine leukemic cells (GRK2KO) were generated by CRISPR/Cas9 editing on the mouse cell line TCL1-355 TKO. For *in vivo* migration assays, GRK2KO or MOCK (control) cells were labeled with CTV or CFSE, mixed in a ratio 1:1 and injected through the tail vein. After 20 hours, their localization in PB, bone marrow (BM) and spleen (SP) was evaluated by FC. GraphPad Prism software was used for statistical analysis.

We found that leukemic cells from CLL patients (n=20) express similar levels of GRK2 as B cells from HD (n=6). The presence of a chemical GRK2 inhibitor, CMPD101, impaired the up-regulation of CD86 expression on CLL cells activated through the BCR (n=5, $p<0.05$), and also by co-culturing with autologous activated T cells (n=7, $p<0.05$). CMPD101 increased the *in vitro* migratory response of human and murine leukemic cells towards S1P, while it did not affected migration towards CXCL12 and CXCL13 (n= 9, $p<0.05$). The GRK2-deficient murine cell line allowed us to show that GRK2 deletion increased the *in vitro* response of leukemic cells to S1P (n=6, $p<0.05$), while migration towards CXCL12 and CXCL13 was not affected (n=6, $p<0.05$). As expected, CMPD101 increased S1P response of MOCK but not GRK2KO cells (n=7, $p<0.05$). Interestingly, GRK2KO cells showed a lower spontaneous and LPS-induced proliferation rate when cultured *in vitro* compared to MOCK cells (n=5, $p<0.05$). Finally, we evaluated the effect of GRK2 deletion on the *in vivo* homing capacity of leukemic cells intravenously injected into mice, and found that GRK2KO cells preferentially localized in PB and SP and were underrepresented in the BM, compared to MOCK cells ($p<0.05$, n=5).

Our results suggest that GRK2 inhibition could be explored as a strategy to induce their retention in the blood, increasing their exposure to therapeutic agents and/or to overcome resistance to treatment induced by the protective microenvironment.

159 (62) HUMORAL AND CELLULAR RESPONSE TO THE COVID-19 VACCINE IN IMMUNOCOMPROMISED CHILDREN

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Background: Developing an optimal vaccine-induced anti-SARS-CoV-2 protective immunity depends on a fully competent immune system. Some evidence was gathered on the effects of vaccination outcomes in immunocompromised adults. But few observations have been made regarding immunocompromised children in terms of strength and perdurability. We aimed to determine the humoral and cellular immune response to the 2nd and 3rd COVID- 19 vaccine in immunocompromised children.

Methods: Two cohorts were included (healthy and immunocompromised children), aged between 5-17 years, who received two (n=50) or three doses (n=22) of a first series COVID-19 vaccine (BBIBP-CorV and/or mRNA vaccines). The median time since the 2nd dose until sampling was 462 days and since the 3rd dose was 300 days. Plasma levels of anti-spike IgG antibodies, neutralizing activity, and antigen-specific T cells against the ancestral variant (Wuhan) and the variant of concern Omicron BA.4/5 were analyzed. The anti-spike IgG antibody titer was determined by endpoint titration. Neutralization titer (IC50) for all plasma samples was calculated. Antigen-specific T cells were measured by flow cytometry as a percentage of AIM+ (OX40+CD137+) CD4+and CD69+CD137+) CD8+ T cells after stimulation of PBMCs with peptide megapools.

Findings: Of the participants, 18 (25%) were immunocompromised suffering cancer (ALL, lymphoma, sarcoma, hepatoblastoma), Inborn Errors of Immunity (IgA deficiency, X-inked agammaglobulinemia, common variable immunodeficiency) and LES under treatment with steroids. Almost all children remained seropositive and there were no differences in the titer of anti-spike IgG antibodies between healthy and immunocompromised children for any dose of the COVID-19 vaccines. Plasma neutralizing activity was significantly higher in healthy compared to immunocompromised children after the 3rd dose [1066 (434- 1261) vs 135 (31-813), median (IQR) against Wuhan ($p<0.05$) and 249 (111-481) vs 16 (1-93) against Omicron ($p<0.001$)]. Consequently, we calculated an anti- spike IgG neutralization potency index (IC50/IgG) for each patient and found that immunocompromised children had a significantly lower index against Omicron ($p<0.001$). Nearly 50% of children had detectable CD4+ T cell memory after 2 or 3 doses, but the proportion of children showing CD8+ specific T cells was about 20%. There were no differences in the specific T cell response between groups. Interpretation: Although most immunocompromised children mount a long-term humoral memory immune response to the first series COVID-19 vaccine, the specific T cell-response has a distinct kinetic. Understanding the

complexities of immune memory to COVID-19 vaccines is key to inform vaccination strategies in the pediatric population.

160 (197) IMPACT OF TRISOMY 21 IN TONSILS: ACCUMULATION OF T AND B CELLS WITH PHENOTYPES ASSOCIATED WITH INFLAMMATION AND AUTOIMMUNITY

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Children with Trisomy 21 (T21), also referred to as Down Syndrome (DS) are at a higher risk of contracting serious infections, often with heightened severity. Also, adults with DS exhibit a distinctive range of diseases compared to the general population, including lower occurrences of solid malignancies and an increased prevalence of Alzheimer, autoimmunity, and leukemia. These susceptibility factors arise from anatomical traits but mainly from substantial immune dysregulation early in lifetime. Most of our knowledge about the immune system in DS has been derived from blood studies. To delve deeper into the immunological mechanisms behind the anomalies observed, we sought to investigate the relationship between T and B cells within secondary lymphatic organs (SLOs). Here, we have analyzed tonsils, a readily accessible and underutilized source of human immune tissue, that can serve as a model for human SLO.

We conducted multiparametric flow cytometry and multiplex immunofluorescence to characterize the B and T cell compartments in hypertrophied tonsils in children with T21 compared to age-matched controls (n=10 per group). We examined the frequencies of various cell types including naïve B cells (IgD^+ $CD27^-$ $CD38^-$), germinal center B cells ($CD27^+$ $CD38^+$), plasmablasts ($CD27^{hi}$ $CD38^{hi}$), and memory B cells ($CD27^+$ $CD38^-$), among others. Additionally, we assessed the frequencies of activated non-Tfh cells ($CD3^+$ $CD4^+$ $CD45RA^-$ $CXCR5^-$ $PD1^+$), Tregs ($CD3^+$ $CD4^+$ $CD45RA^-$ $Foxp3^+$) and Tfh ($CD3^+$ $CD4^+$ $CD45RA^-$ $CXCR5^{hi}$ $PD1^{hi}$). We also analyzed the expression of CXCR3 and Tbet as markers associated with a Th1 response. Our findings indicate an important increase in $CD11c^+$ naïve B cells ($p<0.05$) and a significant decrease in IgM^+ memory B cells ($p<0.01$) and in switched memory B cells ($p<0.001$). Additionally, we observed a significant rise in CXCR3-expressing B cells, a population associated with autoimmune conditions. Regarding the T cell compartment, we identified a remarkable increase in the subset of $CXCR3^+$ non- naïve non-Treg cells ($p<0.05$) and a decrease in conventional $CXCR3^-$ Tfh cells ($p<0.05$). To explore the location of Tfh $Tbet^+$ cells (Tbet being the transcription factor associated with Th1 phenotypes, which, like the CXCR3 indicates polarization to Th1), we assessed the density (#cells/mm^2) of Tbet expressing cells in paraffin fixed formalin embedded tonsil sections. We observed a significant increase ($p<0.05$) of $CD3^+$ $CD4^+$ $PD1^+$ $BCL6^+$ $Tbet^+$ cells outside and inside the follicle in samples from individuals with T21. We also found a strong negative correlation between total $CXCR3^+$ B cells (all individuals from both groups were included together) and the size of lymphoid follicles ($p<0.05$), suggesting that this increment in CXCR3- expressing lymphocytes could be leading to an impaired germinal

center reaction. Collectively, our findings suggest that children with DS exhibit an altered T and B cell compartment within the tonsils which impact on its architecture.

161 (116) PATIENTS WITH A MAJOR DEPRESSIVE EPISODE HAVE CIRCULATING CYTOKINE-PRODUCING CD4+ T CELLS WITH LESS CAPABILITY OF CYTOKINE PRODUCTION SUGGESTING A MORE EXHAUSTED PHENOTYPE

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Introduction. Depression is a highly prevalent psychiatric condition occurring early in life and following a relapsing and remitting course. According to the main international classifications, the term depression, typically refers to a Major Depressive Episode (MDE). During the last years, emerging literature associated MDE with increased inflammatory status and unbalanced immune response. Most of the studies have focused on plasma proinflammatory biomarkers, such as IL-6, and C-reactive protein. Despite the extensive research on plasma biomarkers, less exploration has been made regarding the involvement of immune cells in depression. Unpublished cross- sectional study matched by sex and age of our group including patients with MDE in active and remitted condition and healthy controls showed an unbalanced inflammatory condition including cytokines, growth factors, and innate and adaptive immune cells. Remarkably, this study has shown that $CD4^+$T cells have a more activated and exhausted phenotype measured by CD69 and PD1.

Aims. The present work aimed to further dilucidate the contribution of $CD4^+$ T helper (Th) cell subsets (Th1, Th17 and Tr1) in peripheral blood from patients with MDE in an active or remitted condition compared to healthy controls (HC). Individuals were evaluated by psychiatrists using the International Psychiatry Interview MINI to diagnose MDE and the Hamilton Depression Rating Scale (HADRS) to define AD and NAD status. The MDE sample was 26% male and 74% female with 18 to 55-year age range and matched with the HC group by age and gender. Peripheral blood mononuclear cells (PBMC) were isolated from blood samples by Ficoll gradient, and cryopreserved at -80°C or liquid nitrogen until use. Samples were thawed and incubated in IMDM + FBS 10% + P/S 1% complete medium with ionomycin (1 µg/ml), Phorbol 12-myristate 13-acetate (25nM) and GolgiStop (BD BioSciences) (1/1000) for three hours at 37°C, to stimulate cytokine production in T cells, and later stained with the fixable viability dye (Zombie Violet), fixed, permeabilized and intracellular stained with antibodies against CD4, IFNγ, IL-17 and IL-10 and analyzed by flow cytometry. Our results show that the frequencies of Th1 ($IFN\gamma^+$) and Th17 ($IL\text{-}17^+$) cells were not statistically different among groups at the basal stage (AD, N=21; NAD, N=20, and HC, N=18). Nonetheless, we do find a significant ($p<0.05$) less percentage of $CD4^+IFN\gamma^+IL\text{-}17^+$ cells in patients with MDE compared to HC. When we analyzed the CD4+IL-10+ T cells we observed a trend in the reduction of this subpopulation in patients with MDE compared to HC. Interestingly, we also found a significant decrease of $CD4^+IFN\gamma^+IL\text{-}10^+$T cells when compared to both HC

(p=0.01) and AD patients (p<0.05). Altogether, these results indicate that patients with MDE have an altered proportion of circulating cytokine-producing $CD4^+$ T cells with less capability of cytokine production suggesting a more exhausted phenotype.

162 (104) PIMOZIDE: A NOVEL/OLD DRUG THAT ENHANCES CROSS PRESENTATION OF ANTIGENS MEDIATING THE AUGMENT OF ANTIGEN TRANSLOCATION TO THE CYTOSOL IN DENDRITIC CELLS

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Dendritic cells (DCs) are professional Antigen Presenting Cells (APCs) responsible for activating naive T lymphocytes to induce specific response against antigens. In particular, DCs have the special ability to internalize exogenous antigens and cross present (XP) them in MHCI context to induce cytotoxic CD8+ T cell (CTL) response, which can be potentially exploited for immunotherapeutic purposes. Our group is interested in searching for new adjuvants able to improve CTL response. In that context, we performed a high throughput screening (HTS) to identify new compounds that could potentiate XP, using an assay developed in our laboratory based on JAWSII DCs line and soluble OVA as antigen (sOVA). In this work, we analyzed the mechanisms underlying XP potentiation of Pimozide (P), one of the 5 hits identified after HTS. We observed that P does not modify sOVA internalization, and neither MHC I-surface expression in DCs. It has been reported that P accumulates in acidic compartments and acts as a lysosomotropic drug. Thus, we studied if P accumulates in lysosomes. After blocking endo phagosomal acidification, P effect on DCs XP was diminished. We also observed that incubation of DCs with P increased antigen translocation from endosomes to the cytosol, a key step in XP. Taking all these results into account, our evidence suggests that P affects endo phagosomal processing of antigens on DCs. Further studies will be engaged to completely understand the mechanisms involved in enhancing XP.

163 (182) PURINERGIC SIGNALING CONTROLS T-CELL RESPONSE

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In response to hypoxic or inflammatory conditions, immune cells release ATP, which triggers multiple immune effector functions to fight infections. Its life span is determined by CD39 and CD73 purinergic ectoenzymes that coordinate ATP hydrolysis into the potent anti-inflammatory adenosine (ADO). The enzymatic activity of CD39 and CD73 could be boosted by HIF-1, leading to the generation of ADO, which in turn helps to reduce inflammation.

How HIF-1 regulates ADO production by CD39 and CD73 and the impact on T-cell activation will be particularly addressed. We found that *in vitro*-activated CD4 T-cells increase the expression of ATP-receptor P2X7, CD39, CD73, and ADO-receptors A2a and A2b, and HIF-1α compared to naïve cells. CD73 activity inhibition (CD73KO), induced a higher activation state (CD44 and CD69) compared to WT cells (WT vs CD73KO: $p<0,05$). As expected, when WT cells were cultivated in an ATP-enriched medium, to mimic CD73-deficient environment, an increase in the activation markers (CD44, CD69, CD25) and proliferation ability was observed (Medium vs +ATP: $p<0.05$). In contrast, CD4 T-cells cultured in an ADO- enriched medium exhibited a defective activation state (Medium vs +ADO: $p<0,05$) which was reversed by the treatment with ZM-241, an A2a inhibitor (Medium vs +ZM: $p<0.05$). Besides, when the protein responsible for HIF-1 degradation was absent in CD4 T-cells (VHL-KO), CD73 expression increased in both naïve and activated cells (WT vs VHL- KO; $p<0.05$). As expected, these cells exhibited defective activation and proliferation capacity (WT vs VHL-KO; $p<0.05$). However, the immunosuppression caused by HIF-1 can be reversed by adding exogenous ATP (VHL-KO vs VHL-KO + ATP; $p<0.05$).

We detected that *Trypanosoma cruzi in vivo* infection induced an inflammatory and hypoxic environment in the heart and spleen target tissues. Remarkably, we observed an increase in the levels of HIF-1α and CD39, but a decrease in CD73 expression by CD4 T-cells after infection (No inf vs. Inf; $p<0.05$). Effector molecule expression (granzyme B) was higher in the CD73- subset compared to CD73+. In concordance with the findings, CD73KO mice exhibited an improved immune response and reduced cardiac and splenic parasite burden compared to their WT ($p<0.05$).

The above results support the hypothesis that CD39 upregulation on T-cells prevents uncontrolled inflammation by limiting the availability of ATP released in response to acute infection. In turn, CD73 decreased to avoid the anti-inflammatory action of ADO. These findings evidence that the modulation of purinergic signals regulates T-cell functions and influences the outcome of this infection.

164 (40) SNX27: AN EMERGING MODULATOR OF INTRACELLULAR TRAFFICKING AND ANTIGEN CROSS-PRESENTATION BY DENDRITIC CELLS

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Dendritic cells are remarkable for their ability to uptake exogenous antigens, process and present them on MHC class I molecules to trigger potent cytotoxic immune responses. This process called cross-presentation is critical to counteract the harmful effect of numerous pathogenic microbes and tumor cells. The endocytic pathway of dendritic cells plays a central role for achieving an efficient cross-presentation. In this context, sorting nexin proteins play essential functions in the regulation of several aspects of the endocytic route, such as endocytosis, endosomal sorting, signaling and tubulation. In this work, we focused on the study of SNX27 and its role during intracellular trafficking and antigen cross- presentation by dendritic cells. In particular, SNX27 associates with the retromer complex and mediates the recycling of internalized proteins from endosomes to the plasma membrane. Here, we show that SNX27 silencing results in a defect of both soluble and particulate antigen cross-presentation. Regarding the presentation of the intracellular parasite *Toxoplasma gondii*, we found that soluble intravacuolar antigens are significantly affected by the knock-down of SNX27 in dendritic cells. However, the parasite immunodominant and transmembrane antigen GRA6 is presented by a different pathway, which is independent of SNX27 expression. Future and ongoing experiments will helps us to elucidate the intracellular steps of cross-presentation governed by SNX27. Our findings provide evidence that SNX27 plays a central role in endocytic trafficking of dendritic cells and is crucial to guarantee an efficient antigen cross-presentation.

165 (113) UNVEILING THE ROLE OF LIPID METABOLISM IN ENHANCING ANTIGEN CROSS-PRESENTATION BY PERHEXILINE MALEATE-STIMULATED DENDRITIC CELLS

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The immune response mediated by cytotoxic $CD8^+$ T cells (CTLs) plays a pivotal role in both tumor immunotherapy and defense against intracellular pathogens. Dendritic cells (DCs) have the ability to internalize and present exogenous antigens (Ag) via MHC I to activate naïve $CD8^+$ T cells, a process termed cross-presentation. Adjuvants are necessary to robust CTL response, especially in subunit vaccines. However, due to the intricate nature of Ag cross-presentation pathways, identifying therapeutic targets that can serve as adjuvants for generating protective CTL responses has proven challenging. To address this, we previously performed a high throughput screening of drug libraries approved by international agencies. The aim was to identify novel compounds and molecular pathways capable of enhancing Ag cross-presentation in DCs. Our approach involved the adaptation of the B3Z presentation assay using the JAWSII DC cell line and Ovalbumin (OVA) as the soluble Ag. In this study, we elucidate the mechanisms underlying the enhancement of cross- presentation by Perhexiline Maleate (PM), one of the five active compounds identified. Notably, PM does not influence OVA internalization nor MHC I-surface expression in JAWSII DCs. Moreover, incubating JAWSII DCs with PM led to increased translocation of Ags from endosomes to the cytosol ($p<0.001$), an essential step in cross-presentation. To assess the capability of PM-stimulated DCs to activate naïve CD8 T cells, we co-cultured Flt3-L BMDCs pre-treated with PM + OVA with $CD8^+$ T cells purified from OT-I mice. After three days, our observations indicated that PM-stimulated DCs activate naïve $CD8^+$ T cells, triggering proliferation ($p<0.05$), CD44 expression ($p<0.05$), and IFNγ secretion.

PM is known to inhibit the mitochondrial enzyme carnitine palmitoyltransferase 1 (CPT1), thereby reducing fatty acid metabolism. In alignment with this, our prior work demonstrated that PM augmented the presence of lipid bodies per cell, which has previously been related to the ability of DC to cross-present Ags by various researchers. Consequently, we evaluated the involvement of lipid metabolism in the cross-presentation process. Firstly, we found that Etomoxir, another CPT1 inhibitor, enhance OVA cross-presentation by JAWSII DCs ($p<0.01$), implicating CPT1 as a potential target. Secondly, we discovered that both TOFA, an inhibitor of Acetyl-CoA Carboxylase-α (ACCA), and Xanthohumol, an inhibitor of diacylglycerol acyltransferase 1 (DGAT-1) and DGAT-2, counteracted the increase in OVA cross-presentation observed in PM-stimulated JAWSII DCs ($p<0.05$ and $p<0.01$ respectively), without affecting other active compounds.

Further studies will be engaged to completely understand the role of lipid metabolism in the enhancing cross-presentation ability in PM-stimulated DCs.

Antitumor Immunity
Saturday, November 11, 8-9:30h
Chairs: *Andrés Alloati - Roberto Davicino - Eva Acosta*

166 (12) ANAPLASTIC THYROID CANCER CELL-SECRETED TGFB1 CONTRIBUTES TO THE ACTIVATION OF MACROPHAGES THROUGH MODULATION OF SNAIL AND SLUG EXPRESSION

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Introduction: Anaplastic thyroid cancer (ATC) is a clinically aggressive form of undifferentiated thyroid cancer with limited treatment options. Tumor-associated macrophages (TAMs) constitute over 50% of ATC-infiltrating cells, and their presence is associated with a poor prognosis. The mechanisms of how TAMs promote ATC progression are not clear. We have previously shown that soluble factors secreted by ATC cells induced pro-tumor M2-like polarization of THP-1 cells. However, it remains to be identified which ATC cell-derived soluble factors drive macrophage activation. It has been previously reported that transforming growth factor β (TGFβ) induces M2-like macrophage phenotype. Therefore, we investigated the involvement of TGFβ1, its main member, on the phenotype of macrophage activation induced by ATC cell-derived conditioned media (CM).
Methods: THP-1 cells (human monocytes) were treated with human ATC cell lines 8505C or C643-derived CM (ATC-CM) or recombinant human TGFβ1 protein. THP-1 cells exposed to ATC cell-derived CM, were also treated with a TGFβ receptor inhibitor (SB- 431542) 20μM. THP-1 cell proliferation and polarization were assessed by flow cytometry, RT-qPCR and Western blot analysis. TGFβ1 levels in ATC-CM were quantified by ELISA. Gene expression profiles were obtained from the NCBI Gene Expression Omnibus database and analyzed using bioinformatics analysis.
Results: Similar to our previous studies using ATC-CM, recombinant human TGFβ1 treatment significantly influenced the phenotype of THP-1 cells. The changes involved increased expression of Dectin1 and CD163, which are classic M2 phenotype markers of TAMs. In contrast, the levels of CCL13, another M2 marker, were decreased. In addition, TGFβ1 treatment decreased the proliferation of THP-1 cells. Moreover, we showed that TGFβ1 induced mRNA and protein levels of the transcription factors SNAIL and SLUG. Accordingly, TGFβ1 was detected in ATC-CM (DMEM, 10.42±5.4pg/mL; 8505C cell- derived CM, 3251±162.5pg/mL; C643 cell-derived CM, 2752±213.1pg/mL). SB-431542 significantly decreased the Dectin1, CD163, SNAIL and SLUG expression promoted by ATC-CM, whereas increased CCL13 expression in THP-1 cells. We validated the clinical significance of the expression of TGFβ ligands, its receptors as well as SNAIL and SLUG in human ATC by analyzing public microarray datasets, and found that the expression of the main TGFβ ligands, TGFβ1, TGFβ2 and TGFβ3, as well as their receptors were significantly higher in human ATC tissue samples than in normal thyroid tissues.

Conclusions: Our findings indicate that ATC cell-secreted TGFβ1 may play a key role in M2-like macrophage polarization of human monocytes possible involving the up- regulation of SNAIL and SLUG transcription factors. Thus, ours results uncovered a novel mechanism involved in the activation of TAMs by soluble factors released by ATC cells. Our findings have provided novel rationale basis for the development of original therapies for ATC.

167 (180) ANTI-TUMOR POLY ALLYLAMINE NANOPARTICLE-BASED VACCINE PLATFORM

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Nanotechnology has shown promising results for cancer immunotherapy. Nanoparticles trigger the immune response by enhancing antigen presentation on phagocytic cells and activating T cells. Here, we focused on a vaccine PAH nanoparticle platform to control tumor cell growth in a mouse model.

C57BL/6 male mice were immunized with two doses of Np-OVA, OVA or PBS at 14 day-interval through intramuscular (IM), subcutaneous (SC) or combined routes (IM+SC). On day 21, mice were subcutaneously injected with the tumor cell line B16-OVA. Tumors were macroscopically monitored and mice were euthanized when tumor volume reached >1500 mm^3. Humoral (IgG) and cellular (T cells and NK cells) immune responses were evaluated by ELISA and flow cytometry, respectively.

Mice from PBS, OVA and Np-OVA IM groups developed tumors with a mean volume of 737,43mm^3, 999,47mm^3 and 561,84mm^3, respectively. On the other hand, in the Np-OVA SC group, 2 out of 5 mice developed tumors with a mean volume of 154,71mm^3 ($p<0.05$), whereas in the Np-OVA IM+SC group, 1 out of 5 mice developed the tumor, with a volume of 32,61mm^3($p<0.05$). Mice from control groups (PBS and OVA) died at day 14, while all vaccinated mice (Np-OVAIM/SC) survived.

We found higher titers of serum OVA-specific IgG in Np-OVA-vaccinated mice than in control mice (100,2±15, 203,3±103, 781,1±244, 566,7±140 and 470,2±73 for PBS, OVA, Np-OVA IM, Np-OVA SC and Np-OVA IM+SC, respectively.

The analysis of the cellular immune response showed a higher number of $CD4^+IFN^+$ and $CD8^+IFN^+$ T cells in the draining lymph node of mice from the Np-OVA IM+SC group before and after the tumor implantation compared with the PBS group ($p<0.05$). The tumor injection doubled the absolute number of $CD4^+$ IFN^+ and $CD8^+IFN^+$ T cells in vaccinated and non-vaccinated mice. The tumor-infiltrating T cell and macrophage analysis showed no significant differences between the PBS and Np-OVA IM+SC groups. Nevertheless, we found an increase in Nkp46+$NK1.1^+$ cells in the Np-OVA IM+SC group compared with untreated mice ($p<0.05$).

In conclusion, our findings showed that the nanoparticles are potent adjuvants that promoted an anti-tumor response in vaccinated mice, that improved mouse survival, serum OVA-specific IgG, $CD4^+IFN^+$ and $CD8^+IFN^+$ T cells in tumor-draining lymph nodes, and NK tumor infiltration. Overall, this response controlled the growth of the murine melanoma tumor cell line.

168 (49) CARBONIC ANHYDRASE IX AND HYPOXIA-ASSOCIATED METABOLIC CHANGES: A FIRST APPROACH TO THE IMPACT IN TUMOR CELL SURVIVAL AND THE IMMUNE RESPONSE IN LUNG CANCER

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In solid tumors such as lung cancer, tumor survival is, at least in part, sustained by a repertoire of effects induced by pH regulation and glycolysis under low oxygen tension (hypoxia). Then, cancer cells increase lactic acid, glutamine, and CO2 production, resulting in acidification of the tumor microenvironment (TME). Tumor cells regulate the pH through the modulation of extracellular carbonic anhydrases (CA) such as CAIX and exchange H+ from CAIX activity releasing lactate, which could modify the function of immune cells. Hypoxia also induces the recruitment of tumor-associated macrophages (TAMs, which could express CD68 or CD163), and stimulates the immune checkpoint Programmed Death-Ligand 1 (PD-L1). Previously, we analyzed the relevance of hypoxia- associated factors and immune markers in lung cancer patients. We found that hypoxia-inducible factor 1a (HIF1a) and CAIX are differentially expressed in adenocarcinoma (LUAD) and squamous cancer (LUSC), subtypes of non-small cell lung carcinoma (NSCLC) ($p<0.001$). Patients with high expression of CAIX show a decrease in overall survival (Kaplan-Meyer). We also found a strong positive correlation between the expression of HIF-1a vs. CAIX $r=0.22$; HIF1a and PD-L1 ($r=0.48$, $p<0.001$), PD-L1 with CD68 ($r=0.52$, $p<0.001$), and HIF1a and PD-1L with CD163 ($r=0.37$ and 0.62, respectively) in LUAD. PD-L1 and CD 163 also correlate in LUSC patients ($r=0.36$), in which we also found a positive correlation between HIF1a and CAIX ($r=0.22$). As we have reported that coumarin 4-methylumbelliferone (4Mu) modifies HIF1 levels, and is able to inhibit CAIX, we aim to test the effect of 4Mu *in vitro* and *in vivo* using Lewis Lung Carcinoma cells (LLC). We analyzed viability, metabolic activity, CAIX/HIF-1, pH changes, and lactate levels in LLC cells under hypoxia/normoxia. We also evaluate the effect of tumor cells' conditioned medium on macrophages (J774 cells). Hypoxia induces an increase in CAIX levels compared to normoxia ($p<0.05$) and reduces extracellular pH ($p<0.05$). Increased levels of lactate were also detected in hypoxia. We observed that CAIX, pH, and lactate returned to normal in the presence of 4Mu. In addition, while conditioned medium from LLC cells exposed to hypoxia, induced an M2 profile in J774, conditioned medium from LLC exposed to hypoxia + 4Mu, induced an increase in M1 cytokines. We obtained similar results when testing 4Mu effects in vivo. Moreover, we observed an increase in the levels of tumor- infiltrating CD8 after 4Mu treatment ($p<0.01$). We suggest that HIF1a/CAIX markers are relevant for NSCLC patients' prognosis and possibly for immunotherapy success. Besides, in our hands, 4Mu could contribute to regulating CAIX and preparing the TME in favor of a suitable antitumor immune response.

169 (71) CELL SURFACE DENSITY OF MICA EXHIBITS FLUCTUATIONS ALONG G1 AND G2/M PHASES OF THE CELL CYCLE BUT IS INDEPENDENT OF THE TP53 STATUS OF THE CELL

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MHC Class I chain-related A (MICA) has emerged as a target for cancer immunotherapy because it enhances NK-mediated cytolysis through the NK-activating receptor NKG2D. The expression of MICA is restricted to tumor cells and has been described to be affected by several drugs and compounds used to treat cancer patients. Most importantly, recently MICA has been validated as target for monoclonal antibody (mAb)-mediated tumor cell elimination. These mAb-mediated treatment alternatives trigger antibody-dependent cell-mediated cytotoxicity (ADCC) and induction of tumor cell death. However, mutations in the tumor suppressor gene *TP53*, common in over 50% of colorectal carcinomas, have been shown to confer resistance to tumor cell apoptosiscontributing to malignant progression. Furthermore, since gene expression can exhibit fluctuations at different phases of the cell cycle, expression of MICA and subsequent susceptibility to MICA-targeted, mAb-mediated ADCC might be different in the G1 vs. G2/M phases of the cell cycle.Thus, the objective of this work was to investigate the expression of MICA inthe wild type (wt) and $p53^{-/-}$HCT116 human colon adenocarcinoma cell line in the G1 and G2/M phases of the cell cycle. Accordingly, wt and $p53^{-/-}$HCT116 cell cultures were synchronized for 48 h in serum-depleted media and were thereaftercultured in complete media for 24 h. Then, expression of MICA was assessed in cells in G1 vs G2/M phases, which were identified by flow cytometry according to the differential DNA content using 7-AAD. Since efficacy of ADCCdependson target antigen density, we relativized MICA expression at G1 and G2/M to cell area using the FSC parameter, given that cells in G2/M are larger than cells in G1. No significant differences were observed in the relative expression of MICA between wt and $p53^{-/-}$HCT116 in either the G1 or G2/M phases. However, we observed that both cell lines exhibited significantly higher amounts of MICA expression in G1 vs G2/M phase (Mean$\pm$SD: $G1_{wt}$: 3.24$\pm$2.48, $G2_{wt}$: 1.21$\pm$1.01, $p<$ 0.05; $G1_{p53-/-}$: 3.25$\pm$4.04, $G2_{p53-/-}$: 1.33$\pm$1.17, $p<$ 0.01). These results demonstrate that the expression of MICA fluctuates throughout the phases of the cell cycle regardless of the p53 status of the cell. Accordingly, cells in the G1 phase of the cell cycle might be more susceptible to ADCC with anti-MICA mAb. Furthermore, as wt and $p53^{-/-}$ HCT116 cells expressed similar amounts of MICA, this indicates that the potentialmAb-mediated therapies would be equally effective in tumors with wt or mutated TP53.

170 (13) COMBINED GLYPHOSATE AND CHLORPYRIFOS BASED PESTICIDES ALTER BOTH INNATE AND ADAPTIVE IMMUNE EFFECTOR FUNCTIONS

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Glyphosate- and Chlorpyrifos-based pesticides (GbP and CbP respectively) are widely used in agriculture and are often applied to the same fields. There is evidence suggesting that GbP are genotoxic and that both GbP and CbP are associated with an increased frequency of malignancies observed in highly fumigated rural areas. However, their effect on immunosurveillance has been poorly explored. We have previously shown that GbP and CbP individually alter NK cell function by reducing IFN-γ productionafter cytokine stimulation, and that the combination of GbP+CbP affects T cell proliferation and the Th1 differentiation profile. Here, we focused on the mechanisms involved in the effects exerted by GbP and CbP on both innate and adaptive immunecompartments. First, NK cells isolated from healthy donors were stained with CFSE and exposed to Gb, CbP or GbP+CbP, after which they were co-cultured for 5h with eFluor670dye labelled K562. Cells were harvested and stained withZombie Aqua Viability Dye and analyzed by flow cytometry. GbP but not CbP treatment showed a reduction in NK cytotoxicity compared to control cells ($p<0.01$). Notably, GbP+CbP was statistically more potent than GbP orCbP alone ($p<0,05$). Also, NK treated with GbP+CbP formed less NK-K562 conjugates, indicating that theirmechanism of toxicity involvesthe NK cell immunologicalsynapse.Regarding the adaptive immune response, PBMC isolated from healthy donors were cultured for 24h in the presence of GbP, CbP and GbP+CbP and stained with the dichlorofluorescein-diacetate(DCF-DA) probe to measure intracellular H_2O_2 levels by flowcytometry. We observed that both GbP and CbP causeda dose-dependentincrease in intracellular H_2O_2. Also, GbP+CbP produce a greater increaseinH_2O_2 levels than each pesticide alone.Moreover, PBMC were stained with CFSE and stimulated with PMA/Ionomycin in the presence of GbP+CbP. After 4d of culture, cells were stained with anti-CD3 and Zombie Aqua viability dye for flow cytometry analysis. We observed that GbP+CbP inhibited T cellproliferation($p<0,01$), an effect that could be reversed by catalase ($p<0,001$)indicating that a pesticide-mediated increase in oxidative stress is among the underlying mechanisms.Altogether, our results suggest that GbP+CbP strongly impact on NK and T cell function, both of which are crucial cell populations involved in the immunosurveillance against tumors.Since the concentrations of GbP and CbP used here were always sub-apoptotic and far below the Non-Observed Adverse Effect Level (NOAEL) for each active principle, this work reinforces the necesity of reevaluating their safety when using commercial formulations.

171 (199) EFFECT OF SLPI ON THE GENERATION OF DENDRITIC CELLS DERIVED FROM MONOCYTES WITHIN TUMOR MICROENVIRONMENT.

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The Pancreatic ductal adenocarcinoma (PDAC) tumor microenvironment is characterized by being highly immunosuppressive. The number of dendritic cells (DC) present there is usually scarce, however, its increase is correlated with better prognosis of the disease. Previous work in our laboratory showed that the secretory leukocyte protease inhibitor (SLPI) inhibits the generation of dendritic cells derived from monocytes (MoDC) by increasing the expression of CD14 and decreasing that of CD1c. In addition, it has been reported that SLPI favors cell proliferation, migration, and invasion in different PDAC tumor lines. The objective of this work was to determine the role of SLPI in the generation of MoDC in different pancreatic tumor microenvironments. To this end, the expression of SLPI in tumor biopsies from patients with PDAC was analyzed by immunohistochemistry, and the production of SLPI in two pancreatic cancer cell lines by Elisa sandwich. Monocytes were cultured in differentiation medium (RPMI 1640, FBS 10%, IL-4 0.01 ug/ml, GMCSF 0.036 ug/ml) and in the presence or absence of SLPI-producing and non-producing cell lines. Additionally, we evaluated the differentiation measuring the expression of CD1c and CD14 by flow cytometry. Moreover, eleven biopsies were sampled and immunostained for SLPI, in three of them, SLPI was not found within the total of the tumor cells. In contrast, in the remaining eight, the percentage of tumor cells that expressing SLPI oscillated between 15% and 100%. Then, the presence of SLPI in the supernatant of the tumor cell lines, MIAPACA-2 and BXPC-3, was measured resulting in levels up to 4.6 ng/mL in BXPC-3, whereas, in the supernatant of MIAPACA-2, SLPI was not detected. Finally, monocytes from 4 healthy donors with MIAPACA-2 or BXPC-3 were cultured in differentiation medium. Regarding the expression of CD14 and CD1c; on the 5th day of the co-culture with the cancer cell lines, a greater number of CD14+ cells were observed in contrast to the control (no tumor cells), which was not significant ($p=0.0625$; Wilcoxon matched-pairs signed rank test). A similar but opposite trend (reduction) was seen when CD1c expression was analyzed in monocytes co-cultured with MIAPACA-2. These results would indicate that the non-SLPI-producing cell line MIAPACA-2, inhibit monocyte differentiation to DCs through an SLPI-independent mechanism.

172 (123) EXPLORING 4-METHYILUMBELLIFERONE AS AN IMMUNE MODULATOR IN GLIOBLASTOMA TREATMENT

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Glioblastoma (GBM) is the most aggressive primary brain tumor presentingtherapeutic obstacles due to its complex immune microenvironment. The immune response against GBM initiates with microglial cells orchestrating early defenses. Subsequently, the blood-brain barrier's disruption allows neutrophils and γδ T cells to infiltrate the tumor site. Temozolomide (TMZ) remains as the first-line drug forGBM treatment despite its limited efficacy and adverse effects, underscoring the need for innovative therapies.In light of this, we previously demonstrated the anti-tumor effect of 4-methylumbelliferone (4MU), a natural compound without reported adverse effects, on GBM cells. Considering the previous findings, this study aimed to evaluate the impact of TMZ, 4MU, and their combination on immune cell populations tied to the anti-GBM response.In vitro assays encompassed BV2 microglial cells, neutrophils, and γδ T cells isolated from GBM patients. Metabolic activity, cell proliferation, and cell death were assessed through the XTT assay, BrdU incorporation, and PI staining, respectively. Additionally, we measured cytokine secretion (IFN-γ and IL-12) using ELISA and γδ T cell activation by flow cytometryfor CD69 expression. Our results unveiled significant findings. Both TMZ and 4MU diminished metabolic activity and BV2 microglial cell proliferation ($p<0.05$). Notably, the different doses of TMZ induced nearly 40% of cell death, while 4MU triggered 17% only at higher doses ($p<0.0001$). The effect of the TMZ-4MUcombination resembled TMZ alone. Furthermore, 4MU enhanced IFN-γ and IL-12 secretion, whereas TMZ inducedonly IL-12 production. The combination yielded intermediate values ($p<0.05$). Within neutrophils, 4MU did not influence cell death, contrasting TMZ substantial elevation ($p<0.05$). Metabolic activity assays depicted an ascending trend with 4MU and a descending trend with TMZ. The combination exhibited intermediate effects between the single drugs. Notably, γδ T cells remained unchanged by TMZ treatment, while both 4MU alone and combined with TMZ triggered its activation ($p<0.05$). Besides, we extended our study to conditioned media assays. Media from GBM cells (U251, U251-R, LN229, and LN229-R) treated for 48 h with 4MU, TMZ, their combination, or vehicle, was assessed on γδ T cells. Media from all GBM cell lines augmented γδ T cell activation compared to control conditions ($p<0.05$). Notably, TMZ-treated GBM cell media maintained or diminished CD69+ cell percentage, whereas 4MU or combined treatment notably enhanced this parameter ($p<0.05$). In summary, our study highlights the potential of 4MU as a positive immune modulator in GBM therapy. Its impact on immune cell response highly differentiated from TMZ effects, highlights the value of diversified therapeutic approaches. This study opens avenues to changetreatment strategies that harness the distinct attributes of 4MU and TMZ, potentially leading to enhanced efficacy and minimizing adverse effects.

173 (181) EXPLORING EXPRESSION PROFILES AND SIGNALING PATHWAYS OF INFILTRATING MACROPHAGES IN THE TUMOR MICROENVIRONMENT OF ANAPLASTIC THYROID CANCER

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Immune cells are recruited into the tumor microenvironment (TME) during tumor progression. Anaplastic Thyroid Cancer (ATC), is heavily infiltrated by tumor-associated macrophages (TAMs), making them attractive targets for therapy. We have previously reported that treatment of THP-1 cells (a human monocyte-like cell line) with conditioned media derived from ATC cells induces their activation towards a pro-tumoral phenotype, accompanied by an increase in the immune checkpoint marker TIM3. The Wnt/β-catenin pathway plays a crucial role in the pathogenesis of various cancers, and numerous therapeutic strategies have been developed to inhibit this pathway. However, the role of the Wnt/β-catenin pathway in TAMs phenotype in ATC remains unexplored.

To first characterize gene expression programs of ATC-associated TAMs, we analyzed single-cell RNA sequencing data from ATC and adjacent normal thyroid human biopsies reported by Lina Lu et al (GSE193581). The tumor immune landscape showed an enrichment of monocytes and macrophages compared to normal tissue (27% vs. 5% and 20% vs. 1.3%, respectively). ATC-infiltrating macrophages exhibited upregulated expression of pro-angiogenic genes (*Vegfa, Tgfbi,* and *Hif1a*) and M2-associated genes (*Cd163, Cd68*, and *Col1a2*), unlike macrophages from adjacent tissue. ATC-infiltrating monocytes displayed gene expression patterns associated with inflammatory responses, angiogenesis, and TNF/IL6 production. Interestingly, the expression of β-catenin (*Ctnnb1*) and Tim-3 (*Havcr2*) was higher in macrophages, monocytes, and dendritic cellscompared to NK, T, or B cells. While TAMs from ATC and normal tissue showed comparable expression levels of these genes, a significant positive correlation between Ctnnb1 and Havcr2 expression emerged in the ATC context, absent in normal-adjacent TAMs. A similar correlation was observed between Havcr2 expression and Vegfa, a key pro-angiogenic mediator. To validated the sc-RNAseq results, THP-1 cells were treated with conditioned media derived from 8505C cells (human ATC cell line) or control medium for different times. PCR analysis revealed upregulation of β-catenin and associated pathway genes (*C-myc* and *Axin2*). Notably, β-catenin (*Ctnnb1*) expression increased at 30 minutes, followed by Axin2 and C-myc at one hour, persisting at 6 and 24 hours. Our study reveals the complex immune cell and signaling pathway dynamics in the anaplastic TME, highlighting the importance of the Wnt/b-catenin pathway.

174 (211) GALECTIN-1 ORCHESTRATES A HIERARCHICAL TUMOR TO STROMAL EXTRACELLULAR VESICLE SYSTEM THAT FOSTERS IMMUNE SUPPRESSION AND METASTASIS

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Tumoral extracellular vesicles (EVs) contribute to the differentiation and expansion of immunosuppressive cell subsets. Besides, EVs from tumor-associated myeloid cells can increase their immunosuppressive phenotype in an autocrine manner. These two processes can lead to enhanced tumor immune escape and metastasis. Nevertheless, the hierarchical communication between tumor and stromal EVs and the molecular mediator of their release remains uncertain. We previously showed that Galectin-1 (Gal1) is crucial in deactivation of anti-tumor responses and tumor neovascularization; Here, we explored Gal1 role in EV orchestration of immunosuppressive tumor microenvironment. We generated immunosuppressive myeloid cells by culturing mouse BMDCs with GM-CSF for three days in the presence of Gal1. Additionally, we cultured 4T1 WT and *Lgals1* knocked-out cells. We purified small EVs by size exclusion chromatography from conditioned medium of immunosuppressive myeloid cells (control and Gal1-treated) or 4T1 cells (WT or Gal1 KO). ELISA, flow cytometry, miRNA sequencing, proteomic and metabolomic analyses verified EV identity. We performed functional assays by co-culturing BMDC, activated T or B cells with either 4T1 or myeloid EVs. Reciprocally, we tested myeloid-derived EV role in 4T1 metastatic potential *in vitro*, co-culturing tumor cells with myeloid EVs and *in vivo* in an experimental metastasis model by injecting control or Gal1-conditioned myeloid EVs together with 4T1 cells via the tail vein of BALB/c mice. Gal1 is both in the cargo and corona of 4T1 EVs as revealed by flow cytometry and ELISA (p=0.0032) and4T1 cells treatment with an EV biogenesis inhibitor decreased Gal1 secreted levels (p=0.037). When exposed to tumoral Gal1+ EVs, BMDCs switched their differentiation pathway to a M-MDSCs phenotype (CD11b+Ly6G-Ly6Chi) and showed higher PD-L1 (p=0.005) and VISTA (p=0.003) expression. Moreover, 4T1Gal1 KO EVs failed to inhibit CD4+ and CD8+ T and B cell proliferation and activation. To analyze Gal1 contribution to MDSC-EV autocrine signal, we added EVs from Gal1-treated immunosuppressive myeloid cells to new BMDCs and observed CD11b+ cell expansion and higher VISTA expression on cells with M-MDSCs phenotype. Moreover, these EVs showed greater T cell-suppressive capacity *in vitro* and different protein, metabolic, and miRNA cargo compared to control EVs. Finally, EVs from Gal1-treated MDSCs enhanced the metastatic potential of 4T1 cells *in vitro* and *in vivo*, shown by higher migration capacity in a wound healing assay and higher lung metastatic nodules in experimental metastasis. We propose that Gal1+ tumoral EVs interact with myeloid cells and potentiate their immunosuppressive properties, including the release of EVs with autocrine and paracrine functions that increase tumor progression and immune escape. Targeting Gal1 as a molecular mechanism of EV-mediated tumor development may help overcome therapy resistance.

175 (19) IMPACT OF BRAFV600E MUTATION ON IMMUNOGENIC CELL DEATH IN MELANOMA

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The BRAFV600E mutation is of paramount clinical significance in melanoma, manifesting in a considerable proportion of cases (around 50%), and correlating with escalated tumor growth, progression, and diminished overall survival rates. This prominence has propelled us into a targeted exploration of its potential impact on immunogenic cell death (ICD), a distinctive type of cellular demise that not only orchestrates cancer cell elimination but also prompts robust immune responses through the release of danger-associated molecular patterns (DAMPs).The primary focus of this study was to unravel the potential influence of this mutation on both the induction and the underlying molecular events associated with ICD. Two distinct melanoma cell lines were utilized: one harboring the wild-type BRAF (SK-MEL-2) and the other carrying the BRAFV600E mutation (LU1205). These cell lines were subjected to treatment with doxorubicin, an inducer of immunogenic cell death (ICD), as well as cisplatin, a non-ICD inducer.Remarkably, the cell line harboring the BRAFV600E mutation displayed heightened resistance to both types of treatments, when compared to the wild-type counterpart. This common resistance prompted further exploration into the relationship between the mutation and the genetic signature associated with ICD. Through comprehensive in silico analysis using data sourced from the cBioPortal(TCGA, PanCancer Atlas), a significant observation emerged: the BRAFV600E mutation correlated with heightened expression of IFNAR1 and IFNAR2 receptors, pivotal players in the release of danger-associated molecular patterns (DAMPs) and subsequent immune activation.To authenticate these results, a reporter cell line was established by introducing the pMX2-EGFP plasmid via stable transfection, followed by selective pressure with geneticin. This engineered reporter line provides a convenient avenue to confirm the mutation's influence on type 1 interferon pathway regulation during the induction of ICD. The validation process encompassed exposure to interferon alfa (IFNα) treatment, along with the employment of a PDT-based ICD-inducing technique utilizing Me-ALA as a photosensitizing pro-drug, as previously elucidated by our research team.In conclusion, the BRAFV600E mutation's clinical significance in melanoma, its impact on immunogenic cell death (ICD), and its association with resistance to treatment underscore the complex interplay between genetic alterations and immune responses. The observed correlation between the mutation and heightened expression of IFNAR1 and IFNAR2 receptors suggests potential avenues for targeted therapies. These findings deepen our understanding of melanoma's biology and provide insights for future research aimed at improving treatment strategies for patients with this mutation.

176 (80) INCREASE OF CD4+ CELLS AS A POTENTIAL BIOMARKER TO PREDICT THE DEVELOPMENT OF HEPATOCELULLAR CARCINOMAIN PATIENTS WITH NON-ALCOHOLIC STEATOHEPATITIS

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Hepatocellular carcinoma (HCC) is the fourth-leading cause of cancer deaths worldwide. This high mortality rate observed particularly in low-income countries, is related to late diagnosis. On the other hand, the fastest growing cause of HCC is the Non-Alcoholic Fatty Liver Disease (NAFLD) that is found in 25% of the world population and encompasses a spectrum of diseases including Non-Alcoholic Steatohepatitis (NASH) characterized by steatosis, inflammation, hepatocellular damage, and fibrosis that increase the risk of developing HCC. Despite the growing evidence that innate and adaptive immune mechanisms are critical components in the NAFLD progression, the composition of the immune microenvironment in the NASH liver preceding HCC is still unclear. To study the phenotype and spatial disposition of immune cells infiltrating the liver of NASH patients to identify biomarkers that can predict the development of HCC. NASH patients were retrospectively selected by having or not subsequently developed HCC according to clinical and pathological reports from Hospital Privado (Argentina), Hospital Universitario Santa Fe Bogota (Colombia), and Erasmus MC (The Netherlands) and two groups were defined: NASH pre-HCC (n=11) and NASH control biopsies (n=13). Formalin-fixed paraffin-embedded (FFPE)non-tumoral liver biopsies were obtained from each patient and a multiplex immunohistochemistry (IHC) was performed to stain for CD4, CD8, PD1, PD-L1, FoxP3, and CXCR6markers using the Opal 7-ColorManual IHC Kit. The slides were scanned using the multispectral scanner Phenoimager Fusion (Akoya Biosciences) and the images were analyzed using the In Form Advanced Image Analysis Software. The images were obtained and quantitatively analyzed selecting 5 to 10 areas within each tissue where inflammatory infiltrate was observed. Images were spectrally unmixed and each cell was segmented and phenotyped to get individual cell data. The phenotypic analysis from a total of 291908 different immune cells using markers such as CD8+, CD8+ CXCR6+, CD4+ FoxP3+ (Tregs), and CD4+ PD1+ showed that the cell density (cells/mm^2) was not significantly different between groups. Interestingly, CD4+ cell density was higher in NASH pre-HCC patients compared to the NASH control group ($p=0.039$). We also performed a Nearest-neighbor analysis to measure the distance between the different cell phenotypes within the tissue but we did not observe differences. Multiplex immunohistochemistry allows the study in depth the composition and spatial distribution of immune cells within liver biopsies of NASH patients that precede HCC development. In this study, we detected the presence of aninflammatory infiltrate with a higher number of CD4+ cells in patients with NASH who later developed HCC. This finding could have an impact on clinical decision-making and could help to reduce the number of patients who evolve to HCC by predicting their risk of cancer development.

177 (73) LACK OF CD39 DRIVES TERMINAL EXHAUSTION IN CD8+ T LYMPHOCYTES WITH A CYTOTOXIC PHENOTYPE.

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CD39 is an ecto-enzyme that participates in the generation of adenosine (ADO),animmunosuppressive moleculethat interferes with many anti-tumor immune responses.In this study, we assessed the influence of CD39 expression on tumor progression and its effect on CD8+ exhausted T Lymphocytes (LiT) within the tumor microenvironment. For this, C57BL/6 wild type (WT) and CD39 KO mice were injected subcutaneouslywithMC38tumor cells ($0.5x10^6$). By day 17 we evaluated different tumor–infiltrating (T-I) immune cell populations. Additionally, we examinedthe expression of inhibitor receptors (iRs) (PD-1, 2B4, Lag3 and TIM-3), transcription factors (TF) (Eomes.Tbet, IRF-4 and TOX) linked to exhaustion and cytotoxic related molecules, on T-ICD8+ T cells by flow cytometry. CD39KO mice exhibited a reduce tumor volume on days 12,14 and 17 days post tumor injection ($p<0.05$) than tumor-bearing WT mice. We observed no significant differences on T-I immune cell populations (B lymphocytes, CD4+ and CD8+ T cells, Treg, and Macrophages) among CD39KO and WT mice. WhileCD39KO mice showed similar frequencies of T-I CD8+ T cells than WT mice, the former exhibited higher % of TI-LiTCD8+ co-expressing 4 IR ($p<0.05$), higher expression (measures as MFI) of Eomes, Tbet and IRF4 ($p<0.05$, $p<0.0001$), and higher% of Granzyme B (GrB) and Perforin expressing T-I CD8+ T cells ($p<0.01$). It has been reported that intermediate expression of PD-1 (PD-1^{int}) correspond to pre-exhausted LiT CD8+ phenotype, while high PD-1 expression (PD-1^{high}) denotes a terminal exhaustion phenotype. Thus, we detected that CD39 KO mice exhibited a higher % of TI-CD8+PD-1^{high} T cells than WT mice($p<0.05$). In concordance, within the TI-CD8+PD-1^{high} T cells we found higher co-expression of IRs ($p<0.05$), higher expression of TF associated to exhaustion and increased % of GrB and Perforin compared to TI- LiT CD8+PD-1^{int}.Furthermore, CD39KO mice demonstrated a higher percentage of LiT CD8+PD-1^{high} expressing GrB and Perforin than WT mice ($p<0.05$, $p<0.01$). Together, the lack of CD39 leads to a decline in ATP metabolism, resulting in a reduction of ADO. Although the frequency of immune cells within tumor microenvironment remains unaltered, the absence of CD39 fosters an increase of terminally exhausted CD8+ T lymphocytes exhibiting a cytotoxic phenotype. This T cell population may contribute to better control of tumor progression.

178 (18) MICRORNAS INVOLVEMENT IN DENDRITIC CELL IMMUNE FUNCTIONS IN THE CONTEXT OF IMMUNOGENIC CANCER CELL DEATH

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The interaction between tumor cells and immune cells is a critical aspect of cancer progression, and microRNAs (miRNAs) have been identified as important players in this crosstalk. The present study aims to investigate the role of miRNAs in dendritic cell (DC) immune functions in the context of immunogenic cancer cell death (ICD), which is a type of cell death induced by certain cancer therapies that activates the host immune system. Here, we applied a pre-established model of ICD induced in tumor cell lines by the combination of retinoic acid (RA) and interferon-α (IFNα). Immunogenic tumor cell lysates (iTCLs) obtained from cells treated with RA/IFNα were used as a tumor antigen/adjuvant source for DC loading. Next-generation sequencing was employed to identify differentially expressed miRNAs in iTCLs and iTCLs-pulsed DCs, and validation assays were conducted to confirm the presence of specific miRNAs in recipient DCs. We found that two miRNAs, miR-4284 and miR-212-3p, previously described upregulated in tumor cells during ICD, were present in iTCLs and could be displayed in their immature and/or mature forms in iTCLs-pulsed DCs. The integration of miRNA-mRNA functional networks revealed potential targets involved in cell surface receptor signaling pathways, particularly the IL-1 pathway. The secretion of cytokines linked to the IL-1 signaling family was further confirmed using a bead-based multiplex assay. These findings suggest that intercellular communication involving miRNA during ICD could modulate immune mechanisms, with an impact on DCs functions. This study suggests that miRNAs, among other damage associated molecular patterns, play a role in modulation of DC immune functions in the context of ICD in particular by targeting genes related to cell surface receptor signaling pathways, particularly the IL-1 pathway. However, the molecular mechanisms underlying miRNA transfer from dying tumor cells to DCs remain to be elucidated.

179 (146) PHENOTYPIC CHARACTERIZATION AND GM-CSF PRODUCTION OF VIRTUAL MEMORY CD8$^+$ T CELLS IN TUMOR MICROENVIRONMENTS

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Virtual memory CD8$^+$ T cells (T_{VM}) represent a subset of T cells exhibiting memory phenotype (CD44^{hi} and CD122^{hi}) in the absence of prior antigen exposure. This study delved into the tumor infiltration and phenotypic traits of these cells using different tumor models in the brain. In the GL261 glioma model, we report that both T_{MEM} and T_{VM} cells are highly representative in the tumor in similar percentages surpassing T_N cell frequency ($p<0.001$). The IL-2R chain (CD25) is up-regulated after TCR engagement. Accordingly, CD25 expression was markedly higher in T_{MEM} compared to T_{VM} and T_N cells ($p<0.0001$). Furthermore, diminished CD62L expression was highly noted in T_{MEM} ($p<0.0008$) and also in T_{VM} ($p<0.03$) although in a lesser extend respect to T_N cells. This data demonstrate a more effector phenotype in T_{MEM} cells present in the tumor. In terms of chemokine receptors, T_{MEM} exhibited elevated CXCR3 ($p<0.001$) and XCR1 ($p<0.0001$) expression in contrast to T_{VM} and T_N cells that do not express these receptors, indicating that the three type of T cells may use different set of chemokine receptors to ingress to the tumor. Interestingly, elevated Ki67, a proliferation marker, was evident in both T_{VM}($p<0.02$) and T_{MEM}($p<0.0005$) compared to T_N cells suggesting a more active division state in both type of memory CD8$^+$ T cells. We used FROG+ mice, where active (TOMATO) and past (GFP) GM-CSF production could be simultaneously analyzed. We focus our attention on GM-SCF production in T_N, T_{VM}, and T_{MEM} cells in two orthotopic glioma (GL261 and SB28) murine tumor models and also in blood-borne metastases (melanoma (B16), lung (LLC), and breast (PYMT)) in the brain. In analyzing the glioma tumor cell lines, GL261 exhibited a higher percentage of past GM-CSF$^+$ T_N, T_{VM}, and T_{MEM} cells compared to SB28, revealing distinct cellular characteristics in the orthotopic gliomas T cell infiltration. As for the metastases models, immune infiltrate in the B16 tumor displayed an even distribution of T_{VM} and T_{MEM} cells, whereas the other tumor models favored T_{MEM} predominance. Irrespective of the tumor models, the proportions of both past-producing and active-producing GM-CSF T_{MEM} cells is always higher compared to T_{VM} cells. Notably, the brain metastatic breast tumor model induced higher proportions of both past GM-CSF$^+$ and active GM-CSF$^+$ cells relative to the other tumor lines. This study demonstrates how both type of memory cells, T_{VM} non TCR-driven and conventional T_{MEM} (tumor specific?) cells can co-exist and be actively part of the tumor microenvironment by producing cytokines and adopting different phenotypic and functional features. This data encourages us to deeply investigate the antitumor role of both type of memory CD8$^+$ Tcells in controlling tumor growth by TCR-depending and independent mechanisms.

180 (47) SIMULTANEOUS INHIBITION OF MTORC1 AND PPARG REPROGRAMS MYELOID CELL DIFFERENTIATION IN THE TUMOR MICROENVIRONMENT OF MOUSE B16 MELANOMA-BEARING MICE

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Monocytes are highly plastic cells that can differentiate into either macrophages (Mo-Macs) or dendritic cells (Mo-DCs). In the tumor microenvironment, monocytes are the major precursor of tumor-associated macrophages (TAMs), which are known for their ability to promote tumor growth, angiogenesis, invasion, and metastasis. Therefore, the proportion of TAMs in solid tumors is generally associated with tumor progression and poor prognosis. We have previously reported that simultaneous pharmacological inhibition of mTORC1 (Temsirolimus) and PPARg (GW9662) in human monocytes exposed to GM-CSF switches their differentiation from Mo-Macs into highly immunogenic Mo-DCs. Considering these observations, we hypothesized that pharmacological inhibition of these metabolic pathways in vivo might reduce TAM infiltration and enhance Mo-DC differentiation in tumor-bearing mice. To test this hypothesis, C57BL/6 mice were subcutaneously injected with $3x10^5$ B16 melanoma cells and, after 6 days, randomized to receive daily intraperitoneal injections of: a) saline (Control), b) GM-CSF 0,5 ug (GM), c) Temsirolimus (1mg/kg) plus GW9662 (1mg/kg) (TGW) or d) GM-CSF plus Temsirolimus plus GW9662 (GMTGW) (n=3-5 per group). On day 12 of the experiment, mice were euthanized to analyze the tumor immune cell infiltrate by FACS. We observed that mice treated with the drug combination, either in the absence or presence of GM-CSF, had smaller tumors compared to control mice, albeit not reaching statistical significance when comparing each group individually to untreated mice (Control = 879 ± 422, GM = 646 ± 218, TGW = 282 ± 84, GMTGW = 263 ± 47 mm^3; mean ± SEM). However, statistical significance was achieved when comparing mice that received the drug combination, regardless of GM-CSF treatment, vs. untreated or GM-CSF treated mice (p=0.02). Tumors were then surgically removed and minced according to standard protocols to generate single-cell suspensions for FACS analysis. We found that mice treated with the combination of Temsirolimus and GW9662, whether or not they received GMCSF, had a lower proportion of $F480^+$ TAMs among tumor-infiltrating $CD11b^+$ myeloid mononuclear cells (p<0.001) (27 ± 2.4 vs 14.8 ± 1.4 %). Remarkably, we observed that these changes were also associated with a decrease in the proportion of infiltrating monocytes ($Ly6C^+F480^-$) (p<0.001; 37.3 ± 3.2 vs 18.9 ± 1.9) and a significant increase in the proportion of $MHCII_{high}$ $CD11c_{high}$ $Ly6C^-$ cells (p<0.001; 9.2 ± 0.6 vs 21.24 ± 1.5 %), a phenotype compatible with dendritic cells. Our results suggest that simultaneous inhibition of mTORC1 and PPARg triggers the reprogramming of tumor-infiltrating myeloid mononuclear cells, decreasing the number of TAMs and Ly6C monocytes, commonly associated with tumor-suppressive functions, while enhancing the local infiltration by DCs.

181 (4) SPECIFIC RECOGNITION OF EXTRACELLULAR VESICLES BY ANTIBODIES MODULATES THE FUNCTION OF IMMUNE CELLS IN A FC-GAMMA RECEPTOR-DEPENDENT MANNER

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Extracellular vesicles (EVs) are nanometer-sized, lipid membrane enclosed vesicles involved in cell-to-cell communication that influence both physiological and pathological conditions. Owing to their ability to transfer bioactive components and surpass biological barriers, EVs are increasingly explored as therapeutics. However, whether EVs can form immune complexes binding antibodies (IC), and if so, what function might exert, is still unknown. To determine the functional consequences of antibody recognition of an EV-surface antigen on Fc-γ receptor (FcγR) carrying immune cells. A B-cell lymphoma cells (Ramos cells) that release EVs carrying CD20 and the therapeutic anti-CD20 antibody Rituximab (RTX) were used to generate IC (EVs+RTX). EVs were isolated by ultracentrifugation technique and characterized by western blot, beads-based flow cytometry, Nanoparticle-tracking analysis (NTA) and Transmission Electron Microscopy (TEM). Formation of the IC was confirmed by flow cytometry and labeling with protein A–immunogold microscopy. Purified neutrophils were exposed to EVs and/or IC and reactive oxygen species (ROS) production was analyzed. NK cell-mediated cytotoxicity was evaluated on labeled-K562 cells in presence of EVs and/or IC by flow cytometry. Isolated EVs derived from Ramos cells presented bona fide characteristics of EVs, ie, cup-shaped appearance in electron microscopy, enrichment for EVs markers proteins ALIX, CD63, CD81, HLA-DR and CD107a and a size distribution of 90,53 ± 27,01 nm. Importantly, the EVs also carried the B-cell plasma membrane protein CD20 and mirrored the expression of this protein in the parental cells. We confirmed that these EVs, upon exposure to the therapeutic anti-CD20 antibody, formed immune complexes, as revealed by immunogold/TEM (n=1) and beads-based flow cytometry (n=5). We also found that the exposure to EVs promoted both spontaneous and PMA-induced ROS production in neutrophils ($p<0.01$, n=9) that was enhanced in the presence of the IC (n=4). While isolated EVs impaired the NK cell-mediated cytotoxicity ($p<0.001$, n=9), the addition of the IC promoted it ($p<0.01$). Blocking NK cells with CD32 and CD16 neutralizing antibody previous to the IC stimulation diminished the cytotoxicity rate showing that the effect observed is, at least in part, due to CD32 and CD16 specific activation. Collectively, our data describe that specific recognition of an EV antigen by antibodies modify the functionality of the vesicles upon interaction with Fc-gamma receptor bearing cells. Further studies are needed to assess how this novel concept combines the effect of EVs as delivered agents with monoclonal antibody therapy.

182 (82) THE NKG2D LIGAND MICA FOSTERS TUMOR IMMUNESURVEILLANCE OF NK CELLS IN A BREAST CARCINOMA MODEL THROUGH INHIBITION OF MDSC EXPANSION RESULTING IN DIMINISHED METASTASES

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The MHC class I chain-related protein A (MICA) is as a cell surface molecule recognized by the NKG2D activating receptor found on the surface of natural killer (NK), γδ T, and $CD8^+$ T cells. Being predominantly expressed by tumor cells of diverse phenotypes, MICA has emerged as a potential therapeutic target in immuno-oncology. To investigate the impact of MICA expression on myeloid suppressor cells (MDSC), female BALB/c mice were challenged with MICA-expressing (4T1-MICA) or control 4T1 murine triple-negative breast carcinoma cells. First, we observed that subcutaneous injection of 30,000 cells led to the development of aggressively growing tumors, lung metastases, and splenomegaly only in mice challenged with 4T1 cells but not in mice challenged with 4T1-MICA cells. To achieve sustained tumor growth, it was necessary to challenge mice with 250,000 4T1-MICA cells. Both 4T1 and 4T1-MICA tumors exhibited similar growth rates until day 7. Then, 4T1-MICA displayed a regression and resumed to grow only after day 15, which suggests that MICA expression facilitated tumor growth control. Notably, 4T1-MICA tumor-bearing mice did not exhibit the splenomegaly characteristic of 4T1 tumor-bearing mice (spleen weight/mean± SD 4T1: 513.2±125.2 mg; 4T1-MICA 309.9±51.02 mg; $p<0.05$). This reduced splenomegaly in 4T1 tumor-bearing mice was accompanied by a diminished content of granulocytic MDSC (frequency of $Ly6G^+Ly6C^-CD11b^+$ cells/mean± SD 4T1: 28.7±8.1%; 4T1-MICA 10.1±7.5%; $p<0.01$) and monocytic MDSC (frequency of $Ly6C^+Ly6G^-CD11b^+$ cells/mean± SD 4T1: 3.6±1.8%; 4T1-MICA 0.9±0.5%; $p<0.01$). Furthermore, ex vivo analysis demonstrated that splenic NK cells from 4T1 tumor-bearing mice exhibited a lower percentage of degranulation in response to stimulation with YAC-1 cells than splenic NK cells from 4T1-MICA tumor-bearing mice (frequency of $CD107a^+$ NK cells relative to unstimulated cells/mean± SD 4T1: 1.0±0.2%; 4T1-MICA 1.5±0.2%; $p<0.01$). Also, splenic NK cells from 4T1 tumor-bearing mice exhibited a lower frequency of IFN-γ-producing NK cells in response to stimulation with cytokines than splenic NK cells from 4T1-MICA tumor-bearing mice (frequency of IFN^+ NK cells relative to unstimulated cells/mean± SD 4T1: 1.3±0.2%; 4T1-MICA 2.8±1.4%; $p<0.05$). This less immunosuppressive environment in spleens of 4T1-MICA tumor-bearing mice resulted in a significantly lower lung metastases (number of macrometastases in lung/mean± SD 4T1-MICA: 0.2±0.4%; 4T1 6.2±4.4%; $p<0.01$). We conclude that MICA expression in the 4T1 model enhances tumor immunosurveillance by preventing the expansion of splenic MDSC and increasing the effector activity of NK cells.

183 (141) THE TAM AXIS AND A PARTICULAR NEOPLASM GENE PROGRAM WERE INCREASED IN MONONUCLEAR MYELOID CELL FRACTION OF PATIENT WITH ACTIVE LANGERHANS CELLS HISTIOCYTOSIS

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Langerhans cell histiocytosis (LCH) is a chronic inflammatory disease with neoplastic characteristics, defined by the invasion of pathological myeloid CD207$^+$ and/or CD1a$^+$ cells into different tissues with a preponderance to the bone, skin, lung, and lymphoid node. Clinical presentation includes unisystem or multisystem forms, depending on how many organs are involved. The etiology of LCH is unknown and pathological histiocytes may result from malignant transformation of myeloid precursors and/or a persistent unbalanced inflammatory environment. The tyrosine kinases receptors TYRO3, AXL, and MERTK (TAM) and their ligands GAS6 and PROS1 are immunoregulatory axes that participate in the maintenance of immune homeostasis. However, the overexpression of these receptors has also been associated with oncogenic processes due to alterations in the canonical signaling pathways, promoting genes for survival, migration, proliferation, and epithelial-mesenchymal transition, causing the persistence of the neoplastic phenotype. Our aim was to compare the expression levels of TAM receptors and their ligands in circulating blood monocytes and dendritic cells of patients with LCH, based on CD11b, CD11c, CD1a and CD207 measured by flow cytometry. Additionally, a panel of pro-tumorigenic and regulatory genes expression (BIRC5, IL7R, SNAIL, NCAD, MMP9, MMP1, JAG2, SOCS1 and SOCS3) were evaluated in sorted mononuclear myeloid cells by qPCR. Sorting was performed by depletion of lymphocytes with a-CD3/CD19 PE selection cocktail in the mononuclear fraction. Cells were isolated from pediatric patients with confirmed diagnosis of LCH stratified as unisystem or multisystem with active disease (AD) or non-active disease (NAD) and adult controls. We evaluated the expression of TYRO3, AXL and MERTK receptors and their ligands GAS6 and PROS1 on CD207+ and/or CD1a+ cells of the monocytes and dendritic cells of patient with LCH. We observed an increased level of AXL, TYRO3, MERTK, PROS1 and GAS6 ligands in patients with circulating monocytes and DC expressing CD207+ CD1a+ cells (AD, N=21) compared to NAD patients (N=14) and healthy controls (N=8) with statistical significances of $p < 0.01$ and $p < 0.05$. Furthermore, the sorted myeloid cell fraction of patients with active LCH (skin N=5, bone N=5, and MS N=6 compromise) have showed a distinctive transcriptional profile, with higher expression levels of genes involved in survival, migration, and invasion accompanied with a reduced level of the anti-inflammatory SOCS1 and SOCS3, compared to control RNA (N=6).Our results show a differential expression of the three TAM receptors and their ligands in patients with active LCH, which also showed a particular gene program associated with neoplasm promotion and loss of the suppressor of cytokine signaling.

184 (92) UNRAVELING THE IMMUNOPEPTIDOME IN THE CONTEXT OF *TRYPANOSOMA CRUZI* INFECTION AND CANCER FOR THE DEVELOPMENT OF NEW ANTI-TUMORAL STRATEGIES

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The specificity of the immune system has been evolutionarily shaped by pathogens. This underlies the potential overlap between immune targets from infectious agents (Ags) and neoantigens expressed by cancer cells. Our work aims to analyze the potential cross-reactivity between Ags of *Trypanosoma cruzi* – the etiological agent of Chagas disease – and Ags of murine tumor cell lines (AgTs) of C57BL/6 (B16-F10, MC-38, MCA-205 and LL-2) and BALB/c (4T1, TRAMP-C2 and CT-26) mice, using a bioinformatics approach. Whole exome sequencing of paired tumor and normal samples and gene expression of the tumor cell lines were downloaded from the European Nucleotide Archive (ENA). Somatic variants were detected following Genome Analysis ToolKit (GATK) best practices. MuPeXI version 1.2.0 with Variant Effect Predictor version 102 and NetMHCpan version 4.1 were used to extract and rank the neoepitope candidates of 8 to 11 residues. Briefly, candidate peptides expressed in the normal proteome or showing a low likelihood of binding to MHC I predicted with NetMHCpan version 4.1 (rankEL > 2) were filtered out. Then, pBLAST was employed to search the neo-peptides in the proteome of the *T. cruzi* CL Brener strain, either with 100% identity or tolerating only amino acid substitutions at MHC I anchor positions in the binding cores peptides' for the residues that are frequently found in the corresponding alleles (mimotopes) ($H2\text{-}D^b$ and $H2\text{-}K^b$, and $H2\text{-}D^d$, $H2\text{-}K^d$ and $H2\text{-}L^d$ for C57BL/6 and BALB/c tumor cell lines, respectively). The binding of the mimotopes was verified with NetMHCpan 4.1 and those equal to the normal peptide were excluded. Shared neo-peptides and mimotopes were found for all tumor cell lines except the poorly immunogenic TRAMP-C2 cell line. Interestingly, many of the AgTs have been found to be key proteins or factors relevant to various types of neoplasms, including PSMB8, TARS1, GIMAP1, SPB10, LIN7C, OR10B1, STRA6L, TAS2R102, TOP2B, GPR35, WDFY4, GCLC, IKZF4, PTGS2, ZFAND4, MYO1G, SLC24A4, NUP16, and ABCA13. Moreover, many peptides were shared with *trans*-sialidase (Ts), a parasite protein that has been studied as a potential target for vaccines to prevent Chagas Disease. The identified neo-peptides cross-reactive with *T. cruzi* were found to be unique to each tumor cell lines. Furthermore, none of these neo-epitopes were present in the IEDB database, revealing that they have not been evaluated in immunogenicity assays. Nevertheless, a peptide of the Ts Ag, similar to a neo-peptide of the B16-F10 tumor, was experimentally validated as an epitope. Much remains to be deciphered regarding the basis of TCR cross-reactivity; meanwhile, the above preliminary results suggest our approach as a rational way to elucidate the relevance of this mechanism in the well-documented anti-tumoral immunity mediated by *T. cruzi* infection, which we have already evaluated *in vivo*.

185 (24) UPREGULATION OF THE EXHAUSTION-ASSOCIATED RECEPTOR TIM-3 ON NK CELLS REQUIRES THE COOPERATION BETWEEN TUMOR AND ACCESSORY CELLS

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Immune checkpoint inhibitors have revolutionized immuno-oncology (IO). Recently, natural killer (NK) cells have been positioned at the forefront of IO strategies. However, within the tumor microenvironment (TME), NK usually develop an exhausted phenotype, characterized by an upregulation of NK cell inhibitory checkpoints (NKCIC). For instance, we observed that TIM-3 is upregulated on tumor-infiltrating NK cells from patients with clear cell renal cell carcinoma (ccRCC). Although the blockage of some of these NKCIC results in a reinvigoration of their effector functions, the actual stimuli and mechanisms that induce the expression of NKCIC remain poorly defined. Accordingly, our aim was to elucidate the stimuli that trigger the increased expression of several NKCIC such as TIM-3, LAG-3, TIGIT, PVRIG and PD-1. When NK cells isolated from healthy donors (HD) were stimulated with optimal doses of IL-15, a cytokine that promotes NK cell survival and proliferation, for short (2 d) or long (14 d) periods, we did not observe appreciable changes in the frequency of NK cells ($CD3^{-}CD56^{+}$) expressing NKCIC, evaluated by flow cytometry. We also didn´t observe appreciable changes when isolated NK cells from HD were cultured with an ccRCC cell line (786-O) for 2 d or 5 d. However, when peripheral blood mononuclear cells (PBMC) from HD were cultured with 786-O cells for 2 d, a significant increase in the frequency of $TIM\text{-}3^{+}$ NK cells was observed (Mean ± SD, NK: 19,0 ± 17,4 vs NK+786-O: 53,3 ±14,3, p<0,001). Also, cell contact with tumor cells was essential for the induction of TIM-3 expression since the effect was prevented when PBMC were separated from 786-O cells by transwells (Mean ± SD, NK+786-O: 53,3 ±14,3 vs NK+786-O (Tw): 32,7 ± 23,7 p<0,005). These results suggest that the induction of the expression of this NKCIC requires the participation of accessory cells present in PBMC. We conclude that the induction of NKCIC expression would not be a consequence of NK cell activation. Instead, TIM-3 upregulation requires the contact between NK and tumor cells, and a cooperation with other immune populations that usually can be found in the TME. Moreover, a differential regulation is required for the increased expression of other NKCIC such as TIGIT, LAG-3, PVRIG and PD-1.

Transplant Immunology
Saturday, November 11, 8-9:30h
Chair: Mercedes Borge

186 (118) ASSESSMENT OF IL33/ST2 AXIS IN HEPATIC CHRONIC REJECTION. INITIAL RESULTS

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Introduction: The interleukin-33 (IL-33)/suppression of tumorigenicity 2 (ST2) axis modulates immune response and inflammation, and it is critical in mediating hepatic fibrosis. Previously, we demonstrated that IL-33 is involved in the immunopathology of liver fibrosis. Allograft fibrosis is the hallmark characteristic of the progression toliver chronic rejection (CR), an irreversible, progressive disease that leads to graft loss, requiring re-transplantation.
Considering that the biological mechanisms underlying this process remained not completely understood, our aim was to study the role of IL-33/ST2 axis in the fibrogenic mechanism involved in the CR after liver transplant (LT) in humans.
Materials and Methods: Liver biopsies and tissue samples were obtained from LT patients without any histopathological signs of rejection [Non-rejection (NR)=9, patients with chronic rejection [CR=4] and control samples from donor allograft [Control=9]. The frequency and distribution of immune cell populations were evaluated by immunohistochemistry. The expressionlevels of cytokines, Alpha Smooth Muscle Actin (α-SMA), ST2 were measured by qPCR. Histological analyses of collagen, iron and glucopolysaccharides deposition were performed by trichrome, Perls iron and PAS staining. Multiple comparisons of the immunological parameters, data collected from the laboratory (Total Bilirrubine (TB) and liver enzymes such us AST, ALT and ALP) as well as the histopathological analysis (METAVIR score) were made using the Kruskal–Wallis with Dunn's post-test. Correlations were evaluated with the Spearman rank correlation test. The protocol was approved by the Institutional Review Board of HUFF (DDI [1490] 2419).
Results: Intrahepatic CD3 at the lobular and periportal area were increased in CR compared with NR group. Hepatic levels of IL-33 evidenced a slight increase in the CR group with respect to the control group, but this difference was not statistically significant. However, the expression of total ST2 was significantly increased in CR compared with the control group ($p< 0.05$).ST2s, a soluble form of the receptor which acts as decoy factor was not different among groups. Moreover, ST2 levels correlated positively with alteredlevels of functional liver markers as TB, AST, ALT and ALP ($p<0.05$), suggesting that ST2 expression is modulated by hepatocellular and cholestatic damage. α-SMA expressed by hepatic stellate cells reflects their activation and has been directly related to experimental liver fibrogenesis. Levels of α-SMA and TGF-ß, were increased in the CR group compared with controls and the NR group($p>0.05$). Moreover, the

expression of α-SMA positively correlated with METAVIR score in LTx patients ($p<0.05$).
Conclusions: our preliminary result suggests that the IL-33/ST-2 axis is modulated during chronic rejection after liver transplant. Thus, IL-33/ST2 signalling pathway could be considered as a new therapeutic target for the treatment of liver CR.

187 (145) EXPLORING IMMUNOLOGICAL FEATURES OF AN EXPERIMENTAL MODEL OF HETEROTOPIC ALLOGENEIC INTESTINAL TRANSPLANTATION IN RODENTS: FROM BLOOD TO GRAFT.

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Graft rejection is one of the most common complications associated with intestinal transplantation (ITx) and correlates with graft injury, leading to microbial translocation and subsequent sepsis. High doses of immunosuppressants are needed in order to prevent the allogeneic response, but have a negative impact in the long term. Goblet cells (GC) actively secrete mucus to the intestinal lumen, serving a vital role in mucosal protection, therefore, their depletion potentially increases microbial translocation risk. Regulatory T cells (T regs) are major players in the induction of graft tolerance. On the other hand, donor-specific antibodies (DSAs) have a key role on antibody-mediated rejection.

Our aim was to characterize an experimental ITx model in rodents employing tacrolimus, a widely used immunosuppressive agent in clinical settings, evaluating the frequency of T regs in the graft, and establishing its correlation with the rejection kinetics, GC quantity, and DSAs presence in recipients' sera.

Allogeneic heterotopic ITxs were performed in rats (Sprague Dawley strain as donor, Wistar as recipient; n=6); tacrolimus 0.6 mg/kg/day was administered subcutaneously for 7 days as immune suppressive therapy. Graft samples were taken at 0, 7, 14, 21 and 28 postoperative days (POD). Wu' score was used for histopathological diagnosis of acute cellular rejection (ACR). Flow cytometry assays were used to determine frequency of Tregs in graft (defined as $CD4^{+}CD25^{high}FOXP3^{+}$) and DSAs levels in serum. Alcian Blue staining was used to count GC in graft samples.

Histopathological analysis demonstrated that tacrolimus-treated group developed moderate-severe ACR between 21 and 28 POD ($p<0.0001$, Kruskal- Wallis test). A variable course between rejection progression and Tregs frequency was observed. In samples showing a high rejection score, a decline in the frequency of Tregs cells was observed between days 21-28 post- transplantation. Furthermore, a significant correlation was observed between rejection severity and GC loss ($p<0.0001$, linear regression test). Less than 40% of the cases studied showed detectable levels of DSAs, presenting different kinetics with variable increases of IgM and IgG antibodies overtime. In these cases, Treg frequency reached the highest levels, however histopathological damage was evident.

In our model of heterotopic allogeneic intestinal transplantation, we observed a decrease in pro-homeostatic cells, such as regulatory T cells and Goblet cells, along ACR progression. Furthermore, *de novo* generation of DSAs may also contribute to graft injury.

188 (192) IL-22/IL-22BP AXIS IN INTESTINAL TRANSPLANTATION REJECTION: BIOLOGY AND INTERVENTIONS

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Acute cellular rejection (ACR) remains as one of the main causes of graft loss and death in intestinal transplant (ITx) patients. ACR promotes intestinal injury, disruption of the mucosal barrier, bacterial translocation, and organ dysfunction. In this context, epithelial regeneration is critical and the axis IL-22/IL-22BP/IL-22Rc plays an essential role in that process. Our aim was to evaluate the status of the IL-22/IL-22Rc/IL-22 Binding Protein (BP) axis in ITx patients and the regenerative effect of recombinant IL-22 in an experimental ITx model.

Material and methods:

Humans.IL-22, IL-22Rc, IL-22BP mRNA expression from intestinal biopsies [non-Rejectors (NR) =7; Rejectors (R)=5; non-ITxpatients=8] were measured by qPCR.

RatsITxl.-Allogeneic ITX (Sprague-Dawley to Wistar) was performed. All recipients received suboptimal immunosuppression (tacrolimus 0,6 mg/day) from 1 to 7 post-operative days (POD). This condition allows the development of graft rejection from 8 POD, and it was used to evaluate the effect of the intervention with rIL-22 (30 ug/day, 3 doses, N=3). As control group, ITx rats received vehicle instead (n=7). intestinal grafts were collected before the ITx and at 7, 14, 21 and at the clinical endpoint time, determined by clinical score. Clinical Score, Recipient survival, histopathological analysis assessing ACR (H&E), goblet cell number (Alcian Blue), kinetics of CD4 and CD8 frequencies (flow cytometry) were assessed. GraphPad was used to performed statistical analysis.

Results:

Human mucosal IL-22 expression was reduced during ACR (p=0,125) whileIL-22Rc levels did not show differences between NR and R (p=0,931). IL22-BP blocks IL-22 signaling, and its expression was increased during rejection (p=0,097).

When we evaluated the effect of the rIL-22 in ITx rats we observed similar clinical score and recipient survival in both groups. However, a tendency to gain weight was observed in the rIL-22 treated (104.0±1.7 g) vs. control (98.0±4.8g) group at 21 POD. From 14 POD a prominent histological architecture distortion was observed in the control group while rIL-22 group showed well-preserved architecture. Indeed, at 21 POD, 3/7 (43%) control rats presented severe ACR while 0/3 (0%) of rIL-22 treated rats did not show signs of severe rejection. At this timepoint, the number of goblet cells were higher in the rIL-22 treated group compared to the control group (17±4 vs. 9±3, respectively, p=0,230). Regarding immune cells, a significant reduction of intestinal CD4+ cells frequency was

observed in rIL-22 group vs. control group (32±18 vs.67±4, p<0,05). CD8+ cell frequency was similar in both groups (rIL-22: 54±13 vs. Control:52±11, p=0,928). Conclusion.rIL-22 treatment delays the progression to an aggressive ACR. This phenomenon could be due to a protective or regenerative effect exerted by IL- 22 in the intestinal mucosa. Thus, the intervention with IL-22 analogues could potentially be used as a new complementary therapeutic approach upon ACR.

189 (128) IMMUNOLOGICAL ASPECTS OBSERVED IN AN EXPERIMENTAL MODEL OF ABDOMINAL RECTUS FASCIA TRANSPLANT IN RATS WITHOUT IMMUNOSUPPRESSION, INITIAL RESULTS.

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Background: At the end of intestinal or multivisceral transplant, it is essential to perform a primary closure of the abdominal wall. Synthetic meshes can increase the risks of infections and predispose to tissue reactions, present high costs and low availability on the market. The non-vascularized Transplantation of the Abdominal Rectus Fascia (TxARF) has been proposed as alternative. However, the absence of an experimental model, prevents a better understanding of the immunological phenomena of TxARF and its possible clinical implications.

Aim: Was to develop and characterize an experimental model of TxARF in rats and to study the impact of immune response in an allogenic model.

M&M: The TxARF is performed by applying the "Miami technique". 18 TxARF were performed, 9 were isogenic (ISO) and 9 allogeneic (ALLO) transplants, no immunosuppressive treatment was used. Sprague Dawley and Wistar rats were used. Non-implanted ARF were taken as Control (Ct). Pet Scan of the abdominal wall of some recipients was performed at day 30 and 120. Recipient rats were sacrificed at 30 or 120 postoperative days (POD) and graft samples were taken for H&E, Masson's Trichrome, and immunohistochemistry for CD3+ cells. Relative amounts of CD4+ and CD8+ T cells were determined by flow cytometry. Turnover of hematopoietic cells in the graft was analyzed by flow cytometry using GFP+Wistar rats as recipients. Serum samples were taken for donor specific antibody (DSA) analysis. Statistical tests of Normality, One-way ANOVA tests and Kruskal-Wallis tests were used for the analysis of histopathological results.

Results: Survival at day 30 and 120 was 100%. No adhesions were observed between the graft and the abdominal viscera neither in ISO or ALLO transplants. New blood vessels were observed in all samples. At 30 POD, the mean percentage of collagen fibers stained by Masson's Trichrome was more than 10 times higher in the TxARF ALLO and ISO groups than in the Ct ($p<0.0001$). An increase in the percentage of CD3+ cells is observed in TxARF groups compared to the Ct ($p=0.0221$). The hematopoietic cells found in the graft at 30 POD were mainly from the recipient. The proportions of CD4+ and CD8+ cells in the TxARF were similar to those identified in the Ct. DSA in ALLO TxARF at 30 POD were positive in 30% of samples analyzed. No tissue inflammatory activity in the graft or abdominal wall was identified by PetScan in any of the ALLO TxARF rats at 30 and 120 POD.

Conclusion: The results prove the feasibility of this experimental model of TxARF. The increase in the collagen fibers and CD3+ cells in this model without immunosuppression, and the absence of tissue inflammatory activity by PetScan and low DSA titres in the ALLO TxARF group, suggests a non-immunological origin of the fibrosis observed in the graft; whose cause remains to be determined. This new experimental model will contribute to better understanding the biology underlying the TxARF to increase its clinical applications.

190 (204) SLPI: A BIOMARKER OF INJURY IN KIDNEY TRANSPLANTATION THAT ASSOCIATES WITH B AND PLASMA CELL ACTIVATION

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Antibody-mediated rejection (ABMR) is the most common cause of immune-mediated allograft failure after kidney transplantation which compromise graft long term survival. The mechanism of kidney damage by donor specific antibodies is clear. However, it is undetermined the requirements that lead to B cells and plasma cells activation at any time after transplantation in immunosuppressed transplant patients. Subclinical damage after the transplantation may release damage- associated molecular patterns that contributed to activate, shape and amplify the allograft immune response. The aim of the present study was to evaluate whether secretory leukocyte proteinase inhibitor (SLPI) could be a biomarker of injury in kidney transplantation and whether it associates with a microenvironment prone to activate B cells. For this study, 184 subjects were recruited (17 healthy subjects- HS-, 32 pre-transplanted and 133 kidney transplanted stable patients. Plasma SLPI, APRIL and BAFF were measured by sandwich ELISA. Cognate receptors for BAFF and APRIL (BAFFR, BCMA, HSPG, TACI) were measure by RT-PCR. The levels of SLPI in pre-transplanted and transplanted patients were significantly higher than the levels detected in HS ($p<0.0001$). However, the levels of SLPI in the transplanted patients were lower compared to those found in the patients on the waiting list ($p<0.0001$). There was a direct correlation between plasma creatinine and SLPI ($r=0.54$, $p<0.0001$) and an indirect correlation between SLPI and MDRD index ($r=0.40$, $p<0.0001$). Transplanted patients treated with belatacept
+ MMF + steroids showed lower levels of SLPI than those treated with tacrolimus
+
MMF + steroids or SRL + MMF + steroids ($p<0.001$). We did not find differences in plasma BAFF concentration regardless of the treatment or condition. However, we found out that APRIL concentration was higher in belatacept and tacrolimus-treated patients than HS ($p<0.05$ and 0.01, respectively). The analysis on the transcripts expression of receptors shows that BAFFR and BCMA were lower in either betalacept and tacrolimus-treated patients than HS ($p<0.01$ and 0.05, respectively). However, for TACI and HSPG the relative expression of transcripts was lower in belatacept treated patients than HS or tacrolimus treated patients ($p<0.01$) and there was not differences between HS and tacrolimus treated patients. Finally, we found out that there was a direct correlation between SLPI and APRIL ($r=0.50$, $p<0.0001$). Overall these results shows that SLPI could be used as a biomarker of injury that associates with a microenvironment prone to activate B and plasma cell. Furthermore, the low levels of B-cell stimulating factors receptors in belatacept-treated patients suggest that blocking the

costimulatory signal render cells with less ability to produce antibody and ABMR than calcineurin inhibitors.

191 (97) THYMIC MORPHOLOGY AND FUNCTIONALITY ARE AFFECTED BY IMMUNOSUPPRESSIVE THERAPY ADMINISTERED AFTER PEDIATRIC LIVER TRANSPLANTATION

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In the last years, pediatric liver transplantation has become a surgery with excellent success and limited mortality. Graft and patient survival have continued to improve as a result of progress in immunosuppressive therapies. However, children's thymic functional capacity and therefore, peripheral T cells repertory could be disturbed by these therapies, and this could lead to a major incidence of infection and graft rejection. Nowadays, there are no global reliable strategies to evaluate thymic function after solid organ transplantation. Thus, we aimed to explore by non-invasive techniques, how the morphology and functionality of the thymus is affected in pediatric patients undergoing immunosuppressive therapies after liver transplantation.

During the last year, 9 pediatric patients undergoing liver transplantation were included in the study (mean±SEM; 6,25±1.8 years). Blood samples were collected previous to the transplant (pre-T), and 1, 3, 6, and 12 months after liver transplantation (post-T). Immunosuppressive protocols included calcineurin inhibitors, meprednisone, and basiliximab with mycophenolate eventually added if needed. Blood samples were assessed for recent thymic emigrants (RTEs, detected as CD4+CD45RA+CD31+ by flow cytometry) and T-cell receptor excision circles quantification (signal-joint TRECs by qPCR). These TRECs are a byproduct of TCR gene rearrangement during thymic ontogeny, so its determination is a reliable method for estimating the amount of newly formed T cells in circulation. Thymus size was also established by ultrasonography.

We found an inverse association between age and RTEs levels, but not TRECs, at any time point. Reduced thymus size was recorded, principally 1-3 months post-T (i.e.: in 2-age matched patients the mean of pre-T thymus volume was 266 mm^2, 252 mm^2 1-month and 75 mm^2 3-months post-T), evidencing a slight recovery after 6-months post-T (150 mm^2). RTEs levels tend to decrease in 80% of patients at 1-month post-T (%, mean±SEM, pre-T: 43.5±7.4, 1-month post-T: 38.1±11.6), and TRECs levels were significantly decreased in 5/8 patients (62.5%) at the same time point (TRECs/150.000 leukocytes, mean±SEM $x10^3$, pre-T: 14.6±4.5, 1-month post-T: 7.06±3.1, $p<0.05$; pairedt- test).

Conclusions: Immunosuppressive treatment affects thymus size and functionality, mostly 1 and 3 months after liver transplantation, when immunosuppression is more intense.

Immunotherapy
Saturday, November 11, 8-9:30h
Chair: Mercedes Borge

192 (14) INNOVATIVE IMMUNOTHERAPEUTIC APPROACH FOR CONTROLLING AMERICAN TEGUMENTARY LEISHMANIASIS

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Leishmaniasis, an expanding parasitic zoonosis in South America, has been designated as one of the neglected diseases by the World Health Organization due to its disproportionate impact on low-income populations with limited access to healthcare. Within Argentina, leishmaniasis endemically affects regions encompassing the provinces of Salta, Jujuy, Tucumán, Catamarca, Santiago del Estero, Chaco, Formosa, Misiones and Corrientes. *Leishmania amazonensis*, one of the etiological agents of leishmaniasis in our country, is notable for its ability to induce a wide spectrum of clinical manifestations, ranging from localized cutaneous leishmaniasis to severe forms characterized by mutilation, incapacitation, and often a poor response to treatment. Currently, no vaccine is available for the prevention of leishmaniasis in humans, and the existing treatments, such as Glucantime, are unsatisfactory due to their high toxicity, cost, complex administration and the emergence of resistant strains. Hence, there is a pressing need to explore innovative immunotherapeutic alternatives, especially based on first- generation vaccines. In our research, we assessed the effectiveness of a prophylactic vaccine composed of total antigens from *L. amazonensis* (LTA) combined with a Toll-like receptor-3 (TLR-3) agonist, [Poly (I:C)]. Previous studies have indicated that this prophylactic vaccine formulation can induce a protective Th1-type immune response, characterized by elevated production of IgG2a and IFN-γ, with low levels of IL-4 and IL-10.Female BALB/c mice were infected in the right hind paw pad with $1x10^4$*L.amazonensis* promastigotes and treated with the LTA+Poly (I:C) formulation, administered in up to 5 subcutaneous doses at 7-day intervals. We employed PBS, Glucantime, LTA, and Poly (I:C) as control groups. Parameters such as the swelling at the infection site, weight of lesion, parasite load and humoral immune response were thoroughly analyzed. The evaluation of the statistical significance of the data was carried out with parametric or non-parametric tests. Our findings revealed that Poly (I:C) managed to control the infection, resulting in reduced swelling, lower parasite load, and decreased IgG levels compared to the LTA+Poly (I:C) group, which exhibited contrasting outcomes. This study highlights how the formulation of LTA+Poly (I:C) at a specific concentration may elicit an inadequate immune response. As part of our future perspective, based on these results, we intend to focus on further optimizing the therapeutic dose of Poly (I:C) to conduct in-depth investigations into its response against leishmaniasis.

193 (55) DISPLAY OF VIRAL SUPERANTIGENS ON VIRUS-LIKE PARTICLES FOR THE TREATMENT OF LYMPHOMA AND LEUKEMIA

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Superantigens (SAgs) are a class of antigens that can stimulate T or B lymphocytes through non-conventional interactions. The Mouse Mammary Tumor Virus (MMTV) is a B-type retrovirus that codes for a SAg capable of causing apoptosis of T lymphocytes, but only those clones that carry a certain Vβ chain of its TCR. Virus- like particles (VLPs) are vesicles that mimic the organization and arrangement of viral particles, but in the absence of viral genome and thus are not infectious. Despite this fact, they retain their immunogenic ability due to their composition, size and epitope repeatability. The Junin virus (JUNV) Z protein is crucial for the viral budding and when expressed on cell cultures in the absence of infection, is able to induce the release of VLPs. Previously we have characterized Z VLPs as a vaccine developing platform demonstrating its immunomodulating and self-adjuvant properties. Encouraged by those results, we continued exploring the potential uses of this platform, such as a tool for the delivery of proteins that have biological activity by themselves.

The aim of this work is to design, develop and evaluate a potential treatment for

leukemia and lymphoma, based on MMTV SAgs charged on JUNV VLPs. Our hypothesis is that the different SAgs delivered on VLPs will be able to induce apoptosis specifically to those T cells that carry the reactive Vβ on their TCR.

For that, we have developed plasmids called pZ-SAgs to allow the delivery of BALB2, BALB14, LA y mtv-7 SAgs on VLPs, fused to JUNV Z protein. The transfection of HEK293T cells with those constructions allowed the production of SAgs VLPs that were further purified by ultracentrifugation on sucrose cushions. In order to test the biological activity of these particles, we carried out a preliminary *in vitro* assay co-culturing PBMCs derived from a healthy donor with 200 ng of Z-mtv7 VLPs. Results showed that Z-mtv7 VLPs were capable of inducing apoptosis of approximately 80% of the population without inducing proliferation of those cells, after 6 days of treatment. Given these promising results, we are currently characterizing the specificity of Vβ clones that became apoptotic by RT-PCR and also the apoptotic pathways involved in the mechanism. Moreover, we are also preparing batches of all the SAgs VLPs in order to evaluate their effect on PBMCs of the same healthy donor and also other donors. SAgs VLPs could be an alternative to the traditional treatments for these diseases that may reduce the undesired effects of those therapies due to the specificity of its mechanisms.

194 (206) EFFECTOF *egc* SUPERANTIGENS ON MALIGNANT BAND T CELLS. RATIONALE FOR COMBINATION WITH CURRENT ANTITUMOR THERAPIES

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Lymphomas/leukemias are prevalent T and B-cell hematologic malignancies. Conventional treatment, encompassing radiotherapy and chemotherapy, not only yields limited tumor-specific outcomes but also incurs significant adverse effects. Thus, targeted therapies with less toxicity are essential to improve treatment outcomes. Immunotherapy's rise in oncohematological care and monoclonal antibody integration into therapeutic strategies underscore the need for tumor-exclusive antigens, as most antibodies currently target shared antigens in normal tissues. On the other hand, strategies that attempt to induce a host immune response to the tumor require adequate immunogenicity of the neoplastic cells in context of immunocompromisedpatients. In contrast to conventional antigens, superantigens (SAgs) are viral or bacterial proteins that interact with conventional targets such as variable region of the T-cell antigen receptor (TCR) and different regions of the Major Histocompatibility Complex molecules type II (MHC-II). The aim of this study was to investigate the effects of T SAgs on Hodgkin and non-Hodgkin lymphomas and acute lymphocytic leukemia cell lines. Additionally, we aimed to assess the extent of these effects in combination with low doses of Vincristine (VCR), a vinca alkaloid primarily used as a chemotherapeutic agent with well-described toxicity.We observed an increase in the expression of late activation markers (CD86) on the surface of malignant B cells, as measured by flow cytometry, after 48 hours of treatment with *egc* operon SAgs (SEG and SEI) at 10µg/mL.At 72 and 96 hours, we found a decrease in metabolic activity using the XTT assay for all B cell lines treated with 100µg/mL of SAgs ($p<0.0001$, Dunnett's multiple comparisons test), including the Hodgkin lymphoma KM-H2 cell line. Apoptosis at the same SAg concentration was determined by Annexin V-PE/7-AAD staining at these time points.Furthermore, we evaluated different concentrations of VCR and confirmed its dose-dependent induction of cell death at 48, 72, and 96 hours. We assessed an effective concentration of SAgs with the lowest and least toxic concentrations of VCR [<1µM] to determine if it could enhance the effect on metabolic activity or survival. After
96 hours, the combined treatment of VCR [0.001µM] and 100µg/mL SEI led to a
potentiated decrease in Burkitt's lymphoma proliferation, indirectly assessed through the XTT assay ($p<0.0001$). Jurkat cells express a TCR that is non-

reactive to egc SAgs; however, the effect of these SAgs on T lymphocyte survival was evident at 24 hours and at much lower concentrations (SEG and SEI at 1µg/mL). For this purpose, Jurkat cell line was co-cultured with THP-1 cells or plaque-attached monocytes as antigen- presenting cells (APCs). These results strongly suggest that SAgs T can interact with other non-conventional receptors on the target cells, promoting cell death upon activation and thereby expanding therapeutic oncological possibilities.

195 (159) EXPLORATION OF IMMUNOPHENOTYPIC BIOMARKERS AND CLINICAL RESPONSES IN HER2-NEGATIVE BREAST CANCER PATIENTS UNDERGOING COMBINATION THERAPY OF DURVALUMAB AND BEVACIZUMAB

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The emergence of immune checkpoint inhibitors has transformed the dynamics of cancer treatment, harnessing immune responses against tumors. However, their efficacy in breast cancer, particularly within the HER2-negative subtype, remains partially unknown. To address this unexplored territory, our study investigates the potential of a therapeutic approach involving the angiogenic agent bevacizumab (anti-VEGF) and the checkpoint inhibitor durvalumab (anti-PD-L1).

The primary objective of our study is to investigate responses to the combined regimen of bevacizumab and durvalumab, concurrently identifying potential biomarkers of treatment response. Our cohort comprises twenty-six women ≥18 years with advanced HER2-negative breast cancer, all of whom had received prior bevacizumab-containing chemotherapy regimens. Peripheral blood mononuclear cells (PBMCs) were collected prior to the first durvalumab dose every 4 weeks, followed by immunophenotyping using flow cytometry to identify potential biomarkers of treatment response.

From this cohort, distinct responder and non-responder groups emerged, showcasing a clinical benefit rate of 60% at 2 months and 44% at 4 months.

Employing advanced multiparameter cytometry techniques (FACS and CyTOF) alongside high dimensional analysis, we conducted an in-depth investigation. Our findings reveal that prior to anti-PD-L1 treatment, Responders exhibited an increased frequency of CD8+EOMES+GZMB+KLRG1+ TEM cells (p=0.035), a phenotype associated with senescent cells. Conversely, there was a reduced proportion of CD4+TCF1+CD27+ TCM cells (p=0.035), associated with exhausted cells in comparison to non-responders. Moreover, significantly higher frequencies of CD4+FOXP3+CTLA4+ Regulatory T cells were observed in non-responders, both before (p=0.022) and after the treatment (0.043).

These findings collectively highlight the potential of anti-checkpoint therapy, not only within advanced HER2-negative breast cancer but also as a catalyst for innovative therapeutic paradigms across diverse cancer types. Our study highlights the importance of identifying biomarkers of treatment response to develop personalized treatment strategies that effectively address the unique challenges intrinsic to different cancer subtypes.

196 (162) EXPLORING TREATMENT RESPONSE PREDICTORS IN MELANOMA PATIENTS: HIGH-THROUGHPUT SERUM PROTEOMICS AND IMMUNE CELL ANALYSIS BEFORE AND AFTER CHECKPOINT INHIBITORS THERAPY

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Recently, a prospective multi-center study was performed to identify potential predictive biomarkers of toxicity induced after therapy with immune checkpoint inhibitors (ICI). This published study combined a multi-omics approach including unbiased single-cell profiling of over 300 peripheral blood mononuclear cell (PBMC) samples and high-throughput proteomics analysis of over 500 serum samples to characterize the systemic immune compartment of patients with melanoma before and during treatment with ICIs. In this work, we utilized computational analysis to delve into the extensive volume of generated data, including proteomic data (92 measured proteins using OLink platform) and CYTOF. Our objective was to identify predictive immune biomarkers associated with response. The cohort we analyzed consisted of 94 melanoma patients treated with either anti-PD-1 monotherapy or combined anti-PD-1 and anti-CTLA-4. Patients were divided into responders (R) and non-responders (NR) based on scan results three months after therapy started. Samples were obtained at baseline and at different time-points during the therapy.

In serum samples, we found that R patients have lower levels of 4EBP1 (p.adj= 0,025) and CCL4 (p.adj= 0,034) than NR patients before the start of ICI treatment, but no other significant differences were found at the remaining timepoints. Interestingly 4EBP1, a member of translation repressor proteins that had been linked to adverse clinical outcomes; while, CCL4 is a chemokine known to attract several immune cells to damaged tissue.

One week after ICI therapy, R patients increase the levels of proteins involved in inflammation including TNFRSF9, CCL19, CCL3, MCP3, CCL4, IL12B, TNF, PDL1 (p.adj<0,5) compared to baseline time. Conversely, NR did not exhibit significant changes on these proteins.

As for immune cells, after the initial treatment, R patients show a heightened fold change (FC) in several populations: CD38+Ki67+CD4+ T cells (effsize=0,44), CD38+Ki67+CD8+ T cells (effsize=0,40), CD56+CD16- NK cells (effsize=0,38) and IgD+CD38- B cells (effsize=0,37) in contrast to the FC observed in NR.

Trying to understand the correlation between the different immune parameters measured, we performed multiple correlation analysis. Our results show that in R patients, 4EBP1, correlates positively with Treg cells (R=0,6 p=0.03) while CCL4 correlates negatively with naive CD8+ T cells (R= -0,58 p=0,04).

Our results provided new insights into the search for predictive biomarkers associated with response for optimal patient selection. Further studies should be done to elucidate the immune mechanisms underlying these interactions.

197 (28) *TOXOPLASMA GONDII* SERINE PROTEASE INHIBITOR-1 (rTgPI-1) SHAPING PULMONARY IMMUNE RESPONSE IN ASTHMA

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BACKGROUND: We previously showed that treatment with *T. gondii* serine protease inhibitor-1 (rTgPI-1) significantly reduced experimental asthma. BMDC differentiated in the presence of rTgPI-1 had lower CD80 and CD86 and increased PDL-1 expression which correlated with a lower capacity to activate Th2 and Th17 cells. Also, a diminished cDC2 subset in the lungs of an *in vivo* model of asthmatic mice treated with rTgPI-1 was detected. AIM: To further study the effects of treatment of asthmatic mice with rTgPI-1 on lung dendritic cells and the consequences over the T cell response. METHODS: BALB/c mice were ip. sensitized with OVA/Alum and aerosol challenged. Later, were intranasally treated for 3 days with rTgPI-1+OVA (OPI). Controls included *naive* and sensitized mice treated with OVA (OO). Twelve hours later, activation (CD80, CD86) and tolerogenic (PDL1) markers on DCs (CD11c+MHCII+), and CD4+ T cell proliferation was analyzed in the lung. Also, total lung cells were *ex vivo* stimulated with OVA and the supernatant was used to measure IL-4, IL-5, IL-10, IL-17, and IFN-γ. Proliferation of regulatory FoxP3+CD4+ T cells, and lung resident regulatory CD4+FoxP3+CD103+ T cells was assessed by Ki67 expression 72 hours after the last treatment. RESULTS: Although no differences in PDL1 or CD86 were registered in DCs from OPI mice, a significantly lower expression of CD80 ($p=0.005$) was detected, which correlated with a significantly lower proliferation of total CD4+ T cells ($p=0.01$). Finally, culture supernatants from lungs of OPI mice showed significantly less IL-4 ($p =0.02$) and a trend to decreased levels of IL-5. No differences in IL-10, IL-17 and IFN-γwere detected. Seventytwo hours after the last treatment, there was a significant increased percentage and proliferation of regulatory CD4+FoxP3+ T cells ($p=0.02$ and $p=0.002$ respectively) and regulatory lung resident CD4+FoxP3+CD103+ T cells ($p=0.0002$ and $p=0.0008$ respectively). CONCLUSIONS: rTgPI-1 treatment of asthmatic mice results in semi-mature lung DCs that would have lower capacity to induce Th2 activation and proliferation. The cytokine secretion profile in the lung in response to the allergen correlates with the diminished cDC2 subset previously observed. Finally, rTgPI-1 treatment also induces lung regulatory T cells expansion.

Neuroimmunology
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Chair: Pablo Iribarren

198 (131) ANTI-ACETYLCHOLINE RECEPTOR BLOCKING ANTIBODIES IN AN ARGENTINIAN MYASTHENIA GRAVIS COHORT

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Myasthenia gravis (MG) is an autoimmune disease mediated by autoantibodies that can recognise several proteins present in the neuromuscular junction, being the anti-acetylcholine receptor autoantibodies (ACRA) the most frequent. There are three types of ACRA, according to their pathogenic mechanisms: binding, modulating and blocking autoantibodies. Blocking antibodies (bACRA) are directed to the acetylcholine binding site, interfering directly with the normal signalling. Unlike binding (biACRA), which are known to activate the complement system, its role in the disease remains unclear and controversial. In this study, we aim to assess its prevalence and involvement in the pathology of an Argentinian MG cohort.
Serum and plasma samples were obtained from 82 MG patients from April 2016 to September 2022. biACRA and bACRA were measured in serum by RIA. Additionally, complement components C3, C4 (RID) and C5a (ELISA) were determined.The severity of the disease was established through ADL and MGC clinical scores. T-test or Mann Whitney testwere applied for comparison between means or medians, respectively, while Pearson or Spearman r tests were used for correlation analysis;Fisher's exact test was conducted for contingency tables. 41 (50.0%) MG patients tested positive for bACRA. When comparing complement factors between bACRApositive and bACRAnegative populations, we found a statistically significant (ss) difference in the mean value of C4 (25.4 mg/dL and 20.5 mg/dL, respectively) (p=0.006) and the median value of C5a (14.7ng/mL and 20.8 ng/mL, respectively) (p=0.043), and also a positive correlation between C4 and bACRA titre, which altogether could indicate a minor consumption of complement in the positive group. Noss differences were observed when comparingmedian values of ADL, MGC and biACRA titre among groups. Focusing only in the bACRA positive group, both ADL and MGC scores correlated inversely with its titre (p=0.0252, r=-0.354; p=0.030, r=-0,3445, respectively). Finally, no association was found within the presence of blocking antibodies and exacerbations of the disease, nor ss correlations between biACRA titre and bACRA titre in the overall population studied.
In conclusion, this is the first report of bACRA prevalence in Argentinian MG patients, where we found that bACRA seems to have a role mainly blocking the acetylcholine binding site, instead of activating the complement system. On the other hand, we could not find an association with the severity of the disease comparing bACRA positive vs. bACRA negative groups, although in the bACRApositive individuals an inverse correlation of bACRA titre with the clinical scores was observed. Further studies are necessary to determine the importance of bACRA measurement in the diagnosis of MG.

199 (109) CD4+ T CELLS DRIVE CORNEAL NERVE DAMAGE BUT ARE DISPENSABLE FOR CORNEAL EPITHELIOPATHY DEVELOPMENT IN THE CONTEXT OF DRY EYE

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Introduction: Dry eye disease (DED) is characterized by a dysfunctional tear film, ocular surface inflammation and damage, and neurosensory abnormalities. CD4+ T cells promote disease progression but their relative contribution to epithelial and neural damage in the cornea is unknown.

Methods: Surgical DED was induced in recombinase activating gene 1-deficient (RAG1KO) and wild-type (wt) mice. Corneal epithelial integrity and nerve function were measured on days 0, 5, and 10 by fluorescein uptake and mechano sensitivity, respectively. Nerve and epithelial morphology was evaluated by confocal microscopy of corneal whole mounts. CD4+ T cells from DED or sham wt mice were adoptively transferred to RAG1KO mice.

Results: wt and RAG1KO DED mice developed comparable ocular desiccation (-37±17% vs -38±18% tear production, p=0.85) and loss of conjunctival goblet cells (- 38±29% vs -47±22%, p=0.75), two cardinal DED signs. Both strains showed comparable DED-induced changes in corneal epithelial cells: barrier function worsened (day 0: 4.8±1.5 vs 5.2±1.1, p=0.90; day 5: 12.6±3.5 vs 10.0±3.7, p=0.19; day 10: 14.5±3.1 vs 13.3±3.8, p=0.93) while cell proliferation increased (+66±34% vs +75±36% Ki67+ cells, p=0.93). By contrast, corneal mechanosensitivity progressively dropped in wt but not in RAG1KO mice with DED (day 5: -10±11% vs - 0±11, p=0.05; day 10: -21±14% vs +1±7, p=0.01), and corneal nerve morphology analysis accompanied these changes. We observed a larger DED-induced decrease in subapical (-54±18% vs -17±24%, p<0.001), mid-epithelial (-48±24% vs -19±35, p=0.02), and subbasal (-36±16% vs -8±26%, p=0.01) nerve density in wt than in RAG1KO mice. Compared to sham CD4+ T cell-recipient mice, RAG1KO mice that received CD4+ T cells from DED wt mice showed: 1) no change in the proliferation rate (p=0.97) and barrier function (day 0: 5.8±0.6 vs 4.2±1.0, p=0.06; day 7: 6.3±1.1 vs 5.8±0.4, p=0.45; day 14: 4.7±1.2 vs 3.8±0.6, p=0.20; day 21: 4.4±1.4 vs 4.8±0.8, p=0.79; day 28: 3.9±0.5 vs 4.5±0.7, p=0.17) of the corneal epithelium; 2) decreased mechanosensitivity (day 7: -2±8% vs -10±8%, p=0.006; day 14: -2±9% vs -13±10%, p=0.0005; day 21: -1±10% vs -16±12%, p=0.0045; day 28: +10±8% vs -13±9%, p=0.0001); and 3) reduced corneal nerve density at the subapical (-40±18%, p=0.0001), mid-epithelial (-42±24%, p=0.001), and subbasal (-32±27%, p=0.009) levels.

Conclusion: In the absence of CD4+ T cells, the development of corneal epithelial damage remains unchanged in DED while there is no neurodegeneration. Also, adoptive transfer of DED CD4+ T cells to RAG1KO mice induced the progressive loss of corneal nervedensity and function but had no effect on the epithelium. Thus, our results indicate that CD4+ T cells drive DED-associated corneal neurodegeneration but are not necessary for the development of epithelial damage.

This finding has profound implications in the current therapeutic approaches to DED that target corneal epitheliopathy but overlook nerve damage.

200 (60) HUMAN METAPNEUMOVIRUS INFECTION INDUCES BLOOD-BRAIN BARRIER PERMEABILITY AND LONG-TERM NEUROLOGICAL SEQUELS IN MICE

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Background: The human metapneumovirus (hMPV) is an important viral agent known for causing acute lower respiratory tract infections. This virus mainly affects the pediatric population, causing symptoms like bronchiolitis and pneumonia. However, some of these patients develop neurological manifestations, such as encephalitis. Even more, some of these patients developed severe neurological sequels after the clearance of the virus. Therefore, this work evaluates the consequence of a pulmonary infection with hMPV on the central nervous system (CNS). Methods: To assess the effects of the infection with hMPV on the CNS, four- to six-week-old female BALB/c mice were challenged intranasally with non- infectious control (mock) or hMPV, a clinical isolate from Chile. After 3- and 6-days post-infection (dpi), lung, serum, and brain samples were collected. Additionally, an Evans Blue assay was performed at 3 dpi. Finally, Marble Burying and Open Field tests were performed at 28- and 45-dpi to evaluate the behavior of the infected mice. Results: Even though it was possible to detect viral load in the lungs of mice infected with hMPV, it was not detected in their brains. However, an increased concentration of IL-6 and IFN-□ in the sera of infected mice was detected that could affect the CNS. Along this line, an Evans Blue assay was performed and found that the mice infected with hMPV showed increased blood-brain barrier permeability (BBB). Along with this, there is an alteration in claudin (CLDN)-1, CLDN-5, and ICAM, molecules found in the BBB, and an increase of IL-6 and IL-1□ and monocytes, neutrophils, and T cells in the brain. Finally, a behavioral impairment can be observed at 28- and 45-dpi in the hMPV-infected mice. Conclusion: These results suggest that the infection with hMPV can cause alterations in the brain of infected mice, possibly as a consequence of the increased cytokines in the sera. Even more, the infection with hMPV can induce cognitive sequels in the long term.

201 (134) KETAMINE REDUCES DEPRESSIVE BEHAVIOUR TOGETHER WITH A REDUCTION OF THE IMMUNE RESPONSE IN A MOUSE MODEL OF INFLAMMATORY DEPRESSION

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Background and Aim. Ketamine is an NMDA receptor antagonist showing rapid and robust efficacy as an antidepressant in treatment-refractory patients when administered at sub- anesthetic doses. The mechanism by which ketamine decrease depressive symptoms is not explained entirely by its effect on neuronal NMDA receptors. Evidence supports the role of the innate and adaptive immune systems in the development and maintenance of depression. In numerous studies, patients with depression, showed increased levels of IL-6 and C-reactive protein. In agreement with this, results of our group showed that depressive patients have higher plasma levels of IL-6 and IL-12 and activation of circulating monocytes. We hypothesized that ketamine has an impact on the immune system that contribute to its antidepressant effect. Thus, the aim is to study the immune and behavioral effects of ketamine in a mouse model of inflammatory depression.
Methods. Adult male C57BL6 mice were injected with 10 mg/kg LPS i.p., and 24 h later, 10 and 20 mg/kg i.p. of ketamine were administered. Behavioral tests to measured anhedonia (sucrose preference) and despair with a tail-suspension test (TST) were performed 36 h after ketamine administration. At 72 h from the beginning, 100 µl of blood was extracted from the submaxillary artery. Mice were sacrificed by CO2 and immediately, cardiac reperfusion was performed, and spleen and brain were removed for immunological analysis by flow cytometry.
Results. LPS induced a depressive state evidenced by an increase in the immobility time of TST (1.5 fold) and reduced consumption of sucrose in the anhedonia test compared to controls (CTL) (LPS, N=15 vs. CTL, N=14, $p<0.01$). Ketamine (K) 20mg/kg decreased the immobility time (K20, N=10 vs. LPS, N=15, 1.5-fold $p<0.05$), and significantly improved anhedonia compared to LPS-mice. Depressive behavior was accompanied by immune changes. In the blood of LPS-mice, there was an increase in the % of $MHCII^{neg}Ly6C^{+}Ly6G^{+}$ myeloid cells (CTL, N=14 vs. LPS, N=12, $p<0.01$) which was reversed to control levels by both doses of ketamine (K10, N=11, $p<0.001$ and K20, N=9, $p<0.01$). We also observed an increase in the % of $CD45^{high}$ brain infiltrate (CTL, N=14 vs. LPS, N=12, 2-fold $p<0.01$), represented mainly by $CD11b^{+}Ly6C^{+}$ monocytic cells in LPS-mice. Interestingly, ketamine 20 mg/kg reduced this brain infiltrate similar to control levels (LPS, N=12 vs. K20, N=10, $p<0.01$). Moreover, the expression of CD11b in $CD45^{low}$ microglia showed an increment in LPS-mice compared to controls (CTL, N=14 vs. LPS, N=12, MFI=1.4-fold, $p<0.01$). Notably, ketamine resulted in a decrease in CD11b expression (LPS, N=12 vs. K20, N=10, $p<0.05$), indicating a reduction of microglia activation.
Conclusion. Ketamine reduces depressive behavior mediated by LPS together with a reduction of peripheral inflammation, brain infiltrate, and microglia activation, suggesting that the antidepressant effect of ketamine could be mediated, in part, by this effect.

202 (210) THYMIC STROMAL LYMPHOPOIETIN (TSLP) PROTECTS U251 TUMOR CELLS, PREVENTING U251 APOPTOSIS IN 3D CULTURE AND INDUCING PDL-1 EXPRESSION IN NEUTROPHILS COCULTURED WITH U251 TUMOR CELLS

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Glioblastoma (GBM) is the most devastating brain tumor, with an associated poor prognosis. Despite the advances in surgery and other treatments, the survival of GBM patients has not significantly improved in the past three decades. Thymic stromal lymphopoietin (TSLP) is a cytokine primarily produced by activated epithelial cells and it has been shown to be a key factor in maintaining immune homeostasis and regulating inflammatory responses at mucosal barriers. Furthermore, recent studies have found the participation of TSLP in inflammatory diseases and cancer. Previous results in our group showed that TSLP has an influence on the tumor microenvironment, based on our previous results, the aim of this work wasto elucidate the relevance of TSLP in the interaction between neutrophils and GBM cells. First, we wondering whether the TSLP was able to modulate the apoptosis ofU251GBM cell line. For this purpose, we induced apoptosis with 15 µM of temozolamide (TMZ) onU251 cells cultured as spheroids (3D) in the presence or absence of TSLP (25 ng/ml). We evidenced a decrease in the apoptosis of 3D U251 culture (NA/BrEt staining) when the cells were cultured in the presence of TSLP for 24 hs ($p<0.05$). Next, we evaluated the role of TSLP on PDL-1 expression (flow cytometry). To accomplish that we performed a co-culture of U251 cells with neutrophils from healthy donnors in presence or absence of TSLP (25 ng/ml). It was observed an increase in the expression of PDL-1 molecule in the neutrophil sub-population of the co-culture when they were incubated with exogenous TSLP during 24hs ($p<0.01$). Our findings suggest that TSLP display a protumoral role, by preventing apoptosis in the presence of TMZ and by modulating the inflammatory tumor microenvironment by increasing the expression of PDL-1.

203 (88) TRPV1 SIGNALING FACILITATES AXONAL DEGENERATION OF CORNEAL SENSORY NERVES IN DRY EYE INDEPENDENTLY OF CD4+ T CELLS

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Introduction: Corneal nerve damage causes the most clinically significant symptoms in dry eye disease (DED) yet its pathophysiology remains poorly understood. We have previously shown that type 1 immunity, and more specifically, Th1 CD4+ T cells foster corneal neuropathy. Transient receptor potential vanilloid-1 (TRPV1) channels abound in corneal nerve fibers and respond to inflammation-derived ligands, which increase in DED. TRPV1 overactivation promotes axonal degeneration in vitro but whether it contributes to corneal neuropathy is unknown. Therefore, here we explored the role of TRPV1 in DED-associated corneal nerve damage.

Methods: Surgical DED was induced in TRPV1-deficient (TRPV1KO) and wild-type (wt) mice. Corneal nerve function was measured on days 0, 5, and 10 by mechanical and capsaicin sensitivity and eye-closing ratio as an indicator of spontaneous pain. Nerve and epithelial morphology were assessed by confocal microscopy of corneal whole-mounts. Adoptive transfer of CD4+ T cells from both strains and pharmacological TRPV1 inhibition in wt mice were also evaluated. Gene expression was measured in the trigeminal ganglion by RT-qPCR.

Results: wt and TRPV1KO mice developed comparable ocular desiccation and corneal epithelial damage. Contrasting with wt mice, corneal mechanosensitivity in TRPV1KO mice did not worsen with disease progression. Capsaicin sensitivity increased in wt mice with DED, and consistently, wt but not TRPV1KO mice with DED displayed signs of spontaneous pain. Wt mice with DED exhibited nerve degeneration throughout the corneal epithelium whereas TRPV1KO mice only developed a reduction in the most superficial nerve endings that failed to propagate to the deeper subbasal corneal nerves. Pharmacological blockade of ocular TRPV1 activity reproduced these findings in wt mice with DED. Although TRPV1KO mice with DED had fewer pathogenic Th1 and Th17 CD4+ T cells in the lymph nodes, conjunctival immune infiltration was comparable between strains. Moreover, CD4+ T cells from wt and TRPV1KO mice with DED were equally pathogenic when transferred into T cell-deficient mice, confirming that TRPV1 activity in T cells is not involved in corneal neuropathy. Gene expression of sensory channels TRPV1, TRPM8, and Piezo2 and pro-regenerative activating transcription factor 3 increased in the trigeminal ganglia of wt but not in TRPV1KO mice with DED. By contrast, TNF expression comparably increased in both strains.

Conclusions: Although ocular desiccation is sufficient to trigger superficial corneal nerve damage in DED, proximal propagation of axonal degeneration requires TRPV1 signaling. Conversely, local inflammation sensitizes ocular TRPV1 channels, which are also involved in ocular pain, a key symptom of the disease. Thus, corneal nerve TRPV1 overactivation constitutes a pathogenic event in DED-associated corneal neuropathy and a potential therapeutic target that is independent of the effect of CD4+ T cells on corneal nerves.

204 (75) TYPE 2 IMMUNITY FAVORS CORNEAL NEUROREGENERATION IN A CORNEAL CHEMICAL INJURY MODEL

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Introduction: Corneal nerves are affected by poorly understood mechanisms in inflammatory ocular surface disorders, causing ocular pain. We have shown that type 1 immunity, and more specifically, Th1 CD4+ T cells foster corneal neuropathy. Since type 2 immune responses promote neural recovery in the brain, here we hypothesized that type 2 immunity in the ocular surface could favor corneal nerve regeneration.

Methods: OTII [transgenic for an ovalbumin (OVA)-specific T cell receptor] and Balb/c mice were immunized with 25 µg OVA+5 µg alum (2 i.p. doses, days -14 and -7) to induce type 2 immunity; then, corneal damage was induced from days -6 to 0 by instilling 0.1% benzalkonium chloride (BAK), a commonly used eye drop preservative with known neurotoxicity; and finally, either OVA or saline were instilled daily in both eyes for 2-3 weeks. Corneal neurodegeneration was assessed by functional assays [corneal mechanosensitivity (CMS), capsaicin sensitivity, eye closing ratio) and confocal microscopy (βIII tubulin staining).

Results: On day 1 after BAK exposure, both control and immunized OTII mice had comparable corneal epitheliopathy (staining MFI: 10.48 vs 12.37, p=0.15) and CMS (-59±7% vs -56±8%, p=0.93). Compared to saline, ocular OVA challenge over 20 days accelerated CMS recovery ($p<0.05$ at every 5-day interval, $p<0.0001$ for overall effect, day 20: -64±6 vs -46±10%, $p<0.001$). In control and immunized Balb/c mice exposed to BAK, CMS also declined comparably between groups (-21±8 vs -18±6%, p=0.99) and then recovered faster in OVA-challenged mice (day 15: -15±9 vs +1±5%, p=0.02 for overall effect). While Balb/c mice recovered their baseline CMS after 15 days of OVA challenge, OTII mice did not fully recover after 20 days of OVA challenge and lost more CMS than Balb/c mice. Capsaicin sensitivity (induced pain) was not modified by OVA challenge while eye-closing ratios (spontaneous pain) were different between groups (p=0.04). Confirming the functional data, corneal nerve density was higher in OVA-challenged than in control mice (804±198 vs 927±223 intersections, p=0.004; +42±22% in nerve area, p=0.002). Of note, alum- based immunization per se increased CMS before BAK exposure only in Balb/c mice (day -1: +9±7%, $p<0.001$). As expected, alum-based immunization increased splenic IL4+ (Th2) CD4+ T cells, confirming the immune skewing.

Conclusion: Type 2 immunity promotes corneal neuroregeneration, the more so in Balb/c mice possibly due to their Th2 bias. Conversely, the greater corneal nerve impairment in OTII mice probably relates to Th1-skewing of the BAK-induced immune response. Finally, the effect of systemic alum-based immunization without ocular challenge suggests that an increase in the Th2 tone is sufficient to modify corneal nerve function. Although the underlying mechanisms remain to be investigated, these findings have profound therapeutic implications.

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