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ABSTRACT BOOK

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NOVEMBER 23 AND NOVEMBER 25, 2021.

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1.- Autoimmunity

HLA-DR-associated peptidome analysis reveals novel CD4+ T-cell epitopes in rheumatoid arthritis

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Introduction: Rheumatoid arthritis (RA) is an autoimmune disease, initiated and perpetuated by CD4+ T cells recognizing poorly characterized autoantigenic peptides (epitopes) presented by human leukocyte antigen (HLA) class II molecules on the surface of antigen presenting cells (APCs). In this work we focused on identifying synovial epitopes recognized by CD4+ T cells from RA patients. Methodology: HLA-DR/peptide complexes were isolated from dendritic cells carrying RA-susceptible HLA-DR molecules (n=10) and pulsed with synovial fluid (n=15) or synovial tissue (ST; n=1), as well as directly from RA ST (n=3). Peptide sequencing was performed by high-resolution mass spectrometry and the immunostimulatory capacity of selected peptides was evaluated on peripheral blood mononuclear cells from RA patients (n=25) and healthy subjects (n=10) by flow cytometry. Results: Between 103 and 888 HLA-DR-naturally presented peptides were identified per sample. Six peptides, deriving from gelsolin, histone H2B, citrullinated (cit)-H2B, cit-proteoglycan (PG)-4, histone H4, and myeloperoxidase (MPO), were able to increase CD40L expression and IFN-γ production by CD4+ T cells from RA patients; and three, deriving from gelsolin, cit-PG-4, and MPO, specifically triggered proinflammatory responses on RA CD4+ T cells. Finally, both the frequency of MPO-specific IFN-γ-producing CD4+ T cells and H4-specific TNF-α-producing CD4+ T cells were correlated with disease activity. Conclusions: We significantly expanded the peptide repertoire presented by RA-associated HLA-DR molecules, identifying six new epitopes recognized by RA CD4+ T cells, information that contributes both for a better understanding of the disease immunopathology and the design of antigen-specific immunotherapies.

Financing: Funding: Fondecyt 1181853, Redes 180028, ANID-PFCHA/DoctoradoNacional/2018-21181538

Keywords: Rheumatoid arthritis, HLA-DR-associated immunopeptidome, CD4+ T cell epitopes

SCFAs deficiency and decreased expression of GPR43 on colonic intraepithelial lymphocytes promotes a pro-inflammatory environment in gut mucosa during CNS autoimmunity.

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Microbial metabolites produced in the intestine, have significant effects on inflammatory diseases throughout the body. Short-chain fatty acids (SCFAs) emerge as regulators of CNS inflammation with protective effects on experimental autoimmune encephalitis (EAE). However, detailed roles of SCFAs and their receptors in regulating autoimmune CNS inflammation have been unclear. Here we aimed to study the dynamics of gut mucosa lymphocytes during EAE and the relevance of the GPR43 expressed on T-cells.Our data shows that colonic intraepithelial lymphocytes (IEL) composition changes during EAE development. Specifically, TCRαβ+CD4+ and TCRαβ+CD8αβ+ frequencies are increased, and IFNγ production is augmented in EAE mice compared to healthy control. Moreover, natural TCRαβ+CD8αα+ IELs, a population with suppressive potential, is reduced upon EAE development. These changes were accompanied by a decreased expression of the short-chain fatty acid receptor GPR43 in colonic TCRαβ+CD8αα+ IELs and a reduced content of propionate (C3) in EAE mice compared to controls, suggesting an important role of gut microbiota composition in IELs function. Accordingly, in vitro experiments shows that GPR43 stimulation in this population is necessary for IL-10 production and PD-1 expression. The accumulation of IELs into the gut mucosa and the posterior migration to the CNS appears to be mediated by the kinetics expression the chemokine receptor CXCR3. Thus, our data shows that adaptive lymphocytes distribution are altered in colonic gut mucosa during autoimmune neuroinflammation and suggests that changes in the gut environment are relevant in the development of CNS autoimmunity.

Financing: FONDECYT 11190251

Keywords: multiple sclerosis, SCFAs, gut mucosa

INTERFERON-GAMMA ENHANCES MONOCYTE-DERIVED DENDRITIC CELL EXPRESSION OF PROGRAMMED DEATH LIGAND 1 IN MULTIPLE SCLEROSIS

INTERFERON-GAMMA INDUCES A TOLEROGENIC PHENOTYPE AND FUNCTION IN BONE MARROW-DERIVED DENDRITIC CELLS

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Introduction Multiple Sclerosis (MS) is an autoimmune disease of the central nervous system. Our previous results have shown that interferon-gamma (IFN-g) suppresses experimental autoimmune encephalomyelitis (EAE), a mouse model of MS, by inducing a tolerogenic phenotype in antigen-presenting cells. Here, we analyzed the in vitro effect of IFN-g on the differentiation and tolerogenic function of murine bone marrow-derived dendritic cells (BMDCs). Methodology BMDCs precursors from mice were differentiated into BMDCs using rhGM-CSF and rhIL-4 (20 and 10 ng/ml, respectively) in the presence/absence of increasing concentrations of IFN-g. Untreated (UN) or IFN-g-treated immature DCs (iBMDCs) were harvested or stimulated for 24 h with lipopolysaccharide (LPS, 1mg/ml) to obtain mature DCs (mBMDCs). Total CD4+ T cells from spleens of 2D2 mice (neuroantigen-specific TCR transgenic mice) were co-cultured with IFN-g-iBMDCs in the presence/absence of neuroantigen. Phenotypic characterization of DCs and frequency of regulatory T cells (Tregs) was determined by flow cytometry. Results IFN-g-iBMDCs expressed significantly higher levels of Programmed Death Ligand 1 (PD-L1) than UN-iBMDCs. There was no significant difference in the expression of PD-L2, CD80, and CD86 between IFN-g-iBMDCs and UN-iBMDCs. A significantly higher frequency of cells expressing MHC-class II molecules but significantly lower expression of these molecules was detected in IFN-g-iBMDCs compared with UN-iBMDCs. The tolerogenic phenotype of IFN-g-iBMDCs was stable after LPS stimulation. Interestingly, IFN-g-iBMDCs induced a significantly higher conversion of Tregs than UN-iBMDCs. Conclusions Our results suggest that IFN-g induces a tolerogenic phenotype and function in BMDCS. Further assays will be performed to determine the tolerogenic role of IFN-g-iBMDCs in EAE.

Financing: ANID/CONICYT FONDECYT 1191874

Keywords: Interefron-gamma, Tolerogenic Dendritic Cells, EAE

INTERFERON-GAMMA ENHANCES MONOCYTE-DERIVED DENDRITIC CELL EXPRESSION OF PROGRAMMED DEATH LIGAND 1 IN MULTIPLE SCLEROSIS

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Introduction Multiple Sclerosis (MS) is an autoimmune disease of the central nervous system. Our previous results have shown that interferon-gamma (IFN-g) suppresses experimental autoimmune encephalomyelitis (EAE), a mouse model of MS, and induces a tolerogenic phenotype and function in bone marrow-derived dendritic cells. Here, we analyzed the in vitro effect of IFN-g on the differentiation of monocyte-derived dendritic cells (moDCs) of MS patients. Methodology Monocytes were isolated from either untreated MS patients (n=4) or healthy controls (HC, n=4) peripheral mononuclear cells by CD14-specific immunobeads. moDCs were differentiated using rhGM-CSF and rhIL-4 (1000 U/ml) in the presence/absence of increasing concentrations of IFN-g. Half of medium was exchanged on day 3. On day 5, immature DCs (iDCs) were harvested or stimulated for 24 h with lipopolysaccharide (LPS, 1mg/ml) to obtain mature DCs (mDCs). Phenotypic characterization of DCs was performed by flow cytometry. Results No statistical difference was observed in differentiation viability and yield between MS patients and HC. IFN-g-treated moDCs from MS patients and HC resulted in lower expression of the maturation marker CD83 compared with mDCs. Interestingly, IFN-g significantly enhanced the expression of co-inhibitory Programmed Death Ligand 1 (PD-L1) in a dose-dependent manner in MS and HC, compared with iDCs. Instead, IFNg-treated moDCs expressed significantly lower co-stimulatory molecules CD80 but similar levels of PD-L2, CD86, and HLA-DR than mDCs. Conclusions Our preliminary results suggest that IFN-g would induce a tolerogenic phenotype in moDCs from MS patients and HC. A larger sample size and functional assays are necessaries to corroborate these conclusions.

Financing: ANID/CONICYT FONDECYT 1191874

Keywords: Interferon-gamma, Dendritic Cells, Multiple Sclerosis

2.- Immunity and Infection

Role of the RNA modification N6-methyladenosine (m6A) during HIV-1 replication in monocyte-derived human macrophages

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Chronic inflammation is a hallmark of people living with HIV despite efficient viral suppression by ART, being the main cause of mortality and development of non-AIDS comorbidities. Growing evidence suggests that active transcription of the integrated HIV-1 provirus and the presence of the viral RNA in the cytoplasm of infected macrophages significantly contributes to the establishment of this pro-inflammatory state. Moreover, reports have shown that the post-transcriptional RNA modification m6A would play a crucial role regulating viral RNA sensing and activation mechanisms in infected cells. The present work aims to determine whether the presence of m6A in HIV-1 RNA is involved in viral replication and macrophage activation mediated by innate immune sensors. In this context, during the characterization of HIV-1 infection in Thp-1 macrophages, we observed a peak of viral RNA at 48 hpi with a subsequent decrease in RNA levels until 5 days of infection despite a sustained expression of the viral p24 protein. We also observed induction of the mRNA of IFN-b and CXCL10 with peaks at 72 hpi and 48 hpi, respectively, with a subsequent decrease in these RNA expression levels. Finally, we have observed a decreased expression of the m6A methyltransferase METTL3 during the 5 days of infection in macrophages and the induction of M1 activation markers such as CD-163, MHC-II and CD-80. We are currently investigating the role of the m6A modification in the post-transcriptional control of the HIV-1 RNA and the immune activation triggered by the sensing of the viral transcript.

Financing: This work was supported by ANID through Fondecyt Program Grant N° 1190156 (to RSR) and CAS holds a ANID Doctoral Fellowship N° 2021- 21211369.

Keywords: HIV-1, RNA, inflammation

The immunometabolite itaconate fuels iron scavenging by Pseudomonas

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Introduction - To restrict pathogen survival, phagocytes secrete many noxious determinants, like the metabolite itaconate and proteins that interfere with the assimilation of iron by these organisms. The extraordinary ability of certain opportunists like Pseudomonas aeruginosa to thrive in the human lung indicates that these organisms readily evade these host defense systems. Here, we found that by exploiting the release of itaconate by macrophages P. aeruginosa not only selects for strains that outcompete the capability of lung phagocytes to sequester iron, but also increases iron scavenging by synthesizing siderophores. Methodology - WT and itaconate-null (Irg1-/-) mice were infected or not with either P. aeruginosa PAO1 WT, Δict PAO1, or P. aeruginosa isolates. Airway itaconate was measured by HPLC-metabolomics. The transcriptomic program of lung cells and bacteria during pneumonia were determined by single-cell RNA-Seq and RNA-Seq, respectively. Iron and heme were assessed by atomic absorption spectrometry. P. aeruginosa pyoverdine release was tracked by absorbance (OD400/OD600). Results – Our data indicate that by inducing itaconate synthesis in myeloid cells P. aeruginosa outcompetes the ability of host phagocytes to sequester both iron and iron-containing molecules, like heme. We demonstrate that the metabolic stress induced by itaconate on P. aeruginosa prompts the expression of the ict locus, which, in addition to catabolize itaconate, increases siderophore release and iron scavenging by these organisms. Conclusion – This study establishes that the macrophage immunometabolite itaconate can be harnessed by pathogens to outcompete host iron immunity. This works provides with new targets for the eradication of pneumonia.

Financing: S.A.R. is supported by a Vertex Research Innovation Award (RIA, PG010094) and a CFF Pilot and Feasibility Award (RIQUEL21I0). A.P. is supported by NIH 1R35HL135800.

Keywords: immunometabolism, iron, infection

COVID-19 patients with glucidic metabolic disorders promotes the formation of neutrophil extracellular traps

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Background: Severe Coronavirus 2019 disease (COVID-19) infection has been associated with neutrophilia and the formation of neutrophil extracellular traps (NETosis). In addition, glucidic metabolism is affected during acute COVID-19, and diabetes mellitus type 2 (DMT2) has been associated with worse prognosis. However, whether glucidic metabolism is associated with NETosis after COVID-19 remains unknown. In this study, we evaluated the association between NETosis and alterations in glucidic metabolism in COVID-19 patients. Methods: 60 COVID-19 patients were recruited from Víctor-Rios-Ruiz Hospital, 6 months after acute phase. Serum samples were used to determine glycemia, insulin and HOMA. Vital NETosis was measured in neutrophils from patients using flow cytometry with SytoxBlue and Live/Dead dye, whereas the effect of plasma from patients on healthy neutrophils was analysed using Incucyte with SytoxGreen. Results: In our cohort, 19 patients had insulin resistance (IR) or DMT2 before COVID-19, whereas 41 reported no previous disease. Six-months after acute infection, 26 from those 41 patients with no previous alteration developed IR, thus we had 45 patients with metabolic alteration and 15 patients with normal parameters. Our analysis showed that COVID-19 patients with glucidic metabolic disorders have a significant increment of vital NETosis and their plasma significantly increased NETosis in healthy neutrophils at 2 and 6-hours after exposure.Conclusion: These results suggest that patients develop IR as a result of COVID-19 pandemic and that glucidic metabolic alterations promote NETosis that may contribute with COVID-19 severity. Acknowledgment: Thanks to my lab partners and teacher for their support.

Financing: Proyecto ANID COVID1005

Keywords: COVID-19, Neutrophil extracellular traps, Metabolic disorders

iNKT cells activation by AH10-7 promotes class-switch recombination of B cells towards IgG2c and a potent germinal center response

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An efficient B cell activation requires the interaction with T helper cells (Th) and cytokines delivery by these cells to define the isotypes that will produce by B cells. Invariant Natural Killer T cell (iNKT) also can participate on B cell activation through glycolipids, however, α-Galactosylceramide (αGalCer), the activator ligand for excellence, generate a high production of diverse cytokines that mean an ambiguous contribution on the isotypes that will be produced. αGalCer-analogue AH10-7, produce a TH1-LIKE activation, however, there is no evidence that connect this differential activation with a class-switch recombination (CSR) well-defined. Liposomes (LP) with anchored ovalbumin (OVA) and AH10-7, or without ligand, were generated, then administrate intravenously on C57BL/6 to measure serum isotypes and the splenic B cell activation and CSR. LP/OVA/AH10-7 administration produces high IgG2c and Ig3 serum levels on relation to the treatment without ligand, 7 d.p.i. Major levels of γ2c-GLTs/IgG2c transcription on spleen were induced 3 d.p.i when AH10-7 is provided in comparison with LP/OVA. AH10-7 also produces an important expansion of switched-B cells (IgM- IgD-) that express surface-IgG2c and high levels of germinal center markers correlate with a relative avidity of IgG2c increased 42 d.p.i along with an expansion of antibody secreting cells derived from bone marrow. iNKT activated by AH10-7 can enhance the transcription and circulating levels of IgG2c associated with an effective induction of B cells of germinal center and long-lived plasma cells.

Financing: This research was funded by FONDECYT 1211959, Millennium Institute on Immunology and Immunotherapy P09/016-F and ANID Doctoral Fellowship 21180465.

Keywords: switched-B cells, iNKT ligands, nanoparticles

Dysregulated Immune Responses in COVID-19 Patients Correlating With Disease Severity and Invasive Oxygen Requirements

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The prognosis of severe COVID-19 patients has motivated research communities to uncover mechanisms of SARS-CoV-2 pathogenesis at a regional level. Here, we aimed to understand the immunological dynamics of severe COVID-19 patients with different degrees of illness, and upon long-term recovery. We analyzed immune cellular subsets and SARS-CoV-2-specific antibody isotypes of 66 COVID-19 patients admitted to the Hospital Clínico Universidad de Chile, which were categorized according to the WHO ten-point clinical progression score. These included moderate patients (score 4- 5) and severe patients under high flow oxygen nasal cannula (score 6) or invasive mechanical ventilation (score 7-9), plus convalescent patients and healthy controls. Furthermore, severe patients that recovered from the disease were longitudinally followed over 300 days. Our data indicate that severe COVID-19 patients display increased frequencies of plasmablasts, activated T cells and SARSCoV-2-specific antibodies compared to moderate and convalescent patients. Remarkably, within the severe COVID-19 group, patients rapidly progressing into invasive mechanical ventilation show higher frequencies of plasmablasts, monocytes, eosinophils, Th1 cells and SARS-CoV-2-specific IgG than patients under high flow oxygen nasal cannula. These findings demonstrate that severe COVID-19 patients progressing into invasive mechanical ventilation show a distinctive type of immunity. Additionally, patients that recover from severe COVID-19 begin regaining normal proportions of immune cells 100 days after hospital discharge and maintain high levels of SARS-CoV-2-specific IgG throughout the study, which is an indicative sign of immunological memory. Thus, this work can provide useful information to better understand the diverse outcomes of severe COVID-19 pathogenesis. (Manuscript accepted for publication)

Financing: COVID-ANID grant 0752. FONDECYT postdoctoral fellowship 3190856. HHMI grant 55008744. FONDECYT grant 1200793. ECOS grant 180052. FONDECYT grant 1191438. ANID/ FONDAP 1500002. Millenium Science Initiative P09/016-F.

Keywords: COVID-19, oxygen therapy, immunity

Anti-inflammatory effect of boldine on macrophages stimulated with periapical exudate from patients with asymptomatic apical periodontitis

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IntroductionAsymptomatic apical periodontitis (AAP) is a chronic inflammatory condition characterized by destruction of the apical periodontium due to a polymicrobial infection of the endodontic dental canals. Boldine, an alkaloid identified in boldo (Peumus boldus), could have potential as a new therapy to treat AAP due to its anti-inflammatory properties. We aimed to assess its anti-inflammatory effect in human macrophages.Methodology In this in vitro study THP-1-differentiated macrophages were exposed to different concentrations of boldine (0-1000 ng/ml) for 24 hour and their viability and cytotoxicity were analyzed. To explore the effect of boldine by emulating endodontic conditions, cells were stimulated with periapical exudates (1:20/1:200) from AAP teeth and simultaneously exposed to boldine (100 mg/ml) and with no boldine for 2 hrs. Afterwards, cells were pretreated with boldine for 2 hrs and subsequently stimulated with 100 ng/ml commercial LPS from E. coli for 2 hrs. A second group of cells were simultaneously stimulated with boldine 100 ng/ml and LPS from E. coli for 100 ng/ml 2 hrs. TNF-α and IL-6 mRNA levels were determined by qPCR. Statistical analysis were performed with STATA V12. Results and ConclusionsBoldine up to 100 ng/ml were safe based on the conserved macrophages' morphology, viability, and lack of cytotoxicity. Cells stimulated with apical exudates and LPS from E. coli demonstrated significant increases in the gene expression of TNF-α and IL-6 ($p<0.05$). Conversely, pretreatment with boldine and simultaneous exposure to bacterial stimuli reduced gene expression of TNF-α and IL-6 ($p<0.05$). Sustaining its potential as intracanal medication in periapical diseases of endodontic origin.

Financing: Proyecto FONDECYT 1200098

Keywords: Anti-inflammatory, Boldine, Macrophages

HERPES SIMPLEX VIRUS TYPE 1 MODULATES NEUTRAL LIPID METABOLISM IN DENDRITIC CELLS

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Herpes simplex virus type 1 (HSV-1) is a prevalent pathogen in the human population that causes lifelong infections by establishing latency in neurons. HSV-1 infects dendritic cells (DCs), interfering with their viability, maturation profile and capacity to present viral antigens to T cells, thus impairing an effective immune response against this virus. On the other hand, neutral lipids, such as triglycerides and cholesterol esters can be stored in lipid droplets (LDs), which are organelles that are enveloped by a phospholipid monolayer and surrounded by proteins. LDs have been mainly described as a source of energy; however, LD accumulation impairs antigen presentation by DCs. A recent study reported that early HSV-1 infection modulates LD accumulation in astrocytes. However, LD formation has not been reported in DCs. Here, we report that HSV-1-infected DCs undergo significant neutral lipid accumulation that is associated with LDs formation, as determined by flow cytometry, confocal microscopy, and transmission electron microscopy. Additionally, we found by RT-qPCR that HSV-1 infection modulates genes associated with neutral lipid biogenesis. Moreover, we observed that the pharmacological inhibition of pathways related to fatty acid uptake and neutral lipid synthesis in DCs, and not those related to neutral lipid degradation reduce HSV-1 virion formation. Therefore, our results indicate that HSV-1 modulates neutral lipid accumulation in the form of LDs in DCs.

Financing: This work was funded by ANID - Millennium Science Initiative Program - ICN09_016: Millennium Institute on Immunology and Immunotherapy (ICN09_016; P09/016-F) and FONDECYT grant #1190864 from the Agencia Nacional de Investigación y Desarrollo (ANID). MAF is ANID fellow #21191390.

Keywords: Herpes simplex type 1, Immunometabolism, Lipid droplets

Anti-SARS-CoV-2 IgG antibodies levels are associated with COVDI-19 severity and lung structural damage, but not with pulmonary dysfunction 6-months after COVID-19.

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Background: Severe Coronavirus 2019 disease (COVID-19), caused by the severe acute respiratory syndrome corovavirus-2 (SARS-CoV-2), has been associated with an exacerbated immune response. Anti-SARS-CoV-2 antibodies (ab) revelated a memory response and they can play a protective role due to neutralization and viral clearance. However, antibodies can also activate the complement cascade and promote blood clots, supporting the exacerbated immune response. The aim of this study was to evaluate whether the levels of Anti-SARS-CoV-2 ab were associated with COVID-19 severity and pulmonary sequalae 6-months after acute phase.Methods: 60 COVID-19 patients were recruited from Víctor-Rios-Ruiz Hospital, 6-months after acute phase. Serum samples were collected to measure IgM and IgG Anti-SARS-CoV-2 ab levels (Nucleocapsid + Spike) using commercial CLIA assays. Pulmonary dysfunction was analysed by measuring computerised tomography (CT) scan and the diffusing capacity of the lung for carbon monoxide (DLCO) exam.Results: Antibody levels were compared between patients' groups and increased Anti-SARS-CoV-2 IgG levels were found in patients with acute respiratory syndrome (ARDS). Then, we analysed whether the humoral response was associated with lung sequalae and increased Anti-SARS-CoV-2 IgG levels were observed in patients with abnormal CT scan, but not with impaired DLCO. No significant differences were observed for Anti-SARS-CoV-2 IgM levels regarding severity or sequelae.Conclusions: Chilean patients with severe COVID-19 produced higher IgG Anti-SARS-CoV-2 ab levels than patients with mild symptoms, possibly due to an exacerbated immune response. The increased humoral response was associated with structural lung damage but not with lung dysfunction.
Acknowledgments: Víctor-Rios-Ruiz Hospital, PreveGen and UdeC

Financing: Proyecto ANID COVID1005

Keywords: Antibodies, SARS-CoV-2, COVID-19 sequelae

Platelet-derived thrombospondin-1 restrain neutrophilic inflammation during acute lung Pseudomonas aeruginosa infection.

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INTRODUCTION-Pseudomonas aeruginosa (PA) is a major cause of acute lower respiratory tract infections in hospital settings. During acute PA pulmonary infections, thrombospondin-1 (TSP-1) protects the host by preventing excessive neutrophil activation and lung inflammation. TSP-1 can be produced by different sources, including platelets, endothelial cells, and myeloid cells; however, the source of TSP-1 that mediates lung protection during acute PA infection has not been identified. Here, using different conditional mouse models, we determined the contribution of platelet-, endothelial- and myeloid-TSP-1 in host defense and neutrophilic inflammation during acute PA infection. METHODS-Thbs1fl/fl mice were generated using CRISPR/Cas9 technology and bred with PF4cre/WT, Cdh5cre/WT and Lyzmcre/WT mice to yield platelet, endothelial and myeloid TSP-1 deficient mice respectively. PF4cre/WTThbs1fl/fl, Cdh5cre/WTThbs1fl/fl and Lyzmcre/WTThbs1fl/fl mice, and their WT littermate controls; were intratracheally inoculated with PA14 (106 CFU). Bacterial clearance, broncho-alveolar lavage (BAL) protein content and free neutrophil elastase (NE) activity were evaluated at 20h post-infection. RESULTS-During lung PA infection, PF4cre/WTThbs1fl/fl but not Cdh5cre/WTThbs1fl/fl and Lyzmcre/WTThbs1fl/flmice showed increased lung bacterial burden, increased BAL protein content and exaggerated free NE activity compared with their respective WT controls. Although no differences in BAL total neutrophil counts were found in PF4cre/WTThbs1fl/fl mice, these mice showed increased neutrophil extracellular traps (NETs) and degranulation in the airspace, compared with WT controls.CONCLUSION-Platelet-derived TSP-1 contributes to host defense against acute PA lung infection by restraining NETs formation and degranulation. Our data provides novel insights regarding how platelets regulate aberrant neutrophilic inflammation during lung infection.

Keywords: platelets, neutrophilic inflammation, thrombospondin-1

Role of RNA helicase DDX3 on Zika virus replication

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Zika virus (ZIKV) is a potential pandemic virus considered a global threat to public health by the WHO. This pantropic virus infects different cell types from neurons to immune cells including microglia, monocytes, macrophages, and dendritic cells. During ZIKV infection, host cells activate the innate immune response and previous reports suggest that ZIKV uses host proteins to avoid/abrogate cellular defenses to favor its own replication. Indeed, different RNA viruses including HIV-1, DENV and HCV usurp immune response-related cellular factors, such as the RNA helicase DDX3, to promote viral replication. In this work, we explored the involvement of DDX3 during ZIKV replication in a human microglia cell line. We observed that viral RNA and proteins reach a peak at 24 hours post-infection (hpi) decreasing at 48 hpi. On the other hand, activation of innate immune response in infected microglia followed a different kinetic in which the levels of the IFNb and CxCL10 mRNAs reach a peak at 36 hpi.Interestingly, our data show that DDX3 protein levels increase at 12 and 24 hpi and pharmacological inhibition of DDX3 results in a decrease of viral protein levels without affecting the levels of viral RNA, suggesting a possible role of DDX3 during viral RNA translation.Since DDX3 possess a role in the MAVS-dependent immune signaling, we are currently investigating the involvement of DDX3 in innate immune response triggered by ZIKV infection.

Financing: This work was supported by ANID through Fondecyt Program Grant N° 1190156 (to RSR) and THD holds a ANID Doctoral Fellowship N° 2019-21191987.

Keywords: Zika virus, microglia, DDX3

Porphyromonas gingivalis Lipopolysaccharide induces macrophage M1 profile and reduces TLR2 and TLR4 levels not mediated through DNA methylation

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Introduction: Apical Lesion of Endodontic Origin (ALEO) are initiated by endodontic canal infection. It has been proposed that Porphyromonas gingivalis (Pg) and P. endodontalis (Pe) lipopolysaccharide (LPS), could play a role in the progression of the lesion through a pro-inflammatory macrophage polarization (M1) through their recognition by TLR2 and TLR4 receptors.We aimed to characterize the M1 polarization profiles of macrophages differentiated from THP-1 cells, their TLR2 and TLR4 expression with or without previous DNA demethylation following Pg and Pe LPS stimulation.Methodology: THP-1 monocytes (ATCC TIB-202) were differentiated to macrophages with phorbol 12-myristate 13-acetate (PMA) (10 nM) for 24 hours, demethylated with 5-aza-2'-deoxycytidine (decitabine) (500 nM) and stimulated with Pg and/or Pe LPS (10 µg/mL) for 2 hours. Ec LPS and PAM3CSK4 (10 ng/mL) were used as positive controls. Polarization profiles were characterized through flow cytometry and Multiplex; TLR2 and TLR4 surface expression and mRNA levels were quantified by flow cytometry and qPCR, respectively.Results: THP-1 macrophages stimulated with Pg LPS induced a predominant M1 polarization profile based on an increased pro-inflammatory cytokine secretion although also a reduction of CD64 (M1 marker). Pe LPS did not induce a significant response. TLR2 and TLR4 expression were not modified by Pg, Pe LPS stimulation nor DNA demethylation.Conclusions: Pg LPS induce a M1 profile in THP-1 macrophages, unlike Pe LPS that did not induce changes. Neither TLR2 and/or TLR4 stimulation, nor DNA demethylation induce changes in TLR2 or TLR4 mRNA levels.

Financing: Funding by FONDECYT 1160741 and 1200098

Treatment with anti-PD-L1 antibody generates a protective anti-gonococcal response.

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Introduction: Gonorrhea is one of the most common sexually transmitted diseases and is emerging as resistant to most available antibiotics. Also, Neisseria gonorrhoeae has several mechanisms to evade the immune system to avoid protective immunity. We found that PD-L1 expression upon infection with N. gonorrhoeae in vitro suppresses the immune response, and this phenomenon was reverted when PD-L1 was blocked. Therefore, our current aim is to explore the effect of the administration of anti-PD-L1 blocking antibodies in a mouse model of genital gonococcal infection. Methods: Female Balb/c mice were inoculated intravaginally with N. gonorrhoeae. Anti-PD-L1 antibody and isotype control were injected on days -1, 3, 5, 7. We assessed bacterial colonization loads, cytokines mRNA levels, IgG and IgA antibodies levels, and vagina inflammatory score by histopathology. Results: We showed an accelerated clearance of infection with increasing of circulating and local immunoglobulin G antibodies, a decrease of IL-8, IL-17, IL-23, and TGF-β and an increase of IL-6, IL-12, and IFN-γ in mRNA expression in vaginal tissue, and a decrease in the inflammatory score in mice treated with anti-PD-L1 blocking antibody. Conclusion: It was shown that N. gonorrhoeae proactively induces a Th17-driven innate response that it can resist. Blockage of PD-L1 reverses this pattern of host immune response and enables the emergence of a protective anti-gonococcal response. These results may have implications in the potential development of therapeutically approaches against gonorrhea through PD-L1 manipulation.

Financing: Fondecyt Nº1180666

Keywords: Neisseria gonorrhoeae, anti-PD-L1, mouse model

CXCL-9, CXCL-10 and IL-6 are the main circulating inflammatory cytokines associated with long-term pulmonary dysfunction after COVID-19.

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Background: Severe acute respiratory syndrome corovavirus-2 (SARS-CoV-2) is the aetiology agent of Coronavirus 2019 disease (COVID-19). Severe COVID-19 has been associated with sustained pulmonary sequalae after acute phase, however the inflammatory mediators associated with lung pulmonary dysfunction after COVID-19 remains unknown. The aim of this study was to identify cytokines and chemokines associated with pulmonary dysfunction 6 and 12-months after COVID-19 in patients with mild, moderate and severe disease.Methods: 60 COVID-19 patients were recruited from Víctor-Rios-Ruiz Hospital, 6 and 12-months after acute phase. Serum samples were used to determine 6 cytokines and 4 chemokines using Cytometric Bead Array. Pulmonary dysfunction was analysed by measuring computerised tomography (CT) scan, spirometry and the diffusing capacity of the lung for carbon monoxide (DLCO) exam. Results: Our data showed that 22 patients exhibited impaired lung function 6-months after COVID-19 infection. After identifying patients with pulmonary sequalae, we observed that CXCL-9, CXCL10 and IL-6 were the main inflammatory mediators associated with pulmonary dysfunction at 6-months. Finally, from the group of patients with lung sequalae at 6-months, we compared the presence of cytokines between patients who recovered lung function at 12-months and patients who maintained lung dysfunction, observing that CXCL-9, CXCL10 and IL-6 levels at 6-months were augmented in the group of patients that maintained lung dysfunction 12-months after COVID-19.Conclusion: Increased levels of CXCL-9, CXCL-10 and IL-6 are associated with long-term pulmonary sequelae after COVID-19. Further studies are required to identify the pathological mechanism by which these mediators contribute with lung disfunction after COVID-19.

Financing: COVID1005 Project.

Keywords: COVID-19, Pulmonary function, cytokines

3.- Cellular and Molecular Immunology

Effect of GelMA hydrogels on murine Dentritic cells and macrophages phenotype

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Within the area of tissue engineering (TE), the design of scaffolds with optimal characteristics to promote new tissues or tissue regeneration is one of the key components. Gelatin methacryloyl (GelMA) stands as a suitable material for the development of these scaffolds, presenting many advantages derived from its collagen origin, as well as a versatility to generate hydrogels with varied structures and rigidity. This has allowed GelMA hydrogels to be widely studied in TE applications; however, the effect of GelMA hydrogels structure on the immune response has not been extensively studied. Dendritic cells (DCs) and macrophages type 1 and 2 (M1 and M2) are recognized as cells with phagocytic function and the ability to initiate the immune response. As a first approach, we performed in vitro cultures with murine splenocytes in the presence of porcine- and salmon-origin hydrogels. DCs, M1 and M2 populations were characterized by flow cytometry, and the secretion of IL-6 and IL-10 was quantified by ELISA. Results showed no significant changes in the expression of activation markers (MHC-II, CD40, CD86) on DCs cultured with hydrogels in relation to controls. Interestingly, a decrease in the proportion of M1 to M2 was identified with hydrogels. At the cytokine level, an increase in IL-6 production was observed when splenocytes were incubated with hydrogels from salmon origin, with no changes in IL-10. These results suggest that these cells do respond to GelMA hydrogels; however, the specific phenotype induced is yet to be determined including other markers, cytokines, and functional assays.

Financing: Proyecto FONDECYT Regular 1210654Beca doctorado nacional ANID 21210187

Keywords: GelMA hydrogels, Dendritic cells, Macrophages

Intestinal damage promotes the differentiation of pathogenic commensal-specific T cells

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CD4+ T cell responses against commensal bacteria are a hallmark of inflammatory bowel disease (IBD). However, whether commensal-specific T cells are initiators of intestinal inflammation is not known. In this study, we characterized the activation of commensal-specific CBir1 cells in mice undergoing experimental colitis. We found that CD11c+ cells from the colon-draining mesenteric lymph nodes (MLNs) are sufficient to activate naïve CBir1 T cells ex vivo and that the CBir1 cell response is restricted to the colon, where the CBir1 antigen is located. Activated CBir1 cells preferentially acquired an effector rather than regulatory phenotype which was plastic over time. Lastly, we show that Rag1-/-CBir1 cells, while insufficient to initiate intestinal inflammation on their own, can contribute to worse disease outcome in the presence of other TCRs. Our results show that murine CD11c+ cells initiate pro-inflammatory CBir1 cell responses upon intestinal injury and suggest that the commensal-specific T cell responses observed in IBD are a consequence rather than a cause of inflammation.

Keywords: Microbiota, IBD, T cells

Extracellular ATP promotes antigen extraction and presentation in B lymphocytes by signaling through the P2X4 receptor recruited towards the immune synapse

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B-cell activation through the engagement of the B-cell receptor (BCR) with surface-tethered antigens (Ag) leads to the formation of an immune synapse (IS) where lysosomes are locally secreted to facilitate Ag extraction. B-lymphocytes respond to different signals from the microenvironment; however, their impact on B-cell activation is not fully understood. ATP, a danger signal released by stressed cells, has been shown to modulate the function of immune cells, promoting a pro-inflammatory response by activating P2X receptors, an ATP-gated ion channel. Considering that ATP is released under inflammatory conditions and that aberrant activation of B-lymphocytes is associated with inflammatory diseases, in this work, we evaluated its impact on B-cell function, specifically, their ability to extract and present immobilized Ags. B-cells were activated with antigen-coated beads or coverslips, which mimic the IS, allowing us to visualize and quantify intracellular components mobilized at this level. P2X4 recruitment towards the IS and lysosome recruitment were assessed in the presence of ATP (10-100μM). Under these conditions, we quantified Agextraction by measuring the Ag remaining on beads interacting with B-cells, while Ag presentation was evaluated in a co-culture assay with an Ag-specific T-cell hybridoma. Our results show that extracellular ATP increases the ability of B-cells to extract and present Ags without altering lysosome recruitment to the IS. Additionally, we determined that P2X4 is recruited towards the IS upon BCR activation, where it could be promoting the fusion of lysosomes to facilitate Ag extraction. Overall, our results suggest that extracellular ATP locally regulates B-cell activation and function.

Financing: FONDECYT Regular #1180900FONDECYT Regular 1180385Beca Doctorado Nacional ANID

Keywords: B-Lymphocyte, ATP, Immune synapse

Transcriptional gradient of innateness in mouse T lymphocytes

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Innate T cells, such as NKT cells, MAIT cells, gd T cells and some intraepithelial T cells, are populations with diverse developmental pathways, antigen specificities and functional capacities, but they all share the ability to respond rapidly in TCR-dependent and cytokine-dependent but TCR-independent activation. Recently, a transcriptional program that explains a gradient of innateness has been described in human blood lymphoid populations. Here, using the Immunological Genome Project Consortium publicly available bulk RNA-seq and ATAC-seq datasets of several mouse lymphocyte populations, we constructed linear-mixed models of innateness for mouse lymphoid populations. Natural Killer (NK) cells mark the highest end of the scale, as germline-encoded fully differentiated innate lymphocytes, whereas the other end is marked by naive CD4 and CD8 TCRab T cells, as the most adaptive populations. Pathway analysis shows the resulting innateness gradient to contain transcriptional programs related to NK cell functionality, chemotaxis and motility, all traits of innate T cells. Applying our models to conventional CD4/CD8 T cell transcriptional data assigned higher innateness scores to effector and effector memory populations over central memory T cells. A picture emerges, which indicates that T cells innateness is acquired with some types of antigen-experience and parallels with a loss in expansion capacity and a gain in functional maturation ultimately leading to terminal differentiation. Our results also correlate higher innateness scores with lower levels of calcium-dependent T-cell activation, and a higher dependence on protein kinase C phosphorylation pathways. Therefore, these cells have a higher threshold or different requirements for antigen receptor-dependent activation.

Financing: NIH 5R01AI137230-04

Keywords: Innate T cells, NK cells, innate immunity

Unconventional differentiation of memory B cells in the thymus

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IntroductionThe thymus harbors a small population of B cells that participate in negative selection. Several reports show that without immunization, one-third of thymic B cells have undergone Ig-class-switch, a process associated with the differentiation of memory B cells against antigens. We also found in parabiosis models, that 30-50% of donor B cells that immigrate into the thymus acquire a class-switched phenotype, suggesting that a fraction of thymic B cells are programmed towards a memory phenotype by default. However, it is not clear if this process is entirely independent of B cell stimulation by external antigens. Materials and methodsWe generated mice with reduced microbiota through perinatal antibiotic treatment to evaluate the influence of antigenic exposure on the generation of memory B cells (CD73+, CD73+PD-L2+) and class-switched B cells (IgG2b+, IgA+) in thymus and peripheric organs. We also evaluated the phenotype of thymic B cells in neonatal, adult, and aged mice to establish if the appearance of thymic memory B cells is correlated with continuous antigenic exposure in an age-dependent manner. ResultsMice with reduced exposure to antigens from microbiota exhibit a decreased class-switched and memory B cells in spleen and gut-associated lymph nodes. Simultaneously, this subset remains unchanged in the thymus. Furthermore, we observed that IgG2b and IgA appear first in the thymus within the first days of life, whereas in the spleen, they begin to appear four weeks after birth and progressively accumulate with age. ConclusionOur data support that a subset of thymic B cells acquired memory features independent of external antigenic exposure.

Financing: FONDECYT 3170424 (SN), FONDECYT 1180385 (DS), FONDECYT 1191438 (MB), CONICYT AFB 170004 (MR), and CONICYT 22201364 (JC)

Keywords: Thymus, Memory B cells

INTEGRATIVE TRANSCRIPTOMIC ANALYSIS OF THE INTESTINAL IMMUNE RESPONSE IN IRRITABLE BOWEL SYNDROME

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INTRODUCTION: The intestinal mucosal immune response in irritable bowel syndrome (IBS) has not been fully described. OBJECTIVES: To determine the differences of immune response in ileal and colonic mucosa between IBS patients and healthy controls (HC). METHODS: Differential expression analysis was performed by microarray analysis in ileal and colonic mucosa from 5 women with diarrhea predominant IBS (IBS-D) diagnosed by Rome III criteria, and 5 HC. Functional enrichment analysis was performed by GSEA and SEA, in addition to cellular deconvolution analysis and enrichment analysis of cellular signatures of immune response by bioinformatics. An adjusted $p<0.05$ was considered significant. RESULTS: Patients with IBS present differences in the expression of immune response genes. In the ileum, we observed suppression of pathways associated with adaptive immune response (p=3,52x10-20),lymphocyte activation (p=2,27x10-25). In the colon, we observed suppression of humoral response (p=8,43x10-7), immune cell activation (p=6,98x10-5), among others. CONCLUSION: Differences in gene expression between the ileum and colon indicate a suppression of the immune response in IBS.

Financing: FONDECYT 1181699; FONDAP 15130011

Keywords: Irritable Bowel syndrome, Transcriptome, Intestinal immune response

Role of the TRPV1 receptor in narrow band ultraviolet B light mediated inflammation.

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TRPV1 is a Ca2+-permeable channel that it`s functional in CD4+ T cells, where it acts as a non-store-operated Ca2+ channel and contributed to T cell antigen receptor (TCR)-induced Ca2+ influx, TCR signaling and T cell activation.In this proyect, we investigated if the exposure to NB-UVB on Human CD4+ T cells in vitro, promotes the modulation of TH1/TH17/Treg responses, mediated by an increase in intracelular TRPV1- dependent Ca2+ concentration. Human CD4+ T cells isolated from peripheral blood, were exposed to different doses of NB-UVB in vitro and the frequency of TH1/TH17/TReg subpopulations was determined by flow cytometry. Confocal microscopy and Ca2+ sensitive molecular probes were used to verify Ca2+ influx, [Ca2+]i , in CD4+ T cells exposed to NB-UVB.We identified that the exposure of CD4+ T cells to NB-UVB produces an increase in cytoplasmic Ca2+ from extracellular medium, identifying that the intracellular [Ca2+]i observed was dependent on the activity of the TRPV1 channel. Aditionally, we found that exposure to NB-UVB decreases the TH1/TReg ratio. Human CD4+ T cells respond rapidly to NB-UVB treatment increasing [Ca2+]i in a dependent way on the modulation of the activity of the TRPV1 channel. Furthermore, we observed opsin 3 functional expression in memory CD4+ T cells. Further experiments will be needed to determine how the UVB light leads to the activation of the opsin 3. The results obtained approach us towards direct effect of NB-UVB on T cells, modulating the activity of the TRPV1 channel.

Financing: Funded by grants IMII P09/016F and CONICYT Scholarship 21180616

Keywords: Phototherapy, Lymphocytes T CD4+, TRPV1

Viral protein 1 (VP1) and VP2-chimeric protein of Infectious Pancreatic Necrosis Virus elicit distinct immune response in rainbow trout head kidney

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The infectious pancreatic necrosis virus (IPNV) produces a deadly disease in teleost fish. In salmonids, although the number of outbreaks has been reduced due to the use of IPN-resistant broodfish, the emergence of new variants and reports of outbreaks in farmed rainbow trout show that IPNV continue threatening the aquaculture industry. Several IPNV vaccines have been produced and used during fish production; however, they are not protective enough to eliminate the virus and avoid outbreaks. Here, we studied the immunogenicity of two proteins of IPNV in rainbow trout, which can be used as antigens in vaccines. These are the virion-associated RNA polymerase VP1 and a chimeric antigen VP2-Flagellin (VP2-Flg). To investigate the immunogenicity, we performed gene expression profiling in head kidney leukocytes of in vivo primed and control rainbow trout, and in in vitro antigen-restimulated T cells. Results showed that rVP1 increased Th1 type cytokines (ifn-γ, t-bet, and il-12), Th2 type genes (il-4/13a, il-4/13b2, and gata3), and triggered up-regulation of il-10a and tgf-β. In addition, VP2-Flg increased the expression of ifn-γ, il-4/13a, il-10a, and tgf-β. In contrast, the expression of il-4/13b2 and il-2 decreased. The expression analysis in sorted cells demonstrated that CD4-1+ T lymphocytes produced ifn-γ, il-4/13a, il-4/13b2, il-10a, and tgf-b1 after in vitro rVP1 restimulation. Altogether, results showed that VP1 and VP2-Flg are immunogenic proteins in rainbow trout. In addition, VP1 seems to induce the differentiation of CD4+ T lymphocytes producing a group of cytokines that can stimulate cellular immunity, an essential mechanism of the antiviral response.

Financing: This work was funded by Conicyt through de Newton-Picarte Fund for project MR/N02625X/1.

Keywords: IPNV

The signaling pathway cGAS/STING: a new role in the lipidic metabolism acording to sex.

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The cGAS/STING pathway has emerged as a key mediator that triggers inflammatory responses against cytosolic dsDNA by inducing the secretion of proinflammatory cytokines. Although literature is contradictory about its role in inducing fat accumulation. Therefore, we aimed to investigate participation of this pathway in lipid metabolism using WT, cGASKO and STINGKO male and female mice at different ages. It was observed at young ages that male and female KOs had increased body weight. In adulthood and old ages KOs males maintain their weight high in relation to WT, however KOs females equal their weight to WT. At all ages, male and female KOs consume the same amounts of food as WT. When analyzing metabolic parameters such as glucose, no differences were observed at any age, however we observed higher cholesterol levels in male and female KOs at all ages. The triglycerides levels were elevated only in STINGKO females and males at all ages. In behavioral tests, it was observed at young ages that female KOs had better locomotive capacity and higher levels of strength compared to WT and male KOs. Finally, a greater accumulation of fat by male KOs was observed in young and adult stages compared to WT and female KOs. These results indicate that the cGAS/STING pathway would be regulating accumulation of fats in a differential way in males and females during the reproductive stage, thus being necessary for the use of fats as an energy source and having a protective role against obesity.

Financing: Fondecyt 11190258

Keywords: cGAS, STING, Lipidic metabolism

CD73-bearing retinoic acid-induced Treg-derived extracellular vesicles modulate immune responses and prevent alveolar bone loss during periodontitis

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CD73 ectoenzyme promotes immune-regulation converting adenosine-mono phosphate (AMP) into adenosine, which upholds anti-inflammatory responses. T regulatory cell (Treg)-derived extracellular vesicles (TEVs) are enriched in surface CD73, fulfilling immunomodulatory functions in a cell-free manner. Periodontitis, a chronic inflammatory disease, is triggered by a deregulated host immune response responsible for the accumulation of inflammatory mediators and pro-bone-resorptive factors, which result in tooth loss. Therefore, this study aimed to evaluate the effect of CD73-bearing TEVs on T-cell proliferation, activation, and phenotype in vitro and on periodontitis-induced immune response and alveolar bone resorption in vivo.Methods: CD4+ T cells were differentiated into Tregs in the presence of TGF-b+Retinoic Acid (RA-Tregs), and characterized by flow cytometry (FC). From their recovered supernatant, TEVs were isolated by differential centrifugation, quantified, and characterized by Nanoparticle-Tracking-Analysis. CD73 enrichment was determined by WB and FC. TEVs immunosuppressive and modulatory capacities were evaluated in vitro on CD4+ and CD8+ T cells by analyzing their proliferation, and phenotype. Besides, periodontitis inhibition was assessed in vivo using a ligature-induced mice model quantifying periodontal leukocyte infiltration by FC and the extent of alveolar bone loss by morphometric analysis.Results: RA-Tregs showed high expression of CD73. Isolated RA-TEVs contained CD73, displayed canonical EVs characteristics, downregulated CD4+ T cell proliferation, but upregulated CD8+ T cell expansion and activation. During periodontitis, RA-TEVs increased the frequency of CD73+ leukocytes, promoted anti-inflammatory response, and prevented alveolar bone resorption.Conclusions: CD73-bearing RA-TEVs modulate CD4+ T and CD8+ T cell response and prevents periodontitis-induced alveolar bone loss.

Financing: Fondecyt Regular1181780 (R.V) and 1210654 (K.P-L).ANID Schollarship 21180841

Keywords: Extracellular vesicles, Regulatory T cells, Periodontitis

Characterization of two intestinal macrophage subpopulations in oral tolerance and inflammation in zebrafish larvae

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Introduction. Intestinal macrophage subpopulations play key roles during inflammation and oral tolerance processes. These subpopulations have been described in great extent in mammals but are still largely unknown in fish. Specifically, our lab has phenotypically described two specific intestinal macrophage subpopulations in zebrafish: stellate and round macrophages. The aiming of this study was to describe the transcriptional profile of these subpopulations, along with testing their antigen uptake capacity in a tolerance or steady-state context and after ingestion of an inflammatory diet. Methodology. Transgenic zebrafish line Tg(mpeg1:GFP) was used to isolate the stellate and round macrophages from fish intestines by fluorescence activated cell sorting (FACS). Total RNA was extracted from each subpopulation and RNA sequencing was performed. In order to determine their antigen uptake capacity in vivo, time lapse assay by confocal microscopy from Tg(mpeg1:GFP) transgenic larvae fed with red fluorescently labelled inflammatory diet or control diet was performed.Results. The two macrophages subpopulations were successfully isolated, so total RNA sequencing was performed, and results obtained are now being analyzed. Regarding antigen uptake, under steady state condition similar number of rounded and stellate macrophages took up tolerogenic dietary antigens, while during inflammation more stellate than rounded macrophages took up dietary inflammatory antigens. Discussion. Altogether, these results suggest that stellate macrophage are more proinflammatory than rounded subpopulation.

Financing: FONDECYT 1210903Beca ANID 21180135

Keywords: zebrafish, macrophages, inflammation

Immunomodulatory interactions between type I NKT cells and type II NKT cells

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Natural killer T (NKT) cells are unconventional T cells that recognize lipid antigens presented on the CD1d molecule. Based on the characteristics of their T cell receptor (TCR), NKT cells are divided into two subsets, type I NKT cells with an invariant TCRα-chain and type II NKT cells with diverse TCRs. Type II NKT cells have been shown to have immunoregulatory roles in tumor immunity, autoimmunity, and infectious diseases. The interactions between different subsets of NKT cells, as well as the immune consequences after recognition of glycolipids is not yet clarified. Using glycolipids such as sulfatide and a-Galcer to stimulate type II NKT cells and type I NKT cells, respectively, we have observed a modulatory regulation between both subsets in relation to the release of cytokines produced as well as their effect on the generation of different isotypes of anti-OVA antibodies. In general, we have observed that the activation of both subtypes enhances the release of certain cytokines as well as the generation of certain isotypes of anti-OVA antibodies. These results could have implications on their intervention in inflammatory diseases as well as on their possible use as adjuvants in the generation of vaccines.

Financing: Funded by FONDECYT 1211959, Millennium Institute of Immunology and Immunotherapy and ICGEB crp-chl17-06-ec

Keywords: NKT cells, cytokines, antibodies

Study of the immunosuppressive effect of lactate produced by Mesenchymal Stem/Stromal Cells on the differentiation and metabolism of Th1 and Th17 lymphocytes

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Introduction: Mesenchymal Stem/Stromal Cells (MSC) are promising therapeutic tools for autoimmune diseases due to their potent immunoregulatory capacities. Our previous results indicate that the metabolic reprogramming of MSC from OXPHOS to glycolysis significantly improves their immunoregulatory properties. Furthermore, glycolytic MSCs release higher levels of lactate, suggesting that this could be a potential mediator of their stronger immunosuppressive effect.Methodology: Murine Th1 and Th17 cells were cultured with L or D-lactate and co-cultured with MSC pretreated or not with oligomycin to induce a glycolytic metabolism; galloflavin or a specific siRNA against the lactate dehydrogenase (SiRNA-LD) in order to inhibit lactate production. Subsequently, the phenotype of T-CD4 cells was characterized by flow cytometry, RT-qPCR and seahorse. Additionally, we perform a murine model of delayed-type hypersensitivity (DTH), where we evaluate the therapeutic effect of MSC pretreated or not with galloflavin or SiRNA-LD, by the measurement of paw swelling and flow cytometric characterization of blood and lymph node cells.Results: Our results showed that the pharmacological induction of glycolysis in MSC increases their capacity to suppress the differentiation and proliferation of Th1 and Th17 cells, by inducing a quiescent energy state (low OXPHOS and low glycolysis). Additionally, we prove that lactate released by MSC exerts an immunosuppressive effect on Th1 and Th17 lymphocytes. Moreover, lactate production was critical for the anti-inflammatory efficacy of MSC on the DTH murine model. Conclusions: In this work we deepen on the mechanism behind the immunoregulatory properties of MSC and position the production of lactate as an important MSC- suppressive factor.

Financing: Fondecyt Iniciación N°11190690; Fondecyt Regular N°1211353

Keywords: MSC, Lactate, Immunosuppression

Functional and molecular characterization of two phenotypically different intestinal macrophage subpopulations in zebrafish

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Introduction. Specific macrophages subpopulations play key functions to maintain intestinal homeostasis or during inflammation. In fish, the different intestinal macrophages subpopulations are not identified. In this regard, using the Tg(mpeg1:GFP) transgenic line, which labels fluorescently specifically macrophage, we detected two phenotypically distinct populations of intestinal macrophages, one with a round morphology and another with a stellate one. In this work, we aimed to characterize at molecular and functional levels both subpopulations at steady-state and during inflammation.Materials and Methods. The expression pattern of tolerogenic and proinflammatory macrophage gene markers was determined by in situ hybridization. Pro-inflammatory macrophages were detected fluorescently yellow using the double transgenic line Tg(tnfa:eGFP-F/mpeg1.1:mCherry). To analyze macrophage-T cell interactions, double transgenic Tg(mpeg1.1:mCherry/lck:lck-eGFP) larvae were used. In all these cases cells behavior was determined by performing time-lapse assays with confocal microscopy. Results. We observed mRNA expression of tolerogenic marker (tgfb1a) in the intestine, head, and gills at steady-state and during inflammation respectively. The analysis of the expression pattern of pro-inflammatory markers (il1ß and tnfa) is in progress. At steady state, several, but not all, stellate macrophages were observed fluorescently yellow in Tg(tnfa:eGFP-F/mpeg1.1:mCherry) larvae, and the yellow cells increased during inflammation. On the contrary, rounded macrophages showed no co-localization in steady-state, although during inflammation one cell per intestine was observed. Both macrophage subpopulations maintained short- and long-term contacts with T cells at steady-state and during inflammation. Discussion. Our results suggest that stellate macrophages could be a pro-inflammatory subpopulation and rounded macrophages a tolerogenic one. Further characterizations are required to prove this hypothesis.

Financing: FONDECYT 1210903

Keywords: zebrafish, intestinal macrophages, subpopulations

Characterization of the iNKT cell response following stimulation with α-GalCer-derived C6”-modified ligands in partially humanized mice

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Invariant natural killer T cells are unconventional T cells that upon stimulation rapidly secrete a wide array of cytokines. Via TCR ligation, iNKT cells recognize glycolipid ligands presented by surface molecule CD1d. α-Galactosylceramide, the most widely studied iNKT cell ligand, can induce a potent yet diverse cytokine response. For immunotherapy applications, there is interest to synthesize α-GalCer-derived ligands with structural modifications that could endow them with the ability to induce a more polarized cytokine response. Our aim was to evaluate the iNKT cell activation ability of C6”-modified α-GalCer derivatives to identify potent ligands with therapeutic potential. We evaluated the potency of α-GalCer derivatives in vitro by quantification of IL-2 secreted by iNKT cell hybridomas upon ligand stimulation. Additionally, we evaluated bioactivity of these ligands by determination of IL-4 and IFNγ secretion in serum and iNKT and T cell expansion following in vivo administration into mice. Ligands such as AH10-7, AH17-5, AH17-6 and AH17-8 were found to induce a potent Th1-like response in vivo due to high IFNγ secretion levels detected in serum of stimulated animals and by high cytokine production by iNKT cells observed by intracellular flow cytometry. In addition, we characterized iNKT cell clonotypes expanded by αGalCer-derived ligands in partially humanized hCD1d knock-in mice according to TCR-β chain rearrangements expression.Our results highlight differences between mouse and human lipid antigen presentation in the context of CD1d, as well as the immunotherapeutic potential of ligands such as AH10-7, AH17-5, AH17-6 and AH17-8 which could be evaluated in further assays.

Financing: Funded by:Proyecto IMII P09/016-F.FONDECYT Nº 1211959.Beca Doctorado Nacional ANID 21171172.

Keywords: nkt cells, immunotherapy, innate immunity

Regulation of the cytokine expression by the unfolded protein response sensors XBP1 and ATF6 in dendritic cell

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Background. Dendritic cells (DCs) are key in the coordination of the antiviral immune response mediated by CD8+ T. The priming of LT CD8+ requires activated-DCs, and this activation is partially regulated by the unfolded protein response (UPR), which a cellular mechanism that regulates the fidelity of the cellular-proteome. The UPR axis regulated by IRE1 and the transcription factor XBP1s, directly controls the transcription of proinflammatory cytokines and members of the Ag-presentation machinery. Conversely, the UPR sensor ATF6 can also induce the expression of inflammatory factors in infectious settings, but their overall contribution over DCs functions is largely unknown. In this project, we evaluated the role of XBP1s and ATF6 over the cytokine-expression in a specific setting with TLR7-ligands plus palmitic-acid for DCs activation and validate the novel ATF6 knock-out in DCs. Method. We used a primary culture of GMCSF-derived DCs (GM-DCs) from transgenic mice deficient in XBP1 or ATF6, which has been activated with viral-agonist and lipid-acids. We evaluated the three branches of the UPR activation and cytokine expression by qPCR and flow cytometry. Additionally, we validate the ATF6 expression and immune-cell population in ATF6-cKO. Result. We observed that TLR7-ligand plus palmitic-acid, are potent stimuli for GM-DCs activation, triggering PERK, ATF6 and XBP1s activation with massive IL-23 and IL-6 expression-XBP1 dependent manner. Additionally, the deficiency of transcription factor XBP1s and/or ATF6 in GM-DCs decrease the expression cytokines by a transcriptional mechanism. Finally, the frequency of DCs in spleen, lung, and lamina propria didn't change under basal condition in the ATF6-cKO.

Financing: FONDECYT 1200793HHMI 55008744CONICYT/Doctorado Nacional/2018- 21181584

Keywords: ATF6, XBP1, DENDRITIC CELL

Identification the CXCL9-11/CXCR3 chemokine axis in Atlantic salmon

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The migration of immune cells to the site of tissue damage or infection is fundamental for an efficient innate and adaptive immune response. An important part of this movement is mediated by chemoattractant cytokines called chemokines. CXCL9-11/CXCR3 is one of the most studied axes due to its relevance in T cell migration and the Th1 immune response induction. Although the nature and function of chemokines and receptors are well documented in mammals, the identification of chemokine orthologs in Atlantic salmon and the characterization of their role have been more complex because of the whole genome duplication processes and faster of these immune genes. First, a homology search was carried out to identify CXCL9-11/CXCR3 orthologous genes in the Atlantic salmon genome. Next, modeling three-dimensional structure to confirm the identification of the orthologs. In addition, screening was carried out in the different organs of the Atlantic salmon to evaluate the constitutive expression of these genes. Finally, real-time RT-qPCR was performed to quantify the expression of transcription in leukocytes of peripheral blood, spleen, and kidney of fish induced with IFN-gamma. First, sequences encoding chemokines and their receptors were identified and modeled with templates with an identity of between 27 and 48%. Then, ten different organs were analyzed, and the expression of the genes was observed mainly in the spleen, kidney, and peripheral blood leukocytes. These results suggest that chemokines and their receptor are present in the Atlantic salmon genome and have a constitutive expression in fish lymphoid organs.

Financing: Funded by Fondecyt 1201664, Fondecyt 3210634 and Dicyt 022143IB_POSTDOC

Keywords: Chemokines, Atlantic salmon

Modulation of the microbiota and intestinal immunity through IL-33

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Immune tolerance is mediated by various cellular populations among them T regulatory cells (Tregs) are the main subset with undeniable effect. It has been reported that IL-33 modulates the response of the innate and adaptive immune system in the intestine. To understand whether IL-33 affects the immune system and intestinal microbiota, we used wild type(WT) or ST2KO Foxp3GFP reporter mice to investigate the administration of IL-33 and its impact on leucocytes phenotype and frequencies. After 4 days of IL-33 administration, mice were euthanized and mesenteric lymph nodes and the Peyer's patches were collected to study cell composition by flow cytometry. Faecal samples were also collected at day 0 and 4 for analyzing qPCR genes related to family/genus of bacteria and untargeted metabolomics was measure. Our results indicate that WT animals treated with IL-33 show high frequencies of CD8+ T and Treg cells compared with those receiving PBS. Interestingly, ST2KO Foxp3GFP animals only responded to IL-33 by augmenting CD8+ T cells frequencies and not for Treg cells. Furthermore, IL-33 administration in WT animals positively impacted on Bacteroides, Lactobacillus and Clostridium, and negatively on Enterobacteria, supporting the role of IL-33 in inducing immune tolerance.Ã‚Â All together, our study suggest that IL-33 could modulate intestinal immunity by influencing both leucocyte populations and microbiota composition, favoring an anti-inflammatory environment, which could be used as a therapeutic tool for controlling inflammatory diseases, including the possibility of faecal material transplantation

Financing: Regular Fondecyt grant 1106347/1210654

Keywords: immunetolerance, gutmicrobiota

BCL-3 AS AN INTERMEDIARY MECHANISM OF ALTERED TIGHT JUNCTION STRUCTURE AT INTESTINAL EPITHELIA

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The Irritable Bowel Syndrome (IBS) is a brain-gut communication disorder characterized by a systemic low-grade inflammation with increased intestinal permeability due to an altered structure of the tight junction (TJ) complex. An increase IL-6 plasma levels is observed in IBS patients. B-cell lymphoma-3 (Bcl-3) is a transcriptional regulator of inflammatory genes, whose expression is elevated in the intestinal epithelium of IBS patients. The regulatory role of Bcl-3 in TJ proteins-complex structure is unknown. We aim to evaluate in vitro the Bcl-3 role in TJ proteins expression/distribution in intestinal epithelium. A human colorectal adenocarcinoma cell line DLD-1 was stimulated with IL-6 and the Bcl3 expression modulated by transfection with plasmid DNA containing Bcl3 and silencing (siRNA). The expression of Bcl-3, claudin-2 (Clau-2), myosin light chain kinase (MLCK) and pMLC/MLC ratio was determined by q-PCR and western blot, and ZO-1 intracellular distribution, by immunofluorescence. We observed that Bcl-3 expression was early induced by IL-6 in the time course (3h), reached its maximum at 12h. Similarly, in vitro studies show that Bcl-3 was upregulated by its overexpression using expression vector, being this reduced by its silencing. Moreover, increased expression of Clau-2 and MLCK; and pMLC/MLC ratio were observed for Bcl-3 overexpression, being the expression of these proteins unchanged by its silencing. A cytoplasm relocation of ZO-1 was observed under Bcl-3 overexpression, but no changes were observed with Bcl-3 SiRNA. Our results suggest that Bcl-3 has a regulatory role of the structural composition of TJ complex at intestinal mucosa.

Financing: FONDECYT 1181699

Keywords: Irritable Bowel syndrome, Bcl-3, Tight Junctions

Macrophage infiltrates and their relationship with angiogenesis in human peri-implantitis.

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Introduction: Approximately 30% of implants fail due to peri-implantitis, which hallmark is a loss of supportive bone, with large inflammatory lesions mediated by cells of innate immunity, among them macrophages. Therefore, the inflammatory environment could modify their functions, impacting the proper osseointegration of the implant. However,little is known on how macrophages influence the fate of implants. Aims: Characterize the innate inflammatory cell population of epithelial and connective peri-implantitis tissues.Material and Methods: Five gingival biopsies from subjects diagnosed with peri-implantitis and eight from aged otherwise healthy ones were assessed by immunofluorescence to identify immune infiltrate and vascular structures by the markers hMPO, CD16, CD68, α-SMA, and quantified through morphometric analyses.Results: We found more hMPO+ and CD16+ cells near epithelium in peri-implantitis samples than in healthy aged gums and a lesser number in the fibrotic zone. Also, an increased density of CD68+ cells in peri-implantitis was found in the inflammatory zone, close to blood vessels and a few infiltrating the epithelium. The diameter of blood vessels close tothe epithelium decreased compared to those in the fibrotic zone. Discussion: Implant osseointegration involves angiogenesis. The increasing density of macrophages near new vascular structures suggests they modulate angiogenesis; however, their role in the implant fate is unknown. The increased innate inflammatory environment could be regulating the function of macrophages.Conclusion: This immunohistological analysis suggests macrophages participate in angiogenesis on peri-implantitis tissues, probably leading to local differences in vascular structures, which could be a critical insight into its pathogenesis.

Financing: Funding: Fondecyt 1181263.

Keywords: Macrophages, Angiogenesis, Peri-implantitis

Systemic white blood cell changes following Degenerative Cervical Myelopathy (DCM) and surgical decompression: Clinical implications

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Degenerative cervical myelopathy (DCM) is caused by age-related degeneration of the cervical spine, leading to chronic compression of the spinal cord and subsequently inflammation. The current treatment consists of surgical decompression, which can be associated with prolonged neuroinflammation and spinal cord reperfusion contributing to poorer neurological recovery in a subset of patients. The primary objective of this study was to assess whether the natural progression of DCM and surgical decompression are accompanied by hematological changes in the white blood cell composition. If so, treatments could be developed to target the systemic immune response. Gradual compression of the spinal cord was induced in C57B/L mice at the C5-6 level. Composition of circulating white blood cells was analyzed by flow cytometry at 3, 6, 9 and 12 weeks after induction of DCM and at 2 and 5 weeks after surgical decompression. We show that circulating granulocytes had a 2-fold increase at 9 weeks of DCM (**$p<0.01$). At 5 weeks after decompression, the number of T cells and its subpopulations, granulocytes and monocytes were reduced compared with age-matched naive animals ($0.05>p>0.001$). Our data using an animal model of DCM suggest that changes in white blood cell populations are modest and can not be used as diagnose tools. In addition, we provide a better understanding of hematological changes following surgical decompression, which can be used to choose appropriate pre and/or post-operative treatment strategies to target the immune response following surgery.

Financing: 21st Century Research Grant from the Cervical Spine Research SocietyConicyt, Fondecyt de Iniciación 2019, 11190421.

Keywords: Degenerative cervical myelopathy, decompression, white blood cells

Induction of regulatory iNKT cells with glycolipids encapsulated into liposomes, in a humanized model for iNKT cells.

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Invariant NKT (iNKT) cells, a subpopulation of NKT cells, have attracted substantial attention because of their ability to be activated specifically by glycolipid antigens. The activation of iNKT cells (mainly NKT10 cells, a novel iNKT cell subset with IL-10-dependent regulatory function) with α-galactosylceramide (α-GalCer) can protect against inflammatory diseases. Nevertheless, the strong activation of iNKT cells elicited by α-GalCer exhibit limited therapeutic efficacy, mainly due to the induction of a mixed pro- and anti-inflammatory cytokine response and anergy. Since iNKT cells can be differentially activated by α-GalCer analogs, it is highly important to determine which α-GalCer analogues will expand NKT10 cells. In this study, we identified NKT10 cells in hCD1d-KI mice (a partially humanized murine model for NKT cell responses). Hence, we evaluated different experimental conditions, such as immunization schemes, glycolipid activators of iNKT cells, and the use of glycolipid delivery systems. We observed an expansion of NKT10 cells only in hCD1d-KI mice treated with α-GalCer at seven days, very similar to the expansion of NKT10 cells reported during the immunization scheme of 30 days. Finally, we incorporated glycolipids ligands of iNKT cells into liposomes; it was observed that the incorporation of glycolipids into stearylated octaarginine-modified liposomes effectively induced in vitro activation of iNKT cells hybridoma and remarkably increased the in vivo expansion of NKT10 cells, which was superior in the group of mice treated with the α-GalCer analog AH10-7 incorporated in liposomes. Therefore, AH10-7 contained into liposomes could be an excellent candidate for therapies that target NKT10 cells.

Financing: Funded by FONDECYT Nº 1211959, Millennium Institute of Immunology and Immunotherapy P09/016-F, Copec-UC 2017.J.924 and CONICYT-PFCHA/Doctorado Nacional/2017-21170084 to R.G.

Keywords: iNKT cells, regulatory immune cells

ULTRASTRUCTURAL CHARACTERIZATION OF ACTIVATED MUCOSAL MAST CELL IN IRRITABLE BOWEL SYNDROME

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Irritable bowel syndrome (IBS) is a disorder of gut-brain axis interaction, which is characterized by an elevated neuro-immune activation in the intestinal mucosa. Ultrastructural studies of mucosal mast cell in IBS are scarce. We aim at to determine ultrastructural differences of intestinal mucosal mast cell between IBS patients and healthy controls (HC). To this end, samples from ileal and colonic mucosa from IBS patients (n=12, 5 IBS-D, 2 IBS-C; 3 IBS-M, 2 IBS-I) and HC (n=10) were obtained and processed for transmission electron microscopy. Ultrastructural analysis of the images was performed using ImageJ software, based on electron-dense features, shape, size, number, and degranulation pattern of secretory granules, as well as proximity and numbers of nerve endings surrounding mast cells. Mann-Whitney and ANOVA test were used for comparisons, considering significant $p < 0.05$. We have observed an elevated mast cell degranulation in ileum of IBS patients compared to HC ($p=0.0407$), as well as a tendency of anaphylactic degranulation pattern. A scroll and reticulated-granule pattern trends to appear in a higher percentage in IBS patients compared to HC, in both intestinal segments. A tendency to a reduced number and a close proximity of nerve endings surrounding mast cells was observed, in colon of IBS patients vs HC. Our results suggest that intestinal mucosal mast cells in IBS present ultrastructural differences that respond an elevated neuro-immune activation in this disorder. Futures investigations should be conducted to determine the stimuli and mediators that are associated to these ultrastructural features of intestinal mast cell in IBS.

Financing: FONDECYT 1181699

Keywords: Mast Cell; Ultraestructure; Irritable Bowel Syndrome

Exploring the role of PD-L1 and TIM-1 in the regulatory function of Bregs over Monocytes.

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Background: Regulatory B cells (Bregs) are a subpopulation of lymphocytes capable of modulating inflammatory responses, favoring immune homeostasis. In recent years, I have proposed several mechanisms to explain this regulatory function over other immune cells crucial in the immune responses. In this regard, little is known about the mechanism over monocytes regulation. In this work, we explore the role of the classical PD-1/PD-L1 axis in the contact-dependent regulatory function of Bregs and the involvement of a recently TIM-1/TIM-4 axis described. Methods: To confirm this, we co-cultured monocytes with Bregs in the presence of PD-L1 blocking antibodies; and for TIM-1/TIM-4 axis, we used the Huh7.5-TIM-1+ cell line and its siRNA TIM-1 silenced counterpart co-cultured with monocytes and measured the phenotypic profile by Flow Cytometry. Results: Monocytes co-cultured with Bregs downregulate their expression of activation molecules assessed; this effect is partially reverted in presence of PD-L1 blocking antibodies. On the other hand, monocytes co-cultured with Huh 7.5 acquired an activated profile that is further increased when co-cultured with TIM-1 silenced cell line. Conclusions: We establish that the inhibition of Bregs on monocytes inflammatory function is partially explained by the PD-1/PDL-1 axis. On the other hand, we postulate that in a complementary manner, Bregs would inhibit monocytes function mediated by the TIM-1/TIM-4 axis, since we observed an exacerbation of the inflammatory response in the absence of TIM-1, which would act as a regulatory molecule, however, silencing of TIM-1 on Bregs is necessary to confirm this effect.

Financing: Fondecyt 11170800-Beca Doctorado Nacional 21181286

Keywords: Bregs, PD-L1, TIM-1

The IRE1/XBP1 axis activation in DCs regulates intestinal Th17 differentiation

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The intestinal immune system is constituted by different cell types, which act in concert to finely control the initiation of inflammation and tolerance. Perturbations in this equilibrium is associated with the development of intestinal diseases. Type 1 dendritic cells (cDC1) are fundamental for maintaining tolerance in the gastrointestinal tract (GI tract). The sensor IRE1 of the unfolded protein response (UPR) and its transcription factor XBP1, are reported to regulate the functionality of cDC1 across tissues and their survival in the GI tract. However, little is known about how critical is IRE1 signaling in maintaining the tolerogenic roles of cDC1 in the intestine. Using a transgenic reporter mouse (ERAI) which monitors the RNase activity of IRE1 by means of VenusFP expression, we determined that cDC1 are the predominant cellular targets of IRE1 in the small intestine lamina propria (SI-LP) and across organs.By using conditional knock-out mice we determined that mice bearing IRE1 deletion in DC exhibits an increased frequency of T helper 17 (Th17) cells in the SI-LP. Interestingly, deletion of XBP1 in DC shows an opposite effect, as decreased Th17 frequency is observed in the SI-LP of this mice line. These data indicate that the IRE1-XBP1s axis in DCs regulates the homeostasis of intestinal Th17 cells.Ongoing work is focused on addressing the relevance of these molecules in models of intestinal inflammation and in elucidating the molecular mechanisms by which IRE1 in cDCs regulates adaptive immunity in the intestine.

Financing: FONDECYT regular 1200793HHMI 55008744

Keywords: Dendritic cell, IRE1-XBP1, SI-LP

Effect of Il 22 on intestinal microbiota composition at steady state and during inflammation in zebrafish.

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Introduction. Interleukin 22 is a cytokine produced by activated T and NK lymphocytes, which plays a central role in maintaining intestinal homeostasis due to its participation in processes such as tissue regeneration, cell proliferation, and maintenance of the epithelial barrier integrity. Moreover, previous studies have described that the microbiota is related to the development of inflammation, but the role that il22 may exert on the microbiota has not been addressed. In this work we evaluated the composition of the intestinal microbiota in the presence and absence of Il22.Materials and methods. All experiments were performed using either Il 22 mutant (Il22-/-) or wild type (WT) larvae. The steady-state and inflammatory conditions were induced by feeding larvae with a control or harmful diet. Germ-free larvae were obtained after treated embryos with antibiotics. The intestinal microbiota composition in each condition was determined by sequencing the V4 region of the 16S rRNA gene. Results. Il22-/- larvae fed with control diet showed higher number of neutrophils present in the intestine compared to WT larvae. Likewise, Il22-/- larvae fed with the harmful diet had increased number of neutrophils compared to WT larvae. Of note, Il22-/- larvae fed with control diet have similar number of neutrophils in the intestine compared with WT larvae fed with the harmful diet. At the moment, we are analyzing the 16S rRNA sequence obtained in each condition Discussion. Our results suggest that Il22 is indispensable to maintain intestinal homeostasis to the extent that in their absence spontaneous inflammation develops.

Financing: FONDECYT 1210903

Keywords: il-22, intestinal microbiota, inflammation

4.- Comparative Immunology

Development of new Plant-Based Veterinary Vaccines

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IntroductionVeterinary vaccines development is crucial for animal and human health. The use of plant species as an antigen production platform, has proven to be widely advantageous compared to other technologies, due to its low-cost and simple implementation, versatility, safety, fastness, scalability and also, the ability of plants to generate post-translational modifications in the synthesized proteins. Currently, Nicotiana benthamiana has emerged as a promising alternative to generate new veterinary antigens.MethodsAt the Veterinary Vaccine Laboratory of University of Chile, a new platform for the expression of vaccine antigens to different animal species, was developed and implemented using Nicotiana benthamiana. Highly expressed pTRBO vector, was modified to contain the gene sequence coding for various peptide antigens of interest. Plants were transiently transformed with Agrobacterium tumefaciens. Antigens were extracted, purified, and quantified 7 to 10 days post infiltration. Vaccines were formulated with the antigens and inoculated in Balb/c mice, to corroborate the induction of specific immunity in vaccinated animals by indirect ELISA.ResultsPeptide antigens were expressed for different animal species vaccines including Sigma C antigens of avian Reovirus, VP2 in its hyper variable zone of Gumboro disease, H proteins of canine Distemper virus and VP2 of canine Parvovirus. Successful, good yield and low-cost expression compared to other traditional platforms was achieved. Specific immunity was induced by mice inoculation. Thus, veterinary vaccine antigens of interest, was successfully produced using N. benthamiana new expression platform.

Financing: Fondef Idea ID18i10087

Keywords: Vaccine, recombinant protein, N. benthamiana

Cochleate based vaccine against Salmonella for poultry

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IntroductionSalmonella enterica is an important gastroenteric pathogen, generating outbreaks worldwide in the human population. The most common sources of Salmonella are animal products, particularly eggs and poultry meat. Vaccines for poultry are crucial for preventing Salmonella contamination. Therefore, development of new effective vaccines for different Salmonella serovars should be a priority for veterinary industry.MethodsWe tested the safety and efficacy of a cochleate based vaccine against 3 principal Salmonella serovars: S. Enteritidis, S. Typhimurium and S. Infantis for poultry. The vaccine was administered in two doses to Leghorn chickens in the breast muscle., proving to be safe, without causing local or systemic adverse reactions. One week after the last vaccination, all animals in the control group (n=15) and vaccinated group (n=15) were orally challenged with the 3 serovars. Then, the intestines were obtained to count the number of intestinal. In addition, blood antibodies were measured by indirect ELISA and t-cell immune response was characterized by flow cytometry. ResultsA lower number of Salmonella strains were observed in all the intestines of vaccinated chickens compared of the control group, corroborating that the vaccine was effective in reducing the carriage of the 3 Salmonella serovars. In addition, vaccinated animals showed an increase in specific IgY against the serovars and an increase in the CD4+/CD8+ ratio. Therefore, this new cochleate-based vaccine administered parenterally in 2 doses in chickens is safe and effective, inducing a cellular and antibody immune response and decreasing the intestinal colonization of Salmonella strains in vaccinated chickens.

Financing: Fondef Idea ID18i10008

Keywords: vaccined, Salmonella, lipid nanoparticles

5.- Tumor Immunology

Role of CD73 in the Function and Maturation of Natural Killer Cells

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Natural Killer cells (NK) are innate effector lymphocytes that recognize and eliminate transformed cells and thus have a relevant role in antitumor responses. It has been reported that adenosine reduces the proliferation and maturation of NK cells through the engagement of the adenosine receptor A2A. Adenosine is mainly produced in the tumor microenvironment by ATP hydrolysis mediated by CD39 and CD73 ectonucleotidases. Recent evidence demonstrates that in the tumor, NK cells also express CD73. However, no studies have unraveled the role of this ectonucleotidase on these cells. Here we study the expression of CD73 in NK cells and its role in the maturation, phenotype, and function of NK cells. Our results show that spleen NK cells do not express CD73, but this ectonucleotidase is upregulated following ex vivo activation. In addition, we found that NK cells upregulate CD73 expression in the tumor microenvironment and tumor-draining lymph nodes upon transfer into tumor-bearing mice. Interestingly, spleen and bone marrow NK cells from CD73KO mice displayed a more immature phenotype than NK cells from WT mice. Despite this, CD73KO NK cells exhibit improved control of tumor growth compared to WT NK cells. We conclude that CD73 is upregulated in NK cells upon entering the tumor microenvironment and that this ectonucleotidase regulates the maturation and antitumor function of NK cells.

Financing: Funding: Fondecyt 1180385, AFB 170004, ANID 22190463

Keywords: Ectonucleotidase, Natural Killer Cells, Tumor Microenvironmet

Effect of Heat Shock on the immunogenicity of tumor cell lysates useful in immunotherapies against cancer

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Clinical strategies using immunotherapies have demonstrated durable survival benefits in patients with different cancer. At this respect, Heat Shock (HS) treatment of tumor cell lysates are excellent sources for delivering of a wide variety of antigens, inducing a more integral immune response. Previously we demonstrated that melanoma cells treated with HS at 42ºC cause release of DAMPs that induce optimal DC maturation and tumor antigen cross-presentation. In this context, we have used TAPCells a DC-based vaccines loaded with an allogeneic heat shock-conditioned melanoma cell lysate in the treatment of advanced stage patients in a series of clinical trials. We compared tumor cell lines conditioned at 42º for 60 min (HS42) with 47ºC for 30 min (HS47). Our results showed that HS47 induces higher levels of DAMPs and ROS but decreases the viability of melanoma cell lines compared to HS42. Also, HS42 treatment induces the gene overexpression of members of heat shock proteins like HSP70´s and HSP40´s. Finally, we analyzed by flow cytometry the maturation state of DCs stimulated with HS42 or HS47-conditioned melanoma cells lysate. Both lysates induce similar levels of maturation of DCs, but it is necessary to study whether the new HS could improve the cross-presentation of tumor antigens and thus increase the activity of antitumor T lymphocytes. Based on these results, the HS47 treatment of melanoma cell lines do not induce advantages in the immunogenicity of the lysate, so the HS42 treatment remains the optimal protocol to induce immunogenic cell death in melanoma cell lines.

Financing: Financed by grants FONDECYT 1171213, and IMII ICM-ANID ICN09-016

Keywords: Dendritic cell (DC), Danger-Associated Molecular Patterns (DAMPs), High-Mobility Group Box-1 (HMGB1)

Evaluation of the in situ expression of immunological checkpoint markers in gallbladder cancer (GBC)

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Introduction: Gallbladder cancer (GBC) is a rare malignant tumor; however, Chile shows the highest incidence and mortality worldwide. Current treatments are surgery and chemotherapy, both of which are ineffective in advanced stages. It is necessary to find new immunotherapy targets against this cancer and associated biomarkers related to therapy. Methodology: 252 samples were collected from 243 patients with gallbladder adenocarcinoma from the Hospital Base de Valdivia. Epidemiological data were collected, in addition, an automated immunohistochemistry in tissue microarrays was used to evaluate the expression of the immunological markers: PD-L1, TIM 3, A2aR, ICOS, LAG3 and PD-1. The levels of positive cells and intensity of staining were measured from software obtaining 2 independent scores, from whose multiplication, a final score was obtained and indicates the relative expression of each marker. Results: 2 groups of markers were classified based in their level of expression in the GBC tissue. The first group was PD-L1, A2aR, TIM 3 and HLA-ABC. At contrary, the ICOS, LAG3 and PD-1 markers showed a lower expression. A2aR would be associated with a lower survival in GBC patients. The high expression of PD-L1, HLA-ABC and TIM3 was associated with better survival.Conclusions: PD-L1, A2aR, TIM 3 and HLA-ABC are highly expressed in GBC tissue and was related to the survival of the patients. Finally, there is an association between A2aR and TIM 3 and the age, sex and depth of tumor in patients, which suggests that these markers can be useful for prognosis and follow up of potential immunotherapies.

Financing: Acknowledgments: Financed by grants FONDECYT 1171213, IMII ICM-ANID ICN09-016 and Fondo Regional para la Competitividad región de Los Ríos FIC 19-70.

CD49b+ Tr1 cells: a key cellular population and potential target for anti-tumor therapy

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T regulatory cells (Tregs) act as modulators of the immune response and have a detrimental effect on tumor growth. Therefore, the study of its phenotype, metabolism and function is relevant for the development of new antitumor therapies. Tregs population encompasses a subset known as Tr1, characterized by the expression of CD49b and IL-10. Preliminary data from our lab indicates that Tr1 cells are more abundant than Foxp3+ Tregs. With the purpose to interfere with CD49b signaling and evaluate tumor outcome, we administered anti-CD29 antibody intraperitoneally into B16-tumor mice. B16 melanoma cells were injected via subdermal in flank of C57BL/6-Foxp1-GFP reporter animals. Anti-CD29 treatment was given every other day since day 9 post-tumor inoculation. For the tumor and systemic analysis of animals, flow cytometry approaches were used to study cell populations and hematoxylin-eosin staining for histological studies. The frequencies of Tr1 cells increases over time at the tumor site. The administration of anti-CD29 inhibits tumor growth versus PBS control. At the histological level, anti-CD29-treated animals presented a macroscopic reduction without tumor infiltration, which was not observed in control mice. Moreover, we found that the administration of anti-CD29 induces a change in CD4+ and CD8+ T cells phenotype, as observed by the increment on Th1 and Tc cells. Our results suggest that the antitumoral effect of anti-CD29 is mediated, at least, through the enrichment of inflammatory T cells. On the other hand, we present the characterization of Tr1 cells in animals with tumors, introducing CD29 as a novel molecule with antitumoral potential.

Financing: Fondecyt Regular Grant #1210654

Keywords: melanoma, TR1, CD49

Tumor-infiltrating CD4+ T cells recognize tumor cells in human renal cell carcinoma

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Introduction: Tumor-infiltrating lymphocytes (TILs) are associated with a favorable prognosis in several solid tumors. The ability of cytotoxic CD8+ T cells to recognize and destroy tumor cells in human renal cell carcinoma is well documented. However, the ability of CD4+ T cells to recognize RCC cells remain unknown. Methods: Conventional CD4+ T cells (CD4+CD8-CD45RO+CD25-) were isolated from freshly resected RCC tumors using fluorescence-activated cell sorter (FACS) and expanded in vitro for 13-15 days. In parallel, cells from autologous tumor and non-malignant renal tissue (NMRT) were cultured. Then, HLA-II and EpCAM expression was measured in epithelial cells and evaluated by flow cytometry after IFN-γ stimuli. Finally, CD4+ T cells were co-cultured with RCC and NMRT cells during 16 hours, and activation markers (OX40 and 4-1BB) and cytokine production were evaluated by flow cytometry. Target cells were also incubated with an HLA-II blocking antibody.Results: We observed that RCC cells express HLA-II, which is up-regulated after IFN-γ stimulation. CD4+ T cells is up-regulated activation markers and produced IFN-γ and TNF-α upon co-culture with autologous tumor cells, but not NMRT cells in a HLA-II dependent manner. Conclusions: Our findings show that tumor-infiltrating CD4+ T cells specifically recognize RCC cells and may contribute to anti-tumor immunity. E-mail: alladser@cienciavida.org

Financing: Acknowledgments: ANID FB210008, FONDECYT Iniciación 11190897, FONDECYT Regular 1212070.

Keywords: Tumor-infiltrating lymphocytes, CD4+ T cells, renal cell carcinoma

TRIMELVax promotes a rapid influx of neutrophils, monocytes, type 1 macrophages and DCs at the injection site to orchestrate immunity against malignant melanoma.

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TRIMELVax is a new immunotherapeutic technology consisting of a melanoma cells lysate (TRIMEL®) conditioned with heat shock treatment and combined with the adjuvant CCH. This vaccine proved to be effective in prophylactic and therapeutic in vivo experimental protocols, significantly reducing the growth of tumor mass and increasing the survival of vaccinated mice. The outcome was associated with the induction of humoral and cellular immune responses, especially CD8+ T lymphocytes. Although the clinical impact of this vaccine raises great expectations, many of the immunological factors involved in the effect generated are unknown. In this work, we focus on analyzing early events related to the recruitment of innate immune cells to the administration site at different time periods. To achieve this, C57Bl/6 mice were inoculated on the foot pad with TRIMELVax or controls. Animals were euthanized and injection site biopsy samples were taken. An increase in CD45 + populations was observed in mice vaccinated with TRIMELVax compared to mice injected with PBS or LPS. A characteristic kinetics were found for each cell group analyzed, highlighting a rapid and strong influx of neutrophils, followed by a significant increase in type I macrophages, and monocytes at 12 hours that is maintained 24 hours after TRIMELVax administration. In addition, an increase in antigen presenting cells (cDC1, cDC2, mo-DCs and LCs) and a decrease in suppressor myeloid cells were observed. These results suggest that TRIMELVax induce specific recruitment and activation of innate immune system, which might coordinate a more efficient adaptative immune response against malignant melanoma.

Financing: FONDEF ID16I10148, IMII P09016F, ANID BECA No. 21180633.

Keywords: TRIMELVax, Lysates, malignant melanoma

Host CD8+ T cells are essential for the antitumor efficacy of a murine model of adoptive cell therapy

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Introduction: Adoptive cell therapy (ACT) using tumor-specific cytotoxic T lymphocytes (CTLs) has demonstrated effectiveness in hematological and some solid cancers. Still, ACT does not work in most patients with solid tumors. Hence, understanding the cellular mechanisms underlying effective antitumor immunity in ACT is key to develop improved cancer immunotherapies. Methodology: B16F10-OTI tumor-bearing mice were intravenously transferred with OTI CD8+ T cells in vitro-activated with either peptide or anti-CD3/CD28 antibodies. We analyzed tumor growth, survival and tumor-infiltrating lymphocytes by flow cytometry. To evaluate the role of effector cytokines, antibodies blocking TNFa and IFNγ were administrated during ACT. To study the contribution of host T cells to ACT efficacy, we used RAG1KO, CD103KO mice as recipients. Additionally, host CD8+ T cells were eliminated using anti-CD8 depleting antibodies and tumor infiltration host T cells was blocked with FTY720. Results: We observed that ACT with peptide-activated OTI cells led to tumor rejection and promoted intratumoral accumulation of both progenitor (PD-1+TCF-1+) and terminally differentiated cytotoxic (PD-1+GzmB+) host CD8+ T cells. Furthermore, TNFa blocking during ACT abrogated the infiltration of host CD8+ T cells but not transferred CTLs decreasing their therapeutic efficacy and survival, It also happens with the lack of T cells or the depletion of host CD8+ T cells before ACT and in CD103KO mice. Besides, blocking tumor infiltration of host T cells reduced long-term protection in re-challenge experiments. Conclusion: Our findings reveal an interplay among transferred and host CD8+ T cells that underlies effective and long-lasting antitumor immunity in the context of ACT.

Financing: Acknowledgments: FONDECYT 1171703, FONDECYT 11190897, ANID FB210008, PhD. Scholarship ANID 21180968

Keywords: Adoptive cell therapy, Host CD8 T cells

DNA prime-peptide boost immunization elicits robust neoantigen-specific CD8+ T cell responses and therapeutic protection in mouse tumor models

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Introduction: Therapeutic immunization against tumor neoantigens has the potential to induce a very selective and strong T cell-mediated antitumor responses. Therefore, clinically applicable immunization strategies based on neoantigens are needed. Here, using a mouse model of neoantigen we report the administration of a DNA vaccine followed by peptide boost led to robust neoepitope-specific CD8+ T cell response and therapeutic protection.Methods: Mice were treated with prime and boost immunization using an DNA vaccine encoding for full length human GP100 (hGP100) protein and a human GP10025-33 (hGP10025-33) peptide vaccine. Next, effector and memory hGP10025-33-specific CD8+ T cell response was evaluated by flow cytometry. Finally, effect of therapeutic DNA prime-peptide boost in tumor growth and survival of neoantigen expressing tumor bearing mice was analyzed. Also, circulating neoepitope-specific CD8+ T cell response was evaluated by flow cytometry.Results: Adminitration of DNA encoding for hGP100 protein followed by a boost immunization with hGP10025-33 peptide elicited strong GP10025-33–specific CD8+ T cell responses as compared to prime only and others prime-boost combinations. Therapeutic DNA prime-peptide boost immunization led to the rejection of hGP10025-33-expressing EL4 tumors, in contrast to the administration of DNA vaccine alone or mice challenged with EL4 tumors expressing the mouse GP10025-33 epitope, as a model of self-antigen. Moreover, DNA prime-peptide boost against two neoepitopes of MC38 tumor model showed a marked therapeutic effect in tumor bearing mice and neoepitope-specific CD8+ T cell responses. Conclusion: Overall, these results highlight the potential of DNA prime-peptide boost as a promising strategy for therapeutic neoantigen vaccines.

Financing: FONDECYT 1171703, 1212070 ANID FB210008, Master Scholarship CONICYT 2018-2020

Keywords: Neantigens, Tumor, Vaccine

CCR5 and Pannexin-1 content and its relationship with colon cancer progression

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Tumor cells are continually communicating with stromal cells through soluble factors including cytokines/chemokines, growth factors, thus modulating cancer progression. CCR5 is a chemokine receptor, whose expression increases in colon cancer, and its blocking promotes antitumor responses. Alternatively, Pannexin-1 is a plasma membrane channel allowing the passage of small molecules, such as ATP, essential in proliferation and migration. Recently, CCR5 activation has been demonstrated to induce ATP release through Pannexin-1 in CD4+ T lymphocytes, although how these molecules are linked in cancer has not been detailed. The objective of this study was to determine CCR5 and Pannexin-1 content and localization in tumor and healthy mucosa biopsies from colon cancer patients using immunohistochemistry. Moreover, we aimed to demonstrate an association of these molecules with tumor progression. Our results show higher CCR5 and Panexin-1 content in tumor cells compared to healthy mucosa epithelium (n=27; Wilcoxon signed rank test $p<0,05$), although no significant expression differences were observed in stroma from tumor vs. healthy tissue. Higher CCR5 and Pannexin-1 expression associates with advanced stages of colon cancer. Additionally, a positive correlation between CCR5 and Pannexin-1 content was identified (Spearman, $p<0,0001$). Our results suggest that CCR5 and Pannexin-1 protein are related, being expressed both in tumor and stroma cells, thus participating in colon cancer progression to late stages.

Financing: FONDECYT 11190990.

Keywords: colon cancer, CCR5, Pannexin-1

Clinical relevance of Major Histocompatibility Complex Class I-Related Chain A (MICA) allelic variants in Gastric Cancer

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Gastric cancer (GC) is one of the leading causes of death in worldwide. Natural Killer cells play a key role in the antitumor response mediated by NKG2D receptor. One of its main ligands is MHC class I polypeptide-related sequence A (MICA), which is upregulated in cellular transformation. The MICA gene is highly polymorphic, originating alleles that encode protein variants with a controversial role in cancer. The goal of this work was to investigate the MICA allelic variants, their expression and relationship with clinical characteristics in GC.We included 50 patients with GC from Salvador Hospital and 50 healthy volunteers over 18 years without prior gastrointestinal diseases or some type of cancer. We obtained a blood sample to extract genomic DNA and the MICA alleles were identified by PCR-Sequencing based typing. We also obtained a sample of serum to measure soluble levels of MICA by ELISA. The results showed that alleles frequencies of MICA were different between GC patients and healthy volunteers. The MICA*009 allele frequency was significantly higher in patients than healthy volunteers (p=0.007). The regression analysis indicated that the presence of MICA*009 increases the average probability of GC.According to the survival analysis, MICA*002/002 and MICA*002/004 patients showed a higher survival than MICA*002/008 patients and MICA*002/009 (p=0.040). MICA soluble was significantly higher in patients than controls and it was related with the presence of valine in MICA-129. In conclusion, our findings suggest that both the MICA allelic variants and soluble levels could be used as biomarkers in risk and prognosis in GC.

Financing: National Doctoral Scholarship (ANID) 21171812, FONDEF (ANID) ID16I10027 and ID20I10106, ICBM 2020, Faculty of Medicine.

Keywords: MICA, NKG2D receptor, Gastric Cancer

Non-malignant renal tissue is a reservoir of tumor-reactive CD8+ T cell progenitors in human renal cell carcinoma.

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Introduction: Renal cell carcinoma (RCC) is a prevalent cancer and a health problem worldwide. Cytotoxic CD8+ T cells (CTLs) are the main mediators of anti-tumor immunity. Despite the high CD8+ T cell infiltration of RCC tumors, most of patients progresses and only a fraction of them respond to checkpoint blockade immunotherapy. Tumor-reactive CD8+ T cells with a progenitor phenotype, characterized by expression of the transcription factor TCF1, have been identified as continuous source of tumor-infiltrating CTLs providing effective anti-tumor immunity and response to immunotherapy in several solid tumors. However, the presence and localization of tumor-reactive CD8+ T cell progenitors in RCC has not been studied.Methods: We analyzed the phenotype of CD8+ T cells infiltrating tumors and non-malignant renal tissue (NMRT) from RCC patients by flow cytometry. In parallel, cells from autologous tumor and NMRT were cultured.Results: We observed that RCC tumors are infiltrated by CD8+ T cells mainly with a terminally differentiated phenotype. In contrast, the majority of CD8+ T cells in NMRT possess a progenitor phenotype, characterized by TCF1 expression and the absence of the transcription factor TOX1, and inhibitory receptors Tim-3 and CD39, associated with terminally differentiated or exhausted phenotype. Moreover, an important proportion of CD8+ T cells from NMRT specifically recognized autologous RCC cells but not normal renal tissue. These data evidence the presence of a reservoir of tumor-reactive CD8+ T cell progenitors in NMRT that may provide effective and long-lasting antitumor immunity in RCC patients.

Financing: Acknowledgements: ANID FB210008, FONDECYT-11190897, FONDECYT-1212070

Keywords: Renal Cell Carcinoma, Progenitors CD8+ T cells, Non-Malignant Renal Tissue

ANALYSIS OF THE BINDING CAPACITY OF α2-DOMAIN OF MICA TO NKG2D RECEPTOR

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Therefore, MICA is an important molecule in the recognition of malignant cells by immune system. The structure of MICA is confirmed by three regions: intracellular, transmembrane and extracellular. The extracellular part is formed by three protein domains: α1, α2 and α3. The binding to receptor is take place on α1 and α2 domains, where the NKG2D homodimer interacts with the beta lamina and with two alpha helixes, that conform the domains.The study of this interaction is relevant to understand why some MICA polymorphisms would have least affinity and could escape immune recognition. To the date, the role of each MICA domains (α1 or α2) in the binding with NKG2D is not well understood and in this work we presented the subcloning, production and purification of α2 domain of MICA, and the behavior with NKG2Dr. The α2 domain was bond to the NKG2D recombinant protein, in different concentration, at the same way like MICAsp recombinant protein. MICAsp showed an optical density signal higher than α2 domain, however this signal is half to obtained with MICAsp, indicated that this domain provide, at less, the half of capacity of binding. This work showed the possibility of production and purification of α2 MICA domain, and its functionality in the binding to NKG2D, allowing to know the importance of the α2 domain in the union with the receptor.

Financing: This work was funded by the National Agency for Research and Development (ANID) / Scholarship Program / DOCTORADO BECAS CHILE/2021 – 21212223 And FONDEF/ANID Concourse IDeA I+D 2020 (ID20I10106)

Keywords: MICA, NKG2D, α2 domain

Immunomodulation of T Helper Cells by Tumour Microenvironment in Oral Cancer Is Associated With CCR8 Expression and Rapid Membrane Vitamin D Signalling Pathway

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The immune system plays a key role in the protective response against oral squamous cell carcinoma (OSCC); however, the tumour microenvironment (TME) impairs this anti-cancer response by promoting an anti-inflammatory environment. Regulatory T-cells (Tregs) and Th2 effector cells (Teff) are associated with poor prognosis in OSCC. However, the main immunomodulatory mechanisms associated with the enrichment of these subsets in OSCC remain unknown. We characterised Th-like lineages in Tregs and Teff and evaluated immunomodulatory changes induced by the TME in OSCC. Our phenotypic data revealed a higher distribution of tumour-infiltrating CCR8+ and Th2-like Treg in OSCC compared with non-malignant samples, whereas the percentages of Th1 cells were reduced in cancer. We then analysed the direct effect of the TME by exposing T-cell subsets to cancer secretomes and observed the OSCC-secretome induced CCR8 expression and reduced cytokine production. Transcriptomic analysis showed that the co-culture with OSCC-secretome induced several gene changes associated with the vitamin D (VitD) signalling pathway in T-cells. In addition, proteomic analysis identified the presence of several proteins associated with prostaglandin E2 (PGE2) production by rapid membrane VitD signalling and a reduced presence of the VitD-binding-protein. Thus, we analysed the effect of VitD and PGE2 and observed that VitD promotes a regulatory Th2-like response with CCR8 expression whilst PGE2 also modulated CCR8 but inhibited cytokine production in combination with VitD. Overall, our data showed the immunomodulatory changes induced by the TME involving CCR8 expression and regulatory Th2 phenotypes, which are associated with PGE2 mediated VitD signalling pathway in OSCC.

Financing: Fondecyt Regular 1211480

Keywords: oral cancer, CCR8, Tregs

In vitro study of the binding capacity of a fully human anti- Major Histocompatibility Complex Class I Chain-Related A (MICA) antibody to recognize different allelic variants expressed in gastric adenocarcinoma cell lines.

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MICA protein is involved in tumor immunosurveillance and immune evasion processes, and is expressed in different types of solid tumors, including gastric adenocarcinoma (GA), which proposes MICA as a potential therapeutic target in cancer. MICA gene is highly polymorphic, with 150 alleles that code for 87 protein variants with varied secretion characteristics and affinity to NKG2D on Natural Killer (NK) cells. In this work, we aimed to define MICA allelic variants on GA cell lines and analyze the binding capacity of a fully human recombinant anti-MICA antibody (AcHu- αMICA) to each MICA variant. AcHuαMICA has been developed in our laboratory and is directed against a peptide located in the non-polymorphic α1 region. The identification of MICA allelic variants present on GA cell lines was determined by SBT-PCR. Cell surface levels of MICA and AcHu-αMICA binding capacity were determined by flow cytometry. The protein sequencing revealed the presence of MICA* 010:01 allele in AGS cells, MICA*009:01 in MKN-45 cells, and MICA* 008 in GES-1 cells (gastric epithelial cell control). GES-1 presented greater abundance of MICA on the surface with respect to MKN-45. MICA was not detected on the surface of AGS cells. AcHu-αMICA bound to all allelic variants present on gastric cell lines and displaced the binding capacity of a commercial anti-hMICA Ab. Thus, the molecular characterization of MICA on AGS and MKN-45 cells may allow the establishment of different in vitro models to study the mechanisms of GA immune evasion, as well as AcHu-αMICA anti-tumoral mechanisms of action.

Financing: Financing: Proyecto Fondef ID20I101006

Keywords: Cancer gastric, MICA, NKG2D

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