iMedPub Journals

TRANSLATIONAL BIOMEDICINE

2010
Vol.1
No. 3:4
doi: 10:3823/413

First French-Argentine Immunology Congress, 2010 Abstracts

Held on Buenos Aires, Argentina, 2nd-5th november, 2010

1. Amplification of effector T lymphocyte effect by Treg depletion

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Donor lymphocytes infusions (DLI) represent a possible way to induce graft-versus-leukemia (GVL) effect in patients that relapse after allogeneic hematopoietic stem cell transplantation (HSCT). We and others previously described that the subpopulation of CD4+CD25+ immunoregulatory T cells (Treg) could play a key role in the control of alloreactive responses. In experimental bone marrow transplantation, Tregs were described to be present among conventional T cells within the transplant and their depletion from the transplant before infusion leads to significantly accelerated graft versus host disease (GVHD) in different models. Thus, Treg elimination from DLI could be a way to improve alloreactivity and the associated-GVL effect in patients that relapse from hematological malignancies after HSCT. In this study, we have compared in human two ways of Treg elimination and their consequences on the alloreactivity of the remaining T cells. The first one consisted in elimination of CD25+ cells. For the second one, we developed a strategy of positive selection of CD127 expressing-cells that excludes the Treg subpopulation. We observed in vitro and in vivo in a model of xeno-GVHD that Treg depletion through CD127 positive selection as compared to CD25+ cell elimination or non Treg depleted cells, increases the immune response of the remaining conventional T cells

2. Sec22b mediated ER-phagosome fusion regulates phagosomal functions and is critical for cross presentation in dendritic cells

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The processing of exogenous antigens by dendritic cells (DCs) and their presentation on the class I molecules of histocompatibility (MHC) is known as cross presentation. Antigen cross presentation has been involved in establishing cytotoxic immune responses against bacteria, tumors and certain virus that do not infect DCs. The presence of Endoplasmic Reticulum (ER) components in phagosomes has been proposed to be important for cross presentation. However, there is no evidence about the mechanism of recruitment of these ER components to the internalization pathway. There is also no direct evidence that the ER recruitment to phagosomes is required for cross presentation. We show here the ER-SNARE Sec22b is a key player mediating the fusion between the ER membranes and phagosomes in DCs. Using shRNA-mediated knock down of Sec22b expression in DCs, we found that phagosomes fail to acquire ER components efficiently. Cross presentation of olvalbumin (OVA) coated beads or soluble OVA were strongly inhibited, while the classical MHC class II and endogenous MHC class I presentation pathways were not affected. We also observed that ER-deficient phagosomes acquire more rapidly lysosomal markers and display a higher proteolytic activity than normal DC phagosomes. Our results suggest that the fusion of the ER to phagosomes is essential for cross presentation not only by contributing with the MHC class I presentation machinery, but also by delaying phagosome maturation and promoting cross presentation conditions.

3. Role of CD4+IL-9 producing T lymphocytes in human tuberculosis

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Protection against Mycobacterium tuberculosis (Mtb) depends on cell mediated immunity. Thus, activated specific T lymphocytes display a crucial role in the immune response of the host against the pathogen. In particular, CD4+ IFNγ secreting T cells display a main function in this response. However, different subsets of CD4+ T lymphocytes might participate in the immune response to Mtb. Recently, a novel CD4+ T cell subset named "Th9" was described. This population are lymphocytes that secret IL-9 and IL-10 and express the transcription factor PU.1. In this work we investigated the potential function of CD4+ IL-9 producing T cells. Peripheral Blood Mononuclear Cells (PBMC) from tuberculosis patients (TB) and healthy donors (HD) were stimulated with Mtb antigen and the production of IL-9 was determined by ELISA. Our results showed that Mtb-stimulation induced sig-

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doi: 10:3823/413

nificantly higher levels of IL-9 in HD compared to TB patients ($p<0.005$). Moreover, the increase in IL-9 levels correlated with a significant increment of PU.1 ($p<0.05$). Furthermore, Mtb stimulation also significantly increased the percentage of CD4+IL-9+ lymphocytes as detected by flow cytometry ($p<0.001$). Interestingly, IL-9+ IFNγ-, IL-13-, IL-10-, IL-17- were detected after Mtb stimulation. Antigen-stimulation of PBMC from TB and HD in the presence of IL-4 and TGFβ significantly augmented IL-9 levels ($p<0.01$). Interestingly, addition of IL-9 to Mtb stimulated PBMC significantly augmented IFN-γ levels ($p<0.01$), whereas neutralization of endogenous IL-9 markedly reduced IFN-γ production ($p<0.001$). These data suggest that Mtb induces a Th9 population which in turn might contribute to the immune response against Mtb by enhancing IFNγ production.

4. Evaluation of new antigens for the diagnosis of latent M. tuberculosis infection

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According to World Health Organization, one third of the world population is infected with latent M. tuberculosis (LTB). The introduction of the novel IFNγ release assays (IGRAs) using CFP-10 and ESAT-6 M.tuberculosis (Mtb) latency antigens has vastly improved the diagnosis of LTB. However, these tests cannot adequately differentiate between LTB and active disease. Thus, other latency antigens are currently under investigation to improve those clinical assays. Therefore, the main objective of this work was to identify potential specific Mtb antigen candidates to be used in improved diagnosis assays. To this aim, we first obtained recombinant antigens: Rv2624, Rv2626c, Rv2628, HspX, ESAT-6 and CFP-10. Then, we investigated the immune response of BCG-vaccinated healthy donors (HD), tuberculosis patients (TB) and Mtb infected individuals by determining IFNγ production and proliferation. The results indicate that subjects with LTB showed significantly higher IFNγ responses to Rv2626c and HspX than individuals with active TB and HD ($p<0.05$), whereas no significant differences between these groups were found for Rv2624 and Rv2628. At the same time, HD showed a significantly lower ($p<0.05$) response to these same antigens than TB patients. Moreover, Rv2626c, Rv2628, HspX and ESAT-6 significantly decreased cell proliferation in the three groups ($p<0.005$), which is in line with previous reports on ESAT-6. In order to investigate whether PBMCs studies correlated with whole blood (WB) analysis, both IFNγ and TNFα were measured after 24h and 48h of sonicated Mtb stimulation. Our data showed a significant positive correlation between WB and PBMCs results (Spearman test, $p<0.05$ and $R>0.7$).Together, these results indicate that Rv2626c and HspX, antigens that induced the production of significantly different amounts of IFNγ in the three groups, are potential candidates to be used in the optimization of current IGRAs in the diagnosis of latent tuberculosis.

5. TFH phenotype, IL-10 and IL-21 response of stimulated CD4+ T cells in XLP

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In order to determine the effects of absence of SAP, or follicular T cells (TFH) failure in the generation of hypogammaglobulinemia in XLP, we studied the characteristics of TFH in two XLP patients with the same SH2D1A mutation and different treatment and age of exposure to EBV. TFH phenotype was determined by flow cytometry using monoclonal anti-CXCR5, anti CD57 and anti-PD-1 (associated to active TFH) antibodies. The response of XLP and control (N) peripheral blood mononuclear cells (PBMC) to T cell stimuli was studied after stimulation with phytohemagglutinin and IL-2 for 2 to 16 days. Intracellular IL-10 and IL-21 were assayed after treatment with monensin for 12 hr at different time points in the CD4+ T cell region. Expression of activation markers CD40L and ICOS was measured in the surface of CD4+ T cells. CD4+ T cells co-expressing CD57 and CXCR5 were higher in XLP#9 than in N (% CXCR5+, CD57+/CD4: XLP#9: 2.49+/-0.83; N: 0.48+/-0.07, $p<0.05$), and CD4+T cells co-expressing PD-1 were significantly higher in XLP#4 than in XLP#9 and N (% CXCR5+, PD-1+/CD4: XLP#4: 8.52+/-1.58; N: 2.03+/-0.22; $p=0.007$). ICOS expression was higher in N than in XLP at 2 days of stimulated culture ($p< 0.05$), but after 8 days, ICOS expression reached N values in XLP (22-28%) and remained stable. CD40L followed the same pattern as ICOS. Intracellular IL-10, initially below1% in N and XLP, reached higher values (5-10 %) in XLP after 8 days (XLP#4 and #9 vs N, $p<0.05$). Likewise, intracellular IL-21 (central to TFH function) was significantly higher in XLP than in N after 8-12 days of culture ($p<0.001$). In conclusion, differences between XLP#4 and #9 in the TFH phenotype may be unrelated to SAP deficiency. T cells from XLP patients could respond to T cell stimuli increasing the synthesis of IL-10 and IL-21 even to higher levels than N. However kinetics of this response revealed differences in its profile that may influence the outcome of the humoral immune response.

6. SLAM activation regulates cytokine microenvironment during active tuberculosis

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doi: 10:3823/413

Protective immunity against Mycobacterium tuberculosis (Mtb) requires the generation of cellular immune responses. In this context, IFNγ and TNFα production are crucial to fight this pathogen. Moreover, it has been recently suggested that IL-17 might also contribute to the resolution of tuberculosis. Previously we demonstrated that signaling through the signaling lymphocyte activation molecule (SLAM) increased IFNγ secretion in patients with tuberculosis. Furthermore, we reported that IL-17 decreased SLAM expression. Then, in this investigation we studied the role of SLAM activation on the regulation of the cytokine milieu in tuberculosis. Our results showed that after stimulation with Mtb antigen, most of CD4+ IL-17 secreting T cells expressed SLAM (IL-17+ SLAM+: 94.41%±3.38; IL-17+ SLAM-: 5.59±3.38%, p< 0.005, Wilcoxon Test). Moreover, signaling through SLAM significantly increased the production of IL-17 in tuberculosis patients (Mtb+ α-SLAM relative to Mtb: 246.9±78.23, p< 0.05, Wicoxon Test). In agreement with these data, blocking of SLAM by iRNA, significantly decreased IL-17 secretion. On the other hand, activation of SLAM induced a marked augment of TNFα production in patients with tuberculosis (Mtb: 23872 ± 8442pg/ml; Mtb + α-SLAM: 30715 ±10332 pg/ml, p< 0.05, Wilcoxon Test), but in contrast to IFNγ and IL-17, TNFα didn't regulate SLAM expression on the surface of T cells. Taken together, our present results suggest that SLAM activation leads to the generation of a complex cytokine microenvironment during active tuberculosis, having a crucial role in the homeostasis of the host immune response against the bacteria.

7. Predictive value of anticardiolipin antibodies in an adult hospital of Mar del Plata city

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Antiphospholipid antibodies (αPL): anticardiolipin (αCL) antibodies or lupus anticoagulants (LA) are heterogeneous group of autoantibodies characterized by their reactivity to anionic phospholipids. In clinical practice, both IgG and IgM αCL and LA remain the most established and standardized tests for diagnosis of antiphospholipid syndrome (APS) and to assess the risk of thrombosis. Low specificity (high-false positive rates) and standardization problems remain to be major controversial issues. The aim of this work was to establish the positive predictive value (PPV) and negative predictive value (NPV) of αCL by enzyme-linked inmunosorbent assay in 57 patients with clinical manifestations of the APS. One population consisted of 29 patients with systemic lupus erythematosus (SLE): 9 with SLE and secondary APS and 20 with SLE without clinical history of αPL. The others populations consisted of 9 patients with venous thrombotic events, 20 with arterial thrombotic events and 19 patients with pregnancy morbidity. The NPV and PPV for aCL were evaluated by a 2x2 contingency table analysis. The IgM NPV and PPV for SLE with APS were 71 % and 33% respectively; the IgG NPV and PPV were 78 % and 50 % for SLE with APS respectively. Similar results were obtained between IgM PPV arterial thrombosis (50%) and venous thrombosis (57%).The arterial thrombosis NPV for IgG αCL (44%) and NPV for IgM αCL (47 %) was lower than venous thrombosis NPV for IgG αCL (61 %) and IgM αCL (75 %). The IgM pregnancy morbidity PPV and NPV for αCL were 44 % and 52 % respectively and the IgG PPV and NPV were 40 % and 50 % respectively. The lowest PPV was for IgG αCL in patients with arterial thrombosis (25 %). The highest PPV was for IgM αCL in venous thrombosis (57%). In spite of the small number of patients in this study, these results indicate the necessity to start the analysis of other αPL as β2GPI antibodies to improve the performance of the laboratory assessment for APS.

8. Cationic polymerizable liposomes: Immunoadjuvant effect and cytokine balance

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Vaccine development today encounters a main obstacle which is the need for effective adjuvants suitable for clinical trials. Liposomes are lipid-bilayer vesicles that have emerged as a promising new adjuvant technology: effective antigen-deliver systems that serve to markedly enhance the uptake and presentation of APC. Herein we characterize the adjuvant effect of four liposome formulations: Polimerized (P) and nonpolymerized (NP) with (+) or without (0) positive charges. OVA was ID injected in BALB/c alone, with liposomes or Alum. Specific IgG titres were determined by ELISAS, and DTH response was registered as an index of cell-mediated immunity. Moreover splenocytes of immunized mice were isolated, and cytokine secretion was determined in presence of OVA. NP+, P+ and P0 significantly increase the antibody titre, reaching levels compared with Alum. Also significant DTH response was observed these mice, specialy with cationic formulations. Presence of liposomes induced specific Th1 and Th2 cytokine secretion in the spleen. NP0 treated mice showed a significant secretion of IL-2, TNFα, IL-6, IL-10 and INFγ. In case of NP+, secretion was similar except for de absent of INFγ and the secretion of IL4. Polimerized liposome treated mice, showed detectable levels of TNFα and I-L6 for the P+, and TNFα, INFγ and IL-10 for the P0. Our data suggest that NP+ and P+ liposomes are capable of increase antibody titre and induce cellular immunity and secretion of Th1 and Th2 cytokines. These responses are crucial for vaccines that are required to protect against infection by a number of pathogens. Cytokines such as TNFα, detected in all cases, provide protective immunity against intracellular pathogens such as viruses. Thus, these alternative adjuvants would contribute to improve new generation vaccines, designed with recombinant peptides or proteins for generation of effective humoral and cell-mediated immune response.

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doi: 10:3823/413

9. B cell chronic lymphocytic leukemia (CLL): CXCL12 and nurse like cells (NLCs) enhance the activation of T cells from high-risk or low-risk patients

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The clinical course of CLL is very heterogeneous, with patients who develop an aggressive disease that lead to an early death (high-risk group) and others who survive for decades (low-risk group). Activated T cells from CLL patients provide survival and proliferative signals to the leukemic clone within lymphoid organs. We have previously reported that T cells from low-risk CLL patients show a lower migratory capacity towards CXCL12 (a chemokine produced in lymphoid organs by stromal cells or NLCs) compared to T cells from high-risk patients. Given that CXCL12 also exerts a co-stimulatory activity on T cells, we asked whether this capacity could be impaired in T cells from low-risk compared to high-risk patients favoring the indolent course of the disease in the former group. The aims of this study were: a) to evaluate if CXCL12 increases the proliferation and production of the anti-apoptotic cytokine IFNγ by activated T cells from CLL patients; b) to compare the effects of CXCL12 on T cells from high-risk and low-risk groups and c) to study if NLCs can also enhance the activation of T cells. When we cultured T cells from CLL patients (n=10) with and without anti-CD3 we found a higher proliferation (p=0.004) and expression of IFNγ (p=0.01) if T cells were activated in the presence of CXCL12. We observed a similar co-stimulation induced by CXCL12 on T cells from both CLL risk groups (p>0.05). Finally, we found higher expression of CD25 (p=0.01) and CD69 (p=0.03) when activated T cells were cultured in the presence of autologous NLCs (n=6). Preliminary blocking experiments showed that CXCL12 production by NLC may account for the T cell co-stimulation observed (n=3). Our results suggest that the presence of CXCL12 in lymphoid organs may enhance the activation of T cells. Because no differences were found in the co-stimulation induced by CXCL12 in T cells from both groups of CLL patients, other processes, such as the lower migratory response of low-risk T CLL cells might favor the indolent clinical course of the disease in these patients.

10. CCR4 expression in a case of cutaneous Richter's transformation of chronic lymphocytic leukaemia (CLL) to diffuse large B cell lymphoma (DLBCL) and in CLL patients with no skin manifestations.

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B cell chronic lymphocytic leukaemia (CLL) is characterized by a lymphocytosis of clonal CD5+ B lymphocytes. CLL transformation to Richter´s syndrome (RS) is a highly aggressive syndrome commonly represented by a diffuse large B-cell lymphoma (DLBCL) that arises from the original CLL clone. RS usually develops in lymph nodes and its cutaneous spread is very rare. CCR4 is the specific receptor for CCL17 and CCL22 (two chemokines highly produced in skin) and its expression was associated with normal and malignant T cell homing to skin and in a case of cutaneous DLBCL not related to CLL. Given that a skin RS transformation of CLL to DLBCL was detected in one of our patients, we hypothesized that his circulating CLL cells may express CCR4. We found that 11% of cutaneous CD19+ cells expressed CCR4, evaluated by flow cytometry, and more interestingly, also 7% of his circulating CLL cells were CCR4+ and were able to migrate towards CCL22 in a chemotaxis assay (CD19+ migration index to 0 and 2000 ng/ml of CCL22: 100 vs 145). In order to determine whether CCR4 expression was a special feature of this patient, we evaluated CCR4 expression on circulating CD19+ cells from CLL patients with no skin manifestations and elderly healthy donors. We found that CD19+ cells from these patients express similar levels of CCR4 but in a lower proportion than healthy donors (n=20, p<0.001). Moreover, CD19+CCR4+ cells might be more activated than the CCR4- ones, since the proportion of cells expressing the activation marker CD38 is higher in the former subpopulation (n=18, p<0.001). Since we reported that leukemic cells from CLL patients, independently of the presence of a cutaneous RS express CCR4, we can conclude that the expression of CCR4 on CLL cells seems not to be related to the cutaneous transformation of CLL cells. It remains to be determined whether the expression or functionality of CCR4 is augmented in leukemic cells of CLL patients who develop other skin manifestations.

11. Thioperamide improves the symptoms of allergic mice through the induction of regulatory T lymphocytes in lung

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Histamine (HIS) is one of the most important mediators in the development of allergic reactions. Historically, the pro-inflammatory effect of the amine was associated to its action on H1 receptors; however the use of antagonists for this receptor

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doi: 10:3823/413

showed to be ineffective in the treatment of allergic diseases. In recent years, it was assigned an important role in these pathologies to H4R, which is not clear yet. In a previous work, we demonstrated that the injection of immature dendritic cells (DC) treated with HIS promoted the severity of allergic symptoms through the increase of TCD8+ Tc2 lymphocytes. Here, we decided to evaluate the role of H3R/H4R in allergy using thioperamide (Thi), an antagonist of these receptors, on DC. For this purpose, we use a known murine model of OVA-induced airway inflammation. After 15 days of OVA challenge, mice were injected intratracheally with PBS, DC ($5x10^5$), DC treated with 0.1µM HIS 30 min at 37°C ($5x10^5$) or DC treated with 10 µM Thi 30 min at 37°C ($5x10^5$). 21 days later we obtained the lungs of allergic mice and the suspension of cells were purified by negative selection by using a PAN T antibody. Surprisingly, we found a higher recruitment of regulatory CD4+CD25+Foxp3+ T cells in the lung of allergic mice injected with DCThi (mean of positive cells %±SEM, DCThi: 0.72±0.02 vs. PBS: 0.40±0.11;DC: 0.65±0.05; DCHIS: 0.41±0.05; *p<0.05,n=4) which correlated with increased production of IL-10 by lung DC (mean of IL-10 pg/ml±SEM, DCThi: 3000±470 vs. PBS: 1243±152; DC:904±48; DCHIS: 1230±315; *p<0.05,n=5. Interestingly, we showed a significant decrease in serum of anti OVA- IgE (mean OD±SEM, DCThi: 248.1±16.4 vs. PBS: 367.0±36.0; *p<0.05, n=6) and an inhibition of eosinophils recruitment in lung of mice (mean of eosinophils %±SEM, DCThi: 6±1; PBS: 47±6; *p<0.05, n=6). In conclusion, thioperamide acting on DC could offer an alternative therapy in the treatment of allergy through the induction of a tolerogenic profile

12. Serum levels of RANTES in chronic idiopathic neutropenic patients

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Anti-neutrophil (PMN) cells antibodies (anti-PMN) and circulating immune complexes (CIC) are associated to immune (Im) but not to non immune (N-Im) chronic idiopathic neutropenia (Np). Laboratory findings suggest that N-Im Np is a syndrome mediated by the action of pro-inflammatory cytokines and chemokines (CK). CK play a regulatory function on leukocyte traffic in the recruitment and/or distribution of PMN and natural killer (NK: CD56+CD3-) cells. Sera from patients with Np are evaluated in our laboratory for anti-PMN, CIC and, as complementary study, NK cells. We have found a connection between PMN and NK cell decrease. As a preliminary test we evaluate RANTES serological levels and assess its association with PMN and NK circulating cells. ELISA KIT (Pierce Biotechnology) for RANTES levels was employed. At random, 22/98 sera were evaluated from Np patients previously tested for NK, anti-PMN by flow cytometry and leukoagglutination activity (LAG). RANTES was detected (RA+ >61 ng/mL) in 23% (5/22) sera patients. Laboratory referent values (RV) ranged from 31 to 61 ng/mL; [25 th to 75th percentiles], n=12. According to previous results on anti-PMN and LAG, patients (n=22) were distributed as: A) Im-Np Group (13/22): 54% (7/13) with < 1.5 x 10^9 PMN/l and 92% (12/13) < 0.179 x 10^9 NK/l and 23% (3/13) RA+ sera were detected; B) N-Im Np (9/22): 89% with < 1.5 x 109 PMN/l and 78% (7/9) < 0.179 x 109 NK/l and 22% (2/9) RA+ sera were detected. No connection was found between RANTES levels and PMN and NK circulating cells. Two sera from patients with high RANTES levels (128 and 139 ng/ml from Im-Np and N-Im Np groups respectively) had a background of infections. In summary, no association was observed between RANTES serum levels and PMN or NK circulating cells.

13. Analysis of detection of antibodies to citrullinated peptide (a-CCP) in patients with Juvenile Idiopathic Arthritis (JIA)

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Juvenile Idiopathic Arthritis (JIA) is a clinically heterogeneous group of inflammatory diseases that occur in children under 16 years old, and polyarticular JIA is a subgroup of it. The diagnosis is clinical and the presence of autoantibodies is an unusual finding. The Rheumatoid Factor (RF) is present in 20% of patients. The a-CCP is an autoantibody associated with Rheumatoid Arthritis and its severity; however there are few reports about its usefulness in JIA. Objetive: To assess the sensitivity and specificity of a-CCP in a cohort of Polyarticular JIA patients and to evaluate the correlation between RF and a-CCP. Methods: Serum samples were collected from 67 patients with Polyarticular JIA and 35 controls, 15 with other autoimmune diseases (9 with Systemic lupus erythematosus, 6 with autoimmune hepatitis) and 20 from age-matched healthy controls. Anti-CCP-positivity was correlated positively with the disease characteristics. The median age was 14 and 12 years old respectively. We analyzed by a-CCP ELISA INOVA Quanta Lite CCP3-IGG which cut off value is 15AU and RF by nephelometry where values under 20U/ml are considered negative. Statistical analysis was performed with 2x2 table and Pearson`s correlation. Results: 50% (n=33) of patients with JIA were a-CCP positive, while controls were all negative. The sensitivity was 50%, specificity 100% and positive predictive value 100%. The RF was positive in 30% (n=20) of the patients and none controls. Twelve patients were positive for both autoantibodies. Conclusion: The a-CCP antibody proved to be highly specific to the diagnosis of Polyarticular JIA, but it has low sensitivity, however, it is higher than the determination of FR. A small group of patients were positive for both autoantibodies (RF and a-CCP). We will evaluate a-CCP as a prognostic factor of more severe evolution of the disease.

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14. Presence of Cytomegalovirus protein pp65 in AIDS patients

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Paraguay had 9.438 HIV/AIDS cases by December 2009, 2,585 had developed AIDS, 4,659 were infected, 1,567 have died and 627 were infected with no data about the disease. Target cells of this infection are CD4 lymphocytes, which decrease as viral replication occurs and opportunistic diseases appear cytomegalovirus (CMV) infection among them. CMV primary infection is followed by latency, sometimes with a relapse caused by reactivation of the latent virus but re-infections possibly are due to CMV antigenic diversity. To determine the presence of antigenemia by the identification of a CMV early structural protein (pp65) in the peripheral leukocytes of HIV, infected patients in AIDS stage were evaluated. This is a cross-sectional study carried out in 53 HIV positive patients in AIDS stage that attended IICS/UNA, and/or were hospitalized in the IMT and voluntarily agreed to participate. Samples were collected in the period of May 2008 to August 2009.A pre-coded questionnaire was used for the collection of universal (age, sex, origin) and clinical variables. CD4 lymphocytes were isolated from total blood sample and percent values were determined by flow cytometry (Becton Dickinson). The presence of antigen pp65 was determined by direct immunofluorescence using CMV BriteTM Turbo Kit (Netherlands). The population studied was: 35.6±8.3 years old, 77% under 40 years old, 77% (IC95%: 63.8-87.7) male. Eigthy trhee percent (43/53, IC95%: 69.7-87.7) had symptoms, specially fever and diarrhea in 45% of the studied individuals. Eight percent (4/53, IC95%: 2.10-18.2) had positive antigenemia, ages between 29 and 40 years old and the male female ratio was 3/1. The existence of CMV acute infection was described in this population. HIV-CMV coinfection challenges a deficient immune system and is an element that should be considered by physicians for the treatment and follow-up of patients.

15. The intestinal epithelial cells can induce the integrin CD103 after interacting with chitosan

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The polysaccharide Chitosan (Ch) triggers regulatory cytokine production (IL-10 and TGFβ), modulates the function of mesenteric lymph node (MLN) T cells and promotes tolerance at the mucosal inductive sites. To evaluate the contribution of intestinal epithelial cells (IECs) to the activity of Ch we studied i) the endocytosis of fluorescein isothiocyanate (FITC) conjugated Ch using different cell lines such as Caco-2 (human epithelial colorectal cells), IEC-6 (rat intestinal epithelial cells), RAW 264.7 (mouse macrophage cells) and IECs from normal rats (flow cytometry); ii) the signals derived from IECs after Ch contact: rat IECs isolated 16 h after feeding diluent or 3 mg Ch were co-cultured with spleen (Sp) or MLN mononuclear cells; after 24 h we evaluated the percentage and expression of the integrin CD103 in CD3 positive and negative cells (flow cytometry). Already after 30 min we found an increment in percentage and fluorescence intensity of FITC-Ch+ cells dose and temperature dependent (4°C vs. 37°C) suggesting the endocytosis of Ch. This finding was confirmed by confocal microscopy. The contact with control group IECs induced the expression of the CD103 molecule in CD3 positive and negative cells from Sp and MLN ($p<0.05$). Interestingly, the effect was stronger after co-cultures with IECs isolated from Ch fed rats ($p<0.01$). Moreover, in experiments using transwell inserts the induction of CD103 was abolished, suggesting that the effect relies on the physical contact ($p<0.05$). In gut sections of rats fed Ch, the increment in the number of CD11b/c+ CD103+ dendritic cells in lamina propria was observed (immunohistochemistry). It is well accepted that a subset of MLN dendritic cells which express the integrin CD103 is efficient at inducing gut-homing receptors and regulatory T cell differentiation. Therefore, the ability of IECs to induce the expression of this marker after Ch contact provides a clue to understand the mechanism of its mucosal activity.

16. Tumor infiltrating mononuclear cells derived from tumors induced with LPS-activated B16 cells expres higher levels of IFNγ and reduced levels of IL-10

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B16 murine melanoma cells stimulated in vitro with a TLR4-ligand during 48h prior to their inoculation into TLR4 deficient mice (TLR4lps-del), induce tumors significantly smaller than controls. The apoptosis-proliferation balance of LPS-stimulated B16 cells is not modified and inhibition of tumor growth was not observed in nude mice; thus we hypothesized that TLR4 triggering on B16 cells themselves could induce the expression of pro-inflammatory mediators, that even if they are transitory, could dramatically alter the function of dendritic cells (DCs) present at the site of inoculation and switch the type of immune response elicited against the tumor. TLR4lps-del DCs matured with CpG in the presence of supernantant (SN) from LPS-stimulated (SN B16+LPS) B16 cells were capable of overcoming the inhibition of activation observed when they were matured in the presence of non-stimulated B16-SN. To further analyze the DCs and T cell function in vivo, infiltrating mononuclear cells (TILs) from tumors induced with B16 cells stimulated (B16 + LPS) or not (B16 Basal) with LPS in TLR4lps-del mice obtained at day 20 and 32 post injection (p.i), were cultured in vitro with PMA-Ionomycin

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doi: 10:3823/413

and their cytokine expression was evaluated by intracellular flow cytometry. Interestingly, we observed an increase in CD11c+ IL-12+ cells (2.64%±0.45 vs 0.43±0.02%), higher levels of IFNγ+ cells (11.18±2.81% vs 3.46±0.54%) and reduced levels of IL10+ cells (2.11±0.45% vs 4.35±0.42%, $p<0.05$) in TILs from B16+LPS tumors, in comparison with those induced by B16 cells at day 20 p.i. Similar results were observed when we analyzed TILs isolated at day 32 p.i (IFNγ+ cells: 5.24±3.19% vs 1.66±1.34%, $p<0.05$; IL-10+ cells: 0.19%±0.06 vs 0.31±0.05%). Therefore, stimulation of murine melanoma cells with LPS in vitro before inoculating them into TLR4lps-del, promotes an improvement of the immune response, activating DCs and enhancing IFNγ+ TILs in vivo, at early and later times p.i of tumor cells.

17. Identification of RHD alleles with the potential of anti-D immunization

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The Rh system is highly polymorphic and one of the most clinically significant blood group in transfusion medicine. Reliable routine D typing approaches should be performed to identify some D variants that can cause anti-D alloimmunization upon transfusion. However, extremely weak D expression, termed DEL, may be confound from truly D negative units and used for D negative recipients. The aim of this study was to investigate the presence of RHD alleles in serologically D negative individuals. Blood samples from 661 D negative blood donors were studied. The Rh phenotype was performed by hemmaglutination. The presence of the D antigen was further analyzed by the indirect antiglobulin test. DNA samples were initially screened using a multiplex PCR strategy that amplifies intron 4 and the 3' unstranslated region of the RHD gene. Samples carrying RHD specific fragments were further studied by RHD exon scanning. Uncharacterized samples were analyzed by microarray strategies and sequencing. In the 661 D negative samples studied, 69 (8.93 %) were C positive or E positive. Molecular studies showed that 8 samples carried RHD specific fragments. In 3 C positive samples we detected RHD-RHCE hybrid alleles: one RHD-CE(3-7)-D and 2 RHD-CE(3-9)-D. One C positive sample carried the RHD(361del11) allele. All these alleles were reported to be associated with a D negative phenotype. We also found one C positive sample with the characteristic polymorphism of a DEL(M295I) phenotype and 3 E positive samples carrying an allele that had never been described, named RHD(46T>C). These new variants were serologically retested by adsorption elution assays and proved to be DEL phenotypes. These findings suggest that clinically important variants in D negative individuals are associated with C or E antigens. The detection of DEL alleles in donors is essential to minimize the risk of anti-D alloimmunization.

18. Heterologous antibodies from llama and chicken egg yolk to prevent rotavirus diarrhea

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Group A rotaviruses (RV) are the main cause of diarrhea in human infants. Understanding of the mechanisms of intestinal immunity and their correlation with protection is crucial to develop effective vaccines and complementary and/or alternative passive immunity strategies. Oral administration of specific antibodies (Abs) from different sources such as chicken egg yolk IgY or llama recombinant heavy chain Ab fragments (VHH) could represent effective strategies to prevent gastrointestinal infections in human infants. The aims of this study were i) to evaluate the passive protection conferred by neutralizing VP6-specific VHH fragments or Wa Human RV-specific conventional IgY to gnotobiotic pigs experimentally infected; ii) to study the immunomodulatory effect of heterologous Abs on the magnitude and distribution of systemic and mucosal antibody secreting cell responses. Eight experimental groups of pigs were mantained in isolators and received Abs passive treatments twice a day, for 9 days. Pigs were challenged with Wa Human RV. Animals were monitored for diarrhea and virus shedding. Immune responses were followed by ELISA and virus neutralization in sera, feces and intestinal contents. ELISPOT was performed in systemic and mucosal lyphoid tissues. Non treated and HRV-specific porcine IgG treated groups were used as controls. The passive administration of VP6-specific VHH at a final titer of 4,096 for 9 days prevented HRV diarrhea (100%, 0/4 pigs, $p<0.05$). Wa HRV-specific IgY shown a similar protection against HRV diarrhea, but in a dose-dependent manner. Porcine IgG treated animals were protected against RV induced diarrhea (100%, 0/6 pigs, $p<0.05$). All animals developed high Ab titers and strong ASC responses to RV at the intestinal mucosa. Taken together, these results indicate that passive treatments based on heterologous Abs represent useful strategies to prevent RV-induced diarrhea without interferring with the development of the neonatal immune response.

19. Comparative study of immune response to rubella and measles infection in children living in communities at river banks

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The antibodies's levels against measles, mumps, and rubella in a given populations are of interest as a way to measure objectively

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No. 3:4
doi: 10:3823/413

the proportion of the population that has been immunized. In this cross-sectional study, the levels of protective antibodies against measles and rubella were evaluated. We recruited 132 children of both sexes (0 to 18 years old), in Carmen del Paraná, Itapua District (October-November/2005). Prior the extraction of children's blood samples, informed consent was obtained from their parents/tutors, measles and rubella antibodies were determined by commercial immunoenzymatic assays in serum samples (Rubella IgG, DRG-USA) (Méasles IgG, HUMAN-ALEMANIA). Of all samples 98.5% (95% confidence interval CI: 94.6-99.8) and 79.7% (95%CI: 71.3-86.5) were positive, for protective antibodies against rubella and measles, respectively. The level of susceptibility to rubella was between 3 and 4%, while for measles from 13 to 40%. MMR vaccines is administrated against rubella and measles, but different level of immune response was observed, remarkably, despite vaccination campaigns and programs have become remnants of susceptible groups related to measles, which must be monitored to prevent outbreaks due to these groups.

20. Monophosphoryl lipid A as adjuvant for recombinant influenza nucleoprotein vaccine in mice

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Influenza is one of the most important respiratory pathogens worldwide. Vaccination is the best way to control influenza. Lipopolysaccharides (LPS) trigger innate immune response through activation of Toll-like receptor 4 (TLR4). Such responses may be exploited for the development of adjuvants and in particular monophosphoryl lipid A (MPLA) obtained by controlled hydrolysis of LPS of Salmonella minnesota. The use of influenza A virus recombinant nucleoprotein (rNP) as a vaccine antigen, stems from the fact that NP show less antigenic variation than the influenza virus surface glycoproteins, haemagglutinin and neuraminidase. The object of this work is to describe the production and immunogenicity of rNP from influenza A virus formulated with MPLA as a vaccine. Method: Homologous prime and boost subcutaneous vaccination of BALB/c mice with 10ug of the rNP (control group) and 10ug of rNP formulated with 25ug of MPLA (experimental group) were administered 3 weeks apart. On day 60 post prime, serum samples were collected to evaluate the specific titre of total IgG, IgG1 and IgG2a isotypes. At the same time, culture supernatants of spleen cells were collected, after antigen stimulation to test the presence of IFNγ by ELISA. Result: Subcutaneous injection of rNP with MPLA into BALB/c mice elicited both humoral and cellular immune responses. Animals injected with rNP/MPLA developed NP-specific antibodies, with total IgG titers of 11,200 and increase the ratio IgG2a/IgG1 from 0.045 to 0.96. In addition, the rNP/MPLA vaccine induced a significant different on the production of IFNγ, within control group and experimental group. Conclusion: This study demonstrates that the formulation of rNPr with MPLA induces a strong specific Th1 type immune response, humoral as well as cellular. This is a first step to demonstrate that MPLA can be use as an adjuvant in the formulation of a recombinant NP influenza vaccine.

21. Key transcriptional regulators of probiotic Bacillus subtilis determine the fate of innate immune response

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Probiotic spores of Bacillus subtilis boost the innate immune response by stimulating the three pathways of the Complement System (CS), leading to iC3b deposition although the C5b-9 membrane attack complex (MAC) is not assembled on the bacteria surface. In the present work, we studied i) soluble terminal complement complexes (TCC) generation and ii) Sp-40,40 binding to B. subtilis, a negative regulator of MAC formation. Different isogenic strains of B. subtilis (wild type, spo0A, sinR) were used for coating ELISA plate wells and incubated with normal human serum (NHS) or Mg-EGTA-NHS or EDTA-NHS or zymosan-NHS as positive control. The supernatant was used to assay soluble C5b-9 complexes by an ELISA sandwich. Fixed bacteria were used for surface deposition of CS components and clusterin by ELISA employing a-iC3b, a-C9 and a-Sp-40,40. Controls of bacteria without serum were included. Wild type strain and the sinR mutant incubated with NHS-EDTA inhibited iC3b binding to the bacterium by 98%±2.4% (mean±standard error of the mean, n=3, $P<0.05$), whereas NHS inhibited iC3b binding by only 35%±2.1% and NHS-Mg-EGTA by 87.21%±3.1%. Deposition of iC3b was less efficient in the presence of Mg-EGTA when compared with NHS, but it was not abolished, indicating activation of the classical and alternative pathways at the surface of both bacteria. In all cases, binding of C9 were not detected. The spo0A mutant was unable to activate CS. Sp-40,40 deposition was observed in all strains without significant difference[A450 1.38±0.23 (wt), 1.04±0.08 (SinR), 0.89±0.08 (0A) n=3, $p<0.05$] except for zimosan. To address the possibility that MAC have been formed in solution, we measured TCC. Equally TCC formation was not detected and was statistically significantly different from zymosan-NHS($p<0.05$). Our results suggest that B. subtilis promotes phagocytosis via opsonization but complement lysis resistance, both of which contributed to consider B. subtilis as a promising probiotic bacterium.

22. Role of sphingosine-1-phosphate in neutrophil trafficking to lymph nodes in an immune inflammation model

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Vol.1
No. 3:4
doi: 10:3823/413

We previously demonstrated that when OVA-FITC was injected into the footpad of OVA/CFA immunized mice, the main OVA-FITC+ cells recruited in draining popliteal lymph nodes were neutrophils and this influx was dependent on an antigen-specific response. Moreover, we showed that neutrophils entered into lymph nodes through afferent lymphatics and blood and that this migration was dependent on the presence of immune complexes as well as on an inflammatory condition. On the basis of these findings, we investigated if the phospholipid sphingosine-1-phosphate (S1P) is involved in this traffic. Treatment with the S1P receptor modulator FTY720 inhibited neutrophil influx in draining popliteal lymph nodes of mice immunized with OVA (43±3% vs 9±4%; p=0.02) but not its recruitment to the site of inflammation (23±2% vs 16±3%; p=0.19). To further characterize the role of S1P in neutrophil trafficking from skin (by lymph vessels) or from blood into reactive lymph nodes, we performed adoptive transfer experiments. Bone marrow neutrophils incubated with immune complexes (anti-OVA rabbit sera plus OVA) were differentially labeled either with DiIC(18)-DS or DiOC(18)-SP and simultaneously injected i.v. and s.c. into the footpads, respectively, of FTY720 treated OVA/CFA-immunized mice. The administration of FTY720 caused impaired migration of transferred neutrophils from footpad to popliteal lymph nodes (0.44±0.08% vs 0.21±0.04%; p<0.05) and from blood to lymph nodes (0.56±0.09% vs 0.26±0.07%; p<0.05). In summary, these data suggest that S1P participates in neutrophil migration from a peripheral tissue (by lymph vessels) and from blood into lymph nodes in an immune inflammation model.

23. Relationship between serum prolactin and mammary gland T cell population in early lactating rats

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Mammary stroma is composed of various cells, among them migratory leukocytes. Mammary lymphocytes are important during lactation, supplying maternal antibodies to milk. Recruitment of IgA secreting cells increases from parturition to late lactation. The role of recruited T cells during pregnancy is unclear, but may be related to epithelial cell growth, protection against infections and immune response regulation. This homing pattern suggests that T cell recruitment is controlled by pregnancy- and lactation-specific stimuli. Our aim is to study the relationship between serum prolactin (PRL) levels and mammary T-cell populations in early lactation in order to increase the knowledge in hormone-induced lymphocyte homing events. Female Sprague Dawley (SD) and OFA (SD derived desmoglein 4 -/- hairless phenotype with lactation deficit) rats were sacrificed at day 2 postpartum and trunk blood, mammary gland (MG) and corpora lutea (CL) were obtained to perform FACS, histological and RIA studies. Serum PRL was lower in OFA (16±3ng/ml n=12) than in SD rats (34±7ng/ml n=13; p<0.05). Mammary histology of OFA rats showed impaired development compared to SD (qualitative score, 1 to 5; SD, 4.6±0.4; OFA, 1.7±0.2; p< 0.05). FACS analysis showed lower percentage of MG CD3+ cells (from total leukocytes gating on CD45+ cells) in OFA (32±4%) compared to SD rats (43±4%; p<0.05), while CD11b+ cell levels were similar (SD, 21±1%; OFA, 19±1%). In CL the % of CD3+ cells were similar in SD and OFA rats (SD, 14±1%; OFA, 17±3%). OFA rats showed higher absolute and relative numbers of circulating CD3+ cells compared to SD (SD, 18±2% and 3121±811 cell/µl; OFA, 31±2%, 5491±358 cell/µl; p<0.05), while CD11b+ cells were similar (SD, 43±4%; OFA, 41±3%). These results show that MG T cell population may be specifically affected in early lactating OFA rats and strongly suggest that serum PRL levels may be involved in mammary homing T cell events, probably protecting the gland during lactation development.

24. Stress increases VCAM-1 expression at the maternofetal interface in an abortion prone mouse model

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Numerous mechanisms involved in abortion processes have been described locally at the fetomaternal interface and systemically, largely arising from studies employing the high fetal loss mouse model (CBA/JxDBA/2J, H-2KxH-2d). A 24hs sound stress at 5.5 gestation day (gd) increased the fetal resorption rate (FRR) of DBA/2J mated CBA/J mice but not of BALB/C mated CBA/J females (low fetal loss model, H-2KxH-2d). Sound stress increases fetal loss via inflammatory pathways. Inflammation up-regulates cell adhesion molecules, which induce cell recruitment to the site of inflammation. In this context, vascular cell adhesion molecule-1 (VCAM-1) mediates the adhesion of leukocytes to vascular endothelium after the endothelial cells have been stimulated by cytokines like TNFα Thus, we aimed to study the frequency of VCAM-1+ vessels at fetomaternal interface in stressed and non-stressed pregnant CBA/J female mice mated with DBA/2J or BALB/C males. Pregnant females were divided into control and stressed groups. The latter were exposed to sound stress at gd 5.5 during 24hs. Both groups were sacrificed on gd 6.5, implantation units were removed and cryo-sectioned. The number of vessels per tissue section was determined by PECAM immunostaining. Vessels were classified into small, medium and large according to its size. VCAM-1 expression was analyzed by immunohistochemistry in the cryosections. We ob-

served that both stressed groups had similar number of large vessels. However, stress duplicated the number of large vessels expressing VCAM-1 in the high fetal loss model ($p<0.01$), whereas a not significant increase of VCAM-1 expression was detected in the low fetal loss model. We propose that up-regulation of VCAM-1 at the fetomaternal interface in abortion prone mice contributes to the perpetuation of decidual inflammation, subsequently leading to placental damage and increased fetal loss.

25. Sound stress induces a switch in the expression of oligossacariltranferase isoforms at maternofetal interface in an abortion prone mouse model

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A 24 h sound stress at gestation day (gd) 5.5 increases the abortion rate of DBA/2J mated CBA/J mice (high fetal loss mouse model) but not of BALB/C mated CBA/J females (low fetal loss model). This fetal loss is accompanied by a decreased in protective extra-N-glycosylated antibodies and progesterone (P4) levels in serum. Oligosaccharyltransferase (OST) is responsible for the N-glycosylation of proteins. We previously demonstrated that P4 modulates OST catalytic subunit expression (STT3-A or STT3-B) in vitro with consequences on IgG N-glycosylation. In order to investigate an in vivo modulation of OST isoform expression, we analyzed STT3-A/B expression at maternofetal interface in non-stressed and stressed pregnant CBA/J female mice mated with DBA/2J or BALB/C males. Sound stress (300 Hz in intervals of 15s) was applied at gd 5.5 during 24hs. All mice were sacrificed on gd 6.5, implantation units were removed and cryosectioned. STT3-A/B expression was analyzed by immunohistochemistry and scored from 0 to 4. STT3-A and B reactivity localized preferentially in glands (G), vessels (V) and lumenal epithelium (LE) but with different expression levels of each isoform in each group. In BALB/C mating the highest STT3-A expression was found in G (3.8±0.5) and LE (2.8±0.5) while V staining was weak (0.3±0.3). In contrast, STT3-B expression was similar in every structure (G: 0.7±1.2, LE: 1.0±0.1, V: 0.5±0.5). In DBA/2J mating, similar results were observed for STT3-A expression (G: 3.8±0.5; LE: 3.3±0.9, V:1.3±0.5). However, STT3-B expression was higher in V than in the other structures (G: 1.8±0.9, LE: 1.3±05, V: 3.3±0.5) in this model. Stress increased STT3-B expression (2.3 folds) in the LE of BALB/C and DBA/2J mated females. However, in the latter, stress also decreased STT3-A (0.43 folds). We did not observe any differences in the rest of the tissue. In conclusion, stress differentially modulates the expression of STT3-A and B in the low and in the high fetal loss model. The physiological relevance of this observation is being addressed.

26. Progesterone modulates IgG N-glycosylation in vitro via two mechanisms which involve classic and membrane bound receptors

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Among the immunological effects of progesterone (P4), we reported an in vitro modulation of IgG1 N-glycosylation, which involves the differential expression of UDP-Glc-glucosyltransferase isoforms (GT1 and GT2). GT is part of the glycoprotein quality control mechanism. P4 effects are mainly mediated by intracellular receptors (PRs), which act as transcription factors. In addition, P4 may initiate rapid actions through activation of membrane receptors (mPR), which identities are under study. Considering the diverse effects of P4 doses over 112D5 hybridoma secreted IL-6 levels, IgG1 N-glycosylation, GT1/GT2 expression and total GT activity, we speculated that both types of receptors were involved. PRs were previously found in 112D5 hybridoma. In order to search mPR, cells were incubated with P4-BSA-FITC (0, 10^{-10}, 10^{-9}, 10^{-8}, 10^{-6}, 10^{-5}M) during 30 min at 4ºC and analyzed by flow cytometry. A control was performed pre-incubating cells with P4 10^{-3}M. Only cells treated with P4 10^{-5}M were positive (70±2%). In order to establish the functionality of the P4 binding molecule, we performed P4 and P4-BSA (0, 10^{-5} and 10^{-6}M) cultures and measured IgG1 glycosylation (several lectin-ELISA), GT activity (incorporation of [14C]-Glc to denatured thyroglobulin by microsomes), GT1/GT2 expression (western blot) and IL-6 (ELISA). In comparison to non treated cells, IgG1 N-glycosylation increased with P4 10^{-5}M (23±3%) but diminished with P4-BSA (40±10%). While GT1 expression was reduced with P4 and P4-BSA (50±15%), GT2 was largely increased with P4 (90±16%) and slightly increased with P4-BSA (25±10%). GT activity was induced with P4 (30±10%) and reduced with P4-BSA (30±8%). Secreted IL-6 was undetected in P4 and P4-BSA cultures. As expected, P4-BSA 10^{-6}M had no effect on the studied parameters.These results indicate that 112D5 hybridoma cells are able to express a functional mPR. P4 10^{-5}M acts through PRs and mPR in hybridoma 112D5 whereas the other P4 doses act only through the first ones.

27. Study of the expression and biological activity of matrix metalloproteases and serine proteases involved in the activation pathway of plasminogen at the mouse maternal-fetal interface

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Vol.1
No. 3:4
doi: 10:3823/413

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Repeated pregnancies increase the number of fetal cells in the maternal organism and also induce important changes in the LT subsets of thymus and bone marrow, previously reported by us. We have shown that multiparity status increases the invasive trophoblast tissue (ITT) at maternal-fetal interface in normal and abortive pregnancies. In order to study the local molecular mechanism, we previously detected an enhanced VEGF expression in ITT. Besides its pro-angiogenic function, VEGF increases the expression of metalloproteases MMP2 and MMP-9 and activates serine proteases involved in the activation pathway of plasminogen (PAI- 1, PAI-2, tPA and uPA). Moreover, plasmin cleaves VEGF bound to extracellular matrix (ECM), activates MMPs and degrades ECM. In this work we studied these proteins in our mouse multiparity model. CBA/J female mice were divided in three groups: Primiparous Young: 3.0±0.5 months old; Primiparous Old: 8.5±0.5 months old, both with a first pregnancy, and Multiparous Old: 8.5±0.5 months old with 4 pregnancies. Females were mated with BALB/c (normal crossbreeding) or DBA/2 males (abortion crossbreeding). We analyzed the placental expression of plasminogen by Western Blot (WB) and Immunohistochemistry (IHC) and observed a two fold increase in both MO groups compared to primiparous groups ($p<0.001$). Its expression was restricted to spongiotrophoblast tissue. The expression of PAI-1, PAI-2 and tPA was not modified by multiparity (WB-IHC). In agreement with the increase of plasminogen and VEGF, placental expression of MMP-9 and MMP-2 were increased (WB) only in the normal MO group ($p<0.05$). However, their activities, according to gelatin zymography studies, remained unchanged, indicating other local mechanisms of control. Multiparity upregulates the placental expression of plasminogen, suggesting that placenta represent a new place of extra-hepatic synthesis and its involvement in the trophoblast invasion process.

28. IL-2 reverses established type-1 diabetes by a local effect on pancreatic regulatory T cells

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Regulatory Tcells (Treg) play a major role in controlling the pathogenic autoimmune process in type 1 diabetes (T1D). Interleukin 2 (IL-2), a cytokine that promotes Treg survival and function, may thus have therapeutic efficacy in T1D. Here we show that 5 days of low-dose IL-2 administration starting at the time of T1D onset can reverse established disease in non obese diabetic mice, with long lasting effects. Low dose IL-2 increases the number of Treg in the pancreas and induces expression of Treg-associated proteins including Foxp3, CD25, CTLA-4, ICOS and GITR in these cells. Treatment also suppresses interferon-γ production by pancreas-infiltrating T cells. Transcriptome analyses show that low-dose IL-2 exerts much greater influence on gene expression of Treg than effector T cells, suggesting that nonspecific activation of pathogenic T cells is less likely. We also have observed that in vivo IL-2 effect on Treg and Teff transcriptomes remarkably differ in two mice strains with different susceptibility to develop autoimmunity, suggesting that an altered IL-2 response pattern could be associated with T1D physiopathology. This study, the first pre-clinical data showing that low-dose IL-2 can reverse established T1D, suggests that this treatment merits evaluation in patients with T1D.

29. Opposing effects of dehydroepiandrosterone and cortisol on the functional capacity of M. tuberculosis-stimulated human dendritic cells

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Cell mediated immunity, cytokines induced during the specific immune response and T cell populations are crucial factors for containing M. tuberculosis (Mtb) infection. Recent reports suggest a cross-regulation between adrenal steroids (glucocorticoids an dehidroepindrosterone, DHEA) and antigen-presenting cells (APC) function. Therefore, we investigated the role of adrenal hormones on the Mtb- induced dendritic cell's (DC) functional capacity. To achieve this, we isolated monocytes from healthy donor's peripheral blood by percoll gradients, differentiated them to DC by culturing monocytes with IL-4 and GM-CSF. Afterwards, we assessed i) the phenotype of DC after inducing their maturation overnight with Mtb in the presence or absence of cortisol (10^{-6}M) or DHEA (10^{-7}M) alone or in combination by flow cytometry and ii) the cytokines (IL-12, IL-10, TNFα) produced by DC after the mentioned culture conditions by ELISA. We observed, as expected, a significant increase on the expression of MHC-I, MHC-II, CD86 and CD83 ($p< 0.05$) and the production of IL-12, TNFα ($p< 0.05$) and IL-10 ($p<0.0001$), after Mtb-stimulation. Cortisol treatment significantly inhibited the above mentioned Mtb-induced DC's functions. Interestingly, the presence of DHEA during the culture period enhanced the Mtb-induced expression of MHC I ($p< 0.05$), MHC II ($p< 0.05$), and CD86 ($p<0.05$), without affecting CD83 expression. Moreover, DHEA improved the production of IL-12 in response to Mtb stimulation ($p<0.05$), diminished the IL-10 secretion ($p<0.05$), and could not modify TNFα synthesis. We could not observe a reversion of cortisol's inhibitory effects by DHEA treatment. These data shows for the first time the rele-

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vance of the adrenal axis (especially of DHEA) in the modulation of DC function in the context of tuberculosis, a disease were the induction of a Th1 environment by APC is crucial for the development of an effective immune response to the mycobacteria.

30. Lack of galectin-3 disturbs lymphoid organs homeostasis in the course of Schistosoma mansoni infection leading to disruption of the architecture of B cell compartments

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Galectin-3 (Gal-3) is a β-galactoside binding protein that regulates distinct cell functions. Recently, we showed that Gal-3 controls B cell differentiation in the bone marrow, spleen, mesenteric lymph nodes (MLNs) and peritoneal cavity. Here, we have investigated the role of Gal-3 driving the histological organization of lymphoid organs and its possible influence in the immune response in mice challenged with Schistosoma mansoni. Male C57/bl6 wild type (WT) and Gal-3 knock out (Gal-$3^{-/-}$) mice were infected with S.mansoni and studied 90-95 days pos-infection, during chronic phase of the disease. Histological analysis of lymphoid organs were performed by H&E and immunohistochemistry. Phenotypes were characterized by flow citometry. Propidium iodide was used to DNA-content analysis and mitotic events were defined by microscopy. Institutional guidelines: DAHEICB 009, UFRJ. Gal-$3^{-/-}$ infected mice showed significant histological changes in the lymphoid organs, where lymphoid and myeloid compartments were drastically disturbed. There were high number of lymphoid follicules, exarcebated plasmacytogenesis, disorganized cell cycle and mitotic figures significantly increased in MLNs. Moreover, B220+, CD138+ and Blimp-1+ cells were randomly scattered by the parenchyma, and macrophages were significantly reduced in the spleen and MLNs, where phagocytes were hyporesponsives, lymphocytes more susceptible to pro-apoptotic signals and apoptotic bodies were more evidents. We showed that Gal-3 interferes with histological architecture of lymphoid tissues. Gal-$3^{-/-}$ infected mice had an accelarated B cell differentiation into plasma cells and a delayed monocyte differentiation into macrophages with disturbed cell functions. Thus, we suggested that this molecule regulates the interface of innate and adaptive immunity, controlling cellular events and histological niches.

31. Increased efficiency to perform automated skin fibroblast cell survival assays in 96-well plates: novel applications to detect radiosensitive T–B– severe combined immunodeficiency patients (RS-SCID)

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V(D)J rearrangement in lymphoid cells involves repair of double-strand breaks (DSBs) through nonhomologous end joining (NHEJ). Defects in this process lead to increased radiosensitivity and severe combined immunodeficiency (RS-SCID). The clonogenic survival assay is the gold standard for measuring sensitivity to ionizing irradiation or toward other DSBs-inducing agents like bleomycin (BLM) or etoposide (VP16). Such experiments test at least four different doses of radiation and four different doses of each drug in quadruplicate. Then, in its traditional 10 cm dishes format, this would result in using more than 60 individual plates. This is a time-consuming assay when counting the number of colonies manually under the microscope and a cell-consuming assay due the high number of skin fibroblast required. In the interest of having an efficient cell survival assay in our laboratory to detect RS-SCID we implement a 96-well format assay using an automated cell counting algorithm. Fibroblasts were trypsinized and seeded in quadruplicate into 96-well plates. The cells were allowed to attach overnight and then exposed to radiation (0–6 Gy) or chemical treatment for 24 h (BLM/VP16) at the corresponding concentration. Cells were then incubated for 14 days before they were fixed with 3 % formaldehyde, and stained with 0.25 μM Hoescht 33342. Afterwards, the wells were scanned (30 fields/well) at 10X objective using the INCell Analyzer 1000 (GE) and nuclei were recognized by an algorithm setup on the Multi Target Analysis Module for survival assessment.This novel approach constitutes an efficient screening within the SCID patients lacking T and B cells. This strategy allows identification of RS-SCID differentiating them from RAG-deficient patients. Furthermore it opens the potential application in other radiosensitive conditions such as Nijmegen breakage syndrome or ataxia telangiectasia

32. Evaluation of a Duplex ELISA assay for diagnosis, prognosis and treatment monitoring of Chagas disease patients

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In indeterminate and chronic forms of Chagas disease, current diagnosis is based on the presence of antibodies in the serum using at least two serological assays. However, more rapid tests for diagnosis, disease progression and treatment monitoring.

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doi: 10:3823/413

Accordingly, the aim of this work was to investigate the potential usefulness of T. cruzi whole lysate, recombinant protein JL7, and peptides P013, R13, JL18, JL19, P0β and TcHSP70 as serological markers for human Chagas disease. In this study, we analyzed 228 serum samples from Chagas disease patients classified into four groups according to clinical and conventional serological parameters, and 114 from non chagasic patients including other infectious or autoimmune diseases. We defined the diagnostic sensitivity, specificity, Kappa index, positive predictive value (PPV) and receiver-operating characteristics (ROC) curve of tested substances measured by ELISA. The highest values of diagnostic parameters were achieved for the combined use of T. cruzi lysate and JL7, showing a sensitivity of 99.6% and specificity of 100% with a Kappa index of 0.99. By using the ROC curve to find restricted cut-off values, we observed that the PPV of T. cruzi lysate/JL7 to discriminate between indeterminate and severe Chagas disease was 70.6%, with a sensitivity of 44.4% and a specificity of 82.2%. In addition, JL7 might provide a good marker for monitoring drug treatment efficacy, since 58.3% (7/12 patients) presented a regression in specific antibody levels to this antigen after 24 months after treatment with benznidazole. In conclusion, our results suggest the potential of T. cruzi lysate combined with JL7 for diagnosis of Chagas disease, further providing a prognosis tool for determining clinical progression to cardiac disease, and the potential of JL7 as a biomarker for treatment evaluation.

33. Relative distribution of specific anti-FVIII IgG subclasses in patients with Severe Hemophilia A. Flow Cytometry evaluation and Clinical relevance

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About 25% of patients with Severe Haemophilia A (SHA) develop neutralizing antibodies (I-Ab) against FVIII administered as therapy. Antibodies are also developed against epitopes not associated with FVIII activity. Specific immunoglobulins result from a polyclonal response to FVIII and are predominantly IgG. Previous studies have demonstrated an association between the presence of IgG4 and the failures of the Immunotolerant Treatment Induction (ITI), therapy employed for I-Ab erradication. Here we studied the relative distribution of IgG subclasses of anti-FVIII in 24 SHA patients with I-Ab by Bethesda assay (group 1: 0.5-10 BU/ml, n=11); (group 2: 10-8300 BU/ml, n=13) and in 5 patients during the ITI. We employed a procedure, set up in our laboratory, which combines Flow Cytometry with microspheres coupled with FVIII (FVIII-m) or in Buffer (Control-m). Plasmas were diluted according to the level of total IgG, determined previously, and incubated 2 h at 4ºC with Control and FVIII-m. Antibodies were revealed using biotynilated anti-IgG1, IgG2, IgG3 and IgG4 followed by PECy5-streptavidin. Results showed a predominance of IgG4 in 9/11 patients of group 2 (41.4-89.17%) compared with 2/13 patients of group 1. IgG2 and IgG3 were predominant in group 1. A significant correlation was observed between I-Ab level and IgG2 in group 1 (r=0.6, p=0.02) and IgG4 in group 2 (r=0.71, p=0.005). A patient belonging to Group 1 increased IgG4 percentage from 32.6% to 88.3% when he passed to Group 2. Longitudinal analysis of the patients undergoing ITI showed a gradual decrease of IgG4 percentages that was associated with an improvement of clinical parameters during the treatment. FC method was useful in the evaluation of the distribution of anti-FVIII IgG subclasses. Our results support the notion that a complete monitoring of Severe Hemophilia A patients should include the IgG subclass analysis especially during the ITI.

34. Protons trigger IL-1β production in human monocytes.

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Interstitial acidification (pH5.5-7.0) is a common feature associated with the course of many inflammatory reactions in peripheral tissues. Autoimmune diseases such as rheumatoid arthritis and asthma, as well as the growth of solid tumors are also associated with the development of acidic microenvironments. We have previously reported that extracellular acidosis induces the activation of neutrophils and dendritic cells, supporting the idea that low pH can be recognized by immune cells as a danger signal favouring the initiation of immune responses. Here, we studied the effect of low extracellular pH (pHe) on monocyte function by incubating cells with an isotonic hydrogen chloride solution to allow an pHe of 6.5. We analyzed phenotype and cytokine secretion, in particular IL-1β production which was assessed by ELISA, flow cytometry and confocal microscopy. We observed that low pHe did not induce changes in the expression of CD40, CD86 and HLA-DR. However, cells exposed to pH 6.5 secreted higher levels of IL-1β compared with controls (pH 6.5: 174pg/ml±42 vs. pH 7.3: 82pg/ml±19; n=25; p<0.05). Furthermore, by intracellular staining we detected higher expression of IL-1β in monocytes exposed to pH 6.5 (p<0.05; n=3) (by flow cytometry and confocal microscopy). The increase in IL-1β production induced by extracellular acidosis was found to be associated with a fall in intracellular pH, as assessed by flow cytometry: pH 6.5-treated-cells: 6.8±0.07 vs. resting cells: 7.18±0.03; n=10, p<0.0005). Moreover, we also found that the treatment of monocytes with cycloheximide prevented the rise in IL-1β levels in response to pH 6.5 (p<0.05; n=3), supporting the idea that stimulation of IL-1β production is related to an increased synthesis of the IL-1β precursor: pro-IL-1β. Our results suggest that stimulation of IL-1β production in human monocytes ap-

pears to represent a novel mechanism through which extracellular acidosis contributes to the development of inflammatory processes.

35. Evaluation of hyaluronic acid in bronchoalveolar lavage of patientes with interstitial lung disease

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Interstitial lung disease (ILD) is a heterogeneous group of parenchymal lung diseases with non infectious origin. The lack of uniformity in diagnosis has led to widely varying results in terms of morbidity and mortality. Hyaluronic acid (HA) is one of the main components of the extracellular matrix. Its function varies according to the size. Under physiological conditions HA has a high molecular weigh (HMW-HA) which possesses mainly homeostatic functions. In inflammatory diseases it can also be found as fragments with low MW (LMW-HA). Recent reports have shown that LMW-HA acts as a damage-associated molecular pattern (DAMP). We hypothesise that ILD present alterations in HA turnover, resulting in an increase of bioactive HA fragments that exacerbate inflammation and promote changes in the structure of lung parenchyma. The aim of this work was to assess the concentration of HA in serum and bronchoalveolar lavage (BAL) from patients with ILD and determine the role of HA as a chemotactic molecule for BAL cells. Samples of patients with presumptive ILD were taken by bronchoscopy to confirm diagnosis. As controls we used BAL samples from people with healthy lungs, after surgery for tracheal stenosis. HA concentration was determined by ELISA using a HA-binding protein. HA levels were significantly increased in BAL samples of IDL patients compared to controls, (2037±171 vs 583±57) ng/ml ($p \leq 0.05$). However HA levels in serum has not shown significant differences with the control. Chemotaxis of BAL cells towards HMW-HA and LMW-HA were evaluated and the results expressed as migration index (MI). Patient and control cells showed an enhanced migration to LMW-HA, (1.95 ±0.19 and 1.49±0.05) vs migration to HMW-AH (1.02±0.16 and 1.05±0.05) respectively ($p \leq 0.05$). Our results showed a significant increase of HA levels in BAL samples from patients with ILD. Therefore HA measurement would be used as a new biomarker for diagnosis of these diseases.

36. Hyaluronan oligomers sensitize human leukemia cell lines to the effect of Vincristine

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Hyaluronan (HA) is a glycosaminoglycan constituted by repetitive units of N-acetyl-glucosamine and glucuronic acid. By enzymatic digestion of HA it is possible to obtain fragments between 2 to 7 disaccharides called hyaluronan oligomers (oHA) that are able to block HA action. Several tumors produce high levels of HA which promote survival. This effect has been associated to deregulation of survival pathways, such as PI3K/Akt and MAPK. The aim of this work was to determine if oHA sensitize a human resistant cell line to the effect of Vincristine (VCR) and if Pgp mediates this mechanism. For this purpose, we used both K562 (sensitive) and Kv562 (VCR resistant) cell lines. We analyzed the role of oHA and VCR on K562 and Kv562 cell proliferation showing that oHA inhibited 20% and VCR 90% K562 cell proliferation after 72h of treatment ($p<0.05$ and $p<0.01$ respectively). No effect was observed for any treatment on Kv562. We also studied the effect of the combination of oHA with VCR to determine if oHA were able to sensitize Kv562 to the effect of VCR. Cells were co-incubated with oHA (100-300mg/ml) plus VCR (0.5-1mM) for 48-72h. We showed that oHA 300mg/ml + VCR 1mM inhibited 30% cell proliferation ($p<0.01$) after 48h and 72h treatment, only at 72h oHA 100mg/ml + VCR 1mM inhibited 25% Kv562 proliferation. To analyze if PI3K/Akt and MAPK pathways were implicated, we assessed by western blot the level of those proteins. We found that oHA decreased pAkt/Akt ratio on both K562 and Kv562 and decreased pERK/ERK ratio on K562 ($p<0.01$). Finally, we analyzed if oHA were able to inhibit Pgp. By flow cytometry we observed that oHA inhibited 20% daunorubicine extrusion. This effect was abolished by addition of an antibody against CD44. We conclude that oHA could sensitize Kv562 cells to the effect of VCR through Pgp inhibition and PI3K/Akt modulation. These findings highlighted the potential use of oHA as a coadjuvant for treating resistant leukemia.

37. Analysis of the concentration of endothelial microparticles in patients with venous thromboembolism

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Microparticles (MPs) are plasma membrane fragments released into the extracellular space by endothelial cells, platelets, monocytes, neutrophils, etc. MPs spread the procoagulant activity and promote cell adhesion at distant sites from the cell that originate them, thus contributing to the prothrombotic and procoagulant tendency. MPs harbouring endothelial-associated molecules are called endothelial-derived MPs. Herein we determined MPs in plasma from patients (25-65 age) with venous thromboembolism and we compared the levels of MPs with healthy people of similar age (control group). Samples from patients (n= 47) and control (n= 20) groups were ob-

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doi: 10:3823/413

tained by venipuncture into tubes containing sodium citrate and centrifuged for 15 min at 3000 rpm. Supernatants were removed and centrifugated at 13000 rpm for 1 minute. After this step, supernatants were removed and discarded. Pellets containing MPs were labeled with monoclonal antibodies against two specific markers of endotelial MPs: the adhesion molecule E-selectin (CD62E) and the integrin αVβ3 (CD51), the receptor for vitronectin; samples were processed by flow cytometry and results expressed as percentage of positive MPs. We found that patients exhibited 55±12% and control group 56± 1% of positive E-selectin MPs (p=0.497). Interestingly, after labeling with anti Vitronectin antibody, patients showed 87 +/- 8% of positive MPs while in controls the value was significantly lower (59±6%) (p = 0.0143). The percentage of MPs that express the adhesion molecules specific for mature endothelial cells CD62E was similar. The selective increment in Vitronectin positive MPs in patients with venous thromboembolism could be related to the pathogenesis of the disease, as increased numbers of MPs are indicative of severe inflammatory disorders, vascular injury, angiogenesis, and thrombosis.

38. Concentration of IL-6, IL-12 and IFNγ in serum and placental extracts during porcine gestation

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Mammalian pregnancy requires coordinated interactions between conceptus and gestating endometrium which involve several hormones, growth factors and cytokines that act through specific receptors. Sows have an epitheliochorial, diffuse, adecidua, folded and non-invasive placenta. It is a natural target of the immune system because of its location as a barrier between mother and embryos. The aim of this study was to investigate the development of pig pregnancy through the expression of IL-6, IL-12 and IFNγ in sera and in porcine placental extracts of different gestational periods. Pig placentas and sera were collected (n=40) at 5, 15, 20, 32, 44, 51, 60, 70, 80, 90, 114 Days of pregnancy (at term). The placenta were carefully separated in fetal and maternal portions to form placental extracts called homogenates of fetal placenta (HoFP) and maternal placenta (HoMP). Determination of cytokines by a commercial ELISA kit is carried out. Values found on day 32 of pregnancy in HoFP from IL-6 (878.29 pg/ml), IL-12 (203.90 pg/ml) and IFNγ (505.024 pg/ml) in relation to those dosed in serum ($p<0.001$) and in homogenates of maternal placenta ($p<0.05$) for each cytokine are emphasized. IL-6 is expressed at fixed peaks on day 32 in HoFP and on day 90 in serum decreasing then to basal values at farrowing. IL-12 was found only in high concentrations in HoFP between day 32 and day 44 of pregnancy (228.25pg/ml). From the previous cytokines only IFNγ showed a significant peak on day 20 of pregnancy in HoMP (567.43 pg/ml) and another on day 32 in HoFP. In conclusion, it is underlined the presence of IFNγ at the feto-maternal interface between day 20 and day 32 of pregnancy. It is emphasized a high concentration of the three cytokines on day 32 of pregnancy in feto-placental extracts that agrees with the start point of ossification and development of the feto-immunological system inherent of a successful pregnancy.

39. Expression of the inhibitory receptor CD94/NKG2A allows the identification of different subpopulations of NKT cells

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We have previously analyzed the genotype frequency of KIR genes in a Caucasian and Amerindian control population that were then compared with their frequency in two conditions where NK cells participate, like HCV infection and pregnancy. Because of their random expression, we decided to investigate cell surface expression of KIR and CD94/NKG2A in a control population of NK and NKT cells, by using a wide panel of antibodies directed against NK receptors and examined by flow citometry. We found in peripheral blood a mean frequency of NK cells of 11.05±1 of which NKbright, that seems to represent precursors of NKdim cells, represented 0.68% of total NK cells. Moreover, we identified a frequency of 5% of NKT cells. In concordance with NK cells, NKT cells also expressed, although at lower frequency, KIR genes. In contrast with NK cells, NKT cells did not show cytotoxic activity against the target cell K562. In a cohort of 28 healthy individuals, we were able to differentiate two subpopulations of NKT cells. All 28 individuals expressed the CD3low subset, characterized by a low expression of the CD94/NKG2A heterodimeric receptor (means: CD94 49.7±4.3 and NKG2A 14.1±1.9). Sixteen of them also expressed a CD3high NKT cells subset, which shows a high expression of the inhibitory CD94/NKG2A heterodimeric receptor (means: CD94 85.2±2.1 and NKG2A 53.4±4.3, $p<0.0001$). Because NKT CD3low is present in all individuals, we assumed that represent the precursor of the CD3high subset, which after maturation acquire the highest expression of the inhibitory receptor CD94/NKG2A. This represents the first report showing a phenotypic heterogeneity of NKT cells whose functionally implications are under study.

40. Anti-ManLAM antibodies circulating in Multi Drug Resistant (MDR) and Drug-sensitive tuberculosis (TB) patients exert differential effect on fagocytosis and costimulatory potential of Dendritic Cells

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doi: 10:3823/413

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Mannosilated lipoarabinomannan (ManLAM) is an abundant cell wall glycolipid of pathogenic mycobacteria exerting its immunomodulatory effect through recognition by innate immune receptors of host cells. Anti-ManLAM antibodies (αManLAM Abs) have been detected in serum from tuberculosis patients (TB) but its role in physiopathology remains unknown.
To address the role of αManLAM Abs on dendritic cell (DC) function and factors/mechanisms involved, αManLAM Abs were characterized in serum samples from 20 MDR and 20 Drug-sensitive TB patients and 17 healthy donors (N), thereafter, serum subgroups were employed to perform bioassays on monocytes derived DC from N. Immunochemical characterization showed heterogeneous αManLAM response in terms of serum titers (ELISA), epitope chemistry (competition ELISA) and cell wall epitope-display (bacterial based ELISA) with no differences among patients, but above N ($p<0.01$). Isotypic analysis of αManLAM Abs were performed by FACS on M.tuberculosis 6006 local strain (Mtb-6006) opsonized with pre-absorbed serum on plate bound ManLAM (αManLAM depleted) and staining with antihuman-IgA, -IgE, -IgG and -IgM 2º Abs. Although isotypes showed high variability, IgG and IgM αManLAM Abs were inversely correlated between individuals ($r=-0.39$; $p<0.05$). FITC-conjugated bacteria (Mtb-6006-FITC) opsonized with total or αManLAM Abs depleted sera were used in DC mediated fagocytosis. αManLAM depletion inhibited fagocytosis in those sera with high IgG/IgM ratio, but in low IgG/IgM sera fagocytosis was favored or not affect. Finally, CD86/CD83 (stimulatory) vs PD-L1/PD-L2 (inhibitory) molecules were evaluated on DC matured with Mtb-6006 opsonized with total or αManLAM depleted sera. A strong increase in PD-L1/PD-L2 expression was observed by serum pretreatment only in those sera with high IgG/IgM ratio ($p<0.01$), whereas CD86/CD83 remain unaffected. Taken together, this data suggest an isotype dependent mechanism, presumably mediated by Fc receptors.

41. Production of IL-10 in serum and peripheral blood mononuclear cells of atopic asthmatic pediatric patients

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It has been described that asthma is related to defects in the regulation of the immune response and that IL-10 is a regulatory cytokine involved in the physiopathology of this disease. In order to determine if this cytokine is involved in the response to different bacteria, we studied asthmatic pediatric patients at the time of crisis (Cr) and of stability (St), and healthy controls (He). The concentration of IL-10 was evaluated in supernatants (SN) of peripheral blood mononuclear cells (PBMC) cultured for 48 h or 96 h, with or without stimulation with Streptococcus pneumoniae (Sp), responsible for most of the respiratory bacterial infections in these patients, or with Mycobacterium tuberculosis (Mt), that provokes a respiratory infection but does not affect asthmatic individuals more than healthy ones. IL-10 concentration was also evaluated in serum. All determinations were performed by ELISA. Results: the IL-10 (pg/ml, x+SEM) in SNs was: He: C 13+6; Sp 181+55; Mt 102+24 (n=10); St: C 21+4; Sp 170+49; Mt 141+42 (n=7); Cr C 17+7; Sp 175+28; Mt 187+40 (n=5). In the Sera: He:13+4 (n=10); St:15+5 (n=11); Cr:20+6 (n=11). The concentration of IL-10 in supernatants obtained by culturing for 96 h yielded similar results. No differences were observed in the response of patients at times of crisis and stability. No significant differences were observed in the response to the two bacterial antigens tested, although a slight tendency to a higher response to Sp can be observed in He and St, which is not observed in Cr. These data suggest that IL-10 would not be involved in the response to bacteria, at least to the ones studied. Moreover, no significant differences were observed in the serum IL-10 concentration of the different groups studied. This suggests that serum IL-10 concentration would not be a useful parameter for the clinical control of asthmatic patients.

42. Characteristics of peripheral blood mononuclear cells in patients with specific antibody deficiency

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Patients with specific anti-polysaccharide antibody deficiency (SPAD) suffer frequent infections, 42% of which are pneumonia mostly caused by Streptococcus pneumoniae, which also provokes most of the bacterial respiratory infections in asthma patients (A). B cell abnormalities have been reported in SPAD patients (P). Here we study some cell populations related to innate immunity and activation parameters in order to determine if there are other abnormalities in these patients. Evaluation was performed by flow cytometry in peripheral blood mononuclear cells (PBMC). x+SEM is given. Monocytes play an important role in microbial defense. Two major types of monocytes, CD14+CD16- and CD14+CD16+, have been described and the latter is considered proinflammatory. We evaluated the expression of CD14 and CD16 in PBMC of healthy controls (C) and P. % CD14+CD16+: C 6.6+0.8 (n=5); P: 20.8+6.6 (n=5), data significantly different, $p= 0.0317$. Gammadelta T cells (γδ) have several innate cell-like features and regulate pathogen clearance. We previously detected a significantly lower γδ % in A than in C. Since P share some characteristics with A, we decided to evaluate γδ in their PBMC. γδ %: C: 7.9+1.4 (n=14); P: 2.5+0.6 (n=5). This difference was significant, $p=0.0182$. Considering the association of CD14+CD16+ monocytes and γδ to inflammation, we analyzed

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activation markers in the lymphocyte region of no stimulated PBMC. The early activation marker CD69 showed no differences, CD69% C: 2.9+0.6 (n=15); P: 2.7+0.4 (n=5), but the late activation marker CD25 had a lower expression in P when compared to C. CD25% C: 6.9+1.8 (n=9); P: 3.5+0.8 (n=5), although this difference was not significant. We have detected differences in cell populations other than B cells of SPAD patients: a significant increase in CD14+CD16+ monocytes, for which a crucial role in inflammation and infectious disease in man has been suggested, and a decrease in γδ T cells with respect to normal controls.

43. Different presentation of Autoimmune Hepatitis in IPEX syndrome

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IPEX (Immune, Poliendocrinopathy, Enteropathy, X linked) syndrome is a rare immunodeficiency caused by mutations in Foxp3. Symptoms included autoimmune enteropathy, early onset type 1 diabetes mellitus (TIDM) and dermatitis. We report two IPEX cases diagnosed in our Center with different clinical onsets and outcomes. Case1: Boy born from healthy parents, without family history of immunodeficiency, developed neonatal TIDM. Diarrhea began at 2 years old diagnosed as celiac disease. He was referred to our Unit at 4 years old with fulminant hepatitis (no A B) for hepatic transplantation. In the laboratory findings Coombs positive hemolytic anemia, ASMA autoantibodies, elevated IgG and IgA were observed. IPEX was suspected clinically and was treated with corticosteroids and antithymocyte globulin. He died without response. Punctual mutation (p.F367L) on FoxpP3 confirmed the diagnoses. Case2: Second boy of two siblings born from healthy parents. At 2 month old developed eczema. Chronic diarrhea and failure to thrive began during the second year of age; duodenal biopsy showed eosinophilic infiltration in the lamina propria and villous atrophy. At 4 years old he presented eosinophilia, autoimmune hepatitis (LKM+ autoantibodies) and Coombs positive hemolityc anemia and begun corticosteroids treatment. Insulin dependent diabetes mellitus was diagnosed at 5 years old. He received azatioprime and prednisone as an immunosuppressant during 18 months with good response but after that he developed diarrhea caused by microsporidium, thoracic herpes zoster and two pneumonias. The patient underwent bone marrow transplantation from his HLA matched sibling at 7 years old with reduced intensity conditioning. Actually his on +60 day post transplant. Although hepatic involvement is less common than other autoimmune phenomena on IPEX, our two patients had autoimmune hepatitis with different response to immunosuppressive treatment.

44. Role of Nitric Oxide Synthases in Elastase-Induced Emphysema

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Inducible nitric oxide synthase (iNOS) is overexpressed in the human emphysematous lung. iNOS produces nitric oxide (NO) that in combination with superoxide generates peroxynitrites and induces protein nitration. Although iNOS is a well known pro-inflammatory molecule, its role in this disease is unknown. The aim of this study was to determine whether iNOS contributes to the development of elastase-induced emphysema in mice. iNOS -/- and eNOS -/- mice and mice treated with a pharmacological iNOS inhibitor were intratracheally exposed to elastase. Expression of iNOS, eNOS and inflammatory mediators was evaluated at the protein and mRNA levels. Emphysema was quantified morphometrically. iNOS and eNOS were diffusely upregulated in the lung of elastase-treated mice and a 12-fold increase in the number of 3-nitrotyrosine-expressing cells was observed (80% of alveolar type 2 cells, 20 % macrophages). In elastase-instilled mice, iNOS inactivation reduced protein nitration and increased protein oxidation but had no effect on inflammation, MMP activity and the subsequent development of emphysema. eNOS inactivation had no effect on inflammation and emphysema. In conclusion, in the elastase-injured lung, iNOS mediates protein nitration in alveolar type 2 cells and alleviates oxidative injury. iNOS are not required for the development of elastase-induced inflammation and emphysema.

45. Experimental error control during immune monitoring by flow cytometry of positive reactors to bovine tuberculin skin test

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Flow cytometry (FC) has become the method of choice for studying the immune response to infectious agents. The development of an extensive set of monoclonal antibodies (MAb) to bovine leukocyte differentiation molecules has now made it possible immune response characterization in bovine tuberculosis (Tbc). Before extending these studies it is essential to establish results consistency. Currently indirect labeling (IL) is used in single and multiparameter analysis. This introduces the probability of ex-

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perimental error because of the multiple processing steps needed. The objective of this study was to develop and test a strategy for assuring the reliability of methods for processing peripheral blood mononuclear cells (PBMC) from Tbc positive cattle. PBMC were obtained from 10 Tbc positive cows by density gradient centrifugation (Histopaque1077). Each sample was labeled with four MAb cocktails (antiCD4-IgM/antiCD25-IgG1, antiCD4/antiCD45Ro-IgG, antiCD8-IgM/antiCD25, antiCD8/antiCD45Ro), then they were washed and labeled with antiIgG1-PE/anti-IgM-FITC cocktail. Finally PBMC were fixed, stored and 10000 events were acquired with cytometer FACS-CANTO (Becton-Dickinson). Data were analyzed with FCS Express trial version. A dot plot side light scatter (SSc) vs forward light scatter (FSc) was set to define the mononuclear cell population. Then a dot-plot FSc vs. fluorescence distinguished between unlabeled and labeled cells. Finally a histogram is generated to compare geometric means of fluorescence intensity (GMFs). SAS v 9.2 calculated the paired t test between duplicate GMFs. The variation in labeling between paired samples was very low, p value of paired t test>0.05 in all samples, showing that reliable results can be obtained with minimal differences introduced during sample preparation. When in FC IL is routine, statistical comparison of results from repetitions of the same sample labeled with the same MAb would be strategic as an experimental error control.

46. Molecular Strategies to Detect Gross Gene Lesions in Primary Immunodeficiency (PID) Genes

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The majority of gene disruptions underlying human inherited diseases are microlesions and small deletions (DEL) or insertions (INS), gross lesions are less common, with gross DEL being most frequently found (6%). BTK, CYBB, SH2D1A and Artemis genes showed gross lesions with greatly different incidence (7%, 12.5%, 27.9% and 56% of disease-causing mutations, respectively). In this work, we show molecular strategies to detect and to confirm gross lesions. We studied 2 XLA, 2 CGD-X, 1 XLP and 1 Artemis-SCID patients. The genes were screened by SSCP or RT-PCR, followed by sequencing. When a gross lesion was suspected, additional methodologies were incorporated: PCRs for large gene segments (up to 4 kb), Long Expand PCR for fragments longer than 5 kb and SNP haplotype analysis. In 2 CGD-X patients, that failed to amplify CYBB single exons, PCR for large gene segment showed a 2.3 kb DEL and a 780 bp complex INS-DEL in each other. Screening the BTK gene by using RT-PCR, showed in 2 patients abnormal size fragments, containing each other a DEL and a duplication of exons 2 and 3. These lesions could result from Alu element-induced unequal homologous recombination. A homozygous mutation in Artemis heterozygously carried by her father but not by her mother, suggested DEL inside the maternal allele. By SNP haplotype evaluation we tried to define the size of this DEL, likely arosing from mispairing between 2 highly homologous sequences. In 2 XLP brothers that failed to amplify exon 3 in SH2D1A, Long Expand PCR allowed to counsel their mother, carrier of a 797bp DEL. Once located the deletion breakpoints, specific primers were designed, to perform an accurate genetic counseling for this family. These patients clearly show the importance of having more than one screening strategy for an efficient diagnosis and family genetic counseling. It is also very important to be attentive to the genes having higher frequency of gross lesions to incorporate appropriate study methodologies.

47. Development and validation of an anti-p16 monoclonal antibody for the detection of high risk HPV-associated lesions in cervical biopsies

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Human papillomavirus (HPV) infection is an essential factor for the development of cervical lesions that may lead to cancer. Cervical cancer is an important public health problem in developing countries. However, the diagnosis of HPV is currently based on microscopic observation of the cervix and morphological analysis of lesions in endocervical smears and biopsies, being these methods highly dependent on the criteria and training of the observer. As HPV oncoproteins induce cell cycle deregulation, proteins of the cell cycle may be used as indicators of HPV infection. These proteins may be used in immunochemical techniques to help in the diagnosis of cervical neoplasia. Here, we aimed to develop monoclonal antibodies (mAbs) to proteins that are known to be surrogate markers of high risk HPV-associated neoplasias. p16 is a cyclin dependent kinase inhibitor regulated by retinoblastoma protein (pRb). In high risk HPV-related cervical lesions there is a functional inactivation of pRb by HPV E7 oncoprotein, leading to p16 upregulation. We expressed and purified recombinant p16 protein and produced mAbs using standard technique. From the panel of mAbs obtained, we characterized one anti-p16 mAb that specifically recognized endogenous p16 protein in HPV cancer cell lines, in agreement with reported data, as judged by enzyme-linked immunoassays and Western blots. Importantly, in conventional immunohistochemistry our mAb specifically immunostained paraffin-embedded sections of cervical cancer biopsies, HPV positive, and was not reactive in normal cervical epithelium, in correlation with a commercial anti-p16 mAb immunostaining used as control. Our results suggest that our anti-p16 mAb has high sensitivity for immunohistochemical detection on high risk HPV-related neoplasias, constituting a useful tool to improve diagnostic accuracy at low cost.

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doi: 10:3823/413

48. Hepatic mononuclear cells (HMC) and NKT cells subpopulation involved in the immune response to candida albicans and in the abrogation of intrahepatic tolerance

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The liver is an organ that favours the induction of tolerance rather than the induction of immunity. Here we explored the contribution of total HMC and the NKT cell subpopulation in the balance of inflammatory/antiinflamatory mediators at local level during C.albicans primary infection and NKT cells activation. To this end, HMC purified from control (N) and infected (Ca) animal (day 1), were incubated 24h with or without the fungus(1:1). C.albicans primary infection clearly induces INFγ production by HMC (25.5±6.4 fold increase vs N-HMC production, $p<0.05$) while the release of tolerogenic cytokines such as IL-10 (302±32 pg/ml) and TGFβ (158±23 pg/ml) remained unmodified. After a second in vitro contact with the fungus, the HMC showed an increase in INFγ production (62±6.5 fold increase vs N-HMC production, $p<0.05$) and no significant changes in the production of tolerogenic cytokines. NKT cells play a key role in the regulation of infectious diseases due to its ability to produce INFγ and/or IL-4. NKT cells purified from infected livers (cell sorting) were able to release higher concentrations of INFγ (100±10pg/mL, $p<0.05$) and very low levels of IL-4 (1.2±0.2pg/mL). Moreover, after in vitro reexposure to the pathogen, the INFγ production increased significantly (340±15pg/mL, $p<0.05$), while IL-4 concentration was unmodified. When intrahepatic NKT cells sorted out of uninfected controls were cultured in the absence of any stimuli, we detected very low levels of IL-4 and absence of INFγ production. The basal production of these cytokines was not affected after the challenge in vitro with the fungus, suggesting the need of antigen presenting cells (APC) in NKT cells activation. Co-cultures of the fungus with CD11b/c-depleted N-HMC cells confirmed these results. These findings show that C.albicans hepatic colonization breaks liver tolerance, with the direct involvement of NKT cells. The presence of local APC could be necessary for the activation of these lymphocytes.

49. Granulomatous lymphocytic interstitial lung disease in Common Variable Immunedeficiency in pediatrics population

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Common Variable Immunodeficiency (CVID) is a primary antibody deficiency characterized by recurrent bacterial infections particularly in respiratory tract prone to bronchiectasis. Granulomatous-lymphocytic interstitial lung disease (GLILD) is a rare complication in this illness. We describe the experience in our Unit about patient diagnosed CVID with this pulmonary entity. Out of 41 patients diagnosed CVID, 4 (3 female, 1 male) exhibited histopathologic patterns as GLILD (9.7%). In one patient GLILD was diagnosed concomitantly CVID, the remainder was made later under gammaglobulin replacement (2 received irregular infusion). Cough was a persistent symptom in all patients. Clubbing was found in only one patient. Interstitial diffuse pattern on chest radiography was found in all cases. Nodular lesions were observed in two cases, the remainder bronchoalveolar involvement, ground glass opacification and mediastinal lymphadenopathies were the findings in tomography. Pulmonary function test showed restrictive pattern in 2 patients, obstructive and mixed pattern in two others respectively. Low diffusion lung CO was found in two patients that were evaluated. All exhibited lymphocytic interstitial pneumonia as histological pattern; T cells were predominantly in two cases and a mixture T and B cells in another patient. All have recurrent tract infections previous diagnoses of GLILD. Splenomegaly and autoimmune cytopenias were documented in two patients and 3 had persistent ganglionar hyperplasia. Three patients improved with oral metilprednisone 2mg/kg/day, one of them relapsed with response to a second steroids set, the fourth patient did not improve either with initial EV metilprednisolone pulse or oral corticosteroids therapy. GLILD continue being a rare non infectious complication. Histological analysis is required to verify diagnose in CVID patients with persistent cough and diffuse lung disease. Oral corticoids showed benefits.

50. Enhanced migratory capacity of T lymphocytes in chagasic patients with more severe degree of heart disease

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It is estimated that 30% of individuals bearing Chagas disease (CD) progress to chronic myocarditis, displaying T cell-inflammatory infiltrates in heart tissue. The presence of inflammatory cytokines and the enhanced deposition of fibronectin (FN) in the myocardium may contribute to the recruitment of inflammatory T cells, resulting in the establishment of carditis. In order to further approach the dynamics of T cell migratory events in CD, we performed FN-driven migration assays with PBMCs from chronic chagasic individuals. Migrating T lymphocytes were then evaluated for the cytofluorometric profiles of molecules involved with activation and cell migration. Serologically posi-

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doi: 10:3823/413

tive for T. cruzi (S+) patients without heart involvement were classified as asymptomatic (ASY, n=15) and S+ patients with severe carditis as SEV (n=13). Healthy volunteers acted as a control group (Co, n=15). The percentage of circulating CD3+T cells was decreased in ASY and SEV compared to Co ($p<0.05$ in both cases), although T cell expressing HLA-DR and VLA-4 (an integrin-type FN receptor) was increased, especially in SEV ($p<0.05$ vs Co). SEV also showed an abnormal presence of CD4+CD8+ T cells with activated phenotype in periphery ($p<0.05$ vs Co). In vitro capacity of T cells to migrate through FN showed an increased migratory response that correlated to the severity of heart disease (SEV vs ASY $p<0.05$). The SEV group presented a significantly higher migratory capacity of HLA-DR+VLA-4+ T cells when compared to ASY and Co groups ($p<0.05$ in both cases). Present data are compatible with the existence of disturbances in T cell migration during CD as it was previously showed in an experimental model of T. cruzi infection. Differences in terms of T cell migratory responses in SEV compared to ASY may partly explain why ASY individuals do not evolve to cardiac illness remaining for the lifetime in the latent stage. Financial support: PIP-CONICET (Argentina); Fiocruz, CNPq, Capes and Faperj (Brazil).

51. Distinction between polymorphic variants and pathogenic mutations: case studies on STAT3

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Molecular studies became an important component to define specific primary immunodeficiency diagnoses on patients fulfilling inclusion criteria. But if an alteration is identified at the genomic level, it must be demonstrated to be deleterious, unless it has been previously reported associated with the relevant disease. For many genes, there are mutation databases such as STAT3 base for mutations in STAT3-deficient hyper-IgE syndrome. This gene showed a particularly high proportion of novel missense mutations in our cohort of patients.When the identified change has not been previously reported in a mutation registry, it is critical to verify that the change is not a normal variant, as single nucleotide polymorphisms (SNPs) erroneously thought to be disease-causing changes would lead to false-positive results. To accomplish this, we referred to the SNP database (dbSNP), for identified population-based polymorphisms. The nucleotide changes detected in our patients were not reported in dbSNP, being necessary to perform a population screening by direct sequencing of 100 alleles to rule out that the findings were actually private polymorphisms (allelic variants found only within a restricted population). None of these novel changes were found in 50 healthy control samples. We then evaluated the effect of the nucleotide changes at the amino acid level by bioinformatics tools such as ConSeq, PolyPhen, and SIFT. ConSeq, a tool able to calculate the evolutionary rate at each amino acid in a multiple sequence alignment of homologous proteins, showed that the changes are evolutionary conserved. PolyPhen predicted a possible impact of substitutions on the structure and function of STAT3 by using physical and comparative considerations. SIFT predicted affected protein function based on sequence homology and the physical properties of amino acids. Given the correlation between structure and function, these tools provided overlapping results, adding value to the predictions.

52. Cost-conscious strategy to evaluate AIRE gene

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The autoimmune regulator (AIRE) gene, spans about 13Kb, consists of 14 exons and encodes a polypeptide of 545 amino acids that contains typical domains of a transcription factor. The protein is expressed in the thymus, where it contributes to the expression of peripheral antigens and it is associated with the negative selection of autoreactive T cells. Recessive AIRE mutations lead to autoimmunity targeting endocrine and other epithelial tissues (APECED or APS-1), although chronic candidiasis usually appears first. Objective: to establish a strategy for studying AIRE in 21 patients on suspicion of APS-1, through an efficient way with time and resource savings. Methods: The 14 exons and the intron-exon boundaries will be screened by SSCP (Single-strand conformation polymorphism) analysis followed by direct DNA sequencing. Results: SSCP analysis showed an efficiency of 84.2% to detect gene alterations. However, despite this good efficiency we detected a large numbers of SNPs (Single Nucleotide Polymorphisms), polymorphic variants rather than disease-causing mutations. We then try to implement RT-PCR with sequencing of transcripts, but being very low the expression of AIRE in peripheral blood, we obtained poor results. By direct sequencing of DNA from the 14 exons and the intron-exon boundaries, we found in 7 patients deleterious mutations and surprisingly all but one of them showed a 13-bp deletion in exon 8 in at least one allele (c.1094_1106del13bp). The latter is the most common mutation in the Finnish and British populations. Conclusion: Although greater numbers of samples could be necessary to delineate a definite conclusion, it makes sense to check firstly the exon 8 in our population, being clear the high frequency of the c.1094_1106del13bp mutation. We can evaluate this recurrent mutation by SSCP method and also by RFLP (Restriction Fragment Length Polymorphism), allowing rapid molecular screening for APS-1 in Argentinean kindred.

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doi: 10:3823/413

53. Erythrocyte aggregation in patients with systemic lupus erythematosus

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Systemic Lupus Erythematosus (SLE) is an autoimmune, chronic inflammatory, non-organ specific disease. SLE patients present a high prevalence of thrombotic and arteriosclerotic disease. The aim of this work was to study the erythrocyte aggregation kinetics and the effect of two of the plasma factors (immunoglobulins (Igs) and fibrinogen (F) concentration) on the erythrocyte aggregation, in 39 women with SLE and 50 healthy women of similar age. The present study was approved by the Bioethics Committee of Facultad de Ciencias Médicas, Universidad Nacional de Rosario. Both, patients and control signed an informed consent. Erythrocyte aggregation was estimated by optic method, obtaining two parameters: s0/n0 and 2k'2n0, the first one estimating the size of the aggregates; and the second one characterizing the aggregation process kinetics. Plasmatic fibrinogen was measured by gravimetric method and immunoglobulins by radial immuno-diffusion. For the statistical analysis the Student's t test and the coefficient of Pearson's were used. We found that women with SLE have shown significantly higher values with regard to controls, of: s0/n0 (1.89±0.06 vs 1.80±0.10, $p<0.0001$), 2k2n0 (1.02 ±0.71 vs 0.70±0.49, $p<0.02$), F (mg/dl) (362±84 vs 280±47, $p<0.0001$), and Igs (mg/dl) (1803±521 vs 1411±130, $p<0.005$), Positive correlations were found between F and 2k2n0 ($r=0.56$; $p<0.001$), F and s0/n0 ($r=0.55$; $p<0.002$) and between Igs and 2k2n0 ($r=0.44$; $p<0.02$), Igs and s0/n0 ($r=0.37$; $p<0.05$). The results show that SLE patients red blood cells aggregate at higher rate and the aggregates size are also greater than controls due to an increase of immunoglobulins and plasma fibrinogen. The erythrocyte aggregation increase in these patients could induce a decreased flow that might contribute to the thromboembolic process present in SLE patients.

54. Autoimmune findings in patients with primary immunodeficiencies: ten years follow-up

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Primary immunodeficiencies (PID) are a genetically heterogeneous group of disorders that affect distinct components of the immune system, and are associated with variable autoimmune diseases prevalence and/or autoantibodies occurrence. Clinical features of autoimmune disease and positivity of autoantibodies were recorded in 61 children with PID studied at Immunology Division of Pedro de Elizalde Hospital, with a ten years follow-up. Serum samples were collected to test a panel of autoantibodies: ANA, anti DNA, AMA, ASMA, APCA and ANCA were assayed by IFI, and RF was measured by nephelometry. 7 out of 61 children with PID (11.5%) developed autoimmune disease. Three children with IgAD showed: celiac disease, celiac disease + hypothyroidism and rheumatoid arthritis. Haemolytic anaemia was observed in two children with CVID and ALPS, meanwhile vasculitis and idiopathic thrombocytopenic purpura were recorded in two children with AT and Di George syndrome respectively. New-onset of autoimmune disease had a variable range of age (3 months-16 years) with autoimmune features preceding the PID diagnosis in four children. 10 out of 54 patients (18.5%) with no evidence of autoimmune disease showed positivity for some autoantibody from the assayed panel. Of 25 children with IgAD, 7 (28%) were positive (ASMA: 4, ANA: 2, AMA: 1). Of 7 patients with CVID, 2 (29%) showed ASMA (+). In 1 out of 4 patients (25%) with AT, an APCA (+) was recorded. Patients with HIGM (8), XLA (8), autosomal recessive agammaglobulinemia (1), XLP (1), HIGES (3), Di George syndrome (1), did not show positivity for any of the assayed autoantibodies. IgAD and CVID showed the highest prevalence of autoimmune disease (36% and 37% respectively). This could be related to a common polygenic pattern of inheritance associated with the induction of both diseases.

55. CD127 and CD25 expression in CD4+ and CD8+ T cells in HIV (+) paediatric patients

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HIV infection is associated with a progressive decline of CD4+ T cells, changes in the cellular dynamics and loss of immune functions. Expression of the receptors for IL-2 (CD25) and IL-7 (CD127) defines CD4+ T cell subsets which are differentially depleted during HIV infection. Näive and central memory (N/CM) (CD127+CD25 low/-), regulatory (Tregs) (CD127 low/-CD25+), and effector (CD127-CD25-) T CD4+ subsets, were studied in 34 HIV (+) infected children, 14 of them with severe immunosupression (A: CD4<15%) and 20 without evidence of immunosupression (B: CD4>25%). Percentual T CD4+, T CD8+ and subset levels were assayed by multicolour flow cytometry using monoclonal antibodies CD4, CD8, CD25, CD28 and CD127 (FITC, PE or PerCP).Clinical and virological evaluation was also performed in all patients. Same determinations were assayed in a control group (Co) of uninfected children simultaneously. CD127 low/-CD25+ Tregs cells and CD127-CD25- effectors T cells showed a significant increase ($P<0.05$) in patients with severe immunosupression, A: 9.6 ±4.2% vs B: 7.34±1.2% vs Co: 8.3 ±1.3%, and A: 1.35±1.33% vs B: 0.64 ±0.36% vs Co: 0.64±0.60%, respectively. Conversely, group A showed a non-significant decrease in N/CM T CD4+ levels (A: 85.5±5.4% vs B: 89.1±3.9% vs Co: 89.9±1.6%). A significant positive correlation between T CD8+ and CD127-CD25- effector T

CD4+ percentual levels (r: 0.41, P<0.05) was recorded. Activated CD8+ T cells, CD8+CD28- and CD8+CD127- correlated positively (P<0.05) with CD127-CD25- effector T CD4+ percentual levels (r: 0.49, and r: 0.69, respectively). CD127-CD25- effector T CD4+ cells were not correlated with viral replication level. An extended immune dysregulation associated to severe immunosupression could be responsible for the simultaneous percentual increase in the effector and regulatory CD4+ T cell subsets and also in cytotoxic cells showing signs of immune activation.

56. Yersinia pseudotuberculosis Infection Impairs IL-17 expression and pro-inflammatory cytokines levels of T lymphocytes

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Y. pseudotuberculosis is an enteropathogen that causes gastrointestinal disorders. The resolution of the infection is mediated by CD4+ Th1 cells that produce cytokines such as IFNγ and IL-2 An effective way for Yersinia to subvert the immune response would be to inhibit T-cell stimulation. Studies indicate that T lymphocytes producing IL-17 also play an important role in host protection against infection by extracellular bacteria. Objective: To verify the influence of Y. pseudotuberculosis YpIIIpIB1 infection on the IL-17 and pro-inflammatory cytokines levels of T lymphocytes from BALB/c mice. Methods: Cells were obtained from the spleens and lymph nodes of infected BALB/c mice with Y. pseudotuberculosis on the 7th, 14th, 21th, 28th days post-infection, and stimulated in vitro with PMA and ionomycin. Uninfected mice were used as control. IL-17 and pro-inflammatory cytokines (IL-2, TNFα and IFNγ) expression of CD4+ T lymphocytes was determined by flow cytometry. Results: During Y. pseudotuberculosis infection, were observed significant changes in the expression of the cytokines analyzed. There was a decrease in the IL-2 production, with higher reduction on the 21th days post-infection (1.3-fold lower than the control). The TNFα cytokine measured showed reduction on the 7th, 14th and 21th days pos-infection, with lower levels on the 14th days pos-infection (1.7-fold lower than the control). The IFNγ levels presented decrease on the all days post-infection, with higher reduction on the 14th days post-infection (1.8-fold lower than the control). Were observed reduction also in the IL-17 expression on the 21th days post-infection (1.4-fold lower than the control). Conclusion: Results suggest that the Y. pseudotuberculosis infection can influence the immune response of T lymphocytes by impair the cytokines expression.

57. Effect of cyclopalladated compounds in breast cancer

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The cancer, manifestation originated by the growth not controlled of cells, affects million of individuals. The cancer development is strongly associated to a chronic inflammatory process; the different cytokines production can influence the growth of tumoral cells. Some compound of palladium (II), general formula: [Pd(dmba)(Cl)tu] and [Pd(dmba)(N3)tu] are known by present potentials anti-tumoral. Actually, the cis-platinum is drug most used against cancer. Objective: In this study the activity anti-tumoral of the compound of palladium was analyzed. Methods: Adherent cells obtained from tumor of Ehrlich in its solid form carrier Swiss mice were cultured with [Pd (dmba)(Cl)tu], [Pd (dmba)(N3)tu] and cis-platinum. The cellular viability (IC50) (MTT method), nitric oxide production (NO) (Griess), production of pro-inflammatory cytokines as tumor necrosis factor-alpha (TNFα) and interleukin-12 (IL-12) (ELISA), were tested. The mutagenecity of these compounds was analyzed using Test of Ames. Results: The IC50 (μM) obtained was: 137.65±0.22-[Pd(dmba)(Cl)tu], 146.51±0.22-[Pd(dmba)(N3)tu], cis-platinum-113.21±0.28. All tested compound stimulated NO production, well as TNFα and IL-12 cytokines. The [Pd (dmba)(N3)tu] induced higher NO production (27.29±8.01) than cis-platinum (19.33±9.22). The IL-1β production was similar in the substances, with [Pd (dmba)(Cl)tu] (67.2±55.7), [Pd(dmba)(N3)tu] (46.4±80.72) and cis-platinum (39.5±157.1). The same was observed for IL-12 production, with [Pd (dmba)(Cl)tu] (2101±844.1), [Pd(dmba)(N3)tu] (2253±686.6) and cis-platinum (2233±563.9). The compound and your ligands didn't present mutations, different of cis-platinum, that it caused punctual mutations in the DNA of S. typhimurium. Conclusion: The results indicate the Palladium (II) complexes as promising in the development the therapies for cancer treatment.

58. NK cells in chronic lymphocytic leukemia (CLL): defective expression of CD62L and interferon gamma (IFNγ) production

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Patients with CLL exhibit a progressive deficiency in the immune response which has been associated to autoimmune disorders, recurrent infections and second malignancies. Given the key role of NK cells in immunesurveillance, we have evaluated the expression of CD62L and CCR7, two molecules involved in NK migration to secondary lymphoid tissues in CD56+CD3- cells from CLL patients and healthy age-matched donors. By flow

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No. 3:4
doi: 10:3823/413

cytometric analysis we observed a lower proportion of CD62L+ cells in peripheral blood NK cells from CLL patients (30.4 ± 1.6) compared to healthy donors 44.8 ± 2.6 (results are expressed as the mean ± SD, n= 28 and 45 respectively, p<0.01). In regard to NK cells expressing CCR7, we found a comparable low proportion of positive cells in both groups (6.8 ± 3.4 versus 7.4 ± 2.4, healthy donors vs CLL patients, n= 11 and 9 respectively, not significant), except for three CLL patients who had more than 40% of CD56+CD3-CCR7+ cells. We also analyzed the functional activity of NK cells from CLL patients by evaluating their capacity to produce IFNγ when activated with Poly I:C, a TLR-3 specific agonist. To this aim, we incubated peripheral blood mononuclear cells for 24 h in the presence of poly I:C (50 ug/ml) and determined the proportion of NK that produced IFNγ by flow cytometry. There was no difference in spontaneous IFNγ production between NK cells from healthy donors and CLL patients (% CD56+CD3-INFγ+ cells 1.8 ± 0.3 vs 1.7 ± 0.4, n=17 and 19 respectively). By contrast, NK cells from CLL patients showed an impaired response to poly I:C stimulation (% CD56+CD3-INFγ+ cells 17.0 ± 2.8 vs 9.5 ± 2.5, p<0.01). In conclusion, our findings indicate that NK cells from CLL patients present phenotypic and functional defects that may be implicated in the high incidence of second malignancies in this pathology.

59. Role of the erythrocyte protein Band 3 in autoimmune hemolytic anemia associated to chronic lymphocytic leucemia

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Chronic lymphocytic leukemia (CLL) is the most common cause of autoimmune hemolytic anemia (AHA). We have previously shown that neoplastic B cells are able to bind the erythrocyte protein Band 3 (B3) through its N-terminal domain (B3N), an ability that could allow the neoplastic clone to trigger the autoimmune process. The aims of the present work were: 1) to identify molecules in the cell membrane of leukemic B cells as candidates for being B3N binding site and 2) to investigate the process of binding and endocytosis of erythrocyte-derived vesicles to leukemic B cells. In order to accomplish our first objective, we removed non-integral membrane proteins from DAUDI cells (a human B cell line that we have previously reported to bind B3N) by acidic elution using potasium phosphate 0.1M, sodium citrate 0.05M buffer, pH=2.5. By flow cytometric analysis, we found that this treatment decreased the binding of B3N (50 ug/ml) to cells (MFI control cells: 160±8, MFI treated cells: 70±10, n=5, p<0.05). After neutralizing the acid eluates, they were passed through an affinity column coupled with B3N, and molecules interacting with B3N were recovered and analyzed by mass spectrometry. HMGN2, a nucleosomal protein reported to be secreted by leukocytes, was detected in acid eluates of DAUDI and CLL B cells (n=3). To evaluate the binding and endocytosis of erythrocyte-derived vesicles to leukemic B cells, we used CFDA staining and flow cytometry. We found that 30.4±8.8 % of CLL cells were able to bind inside-out vesicles (with B3N exposed to the outer surface) while only 10.5±1.7 % of T lymphocytes did (n=6, p<0.05). Images obtained by confocal microscopy suggest that the vesicles are found inside the B cells after 2 hours of culture at 37ºC. We propose that leukemic B cells can specifically bind and uptake erythrocyte-derived vesicles to act as antigen-presenting cells of erythrocyte proteins, and that HMGN2 is a candidate molecule to function as a receptor in this system.

60. Regulation of tumor necrosis alpha converting enzyme (TACE) activity in immune cells by Staphylococcus aureus protein A

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S. aureus is a major human pathogen associated to diverse types of local and systemic infections with high morbidity and mortality. Among its many virulence factors, we have described the ability of protein A (SpA) to mimic TNFα responses by interacting with the type I TNFα receptor (TNFR1) on airway epithelial cells. This interaction is critical for development of pneumonia. SpA also induces the cleavage of TNFR1 from the surface of epithelial cells, a process mediated by TACE. This metalloprotease plays a central role in immunity by regulating the release of pro-inflammatory cytokines and limiting inflammation through the shedding of cytokine receptors. The aim of this study was to determine the role of SpA in the regulation of TACE activity in macrophages. We determined that purified SpA induces TNFα production in macrophages. A decrease in TNFα production was observed when the cells were stimulated with a spa null isogenic mutant as compared to wild type S. aureus. Both, the N-terminal and the C-terminal regions of SpA were involved in TNFα secretion. S. aureus and SpA also rapidly induced shedding of TNFR1 and the type II IL-1 receptor (IL-1RII) in macrophages, a process that was mediated by the N-terminal region of SpA. Similar responses were observed in cells stimulated with Lactococcus lactis expressing SpA. Exposure of cells to SpA and L. lactis-SpA in the presence of TAPI-I, a biochemical inhibitor of TACE, resulted in a significantly dose-dependent inhibition of IL-1RII shedding ($p < 0.01$) confirming the role of this enzyme in receptor cleavage induced by SpA. Using mutated proteins we established that the aminoacids of SpA critical for receptor shedding were those known to interact with IgG. In conclusion, we demonstrate the ability of S. aureus protein A to activate TACE in macrophages and to induce the release of both pro- and anti-inflammatory mediators. SpA induction of receptor shedding could contribute to S. aureus evasion of the immune response.

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No. 3:4
doi: 10:3823/413

61. Combination of Cy and AdIL-12 enhances in vivo antitumor immune response by reversion of Tregs and MDSC-driven immunosuppression

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We have previously demonstrated the synergistic antitumor effect of the sequential administration of low-dose cyclophosphamide (Cy) followed by sub-therapeutic doses of adenovirus expressing IL-12 genes (AdIL-12) in a mouse colorectal carcinoma (CRC) model (CT26). Our results suggested that the combined treatment allowed the generation of strong antitumor immune responses. The aim of this work was to investigate the immunological mechanisms responsible for the therapeutic effect of the combination. Methods: Balb/c mice were s.c injected with CT26 cells (day 0), distributed in experimental groups (day 7) and treated with: saline; Cy 50 mg/Kg i.p (day 7); AdIL-12 109 TCID50 i.t (day 8) or Cy + AdIL-12. Tumor volume was measured and samples of peripheral blood, spleen and tumor were taken. $CD4^+CD25^+$ and $CD4^+CD25^-$ T lymphocytes were isolated by magnetic separation. Results: We observed that mice non-responder to the combined treatment showed a proportion of regulatory T cells (Tregs) higher than in responder mice, both in peripheral blood and spleen, as well as in tumor samples ($p<0.05$). Also, the antitumor effect of combined therapy was reverted by in vivo administration of Tregs. The combination Cy+AdIL-12 inhibited Tregs ability to secrete IL-10 and TGF-β and their capacity to inhibit dendritic cells maturation. We also observed that a subpopulation of immature cells with myeloid and suppressive phenotype (MDSCs) was recruited in the spleen of mice during tumor progression. Cy + AdIL-12 induced a significant decrease in the percentage of MDSCs with respect to untreated mice (4.5 % vs 16 %; $p<0.05$). Importantly, depletion of Tregs and MDSCs by combined therapy leads to development of specific IFNγ secreting CD4+ T lymphocytes response, able to eradicate CRC tumors followed to their adoptive transfer. Conclusion: Our results suggest that Cy + AdIL-12 induces a synergistic antitumor immune response against CRC by reversion of Tregs and MDSCs number and function.

62. Omenn syndrome in an infant with atipical complete DiGeorge anomaly

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Omenn syndrome (OS) is characterized by early onset generalized erythroderma, hepatoesplenomegaly, lymphadenopathy and autollogous, oligoclonal and activated T cells. Several genes mutations have been related to OS. We report a patient with clinical features of OS who is found 22q11.2 deletion. First boy borned from healthy and non consaguineous couple. On prenatal control ultrasound intrauterine harmonic growth retardation, hypospadia and strawberry skull were detected. Microcefalia, minimal and transitory septal heart defects, wide forehead, ear anomalies, right atresia choanae, retrognathia and micrognathia were detected on postnatal period. During his first month of age he developed scaly erythematous severe eczema, alopecia, hepatoesplenomegaly, lymphadenopaty. He showed lymphocitosis (10340 mm^3), eosinophilia (2106mm^3), IgE: 18000 UI/ml, and normal IgG, IgA and IgM. Lymphocytic dermis infiltration and small thymus were documented. Omenn syndrome was suspected. Flow cytometry analysis was CD3: 78%, CD4:13%, CD8: 61%, CD16/56: 9%, CD19: 9%, CD3/HLADR: 69%, CD4/CD45RA: 2%, CD4/CD45RO: 99%, TCRαβ: 100%. In vitro proliferation to PHA and CD3 were low. Maternal T-cell engraftment was ruled out. Restricted and oligoclonal TCR Vß repertoire was found. Total B cell $CD27^+$ and $CD27^+$ IgD^- were low. Chromosome 22q11.2 deletion was confirmed by fluorescence in situ hybridization. The complications were P. jirovecii pneumonia, growth failure, laryngomalasia, and flair of his erythroderma. He died by multiorganic failure at 5 month old. We described the first case with complete atipical DiGeorge who presented Omenn phenotype out of 149 patients with 22q11.2 deletion. The specific malformations encouraged us to research on that anomaly. Despite normal immunoglobulins profile, low memory cells and class switch were found. Omenn syndrome features continue being a challenge for molecular diagnoses.

63. Autoimmune hepatitis and HLA DRB1 alleles

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Although the pathogenesis of Autoimmune Hepatitis (AIH) is not completely known, the susceptibility is determined in part by a gene or genes linked to the region HLA Class II. It has a genetic background associated with different HLA DRB1 alleles depending on the ethnic group. The aim of this study was to investigate the contribution of the HLA-DRB1 alleles in development of AIH in Rosario, Argentina. We studied 47 unrelated individuals with diagnostic of AIH and a control group (n= 81) of non-related healthy individuals. Genomic DNA was extracted from peripheral blood using the CTAB method. The alleles were tested for HLA class II genotyping using PCR-SSP. Absence or presence of PCR products was visualized by agarose gel electrophoresis. Differences in the distribution of HLA-DRB1 alleles

between patients and controls were analyzed by or Pearson's t-test with Yates correction or Fisher's exact test. Odds ratios (RO) were calculated. The results in the patients with AIH, demonstrated that the highest frequencies corresponded to the following alleles: DRB1*0401 (21.28), DRB1*0101 (13.83), DRB1*0701 (11.70), DRB1*0301 (9.18) y DRB1*1301 (8.51). In controls the most prevalent alleles were: DRB1*0701 (14.20), DRB1*1103 (12.96), DRB1*0808 (12.35), DRB1*1303 (9.26) y DRB1*1101 (8.2). Our results show that the HLA-DRB1*0301 (RO=35.55), HLA-DRB1*0101 (RO=5.04) and HLA-DRB1*0401 (RO=5.98), can be considered as predisposing factors for the development of AIH. By contrast, HLA-DRB1*0808 (RO=0.036), HLA-DRB1*1103 (RO=0.035) and HLA-DRB1*1303 (RO=0.05) alleles were less frequent among patients ($p<0.001$) and hence more involved in disease resistance. These findings suggest that the polymorphism of HLA-DRB1 contributes to the diverse spectrum of immune responses observed in AIH. The HLA-DRB1 alleles could either act alone or in combination with other genes to confer differential susceptibility and protection in this autoimmune pathology.

64. Role of invariant natural killer T cells during experimental influenza A virus infection

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Invariant natural killer T (iNKT) cells represent a population of non-conventional 'innate-like» γδT lymphocytes that recognize lipid antigens presented by the CD1d molecule. During viral infection, iNKT cells provide innate surveillance against virally infected cells through the swift production of IFNγ. We assessed the in vivo physiological contribution of iNKT lymphocytes on the host (C57BL/6 mice) response and viral pathogenesis during influenza A virus infection (H3N2). Upon infection, iNKT cells became activated in the lungs to rapidly produce cytokines including IFNγ, and surprisingly enough, IL-22, a cytokine known to play key role in mucosal defence. Relative to wild-type animals, C57BL/6 mice deficient in iNKT cells developed a more severe broncho-pneumonia and had an accelerated fatal outcome. During this presentation, we will present the mechanisms by which iNKT cells produce IL-22 and discuss the potential role of this cytokine during the early stage of acute influenza A virus infection. This work is supported by Inserm, CNRS, Univ Lille 2, Pasteur Institute, Lille and ANR MIME (project R08066ES)

65. Exogenous activation of invariant natural killer T cells protects mice from acute Streptococcus pneumoniae pneumonia

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The gram-positive bacteria Streptococcus pneumoniae is the leading cause of pneumonia, meningitis and septicaemia in humans. Because of the growing resistance of the pneumococcus to antibiotic treatments and the incomplete efficacy of vaccines, it is important to gain insight into the mechanisms of host defence during infection. In this study, we assessed whether exogenous activation of pulmonary invariant natural killer T (iNKT) cells, a population of non-conventional lipid-reactive αβT lymphocytes, could strength innate immunity to control acute respiratory pneumococcal infection. To this end, mice were treated with the canonical iNKT cell agonist a-galactosylceramide (a-GalCer) 24 h before S. pneumoniae (serotype 1) challenge. Pre-treatment (24 h) of mice with a-GalCer through the intranasal route resulted in full protection from mortality whilst a-GalCer administered through the systemic route had no effect. The protective effect was associated with a dramatic diminished bacterial load in the lung tissue and with a reduced pneumonia. We further show that, after a-GalCer intranasal inoculation, respiratory CD11chigh dendritic cells play a major role in the activation (IFNγ release) of pulmonary iNKT cells. This resulted in the rapid elimination (2-4 h) of S pneumoniae in the alveolar spaces. We suggest that the accelerated S. pneumoniae clearance is mainly due the recruitment/activation of a particular population of CD11b+Gr1+ cells in the alveolar spaces. Together, our data reveal a new function of iNKT cells in the control of extracellular bacterial infection. This work is supported by Inserm, CNRS, Univ Lille 2 and Pasteur Institute, Lille

66. Candida albicans prevents macrophage infection by HIV

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Candida albicans (CA) is part of the normal microbial flora that colonizes mucocutaneous surfaces of the oral cavity, gastrointestinal tract, and vagina. CA is an opportunistic pathogen which causes either local or systemic infections. It is unclear whether CA is able to modulate the infection of target cells by HIV. The main objective of this study was to determine whether CA modulates the infection of macrophages by HIV. Macrophages (M) were obtained by incubating monocytes (purity >90%) for 5 days with GM-CSF (30 ng/ml). HIV infection was assessed by incubating M ($5x10^5$) with HIV BAL (50 ng/p24) for 2 h, with or without CA in a CA:M ratio of 10:1 and 1:1. Cells were then washed and cultured for different times. HIV infection was assessed by measuring the levels of p24 antigen in cell supernatants by ELISA or by deter-

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mining intracellular p24 antigen by flow cytometry. Infection of dendritic cells (DC), obtained by culturing human monocytes for 5 days with GM-CSF and IL-4 (15 ng/ml) was analyzed in a similar way. We found that CA markedly inhibits the infection of M by HIV when it was assessed at 7 days post-infection: % inhibition > 90 (n=5, p<0.01 vs controls). Interestingly, when infection was measured 15 days post-infection the inhibitory effect was partially overcome (data not shown). CA also inhibited the infection of DC: % inhibition > 90 (n=3, p<0.05 vs controls). By contrast no inhibitory effect was observed using the cell line Jurkat, as target cells. We then analyzed whether CA was able to down-regulate the expression of CD4, the entry receptor for HIV. CA markedly decreased the expression of CD4 in M when assessed at 24 h of culture: % reduction in mean fluorescence intensity for the expression of CD4 = 75 ± 15 (n = 2). Conclusions: Our observations show that CA impairs the infection of M by HIV and that this inhibitory effect is associated to the down-regulation in the expression of CD4.

67. Protective long-lasting immune response induced by nasal administration of a pneumococcal protein and a probiotic in mouse model

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S. pneumoniae (Sp) is an important respiratory pathogen because it causes high morbidity and mortality rates around the world. New strategies for the fight against pneumococci are necessary and some serotype independent pneumococcal proteins are good candidates for mucosal vaccines. Adjuvants must administer pneumococcal proteins to induce a good mucosal immune response. Objective: To assess the protective immune response induced by nasal vaccination with protective pneumococcal protein A (PppA) associated to Lactobacillus casei (Lc) as adjuvant. Swiss albino mice were nasally immunized with PppA (10ug) + Lc (10^9 cells/d/mice) using a protocol that included 3 successive doses with a 14-day interval between them. Groups that received Lc, PppA and PBS were used as controls. Serum (S), bronchoalveolar and nasal lavages (BAL, NAL) were collected at different days (0, 28, 42, 58 and 73). Assays: 1) IgA and IgG anti-PppA antibodies (a-PppA) in S, BAL and NAL; 2) Cytokines: IL-4, IL-10; IL-17, IFNγ and IL-2 were evaluated in S, BAL and NA; 3) Survival Assay: Survival of mice after intraperitoneal challenge (IP) with Sp was evaluated until d 21. Results: PppA+Lc induced high specific IgA and IgG antibody levels in both mucosal and systemic compartments compared with PppA (42d: IgA=BAL: p<0.01; NAL: p<0.05, S: p<0.05). Also, PppA+Lc induced a higher stimulation of the Th populations (Th1, Th2 and Th17) than the PppA and Lc groups, in mucosal compartment. In addition, PppA+Lc significantly increased survival compared to the control (p<0.05, Cox-Mantel test). The specific immune response induced by immunization was maintained in time so immune memory was stimulated. Association of a probiotic and a pneumococcal protein as nasal vaccines would be a good and safe strategy to prevent pneumococcal infection.

68. Immune response in the gut associated lymphoid tissue (GALT) of weaned mice after intragastric inoculation of Shiga toxin (Stx)-producing Escherichia coli (STEC).

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Hemolytic uremic syndrome (HUS) is a life-threatening complication of STEC gastrointestinal infection and the GALT constitutes the first immune compartment encountered by STEC. We have developed a mouse model of HUS after intragastric inoculation of E.coli O157:H7 Stx+ strains (125/99) at weaning. We have previously observed a decrease of B cell percentage in Peyer's patches (PP), with a simultaneous and transient increase in mesenteric lymph nodes (MLN) in 125/99-inoculated mice at 24h post-inoculation compared to controls: mice inoculated with a Stx- E.coli O157:H7 strain (605/03) or PBS (Ctrl). The aim of this work was to further study the activation and trafficking of T and B lymphocytes as a related phenomenon supporting those changes. We also evaluated alterations in the $CD4^+CD25^+$ population with potential regulatory function at 7d post-inoculation. Flow cytometry analysis showed that both 125/99 and 605/03-inoculated mice presented evidence of activation as a decreased CD62L MFI in MLN (mean±SD, n) for T and B cells (B cell: Ctrl=765±39, 3; 605/03=477±22, 3; 125/99=518±112, 3; p<0.005; T cell: Ctrl= 1764±14, 3; 605/03=1156±168, 3; 125/99=1195±144, 3; p<0.002). Besides, an increased percentage of CD69+ B-cells was observed in MLN from both experimental groups (Ctrl=26.5±0.7, 2; 605/03=35.0±4.0, 3; 125/99=40.0±2.7, 3; p<0.01). After 12 h of i.v. injection of allogenic CFSE+ lymphocytes only 125/99-inoculated mice showed an increased percentage of B220+CFSE+ cells in MLN (Ctrl=12.6±0.8, 4; 605/03=13.1±2.7, 2; 125/99=14.7±1.9, 4; p<0.05). Additionally, an increased percentage of CD4+CD25+ cells was observed 7d post-inoculation only in MLN from125/99-inoculated mice (Ctrl=12.0±0.5, 4; 605/03=11.7±1.9, 4; 125/99=15.9±2.1, 3; p<0.02). Although both E.coli strains induced GALT B and T cell activation, our results suggest that only the stimulus caused by the Stx-producing strain was able to induce trafficking of B cells and increase the CD4+CD25+ cells in the MLN

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69. Macrophages modulate neovascular response in an acute model of imflammation. Expression of pro-angiogenic proteins

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Septic shock (SS) induced by bacterial infections, affect mainly the cardiovascular system. This damage is caused by microorganisms themselves or their endotoxins, and may be enhanced by the infiltration of immune cells in the myocardium. The role of macrophages (MP) in inflammatory angiogenesis during SS is almost unknown. We investigated the angiogenic abitility of peritoneal MP in a model of acute inflammation induced by lippopolysaccharide (LPS) from E. coli in BALB/c mice. We demonstrated that MP are able to stimulate neovascularization at $5x10^5$ per site, and treatment with LPS (10 ug/ml) in vitro (LPS-MP) reduces MP number required to produce angiogenesis ($4x10^5$) potentating this effect (number of skin vessels/mm^2) (normal skin: 1.13 ± 0.20, MP: 1.35 ± 0.13, LPS-MP: 2.18 ± 0.19, p <0.01). Furthermore, by Western blot (Wb) (relative O.D.) we observed that LPS-MP also increase the expression of pro-angiogenic proteins at the site of inoculation: MMP-9 (MP: 0.36, LPS-MP: 0.64, p <0.05), CD-31 (MP: 0.73, LPS-MP: 1.01, p <0.05) and VEGF-A (MP: 0.13, LPS-MP: 0.73, p <0.05). In vitro, LPS-MP have increased activity of MMP-9 in comparison with MP without treatment (p<0.05) via nuclear factor kB (NF- kB). In addition we studied the role of MP obtained from BALB/c mice treated in vivo with LPS (1 µg/g, i.p.) (LPSip-MP). We observed an up-regulation of MMP-9 (MP: not detected, LPSip-MP: 1.01, p<0.05) and VEGF-A expression (MP: not detected; LPSip-MP: 1.29, p <0.05) compared to MP from untreated animals. Normal hearts were cultured with LPSip-MP or its supernatants and homogenates showed an increment in proangiogenic molecules expression: CD31 and VEGF-A (p <0.05). We conclude that peritoneal MP are inducers of angiogenesis and infection with LPS enhances their pro-angiogenic effect. In addition, LPS-MP modulates the level of expression of angiogenic molecules in normal hearts and this action enables them as modulators of cardiac angiogenesis during the SS.

70. Primary immunodeficiencies registry immunology group of the "Ricardo Gutiérrez" (hnrg) Children´s hospital

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The primary immunodeficiencies (PIDD) are genetic diseases. There are more than 120 with definitive diagnosis (molecular); others are still a diagnostic challenge. They present a high morbidity and mortality. The early diagnosis allows the prescription of the appropriate treatment to improve the quality and survival. This, in some cases, may help to obtain the cure. OBJECTIVE: To relieve and to communicate the prevalence of different PIDD diagnosed by the Group in 20 years. MATERIAL AND METHODS: restrospective analysis of patients' records. RESULTS: Out of 767 patients with PIDD, 689 were classified (according to the 2009 IUIS classification). Combined deficiencies 2.9%; predominantly antibody deficiencies 73%; other well-defined immunodeficiencies syndromes 14%; disease of immunedysregulation 3.2%; congenital defects of phagocyte 2.46%; defects in innate immunity 0,1%; autoinflammatory disorders 2.46%; complement deficiencies 2.9%. Other patients have immune defects but are not included in the above-mentioned classification (CD4 lymphopenia, alteration in NK cell compartment, associated with genetic syndromes, humoral defects). CONCLUSIONS: Predominantly antibody deficiencies are the most prevalent PIDD, the diagnosis of defects in innate immunity is not much and the PIDD exceed the present classification.

71. CD1d mediated ganglioside presentation by human B cells: novel pathway to recruit NKT cells´ help

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Gangliosides are a family of sialylated glycolipids that are normal components of the cell membrane. Being a ganglioside itself, N-glycolyl-GM3 (NGcGM3) is not expressed in normal human tissues though, owing to a specie-specific genetic mutation that abrogates its biosynthesis. However, it is highly expressed in tumors. As a result, it has been a target of choice for immunotherapy, and it has been proved an antibody response against NGcGM3. B lymphocytes produce antibodies upon antigen (Ag) induced activation and are capable of presenting internalized Ags. This is well established for peptide Ags, yet antibody response against glicolipids remains poorly characterized. Notably, a compartment of T cells, known as natural killer T (NKT), recognize and become activated in response to glycolipid Ags presented by CD1d molecules expressed on the surface of antigen presenting cells. In this study, we aimed to assess whether B cells were competent to present NGcGM3 to NKT cells. To achieve so, we isolated lymphocytes from human sources, sorted the populations of interest and co-cultured them with NGcGM3. By flow cytometry techniques, we show that primary human B cells express CD1d, in resting and activated state. Importantly, we demonstrate that human B cells are capable of presenting NGcGM3 via these CD1d molecules. Upon setting up an assay of isolation and expansion of human NKTs from buffy coats, we prove an enhancement of their proliferation in co-cultures with autolog B lymphocytes in relation to single cultures, when NGcGM3 is added to the media. Taken together, these findings not only aid to establish principles in relation to the mechanism of the humoral response to gangliosides, but also potentially to improve tumor vaccinations´ strategies.

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doi: 10:3823/413

72. Autoimmunity and B cell compartment in pediatric patients with selective IgA deficiency

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Selective IgA deficiency (SAD) is the most common primary immunodeficiency in humans. May manifest clinically with atopy, infection, autoimmunity or be asymptomatic. Aim: To establish the frequency of autoimmunity in 220 children with SAD and to characterize the B cell compartment. Retrospective study of medical records of 220 SAD children (age range: 4-15 ys) was done. Antibodies functionality, autoantibodies, anti-Tranglutaminase IgG, endomysial IgG, T and B lymphocyte phenotype, B memory lymphocyte (BML)$CD27^+$, BML post switch $CD27^+IgD^-IgM^-$, BML pre switch $CD27^+IgD^+gM^+$, transitional BL(BLt) $CD24^+CD38^+$ were performed. Results: 99% patients were symptomatic, 80% of the symptoms began before 4 ys old (median 2 ys, range 0.25-9). Definitive diagnosis of SAD was performed between 3-4 ys after the first visit (median:12, range: 5.8-18); 31.5% of patients presented an autoimmune disease (type I diabetes, Graves disease, precocious puberty, ITP, juvenil rheumatoid arthritis, recurrent autoinmume parotitis) as first manifestation; 21.1% associated celiac disease (CD), in 10 children it was the first manifestation. During follow-up 6/220 had serological markers of organ specific autoimmune disease, two of them asymptomatic. In those CD was confirmed by intestinal biopsy in their evolution. B cell compartment in 35/220 SAD (8 with recurrent infections, 6 with atopy, 10 CD, 10 with autoimmunity) was done. Only patients older than 11 ys old showed a statistically significant decrease of $BMLCD27^+$ (SAD:26.00±11.11 vs N:33.15 ± 7.1 $p < 0.05$), 4 of whom had autoimmunity. Only one patient with CD showed increased BLt. Conclusions: Autoimmunity was a frequent clinical presentation in our population, being the most common CD. The finding of autoimmune disease specific autoantibodies before the onset of symptoms allowed the diagnosis in two cases. B-cell compartment study did not allow us to classify clinical subgroups, although 40% of patients with autoimmunity no CD had BML altered

73. Inflammatory mediators in intestinal coeliac mucosa.

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Elements of both innate and adaptive immune response take part in the mucosal damage in active coeliac disease (CD). Incubation of intestinal biopsy samples with gliadin peptides (particularly, pα31-43), triggers different inflammatory pathways, which lead to increase in intestinal permeability and induction of IL-15, among other effects. Though IL-15 is one of the key cytokines in the early stages of disease there is no a clear link with IL-23, IL-17 and IFNγ production, which may initiate, expand and perpetuate the mucosal damage. The aim of this work was to evaluate the expression of CXCR3, CXCL10, IL-15, IL-15R, IL-17, IL-21, IL-23 by qPCR and/or fluorescence microscopy on intestinal biopsies samples of coeliac and control individuals from paediatric and adult population. By qPCR, expression of IL-17, IL-21 and CXCL10 was significantly higher in active CD compared with control samples. However, there was no difference for CXCR3. CXCL10 was higher in control biopsies stimulated with IL-15 and in coeliac samples incubated with IL-15 and pα31-43. IL-15Ra (RNAm) and the number of $IL\text{-}15^+$ cells in intestinal mucosa were statistically higher in active CD. Control samples could be grouped according to the level of expression of IL-15R. Interestingly, samples with high IL-15R showed the higher expression of CXCL10. Strikingly, the location of $IL\text{-}17^+$ or $IL\text{-}23p19^+$ cells in intestinal mucosa was found different, suggesting different recruitment pathways. In conclusion, several inflammatory pathways are active in CD. IL-15, IL-17, IL-21, IL-23 and CXCL10 could participate in CD pathogenesis driving Th17 differentiation prior to or in parallel to the establishment of the dominant IFNγ pattern. Some of those elements are also present in non-coeliac individuals suggesting that regulatory pathways must be active to preserve the homeostasis in healthy tissues.

74. SLPI (Secretory leukocyte protease inhibitor) induces apoptosis on mammary tumor cells

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SLPI is an 11.7 kDa protein, characterized as an anti-inflammatory agent. It has been described that SLPI is expressed in several tumor cell lines. However, the role of SLPI in cancer is not completely elucidated. Previously studies in our laboratory showed that SLPI over-expressing cells do not develop tumor in mice. The aim of this work was to analyze the ability of SLPI to induce apoptosis of mammary tumor cells. Apoptosis was study on murine F3II cell line (mammary carcinoma) and 2C1 (F3II derived cells over-expressing SLPI). Apoptosis of 2C1 and F3II cells was measured by flow cytometry with FITC-Annexin V Kit and microscopy by ethidium bromide/acridine orange staining. The apoptosis of 2C1 cells was significantly higher than F3II control cells (45 ± 5% vs 16 ± 3%, $p<0.01$). Furthermore, 2C1 cells were much more sensitive to apoptosis induced by serum deprivation (83 ± 4% vs 23 ± 3%, $p<0.005$) and increased 18-fold cas-

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pase 3 activity compared to F3II cells. When F3II cells, cultured with or without serum, were treated with exogenous rhSLPI (4 µg/ml, 48h), apoptosis was increased (Basal: 16 ± 3%, SLPI: 21 ± 4%, p<0.05; Basal without serum: 23±2%, SLPI without serum: 34±2%, p<0.05). Also, an increase (3.5-fold) in caspase-3 activity was observed in cells culture without serum in the presence of exogenous rhSLPI. Similar results were obtained, when a mammary human tumor cell line (MCF-7) was treated with rhSLPI. Remarkably, treatment of 2C1 cells with SLPI siRNA or blocking SLPI mAb partially reversed the cell apoptosis (25 ± 3 and 13 ± 1 % of inhibition, respectively). These data demonstrate that SLPI is a pro-apoptotic factor, at least for mammary tumor cells examined. Furthermore, these results suggest that the apoptotic activity of SLPI on mammary tumor cells may be involved in the lack of growth of 2C1 cells in vivo, previously described in our laboratory.

75. Neutrophil assists the pro-apoptotic activity of SLPI (Secretory leukocyte protease inhibitor) on mammary tumor cell lines

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SLPI is an 11.7 kDa protein that plays an important role in protection against neutrophil proteases during the inflammatory response. In previous results we demonstrated that SLPI induces apoptosis of mammary tumor cells. Moreover, we described that mice mammary tumor cells SLPI overexpressing (2C1), do not develop tumors in Balb/c mice but grow in mice-depleted neutrophils (PMN). The aim of the present work was to examine the contribution of PMN to the pro-apoptotic activity of the SLPI. Apoptosis was evaluated by flow cytometry. Balb/c mice were inoculated or not with 2C1 cells (SLPI over-expressing cells derived from F3II cells). Plasma from those animals were obtained at day 2 and 7 post-inoculation and it assessed for apoptosis activity on 2C1 cells in vitro. Plasma from 2C1 inoculated animals but not control plasma, induced in vitro apoptosis of 2C1 cells (48h: Control: 37±4%; 2C1 plasma: 55±6%; p<0.05; 7d: Control: 53±10%; 2C1 plasma: 83±12%; p<0.05). The apoptotic activity of plasma from 2C1-inoculated mice, was partially lost if animals were previously PMN depleted (Control plasma: 53±12%; PMN depleted plasma: 14±4%, p<0.01). These results confirmed that the in vivo apoptotic effect of SLPI is, in part, mediated through PMN. Then, we set up an in vitro model to test if we can resemble the in vivo situation. For this, human mammary tumor cell line (MDA-MB231), HeLa (cervix carcinoma) and A549 (lung carcinoma) were treated with exogenous rhSLPI in the presence of neutrophils. We observed that PMN increased the apoptosis of the MDA-MB231 induced by SLPI (SLPI: 25±1%; SLPI + PMN: 30±1%, p<0.05). On the contrary, apoptosis of HeLa and A549 cells were significantly decreased when they were treated with SLPI + PMN (HeLa: SLPI: 55±5% vs SLPI + PMN: 42±1%, p<0.05; A549: SLPI: 53±1% vs SLPI+PMN: 44±1%, p<0.05). Overall, these results suggest that the neutrophils help the pro-apoptotic activity of SLPI on mammary tumor cells but not on HeLa or A549 cell lines.

76. Peripheric Blood Mononuclear Cells (PBMC) and polymorphonuclear leukocytes (PMNL) upregulate Secretory Leukocyte Protease Inhibitor (SLPI) expression

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SLPI is a serpin of 11.7 kDa that belongs to the family of four disulfide core proteins characterized by whey acid protein motif. The gene encoding SLPI is modulated at transcriptional and translational levels by pro-inflammatory cytokines. However, there is no data if the SLPI expression can be modulated by leukocytes recruited at the inflammation site. Therefore, the aim of this work was to examine the modulation of the SLPI expression by leukocytes on type II-like alveolar cell line (A549). SLPI was measured by sandwich ELISA in culture cells supernantant of A549, PMNL (resting and LPS-activated), PBMC (resting and IL-2-treated), lymphocyte (resting and IL-2-activated) or cocultures of leukocytes with A549 cells. CS were harvested at 18 h of incubation. SLPI (ng/ml) was present in the CS of A549 (0.06±0.02), PMNL (0.18±0.06), LPS activated-PMNL (0.54±0.06), PBMC (0.1±0.05), but not in IL-2-treated PBMC, lymphocytes or IL-2-treated lymphocytes. The co-culture of A549 with PMNL or PBMC, but not with LPS-activated PMNL, IL-2-treated PBMC, lymphocyte or IL-2-treated lymphocyte increased SLPI production (A549+PMNL: 0.84±0.21; A549+PBMC: 5.02±0.33, p<0.001 and p<0.001). In order to determined whether the increased in SLPI concentration in the co-culture of A549 with PMNL or PBMC were mediated by soluble factor released by leukocyte, A549 cells were cultured with CS derived from PMNL or PBMC. When A549 cells were treated with PMNL or PBMC-derived CS, the SLPI secretion was increased by three fold (p<0.001 and p<0.001). Interestingly, the CS-derived from activated-PMNL or IL-2-treated PBMC was not able to increase the SLPI secretion. However, if activated PBMC were pre-treated with SLPI, the SLPI secretion is restored. Overall these results demonstrate that epithelial cells secretion of SLPI may be upregulated by factors released by neutrophils and PBMC, but not lymphocytes or activated leukocytes.

77. PRE-ELAFIN: a Th2 cytokine profile inducer?

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doi: 10:3823/413

PRE-ELAFIN (skin derived anti-leukoproteinase) is a 9.9kDa serine proteinase inhibitor, precursor of ELAFIN which contains multiple transglutaminase substrate domains. The increased PRE-ELAFIN expression is well documented in inflammatory conditions in epidermis, bronchial epithelial cells, intestinal epithelium and endometrial epithelial cells. The aim of the present work was to investigate whether PRE-ELAFIN modifies the Th1 or Th2 cell pattern of cytokines. First, peripheral blood mononuclear cells (PBMC) were incubated with IL-2 in the presence or not of rhPRE-ELAFIN. After 5 days, proliferation was measured by [3H]-Thymidine uptake. We observed that rhPRE-ELAFIN decreased lymphocyte proliferation induced by IL-2 (control: 76.4±20.6% vs 20.3±2.3%, p<0.05). This effect was dose-dependent. Furthermore, cytokines were measured in the cell culture supernatant with a flow cytometry bead array kit. An increased of IL-4, IL-6 and IL-10 was observed in the culture supernatant of PBMC treated with rhPRE-ELAFIN (IL-4 control: 147±28 pg/ml vs 948±328 pg/ml, p<0.05; IL-6 control: 2370±1024 pg/ml vs 4475±1020 pg/ml, p<0.05; IL-10 control: 99±41 pg/ml vs 430±155 pg/ml, p<0.05), while it did not modify IFNγ levels. The increase production of IL-4 by PBMC treated with rhPRE-ELAFIN was confirmed by performing an intracellular staining of IL-4 on T cells. Overall, these results suggest that PRE-ELAFIN is a Th2 factor inducer.

78. Shiga toxin type-1 induce on LPS-sensitized astrocytes the release of soluble factors that alter the integrity and function of endothelial cells with brain endothelial properties

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Hemolytic uremic syndrome (HUS) is associated to infections with Shiga toxin (Stx)-producing E.coli. 30% of patients show neurophatology related to death. Damage of brain endothelial cells (EC) from the brain blood barrier (BBB) may be involved. Astrocytes (AS) are inflammatory cells surrounding BBB EC. AS interaction with EC determines BBB phenotype and function. Recently, we demonstrated that LPS+Stx induce an inflammatory response on rat AS. Then, the aim was to evaluate the effects of factors released by LPS+Stx-treated AS on EC with brain endothelial properties. EC from human umbilical cord vein (HUVEC) were differentiated to EC with brain properties (HUVECd) using condition medium (CM) of AS. HUVECd were stimulated 24h with CM from untreated AS (CMC) or treated with LPS+Stx (CML+S). We found that HUVECd treated with CML+S caused a 2-fold increase in the expression of the Stx1 receptor compared to CMC, by FACS (p<0.05), correlating with an increased sensitivity to a low dose of Stx1 (p<0.05). Beside, CML+S caused a decreased expression of tight junction protein ZO1 by FACS (MFI: CMC=3±0.2; CML+S=1±0.1;p<0.05), and the loss of peripheral distribution by confocal microscopy. Moreover, CML+S increased translocation of Stx through HUVECd seeded on a transwell, quantified using a Stx-sensible cell line (%death: CMC=18±2;CML+S=39±2; p<0.05). Similarly, PMN migration across HUVECd was increased (PMN x 10^5/ml: CMC=5±0.4; CML+S=10±0.9; p<0.05). CML+S activated HUVECd determined by an increased ICAM1 and vWF secretion (p<0.05), and directly promoted the activation of PMN and platelets (PL) (p<0.05). Furthermore, HUVECd exposed to CML+S induced PMN and PL adhesion (p<0.05). Finally, effects induced by CML+S were not seen when inhibiting AS NFkB activation or blocking AS-derived TNF-α (p<0.05). Data demonstrated that AS treated with LPS+Stx1 release factors (possibly TNFα) that alter the BBB properties and permeability on HUVECd, contributing to EC damage and BBB dysfunction

79. Contribution of neutrophils to the clearance of bacteria in a murine model of tolerance to LPS

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The exposure to repetitive doses of LPS generates a refractory state known as tolerance to LPS. Despite impaired immune functions during tolerance, the clearance of infections is increased. Our aim was to address the contribution of neutrophils (PMN) in the enhanced clearance in a murine model of tolerance to LPS. Daily i.v. inoculation of LPS (5 µg, Tol) or saline (Sn) was administered for 4 days. To determine the ability of PMN to eliminate an infection, we depleted peritoneal macrophages by chlodronate-loaded liposomes treatment prior to an i.p. polymicrobial bacterial challenge (5x10^6 colony formation units, CFU). The remaining CFU in the peritoneum 4h after challenge were lower in Tol (Sn=13125±1773; Tol=6822±1988; p<0.05). This was not due to an increased passage of bacteria to the bloodstream, as CFU in blood was also lower in Tol (Sn=209.1±62.1Tol=33.6±24.5; p<0.05). An increased number of migrated PMN was also found in peritoneum (PMNx10^6: Sn=5.3±0.5; Tol=10.4±1.3; p<0.05). Moreover, the number of PMN in blood was similar in both groups and did not change after bacterial challenge. However, the number of PMN in the marginal pool was increased in Tol as determined by perfusion of vessels (PMN x 10^6: Sn = 0.7±0.1; Tol=1.6±02; p<0.05). To determine the mechanisms involved, phagocytosis of i.p. inoculated FITC-bacteria was evaluated and FITC+PMN were determined by FACS. The amount of FITC+ bacteria/PMN was similar in both groups, but the number of FITC+PMN in Tol was higher (FITC+PMN x 10^6: Sn = 0.6±0.1; Tol = 0.9±0.1; p<0.05). Although both groups showed by confocal

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microscopy the presence of neutrophil extracellular traps (NET) in situ after bacterial challenge, the contribution of NET in clearance was only significant in Tol, showing a two-fold increase in CFU when NET were degraded by S7 nuclease ($p<0.05$). Thus, the enhanced clearance in Tol is related to NET activity and the higher number of PMN that migrate to the infectious site as a result of an increased marginal pool.

80. Effects of All-Trans-retinoic acid on the immunosuppression associated to infectious processes in a murine model

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Immunosuppresssion related to late phases of sepsis is a medical concern associated with high mortality rates. The model of tolerance to LPS mimics several aspects of sepsis-associated immunosuppression. In this sense an increase of myeloid-derived suppressor cells (MDSC, Gr-1+CD11b+CD31+) has been related to the derangements observed in the immune response. All-trans-retinoic acid (ATRA) is a derivative of vitamin-A with immunomodulating properties, that eliminates MDSC, inducing their differentiation. Our hypothesis is that ATRA could restore the immunological competence in the model of tolerance to LPS. We investigated the effects of ATRA on different immune parameters. Increasing i.p. doses of LPS were administered for 20 days and orally doses of ATRA were given for the last 13 days (LPS: 5 to 20 µg/day/mouse, ATRA: 500 µg/day/mouse). Using flow cytometry we observed that ATRA decreased the percentage (%) of MDSC in the spleen (LPS=1.67±0.17, LPS+ATRA=0.97±0.03, $p<0.05$), and increased the % of dendritic cells (CD11c+) in lymph nodes (LPS=1.9±0.1, LPS+ATRA=3.4±0.4, $p<0.05$). This was associated with an enhanced primary humoral immune response against a particulated T-dependent antigen measured by hemagglutination (Titre: LPS=2133±426, LPS+ATRA=6827±1707, $p<0.05$). Whereas LPS increased the anti-inflammatory cytokine IL-10 in plasma, ATRA reversed this effect (pg/ml: LPS=181.6±84, LPS+ATRA=13.5±1.2, $p<0.05$). Additionally, in the site of LPS inoculation an influx of neutrophils (Gr-1+ F4/80-) was observed in LPS-treated mice, concomitantly with a loss of macrophages (F4/80+); ATRA increased the macrophage population, and decreased neutrophils (%Gr-1+ F4/80-: LPS=58.9±1.1, LPS+ATRA=27.6±0.2, $p<0.05$; %F4/80+: LPS=2.1±0.8, LPS+ATRA=6.3±0.1, $p<0.05$). ATRA decreased local inflammation and reversed several parameters associated with the immunosuppression observed in the state of tolerance to LPS, and may be involve in the restoration of the immunological competence.

81. Modulation of bacterial endotoxin-induced tolerance / immunosuppression

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Endotoxin tolerance, defined as a reduced responsiveness to lipopolysaccharides (LPS) after a first encounter with endotoxin, could be involved in sepsis-associated immunosuppression. Previously we demonstrated that RU486, a glucocorticoid-receptor antagonist, induces the disruption of tolerance in mice. The aim of this study was to evaluate the effect of RU486 on both the secondary humoral and cellular immune response in LPS-induced immunosuppresed/tolerant mice (IS). In addition, we evaluated the presence of regulatory/suppressor cells, myeloid-derived GR1+CD11b+ suppressor (MDSC) and regulatory T cells (Treg) and their involvement in the tolerance phenomenon. BALB/c mice immunized with sheep red blood cells were then treated with LPS (IS). Then, mice were immunized again and treated with RU486. Antibodies production was evaluated by hemagglutination on day 7. To evaluate cellular response, IS were treated with RU486 and inoculated with radiated tumor cells and then were challenged with live tumor cells. Tumor presence was observed 10 days later. To elucidate the role of cells in the LPS tolerance maintenance we used cyclophosphamide (C) and gemcitabine (G) that reduces the number of both populations. Treatment with RU486 partially restored both the humoral response (IS: 93±5 vs ISRU486: 225±35; mean ±SEM hemagglutination titre; $p<0.01$) and cellular immune response (tumor growth = IS: 10/11; ISRU486: 3/12; $p<0.01$) in IS mice. Cell number reduction of Treg as well MDSC did not disrupt the LPS tolerance maintenance since all groups survived against a lethal dose of LPS: (MDSC: IS $1x10^8±0.6x10^7$ vs ISG $0.2x10^8±1x10^7$, $p<0.001$; Treg: IS $1x10^7±8x10^5$ vs ISC $0.2x10^7±1x10^5$, $p<0.001$). RU486 partially restores the humoral and cellular immune response in IS, suggesting the involvement of endogenous glucocorticoids (GC). The regulatory/ suppressor cells do not play a major role in the LPS tolerance phenomenon and their presence seems to be a peripheral phenomenon of GC action.

82. Clinical evolution of 30 patients with humoral immunodeficiency with treatment with intravenous gammaglobulin for at least 2 years

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Humoral immunodeficiencies (HI) are a heterogeneous group of diseases with absence response of antibodies. The most frequents are: X-linked agammaglobulinemia (XLA), Common variable immunodeficiency (CVID) and fails to respond to polysaccharide (FRP). These diseases have difference in the age at the beginning, the clinical presentation and the evolution of

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the disease, but the high numbers of infections and the benefit with intravenous gammaglobulin (IG) treatment is common to all of them. Aim: To describe the evolution of 30 patients with HI, receiving treatment with IG. Material and Methods: Retrospective study of 30 patients with HI and IG treatment for at least 2 years of follow up. Results: We analyzed clinics history of 10 patients with XLA, 10 with CVID and 10 with FRP. The average of the age at the diagnostic was 5.4 years (0.083-15). The most frequent clinic manifestations were upper respiratory infections. The average of infection per year was 2.07 and stay at hospital was 0.72 previously at the diagnosis. After beginning with treatment with IG both index decreased to 1.88 and 0.10 respectively. 17 patients (56.7%) had respiratory sequela with bronchiectasis. 10/17 improved or stayed at the same state after the beginning of the treatment .13 patients didn't have sequela at the beginning but 7 of them presented bronchiectasis during evolution. 56% of this last group had bad adherence to the treatment. 6/30 had mild adverse effects, 83% was headache and fever, only one had generalized rash. Conclusion: Our patients presented significance decrease in hospitalizations per year but they had little difference in the number of infections per year. The most patients had improved their sequela after IG treatment but the progression on the pulmonary lesion is related with a bad adherence to the treatment.

83. Autoantibodies against muscarinic acetylcholine receptors in breast cancer. Its role in tumor angiogenesis

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We had demonstrated that autoantibodies from the IgG subtype, present in the sera of breast cancer patients activate muscarinic acetylcholine receptors (mAChR). These receptors are expressed in human breast tumors and in the line, MCF-7, derived from a human mammary adenocarcinoma. Both, the muscarinic agonist carbachol (CARB) and the IgG from patients at T1N0Mx stage (tumor size <2cm, without lymph node metastases) stimulate the proliferation and migration in tumor cells but not in normal in human breast cells, MCF-10A. Angiogenesis is a central step in tumor progression because it promotes tumor growth and metastatic spread, so we investigate the role of CARB and T1N0Mx-IgG in the angiogenic response induced by MCF-7 cells. By western blot, we demonstrated that MCF-7 cells but not MCF-10A cells express vascular endothelial growth factor A (VEGF-A) (1.01±0.01 relative O.D; n=3). Treatment with CARB (10^{-9}M) for 1 h increased VEGF-A expression (2.64±0.26; p<0.001 vs. cell without treatment) and preincubation with the nonselective antagonist atropine (AT) (10^{-6}M) reverted this effect (1.67±0.17; n=3). In an vivo angiogenesis assay in nude female mice, we found that MCF-7 cells stimulated neovascularization (vessels N°/mm^2) (control: 1.12±0.2; n=3; MCF-7: 1.86±0.30; n=15 p<0.05 vs. control), whereas MCF-10A were not angiogenic (1.46±0.38; n=10). When MCF-7 cells were previously treated with CARB (10^{-9}M or 10^{-10}M) an increase in the neovascular response was observed (2.82±0.55; n=14 p<0.001 vs. control or 3.50±0.74; n=10 p<0.001 vs. control). AT reduced these effects (1.42±0.3; p<0.05). We also observed that pretreatment of cells with T1N0Mx-IgG produced and angiogenic effect that was stronger than that of CARB (3.24±0.24; p<0.01 vs. CARB) and was also decreased in the presence of AT (2.06±0.42; p<0.001 vs. IgG-T1N0Mx). We conclude that mAChR activation either by CARB or by auto-Abs may promote the neovascular response in patients with breast cancer.

84. 3-Hydroxy Kynurenine (3-HK) treatment for T. cruzi infection ameliorates the symptoms of chronic Chagas' heart disease controlling the parasite replication and the inflammatory associated pathology

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Chronic Chagas' heart disease, caused by the parasite T. cruzi, is an inflammatory cardiomyopathy that may lead to cardiac dilatation, congestive heart failure, and death. We demonstrated that the therapeutic administration of 3-HK, a catabolite of the tryptophan degradation driven by Indoleamine 2,3 dioxygenase (IDO), decrease the parasitemia of T. cruzi-infected mice and improve their survival. To investigate the effect of 3-HK treatment on the outcome of chronic Chagas' heart disease, BALB/c mice were infected with 500 tripomastigotes of T. cruzi and 5 days post infection (dpi), the mice were i.p. treated with 1 mg/kg/d of 3-HK (3-HK mice) or PBS (control mice) for 5 days (dpi5-10). Electrocardiograms performed at 60 dpi revealed that 3-HK mice showed a significant decrease in the incidence and the severity of the alterations compared with controls (p<0.02). Next, we investigated whether 3-HK treatment could condition the development of a particular immune response able to contribute to the control of the infection or the inflammatory associated pathology. Spleen cells (SMC) from 16 dpi (acute) or 63 dpi (chronic) mice that were treated or not with 3-HK were cultured with an extract of T. cruzi (F105) and the secreted cytokines determined by ELISA. SMC from acutely infected 3-HK mice secreted lower IFN-γ and IL-5 levels (p<0.02) and more than 4-times the TGF-β levels (p<0.001) than those secreted by control mice. During the chronic phase of the infection, SMC from 3-HK mice showed a long term Th1-like response able to secrete the protective cytokine IFN-γ (p<0.02) but not the pathogenic cytokine TNF, while SMC from control mice did not show significant cytokine response against F105 compared with non infected mice. In addition, 3-HK mice presented an important increased in CD4+CD25+Foxp3+, CD4+CD25+GITR+ and CD4+CD25+IL-10+

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Treg populations. Our results show evidence for 3-HK as a novel therapeutic treatment to control both the parasite replication and the inflammatory associated pathology.

85. Microdeletion 22q11.2: experience in 26 patients diagnosed in the city of Córdoba, Argentina

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22q11.2 microdeletion is the most common in humans. The incidence is approximately 1/4000 births. Clinical variability has been described including DiGeorge (DG)/Velo-Cardio-facial (VCF) syndromes and isolated conotruncal congenital heart disease (CCHD). Currently these phenotypes are called 22q11.2 Microdeletion Syndromes. Fluorescent in-situ hybridization technique (FISH) is needed for diagnosis. To estimate the prevalence of microdeletion 22q11.2 on a sample of patients with suggestive clinical features assisted in the city of Córdoba, identify clinical manifestations and diagnose inheritable forms.We studied patients from two institutions from Córdoba that met the following criteria: a) phenotype DG/VCF or two or more of the following clinical signs: isolated CCHD, cleft palate, velopharyngeal incompetence, characteristic facial features, developmental delay, immunodeficiency or thymus hypoplasia. b) Parents of patients with positive microdeletion. Clinical evaluation, high-resolution cytogenetic and FISH techniques for 22q11.2 region were performed. We studied 183 patients: 137 (74.8%) had DG/VCF phenotype, 32 (17%) isolated conotruncal cardiac anomalies and 16 (8.2%) were parents. Microdeletion 22q11.2 was detected in 26/183 patients (14.2%), 11 females, 15 males. Mode of age 6 years: range 2 days to 32 years. In our series 24 patient had DG/VCF phenotype (92%) and 2 isolated CCHD (8%). Six patients were considered to be immunodeficient regardless of their clinical presentation, one of them was confirmed. Six patients had hypocalcemia and two juvenile rheumatoid arthritis. Four patients died due to heart disease. Only one parent was positive for microdeletion. Relative prevalence of 22q11.2 microdeletion was 14.2%. Clinical variability was similar to the bibliography. Only one patient was diagnosed of inherited form. It stresses the importance of early diagnosis to establish anticipatory prevention measures and/or early diagnosis of associated diseases.

86. Novel mutations in TNFRSF13B in pediatric patients with common variable immunodeficiency

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Common variable immunodeficiency (CVID) is a heterogeneous syndrome characterized by impaired immunoglobulin production. The clinical course of CVID is complicated by systemic immunopathology including gastrointestinal, lymphoproliferative, autoimmune, and granulomatous diseases. Mutations in the gene encoding TACI (Transmembrane Activator and Calcium modulator and cyclophilin ligand Interactor, TNFRSF13B) were previously found to be associated with CVID. The aim of the present study was to determine the prevalence of TNFRSF13B mutations in Argentinean patients with pediatric presentation of CVID. We sequenced TNFRSF13B gene in a cohort of 32 Argentinean CVID patients with pediatric presentation of the disease. In patients with novel mutation we study the mRNA and protein expression of TACI by direct sequencing of cDNA or flow cytometry analysis, respectively. Among the 32 studied patients we identified 2 patients carrying a different heterozygous mutation in TNFRSF13B each other. These mutations were not found when studying 80 control chromosomes. One of them, S231R, affected the TACI highly conserved (THC) domain, important for the induction of class switch recombination (CSR). The other novel defect, S144L, is a missense mutation affecting a site previously reported in a CVID patient as a null mutation. We also evaluated the effect of these novel mutations in CSR induction. All patients from our cohort presented TACI variants (T27T, P97P, V220A, P251L, and S277S), not associated with development of CVID in previous reports, at frequencies comparable to those in either our control population and in NCBI build 36.3. In addition, C104R mutation, previously described as the most frequent in CVID, was not found in our patients. The identification of TNFRSF13B mutations, besides aiding in CVID diagnosis, could contribute to understand the mechanism that underlies CSR signaling by TACI.

87. Acute flaccid paralysis (AFP) by polioviruses in patients with primary immunodeficiencies (PID): report of two cases

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AFP is a mode of presentation of polioviruses (PV) infections. Since 1994 no cases of wild polio had been reported in America. Patients with certain PID have an increased susceptibility to polio and non-polio enteroviruses. We report 2 patients with PID and PV infection. Patient 1: boy correctly immunized. At 15 months he presents with right crural monoparesis with decreased tendon reflexes but without sensory loss. Cerebrospinal fluid revealed pleocytosis, elevated protein concentration

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and poliovirus 2 was isolated in culture; magnetic resonance imaging (MRI) showed mesencephalic and thalamic lesions and unilateral commitment of spinal anterior horn; the electromyogram showed pre-ganglionar mielorradicular involvement. His little brother had been recently immunized with OVP. Agammaglobulinemia, absence of B lymphocytes and mutation in BTK confirmed the diagnosis of X-linked Agammaglobulinemia, and the child was given monthly therapy with iv immuno-globulin (IVIG), and physiotherapy. Evolution (18 month) motor sequelae. Patient 2: boy, with a history of sepsis by Candida, and recurrent bacterial infections of the respiratory tract. At the age of 15 months, with diagnosis of sepsis by Haemophilus spp, the immunological evaluation showed severe hypogammaglobulinemia (IgG/A/M: 33/<6/<4 mg/dl), with normal number of B cells and preserved cellular immunity. After second dose of IVIG he presents an aseptic meningoencephalitis. Fifteen days after then, he developed left crural monoparesis with hyporreflexia. MRI showed focal injuries of spinal anterior horn; the electromyogram showed proximal pre-ganglionar involvement Poliovirus type 1, with 96,5% genetic similarity to Sabin 1 vaccine strain, was obtained in stool cultures. Evolution (15 months) motor sequelae. Conclusions: The AFP by PV can be the first clinical manifestation of PID. In patients with PID with neurological symptoms seems necessary a wide microbiological searching including enteroviruses.

88. Characterization of the cells used for adoptive transference in an immunotherapeutic protocol that induced regression of an established murine tumor

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Anti-tumor immunity is often impaired by the growing tumor by the induction of immunosuppression and tolerance. Using an experimental murine fibrosarcoma (MCC) that progresses from a strongly immunogenic to a tolerogenic state, we have previously demonstrated that MCC growth induces a specific immune response that coexists with signs of suppression, like IL-10+ B cells, mainly at the tumor draining lymph node (TDLN). We therefore designed an immunotherapeutic trial consisting of the ablation of TDLN in order to eliminate an incipient focus of suppression, the ex vivo expansion of the ablated TDLN cytotoxic cells with anti-CD3+IL-2, and the re-inoculation of these cells into the donor tumor-bearing mouse by adoptive transference. With this protocol, lower tumor growth, enhanced survival and a high rate of complete tumor remission were obtained. The aim of the present work was to characterize the adoptively transferred cells. Freshly harvested TDLN cells were mostly composed of B, CD4+T and CD8+T cells; ex vivo exposition to anti-CD3+IL-2 increased CD4+ and CD8+T and decreased B cell proportions (% cells before versus after culture: TCD4+: 37±1 vs 74±7***; TCD8+: 17.3±0.5 vs 26.8±6**; B220+CD19+: 44±3 vs 7.3±2***, n=6), and increased the intracellular IFN-γ- expressing T cells (% IFN-γ+/CD4+ cells before versus after the culture: 4.3±1.7 vs 12.0±4.6*; % IFN-γ+/CD8+: 8.9±5.4 vs 20.8±6.8*, n=5). Additionally, cultured cells showed enhanced anti-MCC cytotoxicity evaluated by JAM test. By staining cells with CFSE prior to their adoptive transference, we were able to detect them within the tumor tissue, spleen and distant lymph node on day 7 after the innoculum. Our results suggest that the anti-tumor effects obtained with the treatment are due, at least in part, to anti-CD3+IL-2- induced decrease of B cells and increase of IFNγ+ T cells with enhanced cytotoxic capacity. (*p<0.05, **p<0.01, ***p<0.001).

89. Unveiling antigens in a non-immunogenic spontaneous murine tumor using dendritic cell-based vaccine

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Most attempts to use immunotherapy to cause the regression of animal and human established tumors have not been effective. Former experiments have suggested that this failure could be related to a lack of immunogenicity of spontaneous tumors. We have investigated if this lack of immunogenicity can be attributed to the absence of tumor antigens or to the existences of tolerogenic mechanisms preventing such antigens from initiating an antitumor immune response. We use two different tumors-a non-immunogenic spontaneous lymphoma (LB) and an immunogenic fibrosarcoma (MCC)-, and, dendritic cells (DC) loaded with tumor lysate as immunization assays. When DC were pulsed with LB lysate (DC+LB), no maturation of DC was achieved and no protection against LB after DC+LB inoculation was observed. We also demonstrated that DC pulsed with MCC lysate (DC+MCC) induce the maturation of DC, producing a strong protection against MCC implants after DC+MCC inoculation. When DC were pulsed with both lysates (DC+MCC+LB), we observed a mild maturation of DC, a significant protection against LB (p<0.001) and a reduction of the protection against MCC (p<0.01). As LB and MCC have not common antigens, we suggest that: LB bears specific tumor antigens but lacks other signals to achieve DC maturation that are provided by MCC lysate. In addiction, LB would display active tolerogenic mechanisms which reduce the protection against MCC when DC+MCC+LB (p<0.001) are inoculated in vivo. These tolerogenic mechanisms seem to be associated with the high expression of pSTAT3 in LB cells, since inhibition of pSTAT3 by treating LB bearing mice with the specific inhibitor JSI-124, reduced LB tumor growth associated with the emergence of a specific antitumor immune response. We propose that the high expression of pSTAT3 is one of the reasons by which the LB tumor behaves as a non-immunogenic, and that targeting its expression might promote an efficient antitumor immune response against this spontaneous tumor.

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doi: 10:3823/413

90. T and B cells inmunophenotyping in patients with active pulmonary tuberculosis

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The host immune response to Mycobacterium tuberculosis is particularly important in preventing clinically evident disease following infection, especially cell-mediated immunity. CD4+ T cells have an essential role in this response but are supported by other T-cell subsets such as CD8+, meanwhile B cells influence the inflammatory progression due to accumulation in the site of infection. The objective was to evaluate the T and B cells subpopulations in patients with active pulmonary tuberculosis, without treatment, that attended the National Institute of Respiratory and Environmental Diseases of Asuncion, Paraguay. With previous signed consent, 29 patients and 29 apparently healthy individuals, used as controls, were studied. The quantification of T and B cells was performed in peripheral blood by flow cytometry. Cells marked using the panels: CD3/CD4/CD45, CD3/CD8/CD45 and CD3/CD19, were acquired in a FACSCalibur and analyzed using Cell Quest software. From the studied patients 18 were males. The average age of the patients was 41 years, with a range between 18 and 79, and 35 years for the controls with a range between 18 and 55 years. The TCD4+ cells average in patients was 36% and 38% in controls. Using ANOVA test no significant difference was found (p=0.426). In patients the average of TCD8+ cells was 28% and 30% for controls (p=0.024). The average of T cells in patients was 71% and 66% in controls (p=0.888). The BCD19+ cells average in patients was 125 in patients and 13% in controls (p=0.229). In this study the patients with tuberculosis without treatment showed a significant decrease of TCD8+ cells compared with apparently healthy individuals. Previous studies had suggested that TCD8+ are necessary to control M. tuberculosis infection, therefore a better understanding of the immune mechanisms involved in early stages of the disease is essential for the follow up of the patients or for new alternatives of immunotherapy.

91. Pro inflammatory cytokines in patients with dengue

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Pro-inflammatory cytokines are believed to play an important role in the pathogenesis of dengue infection. There is clear evidence confirming that monocytes/macrophages respond to infection by dengue by stimulating memory cells T (CD4+cells) to produce diverse cytokines. Predominant Type 1 helper responses resulting in IFN-γ and TNF-α production are responsible for cell-mediated inflammatory reactions. The objective of the study was to determine TNF-α and IFN-γ in serum of patients with dengue IgM positive and compare it with patients with dengue negative IgM, which attended the Instituto de Investigaciones en Ciencias de la Salud, from February to April of 2007 during the dengue epidemic. The study was previously approved by Ethics and Scientific Committees of the institution and performed in 163 patient's sera, 143 positive for dengue IgM and 20 negative, from both sexes with an average age of 30 years and a range between 18 and 70 years. The IgM antibody for dengue and the serum levels of cytokines were performed by capture ELISA methodology. The serum level of IFN- was detected in 33 (23%) of 143 patients dengue positive IgM with an average of 381.8 ± 264.7 pg/ml. Using Chi^2 test a statistically significant difference was found (p= 0.007), compared to the values of the 20 dengue IgM negative sera 38.2 ± 4.02 pg/ml. The serum level of TNF-α was detected in 4 (2.8%) of the 143 studied dengue IgM positive patients with an average of 37.5 ± 10.6 pg/ml. Applying Chi^2 test, no statistic significance was found (p=0.59), compared to the 20 dengue IgM negative sera with an average of 17.9± 1.5 pg/ml. Further investigations are necessary to determine the significance of the presence of these cytokines in febrile dengue, because this can trigger a hemorrhagic dengue, from which arises the importance of the TNF- α and IFN- γ dosage for the management of the disease

92. Hepatitis B virus during pregnancy

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The child is infected with hepatitis B at birth, due to exposure to infected blood through mucous membranes or skin lesions. Transplacental transmission occurs in less than 5% of cases when the pregnant woman suffers from acute hepatitis in the third trimester of pregnancy or if she is a chronic carrier with hepatitis Be antigen (HBeAg) positive, which shows the degree of maternal infectivity. Over 90% of children born to a mother HBeAg (+) and only 15% to 20% of those born to a mother with antibodies against antigen hepatitis Be antigen (anti HBeAg) get infected. The objective was to assess how many pregnant women attending for antenatal care to the Institute for Research in Health Sciences in a period of five months of 2009, were asked to determine the surface antigen of hepatitis B (HBsAg). In this retrospective descriptive study, we examined appropriately coded sheets of 73 pregnant women, with ages between 16 and 47 and an age average of 30 years, with prenatal medical order, whose data and results were kept confidential. The HBsAg was requested to 42 pregnant women and was performed by ELISA. Of the 73 pregnant women with prenatal testing order, HBsAg was requested to 42 of them. One of them was positive for HBsAg. Pregnant women who have been infected with hepatitis

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B virus can pass it to child during birth. Therefore, the medical request of this antigen is important during pregnancy because the more advanced the pregnancy at the time of infection, the greater is the probability that the child will get infected. It would also be interesting, if the mother is positive for HBsAg, to assess the degree of maternal infection by testing her for HBeAg and anti HBeAg.

93. Hyper-IgE Syndrome (HIES): clinical and immunological features of 28 patients from a single center

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The autosomal-dominant HIES (AD HIES) is a complex primary immunodeficiency with immunologic, infectious, connective tissue and skeletal abnormalities. NIH score (NHIs) has been the only clinical tool to diagnosis until mutations in STAT3 were identified. A new score for predicting mutation in STAT3 (STAT3s) is available, together with reduction of Th17cells. Objective: To describe the clinical and immunological features of patients (p) assisted in our center with a strong suspicion of HIES and NIHs ≥20. Methods: Data were retrospectively collected. NIHs and STAT3s were performed at last follow up. Th17 were determined by flow citometry. Coding regions of exons 13, 14, 16, 17, 18, 19, 20, 21 and their flanking intron junctions were sequenced. Results: 28p (20 male, 8 female) were identified. The mean age at first immunological consult was 4.56 years (r: 0.21-13.4). The most common infections were pneumonia (82%) and skin abscesses and boils (61%). Eczema and characteristic face were the more frequent noninfectious manifestations (82%) followed by hyperextensibility (62%) and lung cyst formation (57%). The mean age at scoring was 9.75 years (r: 2.25-19.6). Both NIHs ≥40 and STAT3s ≥30 were observed in 54% of the patients. Of 14p evaluated, 5p carried reported mutations and 3p novel missence alterations. All patients with mutation had NIHs ≥40 and STAT3s ≥30. Four of 6p without mutation had STAT3s <30. All 6p evaluated for Th17, showed low values (<0.5%), of whom 4p carried mutation, 1p was not molecularly evaluated, and 1p doesn't have mutation. Vascular abnormality was seen in 1p (carrying STAT3 mutation). One patient died at the age of 17 with severe lung involvement. Conclusion: compared to literature our cohort is younger but the frequencies of clinical manifestations are similar. STAT3s and Th17 <0.5% seem to correlate with the presence of mutation.

94. Griscelli syndrome type 2, a single- center experience

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Griscelli syndrome type 2 (GS2) is an autosomal, recessively inherited disease, characterized by albinism oculocutaneous and hemophagocytic lymphohistiocytosis (HLH), caused by mutations in Rab27a. Hematopoietic stem cell transplantion (HSCT) is the only curative treatment. We describe clinical and immunological features of patients (p) with GS2, diagnosed in our center. Results: 7p with GS2, of 6 unrelated families, were diagnosed between 1987 and 2010. Consanguineous parents were observed in only one family. The mean age at diagnosis was 1.39 years (0.12 -3.6). At diagnosis 3p had albinism oculocutaneous, 4p albinism and HLH. Of these last, 2p showed concomitant central nervous system (CNS) involvement. The mean age at HLH onset was 1.20 years (0.12- 3.8), 2p had more than 1 episode of HLH. Marked impaired NK cell activity was observed on 4/5p. Of 2p who were treated with VP16 and corticosteroids, 1p had partial remission, and other patient, who evidenced neurological HLH, died by sepsis soon after diagnosis. Three patients who were treated with cyclosporine (CSA) and prednisone showed complete remission. One patient presented remission with pretransplant conditioning regimen (Anti-thymoglobulin, Fludarabine, Melphalan). On the second patient with CNS involvement, neurological improvement was obtained after repeated doses of intrathecal methrotrexate, but neurological sequelae were observed. Two patients received HSCT, 1 matched related donor and the other matched unrelated. Both showed active HLH at HSCT. Engraftment occurred in both patients. One patient died by sepsis within 6 months of HSCT, the other patient is alive at 4 months of HSCT, without manifestation HLH. Three patients, without active HLH, are treated with CSA and Prednisone, one patient receives no treatment. Conclusions: the mean age at diagnosis is similar from the description in the literature. HLH remission can be archived only with CSA and Prednisone. HSCT should not be postponed because of active HLH.

95. Homeostatic effect of lipoteichoic acid from L. rhamnosus GG after immunosuppressive UV irradiation

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Immunosuppressive effect of UV radiation (UVR) has been largely described. Following skin UV exposure, immune response is biased towards a Th2/Treg profile. In contrast, probiotics have been largely described as beneficial for the host's immune system. Lactobacilli are the best described probiotics and lipoteichoic acid (LTA) is a major cell wall antigen in this bacteria. Recently, skin homeostasis recovery of UV induced immunosuppressive effect with oral probiotics has been shown, reinforcing the idea of the existence of a gut-skin immune axis. The aim of our study was to evaluate the effect of the oral administration of LTA from L. rhamnosus GG (LrGG) on acute UV irradiated mice focusing on immunological parameters of immunosuppression. SKH:1 hairless mice were divided into 3 groups of 5. One group received 100µl of LTA (1mg/ml) and other group received 100µl/day of PBS for 10 days and the other group was untreated.

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doi: 10:3823/413

Treated groups were irradiated with 400mJ/cm2 of UVB once and sacrificed 24hs later. ConA induced T cell proliferation was evaluated on inguinal lymph nodes (ILN) and spleen cells. IL-10, IL-4 and IFN-γ were measured in the culture supernatants. ILN cells were stained with anti-CD3, CD4 and CD8 fluorescent Abs and epidermal cells with anti-CD3 and γδAbs for FACS analysis. While no difference was found in the proliferative response in either organ, IL-10 production (increased after UV radiation) in ILN was lower in LTA group than in PBS group ($p<0.05$). Additionally, ILN CD3CD4+ cell percentage was higher in LTA group than in PBS group (51.04±3.30 vs 45.77±2.30, $p<0.05$) and epidermal CD3δ+ was lower in LTA group than in PBS group (1.99±0.69 vs 3.02±0.96, $p<0.05$). This study shows that LrGG LTA administration reverts, at least partially, some of the immunosuppressive effects of UVR. Probably, this phenomenon is mediated by the effect LTA has on the GALT, which in turn results in a homeostatic systemic effect observed in the skin draining lymph nodes.

96. Primary immunodeficiencies in children with recurrent infections

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Primary immunodeficiencies are a group of hereditary disorders affecting the immune system and as a result infections are more common. The objective of this study was to evaluate serum levels of immunoglobulins in children with repetitive infections. 22 children were included (7 females and 15 males), with an average age of 5 years, from March of 2006 to April of 2008. With parental consent previously obtained, data was gathered in a file and blood samples were taken. Immunoglobulin's dosage was performed by immunoradial diffusion and chemiluminiscent methodology. The more frequent referred infections were pneumonias. IgA levels were<5 mg/dl in 2 patients (9%); IgM levels were > 250 mg/dl in 11 patients (50%) and IgE levels were > 91 mg/dl in 14 patients (64%). In this series of 17 patients with repetitive infections we had found two patients (9%) with primary immunodeficiency due to IgG deficit. The immunologic evaluation of the patients with repetitive infections is important, because the early diagnosis helps to improve the outcome and avoid complications in the patients.

97. EBV infection and immunodeficiency: atypical evolution of EBV in patients with severe immunological compromise

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Epstein-Barr virus (EBV) can establish persistent infections in immunocompetent hosts. It is known to be involved in several lymphomas and carcinomas and has the capacity to transform B lymphocytes in culture and also it is related to certain Primary Immunedeficiency Diseases (PID). Aim: to determinate if should to be investigated all atypical EBV infection evolution. Retrospective assay of clinical histories of 4 patients with atypical EBV infection evolution. P1: Female, 3 years (y), Burkitt lymphoma. Severe hypogamaglobulinemia, absente response to pneumococo, CD4 lymphopenia, normal proliferative response (PR). CVID phenotype. Treatment (T): iv Gammaglobulin replac ement(ivGGr)+chemotherapy. P2: male 3y, Infectious mononucleosis, hepatitis, Hodking Lymphoma, chronic upper and lower respiratory supurative infections. Severe CD4 lymphopenia, elevated __ Tcells, CD4RA 1%, CD8+DR+51%, absent response to viral antigens. Dead 7y. P3: male 4y, consanguineous parents, 1 year prolonged fever syndrome, infectious mononucleosis, progressive hepatitis, bone marrow failure, coronary vasculitis, hemophagocytic syndrome. Positive EBV:PB, CSF, liver & lung biopsy. Absent CD20-CD19 cells, antibody response & NK function. Low PR to OKT3 stimulation. CD8+DR+ 52-78%. Molecular analysis negative for: SH2D1A, BRIC4, STXBP2 and STX11. T: ivGGr, HLH2004, HSCT. P4: male, 7y, lymphoprolipherative syndrome, chronic upper & lower airway infections, opportunistic infections, thrush, recurrent aphtoid ulcers, multivisceral granulomatous disease, Panuveitis, CNS non Hodking Lymphoma, EBV+ in situ hybridization. Hyper Gammaglobuline IgG, CD4 lymphopenia, low PR mitógenens, positive auto antibodies, low CD40 ligand, normal molecular assay to CD40L, NEMO, SH2D1A. T: ivGGr, chemotherapy, HSCT: died at 15y. Conclusion: It is consistent immunological involvement in all patients. There is none of them with molecular diagnosis. It is of warning an atypical evolution should suggest to investigate PID.

98. Design and characterization of liposomes as vaccine adjuvants

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The great progress in subunit vaccines development requires the design and exhaustive study of new adjuvant formulations. Although liposomes have been widely used as drugs vehicle and immune adjuvants, scarce work exists on the characterization and standardization of these formulations. The aim of this work was to design a liposome formulation containing bovine serum albumin (BSA) as a model immunogen and to set up a protocol of characterization of the formulation. Lyophilization process was also assessed in order to obtain a stable formulation. Liposomes were prepared with dipalmitoylphosphatidylcholine, cholesterol and stearylamine, in a 7:2:2 w/w ratio and antigen was added at 0.23 mg/ml in a solution with sucrose as

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cryoprotectant. The amount of BSA incorporated into lyophilized and non-lyophilized liposomes was analyzed by the bicinchoninic acid assay and competitive ELISA. The ability of the formulations to induce an immune response was assessed by immunization of mice with two doses of 1 or 10 µg of antigen in lyophilized or non-lyophilized liposomes. Liposomes incorporated 33.9% of the offered BSA, 10.2% being on the particle surface. After the lyophilization and resuspension of liposomes, 18.3% of the BSA initially used remained in the particles, being 4.8% of this protein in the particle surface. Nevertheless, both lyophilized and non-lyophilized liposomes induced a humoral immune response significantly different from control without adjuvant and not different among them ($p<0.05$, Kruskall Wallis test). No significant differences were observed in antibody levels when 1 or 10 µg of antigen were inoculated. Our results confirm the efficacy of liposomes as vaccine adjuvants and introduce a protocol to standardize a manufacturing process. They also demonstrated that lyophilization is a suitable alternative for the conservation of vaccines containing liposomes.

99. Further analysis of protection induced by the rTgPI-1 vaccine against Toxoplasma gondii: characterization of the vaccine-induced immune responses in C57BL/6 mice

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We previously showed that the recombinant protein rTgPI-1 (PI) used as an immunogen, resulted a potent vaccine against toxoplasmosis in two mouse strains. Vaccination of highly susceptible C57BL/6 mice induced a reduction of 49% in the parasite load after an oral infection. This protection was achieved only when a prime-boost protocol combining intradermical (ID) and intranasal (IN) immunizations were used. This strategy that included Alum (A) ID and ODN-CpG (C) IN induced the elicitation of a Th1 specific humoral response. The present study was conducted to further characterize the cellular immune response generated by the PI-based protective vaccine. B6 mice were inoculated with 2 doses of [PI+A]ID and boosted with 2 doses of [PI+C] IN (G4) and the control groups received 4 doses of [PI+C] IN (G3) or [PI+A]ID (G2) or 2 [A]ID + 2 [PI+C]IN (G5) or 2 [PI+A]ID + [C]IN (G6) and a naïve control group was also included (G1). Two weeks after the last inoculation, some groups of animals were sacrificed for in vitro splenocyte culture studies (G1-4). After 5 days of in vitro PI stimulation, only G4 showed a significant lymphoproliferative response (Δcpm G4:24813±4209 vs G1:94±1.3a), and a significant increment in both CD4+ (absolute cell number G4:169667±28312 vs. G1:62550±1703a) and CD8+ lymphocytes (G4:92767±16372 vs. G1: 30300±462a) as measured by flow-cytometry. In order to test if both id and in administration of PI (G4) was necessary for protection, a challenge assay was performed including control G1, G5 and G6 groups. We observed that only G4 mice presented a significant brain parasite load reduction (G4:562±131 vs. G1:1910±153b) with an in vivo specific cellular response measured by a DTH assay (mm G4:0.089±0.016 vs. G1:0.031±0.009a) (Data: mean±SEM; ap<0.05; bp<0.01). These results indicate that this PI-based protective immunization strategy, in which PI is administrated by both the ID and IN routes, elicited a humoral and also a strong cellular immune response.

100. Acute Toxoplasma gondii infection modulates systemic immune response against allergen in a murine model of allergic airway inflammation

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We previously showed that acute infection with T.gondii blocks the development of allergic lung inflammation. OVA-sensitization during acute phase results in lower levels of OVA-specific IgE, IgG1 and Th2 cytokines from thoracic lymph nodes (LN). The increase in OVA-specific IgG2a and IFN-γ levels from OVA stimulated LN cells suggests that parasite infection induces a shift towards a Th1 response. Nevertheless, regulatory mechanisms could also be implicated since increases in TGF-ß levels are detected, although IL-10 behaves as a typical Th2 cytokine. The aim of the present study was to evaluate the systemic levels of Th1, Th2 and regulatory cytokines. Adult BALB/c mice were ip sensitized and aerosol challenged with OVA (O). One week before sensitization, the experimental group (OT) was orally infected with cysts from the Beverly strain. Two days post challenge, splenocytes were in vitro stimulated with OVA or total lysate T.gondii antigen (TLA) and supernatant cytokine levels were evaluated. Sensitization during acute T.gondii infection induced a systemic decrease in OVA specific IL-4 (pg/ml: Normal (N):ND; infected (T):ND; O:1341±198a; OT: 469±52) and IL-10 (pg/ml: N:ND; T: 28±28; O: 642±7 c; OT: 371±193c). An increase in allergen specific IFN-γ levels in OT compared to the O group was detected (pg/ml: N:ND; T:ND; O: 106±27a; OT: 322±4a). TGF-ß levels showed no significant differences. Nevertheless, a trend towards increased TGF-ß production was observed in the OT group (OD: N: 0.02±0.09; T:0.06±0.02; O: 0.07±0.03; OT: 0.13±0.05). IFN-γ and IL-10 levels in the infected groups stimulated with TLA are those expected in the immune response against T.gondii (IFN-γ pg/ml: N: 57±57; T: 850±67b; O: 13±4; OT: 694±235b; IL-10: N: 133±93; T: 720±265; O: 29±15; OT: 748±295). (Mean±SEM. a P<0.05 vs all groups; b P<0.05 vs N y O; c P<0.05 vs N y T. ND: not detected). These results show that the systemic cytokine profile correlates with the one observed in lung draining LN.

102. Production of Reactive Oxigen Species by intestinal epithelial cells triggered by interaction with probiotic microorganisms

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No. 3:4
doi: 10:3823/413

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Different probiotic microorganisms may modulate intestinal immune response, however the mechanisms involved remain poorly characterized. Modulation of intestinal epithelial response may contribute to probiotic function. Different intracellular signaling pathways have been shown to be modulated by epithelial-microorganism interaction. Differential targeting to the proteasome of key players of inflammatory pathways such as IkBa protein can be modulated by probiotic bacteria by a mechanism dependent on the production of Reactive Oxygen Species (ROS). The aim of the present work was to set up a system to study this phenomenon on intestinal epithelial cells and test the effect on this pathway by a panel of probiotic bacteria and yeast. We standardized a procedure using Caco-2 human intestinal epithelial cell line, a ROS-indicator dye (C-H2DCFDA) and physiologic inducers of ROS production on Caco-2 cells to quantify the ROS production by fluorescence microscopy. We used this system to test a panel of 20 strains from genus Lactococcus, Enterococcus and Lactobacillus and yeasts from genus Saccharomyces and Kluyveromyces on the induction of ROS. The capacity of these microorganisms to down-regulate the expression of CCL20 was evaluated using a reporter CCL20-luc Caco-2 cell line and flagellin stimulation. Some of the bacterial strains as Lactobacillus brevis ATCC 8287 and Enterococcus faecalis induced a significant two-fold production of endogenous ROS. Although yeast strains did not induce ROS response, they reduced more than 90% the flagellin-induced CCL20 response. A system to evaluate the capacity of microorganisms to trigger ROS production in intestinal epithelial cells was developed. No correlation was observed between the capacity to modulate CCL20 response and ROS production, indicating that other mechanisms triggered by microorganism-epithelial interaction may control this innate activation of epithelial cells.

103. Regulation of IL-1ß release by human neutrophils

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IL-1ß is a pro-inflammatory cytokine which is released after proteolytic processing of pro-IL-1ß. Stimulation of NOD-like receptors leads to the formation of an inflammasome, which recruits and activates caspase-1, the enzyme responsible for the processing of pro-IL-1ß. In previous studies we demonstrated by ELISA that human neutrophils release IL-1-ß by a caspase-1-dependent pathway. Because the mechanisms that lead to IL-1ß release remain controversial and have not been defined in neutrophils, this study was conducted to address this issue. We confirmed by confocal microscopy and flow cytometry that neutrophils synthesize IL-1ß in response to LPS (200 ng/ml), crystals of monosodium urate (MSU; 200 µg/ml) and LPS + 2.5 mM ATP (LPS+ATP) or LPS + MSU. IL-1ß release evaluated by ELISA was inhibited by the proteasome inhibitor MG-132 (% inhibition >70 for all the agonists; $p<0.05$; n=3), suggesting a role for NF-kB in the mechanisms that lead to IL-1ß secretion. Neutrophils from three X-linked chronic granulomatous disease (CGD) patients, which are unable to generate NADPH-dependent reactive oxygen species (ROS), released IL-1ß only in response to LPS+MSU (pg/ml IL-1ß: 410±4) indicating that at least for some agonists ROS play a role in IL-1ß secretion. However, treatment with xanthine/xanthine oxidase (X/XO), an $O2^{\bullet-}$ generating system, partially inhibited IL-1ß release in neutrophils from healthy donors (% inhibition >41% for all stimuli; $p<0.05$, n=6) and in CGD neutrophils treated with LPS+MSU (% inhibition: 74%), suggesting that ROS also exert a negative control over IL-1ß release. Caspase-1 activation was not affected in healthy neutrophils by X/XO treatment, suggesting that ROS negative regulation is not exerted over inflammasome activation. Because ROS are suggested to induce NF-kB activation, our findings suggest that ROS might regulate neutrophil IL-1ß release by both stimulating IL-1ß transcription and negative controlling another step involved in the secretion.

104. Immunochemical characterization of antibodies labeled with vinylsulphonic triazinic dyes

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For several decades, colloidal gold has been the first choice for labeling antibodies to be used in Point Of Care assays. These assays, like dot blot and immunochromatography (IC), require adequate sensitivity and low costs. In this context, triazinic dyes have been proposed as a new generation of protein dyes. It has been previously demonstrated that immunoglobulins (Igs) can be labeled with dichlorotriazones (Procion MX series), and that these Abs can be succesfully used for IC. Nevertheless, neither the sensitivity that can be achieved with these systems nor the stability of the label has been stablished. For the reasons mentioned above, we have optimized the labeling conditions of rabbit Igs raised against human IgG, we have explored the properties of Igs labeled with a vinylsulphonic triazinic dye (Remazol Brilliant Violet 5R), and characterized their properties within a capture dot blot assay. The conditions for optimum labeling have been assessed using a variable-size simplex strategy. Igs were best labeled at pH 10.9, 37 °C, during an incubation of 132 min at a molar ratio of 264:1 (dye:Ig). When these antibodies were used in a capture dot blot assay, linearity was demonstrated and the sensitivity was high enough to detect as low as 40 ng of antigen in the reaction volume (400 ul). The results presented here show that this family of dyes is suitable for Ig labeling. These dyes provide extraordinary sensitivity, and are a

promising replace of colloidal gold. Furthermore, these studies confirm that each subfamily of triazinic dyes requires specific labeling setup, as our optimum labeling conditions are not equal to those reported in literature.

105. Effects of skin exposure to ultraviolet radiation on immune cell populations. Differences between high and low UV doses

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Skin exposure to UV radiation (UVr) is associated with a negative impact on human health, due to the increment in skin tumor incidence, exacerbation of infectious diseases and photoaging. However, there is also evidence showing "immunostimulatory" effects of UVr exposure. The mechanisms involved include production of antimicrobial peptides and activation of TLR-2 pathway, through an increase in Vit D. The aim of the present work is to evaluate the effects on the skin, inguinal and axylar lymph node (LN) cell populations of two UVr exposure models: a single exposure to a high dose (HD: 400mJ/cm^2, proposed as harmful) and repetitive exposures to low doses (LD: 20 mJ/cm^2, proposed as stimulatory). SKH:1 mice were separated into 5 groups of 5. Groups 1 and 2 received a HD and were sacrificed 1 and 8 days (d) post irradiation (PI), respectively. Groups 3 and 4 received LDs for 4 consecutive days and were sacrificed 1 and 8d PI, respectively. Group 5 was the unirradiated control group. Skin histological analysis (H&E), epidermal (Ep), LN T cells and dendritic cells (DCs) populations (CD3, CD4, CD8, CD11c, CD80) were evaluated. Both HD and LDs produced: No changes in Ep DCs number and activation profile at any time; an increment in CD3+CD4+ cell percentage in both LN, 1 and 8d PI ($p<0.05$); an increment in activated DCs in LN 1d PI ($p<0.001$), without changes in total DCs number.HD produced: A reduction in Ep thickness 1d PI ($p<0.05$) with a marked increment after 8d ($p<0.001$); a decrease in CD3+ Ep cell percentage 1d PI ($p<0.05$) leading to a depletion after 8d ($p<0.01$). LDs produced: An increment in Ep thickness 1d PI ($p<0.001$) and 8d PI ($p<0.05$); no changes in CD3+ Ep cells percentage at any time. Concluding remarks: Both UVr exposure models produced direct effects on the skin and on Ep and LN immune cells, leading to an increase in DCs activity and helper T Cells number. We are currently studying in depth the Ag specific immunostimulatory effect of low doses of UVr.

106. Adaptive response to pre anesthetic-surgical stress: comparison between patients treated in Public or Private Hospitals

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Patients experience stress just before undergoing surgery. This may imply either physical or psychophysiological stimulus resulting in adaptive changes to preserve homeostasis. Individual and institutional factors might affect this response. Leukocyte and cytokine response to stressors may lead to immunomodulation. To test the influence of an imminent surgery on representative indicators of the stress and inflammatory response, two groups of elective videolaparoscopic cholecystectomy patients (VLC) treated in a Public (PH, n= 10) or Private (PR, n=30) hospitals, were compared. A group of blood donors was also included: Controls (C, n=16). Controls and VLC patients were evaluated at 7.30 AM. Endpoints included: leukocyte counts, IL-6, cortisol and prolactin levels, and hemodynamic variables (DBP, SBP and HR). Non parametric tests were used for statistical evaluation. Groups were similar regarding age, weight, and gender distribution. There were significant differences among groups in (mean ± standard deviation) Leukocyte/ul, C: 5862±883; PH: 7959±1757; PR: 6670±1551; p= 0.008; Neutrophil/ul: C: 3674±567; PH: 5308±1615; PR:3851±1277; p=0.016; Monocyte/ul: C: 152±109; PH: 474±269; PR: 499±152; p<0.001; prolactin pg/ml: C: 12.2±13.4; PH: 38.5±36.5; PR: 8.0± 5.5; p=0.004 y DBP mmHg: PH 69±7.4; PR 85.3±8.5; p<0.001. There were no significant differences in IL-6, cortisol, HR, SBP and HRxSBP. Both surgical groups showed higher monocyte counts than controls. Higher DBP values were found in PR patients, where DBP> 80 was registered in 16/30 patients vs. PH: 0/10; p=0.003. Leukocyte and neutrophil counts and prolactin levels were found enhanced in PH patients, compared to PR group. However, those values fall to normal limits and will hardly result proinflammatory. Present results show that patients attending a public or a private hospital present comparable adaptive changes in response to pre anesthetic surgical stress, with no detectable hypothalamus-pituitary-adrenal activation.

107. CD4+CD25+Foxp3+ regulatory T cells induced by the immunization with tolerogenic dendritic cells attenuate collagen-induced arthritis symptoms in DBA/1J mice

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Dendritic cells (DC) are professional antigen-presenting cells that maintain immune tolerance to self-antigens by controlling the pathogenicity of auto-reactive T-cells. These cells can be modified ex vivo to induce tolerogenic function and inhibit inflammatory responses in autoimmune diseases. Previous results show that the treatment of DBA/1J mice bone marrow derived DC with a total extract of the parasite Fasciola hepatica (TE) plus CpG (T/C) induces tolerogeneic properties such as a decrease in pro-inflammatory cytokine production, an increases in anti-inflammatory cytokine production and a high expression of indoleamine 2,3-dioxigenasa (IDO). Furthermore, the immunization of DBA/1J mice with T/C-treated DC was able to ameliorate the symptoms of collagen-induced arthritis (CIA). The aim of this work was to evaluate the involvement of CD4+Foxp3+

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regulatory T cells in the mechanism by which the immunization of mice with T/C-treated DC diminishes the symptoms of CIA. The immunization of mice with T/C-treated DC induced an increase in the percentage of CD4+CD25+Foxp3+ T cells, determined by flow citometry in draining lymph nodes (DLN), compare to the control (PBS injected mice). Besides, the recipient mice of T/C-treated DC showed a significant increase in IL-10 production as well as a decrease in INF-γ production detected in the supernatants of DLN of culture compared to the control ($p<0.05$). Additionally, the levels of IL-17 and TGF-β were not modified. In this cultures the IL-17/IL-10, IFN-γ/IL-10, IFN-γ/TGF-β ratios were significantly lower than those obtained with other treatments ($p<0.05$). Finally, an attenuation of the CIA symptoms was observed in the recipient mice of CD4+ CD25+ T cells sorted from DLN of mice that had been injected with T/C-treated DC, compared with control. Our data show that T/C-treated DC are able to induce in vivo a CD4+CD25+Foxp3+ T regulatory cells population, which are functionally effective to prevent the symptoms of CIA.

108. Glycosidic Tn-based vaccines targeting dermal DC favor germinal center B cell development and potent antibody responses in the absence of adjuvant

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The in vivo targeting of C-type lectin receptors (CLR) is an effective strategy for increasing antigen (Ag) uptake and presentation by dendritic cells (DC). We report here the use of glycosylated tumor-associated Tn Ag to target DC through binding to macrophage galactose-type lectin (MGL), in order to induce efficient immune responses. We show the capacity of Tn-glycosylated Ags, and of the MAG:Tn3 therapeutic candidate vaccine, to target human and mouse MGL+ DC, and in particular dermal DC. In mice, MGL+ CD103- dermal DC efficiently captured and processed glycosylated Tn Ag in vivo, inducing a potent MHC class II-restricted T cell response. Intradermal immunization with Tn-glycopeptides induced high levels of Th2 cytokines, even in the presence of unmethylated cytosine-phosphate-guanosine (CpG), and was associated with an increase in the expansion of the germinal center B cell population. Thus, MGL acts as an efficient endocytic antigen receptor on dermal DC in vivo, able to prime Tn-specific T and B cell responses. Moreover, even in the absence of adjuvant, immunization with MAG:Tn3 induced high level of anti-Tn antibody responses, recognizing human tumor cells. In vivo DC-targeting strategies, based on Tn-MGL interactions, constitute a promising strategy for enhancing antigen presentation and inducing potent antibody responses.

109. Systemic cytokines in intestinal homeostasis

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The balance bewteen inflammatory and regulatory cytokines is crucial in the mucosal immunity. Mice (B6) injected with pscIL-12 cDNA plasmid (hydrodynamic gene transfer) increase markedly serum levels of IL-12, IFNgamma and TNFalpha and change the CD4/CD8 cell ratio in spleen (Sp). In this work we compared the effect of systemic IL-12 expression in mucosal (Mesenteric Lymph Nodes (MLN)) and non mucosal lymphoid organs (Sp, Inguinal Lymph Nodes (ILN)). The treatment produced an increase in the Sp weight and a reduction in MLN cellularity/weight ($p<0.05$). The percentage of CD4+ and CD8+ cells expressing the mucosal homing receptor (a4b7) increased in MLN ($p<0.05$). After the treatment, in all immune tissues analyzed, we observed higher expression of the CD69 marker both in CD4+ and CD8+ T cells with a significant increment in the percentage of CD4+CD44+ in ILN ($p<0.05$). Similar results were observed when Sp, MLN and ILN mononuclear cells were cultured in the presence of antiCD3/antiCD28 antibodies. In supernatants of Sp, MLN and ILN mononuclear cells from control group stimulated with antiCD3/antiCD28 antibodies, the levels of IL-10 and IFN-gamma were similar. Interestingly, after pscIL-12 cDNA injection, splenocytes showed a three-fold increase in IL-10 production and a five-fold increase in IFNgamma release ($p<0.05$). In mice deficient of endogenous IL-12 (B6 IL-12 KO) the increment in the activation markers CD69 and CD44 in CD4 and CD8 subsets was not observed in MLN ($p<0.05$). Our results suggest that the exacerbated levels of systemic inflammatory cytokines triggered by IL-12 exert different effects in mucosal and non mucosal environments with MLN cells showing refractory traits.

110. Over-expression of Activation-Induced Cytidine Deaminase (AID) in Chronic Lymphocytic Leukemia (CLL). Link with the nature of the antigenic response

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Despite important progress in CLL treatment, relapse inexorably occurs and disease remains incurable. Studies suggest that disease progression does not occur from passive accumulation of B-CLL cells, but from active generation of subclones derived from a proliferative pool arising through contact with the microenvironment. We recently identified one of these proliferative subsets in peripheral blood (PB) from progressive CLL samples. This small B-cell subpopulation is characterized by an anomalous over-expression of the AID enzyme, a molecule involved in the immunoglobulin diversification process, but also in onco-

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genic events related to lymphoma origins. This proliferate CLL subset depicts an active Class Switch Recombination process, higher levels of proliferation and anti-apoptotic molecules like Ki-67, Bcl-2, c-myc, CD49d and CCL3/CCL4 chemokines. Characterization of this subset led us to postulate the presence of an auto-antigen responsible for a continuously stimulation inner tumoral CLL clone. The role of a putative auto-antigen in the pathogeny of CLL remains unclear as yet. In order to solve this, we aim to characterize the nature of antigen stimulation responsible for preserve this tumoral subset over-expressing AID. In the first time, we established that AID expression is totally dependent of microenvironment interactions. Cultures of CLL B-cells of these progressive patients are unable to preserve AID expression. In contrast, RNA level of this enzyme is maintained when T-dependent or independent responses are emulated. Furthermore, we activated CLL patients following T-dependent or independent in-vitro response evaluating progression and proliferation markers. Results show that classical T-dependent stimulation (CD40L/IL4) as well as innate response by TLRs pathway are not enough to trigger the proliferating CLL sub-population. Our findings outline the relevance of this proliferative sub-population, and link it with an activated microenvironment, highlighting the importance to the nature antigen-stimulation in CLL patients over-expressing AID.

111. Effects of the antibody constant region on antigen recognition

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It is well accepted that specificity and affinity of antigen-antibody interactions are driven by the variable segment of immunoglobulins (Ig), while the constant regions are responsible for effector functions and avidity properties. However, recent reports provided evidence that antibodies with identical variable regions (V), but different constant regions (CH) exhibited important changes concerning the affinity and avidity against the antigen (Ag). These results suggest that the CH could have a role in Ag recognition, but at the present the structural evidences that explain how this phenomenon occur remains elusive. With the purpose to understand the mechanism of this change in the affinity constants of two Ig with identical V regions but different CH, we studied the serum of a patient with an immunocytic sarcoma containing monoclonal antibodies of the IgA1 and IgG1 isotypes. Both Igs, either the entire molecule or the Fab fragment, presented differences in the affinity constants against their Ag evaluated by Surface Plasmon Resonance (SPR). These differences were also obtained at the Fab level, indicating that the CH1 constant domain could have a role in Ag recognition. Using these Ab, we were able to solve the crystallographic structures of the Fab fragments from the IgA1, IgG1 and IgG1 in complex with their Ag (tubulin autoantigen). Also we generated preliminary results by SPR that suggest a possible effect of the glycosylation in the hinge region of the IgA1 in Ag recognition. Additionally, this work reports at a high resolution the first crystallographic structure of a human IgA1 Fab fragment. Overall, our structural and kinetic studies provided evidence on the mechanism by which the constant regions could affect the antigen recognition site.

112. Immunomodulatory properties of a Brucella abortus protein in a food allergy mouse model

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Immunomodulation constitutes a potential therapy for food allergy. It has been reported that a protein from Brucella abortus (Bp) produced a Th1 in vitro and in vivo immune response in mice immunized by oral or intraperitoneal routes. The aim of this work was to use Bp as a mucosal immunomodulator in a cow`s milk allergy mouse model. Balb/c mice were intragastrically immunized with cow`s milk protein (CMP) using cholera toxin (CT) as a Th2 mucosal adjuvant. Mice were simultaneously treated with recombinant Bp intranasally (Bp in), with Bp intragastrically (Bp ig), with E. coli LPS in (control group), with synthetic ODN CpG ig (control group) or with saline (control group). Finally, mice were orally challenged with CMP. Immune response was evaluated with in vitro (serum specific IgE, IgG1 and IgG2a, histamine, IL-5 and IFN-γ secretion by splenocytes) and in vivo assays (clinical score, cutaneous tests and DTH). IL-5 and IFN-γ mRNA expression was assessed in intestinal tissue. We found lower clinical scores and plasma histamine levels in treated mice after the oral challenge than in sensitized animals. Cutaneous tests were positive e in sensitized mice, while negative or mild in the treated groups. DTH was statistically higher in Bp and LPS treated animals (0.20±0.07; 0.16±0.09 vs 0.074±0.04, $p<0.05$). Besides, specific IgE was suppressed [1.06±0.30 vs 0.54±0.21 (Bp in); 0.22±0.2 (LPS); 0.87±0.23 (Bp ig); 0.45±0.18 (CPG)], while IgG2a was increased, in treated mice. IL-5 and IFN-γ were modulated by the different immunomodulators at the protein and mRNA levels. In conclusion, a protein from Brucella abortus, LPS and CpG, were found to modulate the specific Th2 immune response elicited with CMP plus CT, by mucosal administration in Balb/c mice. These results may constitute the basis for a potential immunomodulatory therapy in food allergy.

113. The chemokine receptor CCR1 is involved in the development of Hemolytic Uremic Syndrome (HUS)

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No. 3:4
doi: 10:3823/413

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Hemolytic Uremic Syndrome (HUS) is characterized by thrombocytopenia, microangiopathic hemolytic anemia and acute renal failure caused by gram-negative bacteria producing Shiga toxin (Stx). We previously demonstrated that mice lacking chemokine receptor CCR1 had a higher survival rate to Stx than wt mice. The aim of this work was to analyze the mechanisms involved in CCR1Ko Stx-resistance. Circulating and tissue-associated leukocytes were studied by flow cytometry 72h after Stx intoxication. Stx triggered a loss of peripheral white blood cells (WBC) in wt and CCR1Ko mice. Both circulating neutrophils (PMN) and monocytes (Mo) were strongly reduced in wt mice after Stx. However only Mo were diminished in CCR1Ko whereas PMN number increased after Stx. [(Wt= Basal:WBC:11±2, PMN:1.7±0,5, Mo:0.57±0,13; Stx:WBC:3.4±0.5*; PMN: 0.68±0.09*, Mo:0.11±0.02; CCR1Ko=Basal: WBC:7.8±0.7, PMN:0.7±0.1, Mo:0.41±0.08, Stx:WBC:4.6±0.2*, PMN:1.29±0.20*, Mo:0.15±0.02*) 10^5 cells/ml, (*p<0.05 vs basal in each strain, for all results). In the lung, myeloid cells (MC) and macrophages (Mac) numbers remained unchanged in wt whereas those subsets increased in CCR1Ko mice. [(Wt=Basal: MC: 0.13±0.04, Mac: 0.52±0.14; Stx: MC: 0.10±0.02, Mac: 0.63±0.12); (CCR1Ko=Basal: MC: 0.06±0.01, Mac: 0.48±0.11; Stx: MC: 0.20±0.02*, Mac: 0.83±0.07*)] 10^6 cells/ml. Kidney associated MC and Mac were strongly reduced in Stx-treated wt mice whereas these populations were unaltered in CCR1Ko mice. [(Wt=Basal: MC: 0.04±0.01, Mac: 0.26±0.07; Stx: MC: 0.008±0.001*, Mac:0.06±0.01*); (CCR1Ko=Basal: MC: 0.06±0.01, Mac: 0.31±0.07; Stx: MC: 0.03±0.01, Mac: 0.24±0.07)]10^6 cells/ml. Stx-induced death and renal dysfunction were related with severe loss of peripheral and tissue-associated MC and Mo/Mac populations in wt mice. In contrast, MC including PMN and Mo/Mac were preserved in blood and kidney and accumulated in the lung of Ko mice. This altered redistribution of leukocytes was associated with a better survival and renal function.

114. HIV -mediated up-regulation of invariant chain (CD74) contributes to generalized immune activation in vivo

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HIV-mediated up-regulation of invariant chain (Ii chain) was reported to occur in cells infected in vitro, but no data is available on primary APC cultures obtained from HIV+ subjects. Also, increased plasma levels of MIF protein (for what surface Ii chain might serve as a receptor) have been reported for HIV+ subjects Aim: To analyze i) Ii chain modulation in HIV+ patient´s APCs and its correlation with clinical parameters ii) the impact of MIF-Ii chain interaction on lymphocyte activation M&M: Monocyte-derived macrophages (MDMs) from 40 HIV+ patients were obtained and Ii chain was analyzed in p24+ cells. For in vitro experiments, MDMs from healthy donors were infected with Nef WT and 1Nef viral variants, and treated with MIF. Supernatants from these cultures were used to treat cultured lymphocytes. Activation markers were analyzed by FACS and cytokine levels by ELISA Results: All patients but one, showed Ii chain n-fold up-regulation 1 when infected (iMDMs) vs uninfected MDMs were compared (mean 2.47±1.82). Median Ii chain n-fold up-regulation directly correlated with CD38 and CD38 and HLA-DR expression levels on peripheral B and CD4 T cells, respectively (Spearman's r=0.657, p=0.007, r=0.579, p=0.023 and r=0.657, p=0.007). IL-6 and IL-8 levels where higher in supernatant from MIF-treated p24+/NefWT MDMs cultures when compared to p24+/1Nef MDMs. Moreover, these supernatants induced higher activation of CD4+ T cells. Pre-treatment of MDMs with anti-Ii chain mAb BU-45, before addition of MIF, abrogated this effect Conclusion: Ii chain is up-regulated in iMDMs obtained from HIV+ patients and its expression correlate with higher activation of CD4 T cells. In vitro experimental data support the hypothesis that an interaction between Ii chain and MIF would contribute to generate a proinflammatory environment in a Nef-dependent manner. In summary, these results sustain the idea that Nef-mediated up-regulation of Ii chain plays a relevant role in HIV immunopathogenesis.

115. Purine Nucleoside Phosphorylase Deficiency: a single- center experience

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Purine nucleoside phosphorylase (PNP) deficiency is a combined immunodeficiency presenting with recurrent infections, neurological disorders, malignancies and autoimmune diseases. Hematopoietic stem cell transplantation (HSCT) is the only curative treatment. We report clinical and immunological characteristics of patients (p) with PNP deficiency diagnosed in our center. Results: of 44p with combined T and B cell immunodeficiencies in our registry, 4p (6.8%), belonging to 3 unrelated and non consanguineous families (p1 and p2 were sibling), had PNP deficiency. All patients showed failure to thrive, chronic diarrhea, neurological involvement and recurrent respiratory tract infections. The siblings developed lymphoma B. Increased transaminases and anemia were observed in 4p, one of them with positive direct coombs test. Serum uric acid level was low in 3p. Immunological investigations in all patients showed low lymphocyte count, low T cell, low CD4/CD45RA(+) and low B cell. Three patients had low NK cell. Impaired T cell proliferation was constant. Hypogammaglobulinemia was observed only in one patient and antibodies deficiency in two patients. PNP enzymatic activity in erythrocytes was undetectable in 3p and in

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doi: 10:3823/413

1p very low. Three patients died, 2 by severe infections, 1 by progression of lymphoma. One patient is alive, waiting transplant. Conclusions: the frequency of patients with PNP deficiency in our cohort is similar from the description in the literature. Although anemia, lymphopenia and hepatitis were constant in initial laboratory research, serum uric acid level may be normal. B cell deficiency is variable. Lymphoma is responsible for significant morbidity and potentially fatal outcome in these patients; hence diagnosis and treatment should be established early.

116. Tumor induced senescence T-lymphocytes (TIST) modulate the activation of human monocytes

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We have previously demonstrated that a high percentage of human CD4+ or CD8+ T cells from healthy donors incubated with different tumor cell lines in vitro and then cultured for 7 days undergo tumor induced senescence. We and others have reported the suppressive effects of TIST cells on the adaptive immune system; our current aim is to investigate TIST cell effects on innate immune cells. To this end, autologous monocytes were coincubated with CD4+ or CD8+ TIST or control T cells (CD4+ or CD8+ T cells without tumor co-incubation) in the presence of anti-CD3 mAbs during 40 h, then macrophage classical or alternative activation markers were studied by flow cytometry. CD8+TIST cells induced a reduction in CD14 expression on monocytes compared to monocytes cocultured with controls ($p<0.005$). We also observed that the percentage of CD16+CD14+ was significantly increased in monocytes coincubated with CD4+ or CD8+ TIST in comparison to control T cells (CD4: TIST 28%±11 vs control 9%±2; CD8: TIST 28%±9 vs control 9%±4). In addition, a higher percentage of monocytes coincubated with CD4+ or CD8+ TIST were CD163+ (CD4: TIST 26%±5.6 vs control 13.5%±0.7; CD8: TIST 20%±1.4 vs control 6.5%±0.7) but expressed lower levels of CD206 ($p<0.005$ for CD4+TIST, $p<0.001$ for CD8+TIST) when compared to controls. The expression of HLA-DR molecules were upregulated in monocytes treated with CD4+TIST ($p<0.002$) while the expression of coestimulatory molecules such as CD86 and B7H4 were not affected by the presence of CD4+ or CD8+ TIST. We next assessed by flow cytometry the phagocytic ability as well as nitric oxide (NO) and reactive oxygen species (ROS) production of TIST-treated monocytes. We observed an increase in the percentage of phagocytosing monocytes following CD8+ TIST coculture and a reduction in the percentage of NO and ROS producer- monocytes after culture with CD4+ or CD8+ TIST. Taken together our results demonstrated that TIST cells are able to modulate monocytes/macrophages. We hypothesize that after infiltrating the tumor mass, monocyte/macrophage activity will be affected by not only the tumor microenvironment but also the presence of TIST and this may have important consequences for antitumoral immune response.

117. Early protection induced by the use of a recombinant baculovirus as adjuvant for a foot-and-mouth disease virus vaccine

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Foot and Mouth Disease (FMD) is an acute disease caused by Foot and Mouth Disease Virus (FMDV). During an outbreak, the surrounding cattle must be ring vaccinated with an emergency vaccine. Baculovirus are known for promoting innate immune responses and potentiate the cellular adaptive immune response against soluble antigens.The aim of this work is to investigate the efficacy of baculovirus in developing early immunity, when it is administered together with inactivated FMDV (iFMDV), in the mouse model. To this end a endotoxin-free recombinant pol(-) baculovirus carrying the GFP gene [AcNPV-GFP (pol-)] was used. Groups of 5 BALB/c mice were immunised by i.p. route with a single dose of iFMDV 0.1 µg/dose or iFMDV plus $5x10^7$ pfu/dose AcNPV-GFP (BV) or $5x10^7$ pfu/dose BV or culture medium. Anti-FMDV antibodies and isotypes were detected by solid phase ELISA and protection after challenge was evaluated by viraemia measurement in BHK-1 cell cultures.Mice vaccinated with iFMDV+Bv showed the highest protection percentages at 2 dpv (90%) and had significantly lower viraemia than iFMDV ($p< 0.01$) and culture media ($p<0.001$) control groups. At 4 and 7 dpv protection percentages were lower (60% and 40%) but the viraemia remained significantly lower than that from the control groups.Antibodies titers from group iFMDV+Bv at 7 dpv were significantly higher than those from mice vaccinated with FMDVi ($p<0,001$), BV ($p<0,01$) or culture media ($p<0,001$). All the mice in this group presented IgG2a and IgG2b isotypes. Some pro inflammatory cytokines, as TNFα and IL6, were detected in FMDV+Bv immunized animals at 2dpv. In conclusion, we have demonstrated that BV is capable of enhance early immunity against FMDV. Since no significantly higher antibodies titers were found in these animals at 2 and 4dpv, we believe that other mechanisms are involved in such protection. The presence of IgG2a and IgG2b antibodies at 7 dpv, make us think that a Th1 type immunity is being developed.

118. Evaluation of lymphocyte subsets and cytokine profiles in patients with hyper IgE Syndrome (HIES)

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HIES is a primary immunodeficiency characterized by eczema, recurrent infections, skeletal abnormalities and elevated IgE. Mutations in STAT3 cause autosomal dominant HIES leading to

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doi: 10:3823/413

signal transduction impairment of several cytokine pathways. Reported patients (Pts) present a pro-inflammatory cytokine profile and alteration of some cell populations as IL17 producing T helper cells (Th17). We present the evaluation of T (NKT,Th17) and B cell subpopulations as well as T (INFγ, IL2,IL4) and monocytes (TNFα,IL12) cytokine profiles in 6 Pts with HIES mean (X) [age 9.6 years r:6-13] 4 of them confirmed by STAT3 mutational analysis. Lymphocyte subsets, as well as Th17 cells and cytokine profiles, were evaluated by flow cytometry, the latter upon stimulating cells with PMA/Ionomycin or LPS plus Brefeldin A. Pts with confirmed STAT3 mutation had reduced Th17 X:0.06% (r:0.04-0.09), [age related controls (ARC) (n:9) X:0.56% r:0.17-0.92]; NKT X:0.02% (r:0.00-0.07) [ARC (n:13) X:0.50% r:0.1-1.69] and CD27+memory B cells X: 4.0% (r:2.3-5.8) [ARC (n:18) X: 26.0% r:18.0-34.0].The other two Pts both had reduced Th17 0.02%, while CD27+Bcells were 7.82 and 6.88%. Regarding T cytokine profile, no differences were detected between PTs and ARC: on CD4: (+)INFγ X:13.1% (r:7.4-19.0) (ARC:12.1 r:6.7-17.5), IL2 X:20.5% (r:6.0-28.0) (ARC:28.8 r:25.3-32.3), IL4 X:1.06% (r:0.58-1.54) (ARC:1.97 r:0.97-2.97) on CD8: (+) INFγ X:20.2% (r:8.0-27.0) (ARC:26.0 r:18.7-33.3), IL2 X:5.8% (r:2.0-8.0) (ARC:8.0 r:4.4-11.6. TNFα producing cells were normal in Pts with detected STAT3 mutation X:39.6% (r:35.0-49.0) while + IL12 cells were diminished X:4.9% (r:4.0-6.0) in all Pts studied (n:3) compared with controls for TNFα X:49.0% (r:34.0-68.0) and IL12 X:23.0% (r:18.0-33.0). Extremely reduced Th17 and low CD27+B cells were found in all Pts. Of note, NKT were also highly reduced. Contrary to published data, our Pts did not show a pro-inflammatory profile based on their IFNγ, TNFα and IL12 levels.

119. Influence of cathpesin-L on CD4+ regulatory T cells peripheral homeostasis

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CD4+CD25+Foxp3+ regulatory T cells play a pivotal role in the maintenance of peripheral tolerance and immune homeostasis. We had previously shown that cathepsin-L deficient mice (CTSLnkt) show increased absolute number of CD4+ regulatory T cells in peripheral lymph nodes (LN) whereas the number of CD4+Foxp3+ thymic cells was shown to be decreased. In this study, we investigated the impact of cathepsin-L on CD4+ regulatory T cell peripheral homeostasis by analyzing CD4+CD25+ (Treg) cell turnover in CTSLnkt mice. To determine the proliferation rate of LN Treg subsets, 5-bromo-2- deoxyuridine (BrdU) was injected for 7 days. FACS analysis showed that both the CTSLnkt Treg and CD4+CD25- subsets showed a significant increase in the % of BrdU+ cells as compared to wild type (mean % BrdU+/Treg cells ± DS: 22±3 vs 12±1, $p<0.005$, n=4. Mean % BrdU+/CD4+CD25- cells ± DS: 10±1 vs 4.0±0.1, $p<0.001$, n=4). To evaluate the % of Treg cells undergoing apoptosis annexin V staining was used. An increase both in the % of apoptotic Treg and CD4+CD25- cells was observed in the LN of mutant mice (mean % annexin V+/Treg cells ± DS: 53±3 vs 43±3, $p<0.005$, n=4. Mean % annexin V+/CD4+CD25- cells ± DS: 35±2 vs 25±2, $p<0.001$, n=4). Notably, both mutant and wild type Treg subsets showed increased apoptosis as compared to their CD4+CD25- counterparts. Our results show that the CTSLnkt mutation causes increases in the proliferation but also in the apoptosis levels of both Treg and CD4+CD25- cells. The fact that mutant Treg and CD4+CD25- cells showed increases in proliferation but also in the apoptosis levels, does not support that differences in the proliferation vs apoptosis balance of Treg may be involved in the increases of peripheral Treg numbers in CTSLnkt mice, thus raising the possibility that conversion of CD4+CD25- into CD4+CD25+ cells could be involved. In support of this hypothesis, increased levels of TGF-β were found in cultures of LN CTSLnkt cells using both RT-PCR and ELISA assays.

120. Mannose receptor recycling favors arginase induction and Trypanosoma cruzi survival in macrophages

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Previously we demonstrated that the iNOS/arginase balance was biased towards arginase in Trypanosoma cruzi infected macrophages (Mo) pre-incubated with mannose-BSA (man-BSA, mannose receptor (MR) specific ligand). Moreover, intracellular parasite growth was increased. In addition, the study of MAP kinase intracellular signals showed that pre-incubation with man-BSA but not mannan, induced down-regulation of p-JNK and p-p44/p42 and up-regulation of p-p38 MAPK in T. cruzi infected J774 cells. These results are coincident with previous data published by our group in T. cruzi infected Mo pre-incubated with Cruzipain (Cz). These results suggested that man-BSA and Cz might interact with the same receptor (MR). Moreover, we showed that pre-incubation with man-BSA induced MR up-regulation or increased MR recycling on Mo surface. In this work, we study MR behavior in Cz-Mo interaction. J774 cells were treated with Cz during 2h and then incubated with man-BSA-FITC for 20 minutes. Flow cytometry analysis showed that Cz increased the percentage of FITC+ cells. Therefore, Cz might interact with MR in the same way that man-BSA. Then, we studied whether Cz or man-BSA induced MR up-regulation or increased MR recycling. Therefore, J774 cells were incubated for 1, 2 or 3 hs with man-BSA-FITC at 4°C or room temperature. Then, cells were treated with acid washed and were analyzed by flow cytometry and confocal microscopy. This study showed that the interaction between Cz or man-BSA with MR induced MR increased recycling. Later, we studied the MR behavior during T. cruzi infection in vivo. MR was up regulated in F4/80+ cells from T. cruzi infected mice at 13 and 15 days post infection. Besides, we investigated the effect of MR blocking antibody in T. cruzi infected peritoneal Mo. Arginase activity ($p<0,01$) and growth of intracellular para-

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sites (p<0,01) were decreased. Thus, we postulate that during T. cruzi infection, Cz may contact with MR up-regulating arginase activity and promoting the intracellular growth of parasites. Furthermore, interaction between Cz and MR induces an increase of MR recycling favoring parasite survival

121. IL-4 Regulates activation of peptidoglycan-stimulated microglial cells

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Microglial cells (MC) are involved in responses of the central nervous system (CNS) against infections, aseptic inflammation, injury and neurodegeneration. These cells are accumulated at sites of injury and have the capability of activating when they contact either with endogenous or exogenous signals (pathogens). Our preliminary studies have shown that after prolonged stimulation of MC with peptidoglycan of Staphylococcus aureus (PGN, TLR2 agonist) several neurotoxic factors (IL1β, TNFα, IL6 and nitric oxide (NO)) are produced. IL4 is a cytokine with diverse biological activities, including the induction of alternative activation in macrophages and the inhibition of LPS-induced TLR4 signaling cascade in macrophages and MC. Thus, IL4 may participate in the homeostasis of the CNS by controlling MC responses to inflammatory stimulants. Here, our aim was to study the capacity of IL4 to modulate the PGN-stimulated activation and survival of MC. We first examined (by intracellular flow cytometry and Griess' reaction) the effects of IL-4 on the pro-inflammatory mediators production in PGN-stimulated MC line BV2. We found that PGN and Pam3CSK4 (another TLR2 ligand) increased TNFα and NO production (Medium 0.023 ± 0.041; PGN 1.992 ± 0.170; Pam3CSK4 1.864 ± 0.325; p<0.05). In contrast, IL-4 was able to reduce TNFα and NO production in these cells (IL4 0.069 ± 0.079; IL4+PGN 0.496 ± 0.216; IL4+Pam3CSK4 0.940 ± 0.269 p<0.05). In another set of experiments we found that prolonged stimulation of MC with PGN induced cell death (Annexin V /7AAD staining) and the effect was enhanced by pretreatment of the cells with IL4 (statistical significance is currently tested). These preliminary results suggest that IL4 may regulate potentially harmful responses of PGN-stimulated MC by controlling the production of pro-inflammatory cytokines and reducing the cell number.

122. Immunomodulatory effects of progesterone through the VIP/VPAC system in the trophoblast-maternal leukocyte dialogue

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Successful embryo implantation occurs followed by a local pro-inflammatory and Th1 response, subsequently controlled by a Th2/tolerogenic one. Several data point that progesterone, the main hormone in pregnancy, and VIP (vasoactive intestinal peptide) favor tolerance to fetal antigens displaying anti-inflammatory effects and promoting tolerogenic responses through multiple mechanisms. In the present work, we studied the interaction between VIP and progesterone in the modulation of the maternal immune response using and in vitro model of trophoblast-maternal leukocyte dialogue. We performed co-cultures of trophoblast cells (Swan-71 cell line) and PBMCs from fertile women in the absence or presence of VIP (10^{-7}M) and progesterone (10^{-4}M), after 48h we quantified relevant mediators in this dialogue. Progesterone significantly decreased the expression of T-bet (Western Blot) and COX-2 (RT-PCR), the MMP-9 gelatinolytic activity and the nitrite and MCP-1 production (by Griess method and ELISA respectively) (p<0.05 Student T-test). We did not observe a synergic effect between VIP and progesterone treatment on these mediators. On the other hand, VIP increased IL-10 production while progesterone did not modulate it. To provide further insight into possible interactions between both immunoregulators, we quantified VIP production and its receptors expression (VPAC1 and VPAC2) induced by progesterone. Maternal PBMCs cultured with trophoblast cells in the presence of progesterone induced an increase in the frequency of CD4+VIP+ cells (13.2±1.3%) in comparison with those in the absence of progesterone. Moreover, progesterone also increased VPAC1 and VPAC2 expression in Swan-71 cells determined by RT-PCR. In conclusion, progesterone might contribute to a maternal tolerogenic response triggering direct effects, evidenced by a suppression of all pro-inflammatory tested factors and by indirect effects, increasing the frequency of CD4+VIP+ cells and VIP receptors on trophoblast cells.

123. Both costimulation and the frequency of inoculation are important to reject a murine T-cell lymphoma in an immunization schedule using irradiated whole tumor cells

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Following the evaluation of an anti-tumor cell vaccine we are testing different vaccination schedules for anti-tumoral response optimization. In parallel, we try to dilucidate the mechanisms of the immunological response. BALB/c mice were inoculated i.p. with murine T-cell lymphoma cells (LBC) transfected or not with CD40 and irradiated, or with PBS. An experimental group received 2 doses of cells with an interval of 7 days, whereas another group received a single dose. Mice were challenged 7 days after the last immunization with tumor cells i.p. Our results indicated survival rates from 70-100% for LBC.CD40, 55 to 70% for LBC both immunized with 2 doses, and 33% for LBC.CD40 (1 dose), while none of the mice receiving 1 dose of LBC rejected the

tumor. Mice immunized with 2 doses showed a significant difference in survival respect to the challenged group ($p< 0.0459$) and both groups immunized with 1 dose ($p< 0.0323$). Specific cytotoxicity by Jam's method was 16% for LBC.CD40 (2 d), 13% for LBC (2 d) and 7% for normal mice. Surprisingly, the specific cytotoxicity found in the group immunized with 1 dose of LBC.CD40 was 38 %. In order to study if the generated immune response involved a Th1-Th2 mechanism, the levels of IFN-γ and IL-4 were determined in the supernatant of splenocytes immunized. While there was not any difference among the groups for IL-4, there was an increment in IFN-γ production in all the groups immunized: LBC.CD40 (2 d) 1671pg/ml, LBC.CD40 (1d) 947pg/ml, LBC (2d) 1229pg/ml and normal (34pg/ml). Besides, the percentage of cellular populations was homogenous for CD4, CD8, F4/80, B220, CD49b and Gr1 in spleen and peritoneal cavity. Our results indicate that it is necessary immunization with 2 doses to achieve a high percentage of tumor rejection. A Th1 mechanism is evolved in the anti-tumor response. One dose is not sufficient to reject the tumor, although activate a great number of cytotoxic T-cells; this seems not to be the main mechanism in the rejection of the tumor. However, it is important to note that immunization with cells transfected with costimulatory molecules, even with one dose, provides some protection against tumor.

124. Characterization of exosomes derived from a murine T-cell lymphoma

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Exosomes are 60 to 100 nm lipid bilayer vesicles comprising an enclosed compartment topologically equivalent to the cytoplasm, and with the extracellular domains of transmembrane proteins at their surface. Exosomes originate in the late endosomal compartment by inward budding of the limiting endosomal membrane, thereby generating intracellular multivesicular bodies (MVBs) released in the extracellular medium upon constitutive or induced fusion of MVBs and plasma membranes. LBC cell line is a murine T-cell lymphoma expressing MHC I, CD8, CD24, CD16 and TCR. The aim of this work was to characterize exosomes derived from the T-cell lymphoma LBC to be used as a cell free immunogen. Supernatants of LBC cells cultured until a density of 1.50 to 2.00 E+06/ml were collected and sequentially centrifuged at 300g, 2,000g then at 10,000g and finally exosomes were then pelleted at 100,000g and washed once in PBS. Traditional cultures of LBC cells gave an average yield of (0.37±0.07) µg of protein (measured by Lowry assay) per 1.00 E+06 LBC cells. Exosome preparations, analyzed by electron microscopy, displayed the characteristic exosomal cup-shaped morphology. Beads coated with exosomes were stained with conjugated monoclonal antibodies, and analyzed by flow cytometry. We found exosomes were abundant in the following proteins: MHC Class I, the heat stable protein CD24, the heat shock protein Hsp90 and CD8. It was not possible to detect Hsp60 and Hsp70, even though these proteins are present intracytoplasmatically in LBC cells. Our results demonstrated the presence of proteins with immunological relevance on exosomes derived from LBC cells. Expression of MHC I, the heat stable antigen CD24 or the heat shock protein Hsp90, molecules involved in antigen presentation and ligand-receptor interactions can lead to the activation or modulation of various immune responses and therefore might be useful in developing cancer immunotherapies.

125. IL-17R knockout mice showed increased inflammation and reduced survival during Trypanosoma cruzi infection

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Interleukin (IL)-17 is a proinflammatory cytokine with well-known roles in the control of some bacterial and fungal infections, but the role of IL-17 during parasite infections has been poorly studied. IL-17 is produced by leukocytes from spleen, lymph nodes, peritoneum and liver from T. cruzi infected mice and is detectable in serum during the acute phase of the infection. To address the role of IL-17 during T. cruzi infection, we evaluated the progression of the disease in wild type (WT) or IL-17 receptor deficient mice (KO) infected intraperitoneally with 3000 tripomastigotes of T. cruzi (Tulahuén strain). Infected KO mice showed increased loss of body weight and mortality in comparison to WT mice (survival KO 33% vs WT 66%, $p=0.033$), with no differences in parasitemia (day 18 pi KO: 0.81x10E6 tp/ml vs WT: 0.76x10E6 tp/ml). Histological studies established that, in comparison to WT controls, infected KO mice presented a higher score of liver pathological lesions that correlated with higher levels of ALT and AST transaminases in serum. The evaluation of cell subsets in different organs demonstrated that infected KO mice showed a reduced percentage of neutrophils (CD11bhiGr-1hi) in spleen (day 16 pi; KO: 0.9±0.1 vs WT: 4.3±1.0; $p=0.009$) and liver (day 16 pi; KO: 0.72±0.05 vs WT: 2.5±0.6; $p=0.02$), with no changes in lymphocytes subsets. Neutrophils sorted from spleen and liver of infected WT and KO mice showed a regulatory phenotype characterized by the secretion of IL-10 but not of IFNγ or TNF. The study of the cellular response demonstrated increased levels of IFNγ in serum of T. cruzi infected KO mice in comparison to WT (day 18 pi; KO: 3951 ± 858 pg/ml vs WT: 1405 ± 234 pg/ml, $p=0.008$). Accordingly, infected KO mice presented a higher percentage of IFNγ producing cells in the spleen (day 16 pi; KO: 35.4±1.4 vs WT: 27.7±2.7; $p=0.03$) and liver (day 16 pi; KO: 45.7±8.0 vs WT: 22.9±7.5, $p=0.001$). In addition, CD4+ and CD8+ T cells sorted from the spleen and liver of infected KO mice se-

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creted higher levels of IFNγ and TNF than WT counterparts. Our study demonstrated that IL-17 is required for host resistance during the acute phase of T. cruzi infection. The increased susceptibility of IL-17R KO mice to this parasite infection could be consequence of the reduced numbers of neutrophils in lymphoid and target organs as well as of an exacerbated IFNγ-dependent inflammatory reaction that results in increased tissue lesion.

126. Histone deacetylase inhibitors compromise NK cell viability and effector functions through downregulation of activating receptor expression

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Inhibition of histone deacetylases (HDAC) with HDAC inhibitors (HDACi) is a promising approach for the treatment of cancer patients because epigenetic anomalies are involved in the carcinogenesis process. HDACi increase susceptibility of tumor cells to NK cell effector functions in vitro, but in vivo they can also affect cells involved in immune surveillance such as NK cells. Thus, here we investigated their functional response upon exposure to HDACi. Treatment of human NK cells with Trichostatin A (TSA), a broad spectrum HDACi, reduced the percentage IFN-γ^+, IFN-γ^+CD107a$^+$ and CD107a$^+$ cells from 8.8±2.7%, 9.5±4.8% and 2.5±1.0% to 0.2±0.1%, 0.2±0.1%, and 1.3±1.0%, respectively, in NK cells stimulated with IL-12+IL-15+IL-18, and from 3.3±2.0%, 3.7±3.0% and 12.0±5.1% to 1.6±0.9%, 0.6±0.4%, and 6.2±4.3%, respectively, in NK cells co-cultured with K562 cells ($p<0.05$ in all cases). Inhibition of IFN-γ secretion by TSA was confirmed by ELISA with cell culture supernatants. Part of the effect was due to TSA-induced NK cell apoptosis but ≈50% of the NK cells remained viable after treatment with TSA. Thus, we assessed its effects on NK cell receptor expression. Treatment with TSA for 24h significantly reduced cell surface NKG2D and NKp46 in resting NK cells in 76% and 74% respectively, and the expression of NKG2D, NKp44 and NKp46 in NK cells stimulated with IL-12+IL-15+IL-18 in 88%, 68% and 54% respectively. Also, TSA inhibited expression of NKG2D, NKp30, NKp44 and NKp46 in 79%, 38%, 57% and 64%, respectively, in NK cells stimulated with the cytokines for 4d before the addition of the drug. Expression of these receptors was not restored when TSA was removed from the cultures. Other HDACi with a narrower specificity (sodium butyrate and sodium valproate) induced similar effects. Thus, broad spectrum HDACi compromise NK cell viability, effector function and receptor expression, suggesting that besides their anti-tumor effects, they compromise immune surveillance by NK cells.

127. Autoimmune lymphoproliferative syndrome: a good example of pleiotropy and variable penetrance

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Autoimmune Lymphoproliferative Syndrome (ALPS) is a genetic disorder of lymphocyte apoptosis. Chronic non-malignant lymphadenopathy, splenomegaly, autoimmunity are the main manifestations. Laboratory findings include hypergammaglubulinemia, expansion of circulating TCRαβ+)CD4(-)CD8(-) (TDN), elevated biomarkers (eg. soluble Fas ligand, sFasL) and autoimmune cytopenia. Mutations in the genes encoding FAS, FAS ligand and CASP10 are responsible of most cases. Variable penetrance is not explained. We describe the clinical, molecular and immunologic variability of members of one family. Patient: Boy aged 10 year-old presented chronic splenomegaly, autoimmune cytopenia, autoantibodies (ASMA, anticardiolipins, ANCA-P), elevated TDN (7.6 %), elevated B220 expression in TDN (59 %) and increased sFasL (3381 pg/ml). Fas mediated apoptosis was 6.6 % of normal control and heterozygous germline mutation in FAS gene was identified. His father (no-mutated) had history of non Hodgkin lymphoma, no lymphadenopathy or splenomegaly. Though having normal TDN (1.43 %), sFasL (144 pg/ml) and Fas induced apoptosis, he presented autoantibodies (ASMA anti-actin) and increased B220 expression in TDN (72 %).His mother (mutated) who never showed lymphadenopathy or splenomegaly presented autoantibodies (ASMA anti-actin), normal TDN (1.86 %), increased B220 55 % expression in TDN, elevated sFasL (292 pg/ml) and reduced Fas induced apoptosis (14 %). Both sisters (mutated, 11 y / 7 y) who never showed lymphadenopathy or splenomegaly, presented hypergammaglobulinemia, autoantibodies (ASMA anti-actin), elevated TDN (4.71 / 3.57 %), B220 expression in TDN (85 / 50 %) and increased sFasL (1681 / 944 pg/ml). Fas induced apoptosis was reduced (19 / 10 %).Conclusion: This case showed the variability among individuals in the same family who harbour an identical FAS gene mutation. However, the findings presented by the father support the unknown genetic defects.

128. Multidrug-resistant (MDR) strains of Mycobacterium tuberculosis (Mtb) M and Ra expand IL-17+IFNγ- cells via TGF-β and IL-23-induced inhibition of IFNγ response in MDR-TB patients

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We have previously demonstrated that INFγ+ and IL-17+ T cells are differentially induced by M and Ra strains in MDR-TB patients

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doi: 10:3823/413

and healthy individuals (N). IL-17 is a heterogeneous population that comprises IL-17+IFNγ- and IL-17+IFNγ+ cells. Thus in the present work we evaluated the contribution of both subsets to the whole Mtb-induced Th17 response and their modulation by Th17 inducing cytokines. Peripheral mononuclear blood cells (PMBC) from 6 N and 15 MDR-TB patients were cultured with and without M, Ra or H37Rv strains for 48 hr in the presence or absence of neutralizing MoAbs against IL-23, IL-1β, IL-6 and TGF-β. IL-17 and INFγ expression was evaluated by flow cytometry and results are expressed as % of IL-17+INFγ -, IL-17+IFNγ + and IL-17-IFNγ+ cells within the CD4 subset. Results: a) All strains enhanced %IL-17+IFNγ+cells in MDR-TB and N ($p<0.05$). M and Ra markedly enhanced the %IL-17+IFNγ- ($p<0.05$), being M the highest inducer ($p<0.05$). Higher % IL-17+IFNγ- was observed in MDR-TB compared to N ($p<0.05$) while the %IL-17+IFNγ+ was similar in both groups. B) a-IL23, a-IL-1β and a-IL-6 diminished the percentage of both Th17 subsets in MDR-TB and N ($p<0.05$). In MDR-TB, a-TGF-β diminished %IL-17+IFNγ- and enhanced %IL-17+IFNγ+ induced by M and Ra ($p<0.05$) while no differences were observed in N. C) %IL-17-INFγ+ cells in Mtb-stimulated CD4+ T cells was lower in MDR-TB than in N ($p<005$) and neutralization of IL-23 and TGF-β increased the %IL17-INFγ+ only in MDR-TB patients ($p<0.05$). Conclusions: IL-17+INFg- cells were the main cells involved in M and Ra-induced Th17 response in MDR-TB patients being their expansion promoted by TGF-β. However, neutralization of TGF-β results in an enhancement of IL-17+IFNγ+ and of single INFγ+ cells suggesting that Th17 subsets are plastic to cytokine environment. Interestingly, IL-23 also inhibited the differentiation of Mtb-specific single INFγ+ cells contributing to the impairment of Th1 response observed in MDR-TB patients.

129. Chronic Granulomatous Disease patients expand Th17 cells in response to bacterial and non- specific stimuli

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Chronic Granulomatous Disease (CGD) is a primary immunodeficiency involving lack function to produce reactive oxygen compounds by NADH oxidase complex affecting the ability of children to survive infections. CGD patients are characterized by showing granulomas and recurrent infectious events. Otherwise these patients as a result of increased survival, develop symptoms of autoimmune and inflammatory diseases. Also these patients have blocked tryptophan metabolism as a result of their inborn inability to produce superoxide anion, which favors the imbalances between IL-17 producing T cells (Th17) and regulatory T cells (Treg). The aim of our study was to establish, among different stimulation cultures, the ability of CGD patients to develop a Th17 response. Peripheral mononuclear blood cells (PMBC) from 6 healthy donors and 6 CGD patients: 1 X linked and 5 autosomal recessive, age range: 5-18 years, all asymptomatics, were cultured alone or with OKT3+IL-2+CD28 for 5 days, Staphylococcus enterotoxin B (SEB) for 16 hr, Mycobacteium tuberculosis (Mtb) H37Rv strain for 48 hr, Ionomycin+PMA for 5 days. IL-17 expression was evaluated by flow cytometry on CD4+, CD8+ and gd T cells and results are expressed as percentage of IL-17+ cells within the CD4, CD8 and gd T subsets. IL17+CD4+ T cells were induced by OKT3+IL-2+CD28, H37Rv Mtb strain and Inomycin+PMA stimuli in N and CGD patients ($p<0.05$). IL-17+CD8+ subset was induced only by OKT3+IL-2+CD28 in CGD patients ($p<0.05$). Moreover, IL-17+gd+ T cells were only expanded in CGD patient after stimulation with H37Rv Mtb strain ($p<0.05$). In conclusion CGD patients showed an increased Th17 response to specific and non-stimuli that could be associated to their secondary autoimmune and inflammatory diseases.

130. Multidrug-resistant M.tuberculosis strain M induces low expression of CD69 and IL-2 in circulating CD8+ T cells

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Antigen-specific cytotoxic T cell (CTL) activity has been associated to the lysis of infected macrophages and Mycobacterium tuberculosis (Mtb). Previously, we have demonstrated that the multidrug resistant tuberculosis (MDR-TB) outbreak strain M induces weak CTL response associated with low expression of lytic molecules, when compared with its related strain 410 (non prosperous). The aim of this work was to evaluate the mechanisms employed by M to hamper CTL response. Methods: Peripheral blood mononuclear cells (PBMC) isolated from buffy coats were cultured for 18h or 5d with M or 410 strains. Then we evaluated the surface expression of CD69 and CD25 (early and late activation), CD107 (degranulation marker) and the intracytoplasmatic expression of IL-2 in $CD8^+CD3^+$T cells by flow cytometry. Results: M induced a lower CD69 expression than 410 ($p<0.05$) in 18 h cultures, while CD25 expression in 5d cultures was not significantly different between those strains. Both strains induced a similar CD69 peak of expression (66h), thus M strain was not delaying T cell activation. PBMC preincubated with Mtb strains were activated with anti-CD3 with or not anti-CD28 and no inhibition was detected in terms of CD69 expression suggesting that low CD69 expression was not due to an inhibition of TCR activation by strain M. However, M-induced a reduced IL-2 expression in $CD8^+CD3^+$T ($p<0.05$), cytokine that is regulated upon CD69 signaling and controls upregulation of lytic molecules. Besides, blockade of CD69 with specific monoclonal antibody along 5d cultures inhibited CD107 expression in 410-stimulated $CD8^+$ T cells resulting in a CD107 expression similar to that observed in M-stimulated cells. Conclusions: Our preliminary results indicate that low CTL response induced by M could be due to a low CD69

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expression in M-stimulated CD8$^+$ T cells that could impact on lytic degranulation and IL-2 production, cytokine that is known to modulate lytic molecule expression.

131. Role of C5a anaphylotoxin receptors in the immune response

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Mycobacterium tuberculosis (Mtb), the causative agent of tuberculosis (TB), is capable of establishing a niche inside the macrophage, where it can survive and replicate. Once the infection has occurred, a successful immune response requires the activation of a Th1 response. The complement system, besides its role in innate immunity, acts in the modulation of the adaptative response through the production of proinflammatory anaphylatoxins. Recent studies report that C5a receptor (C5aR) activation in dendritic cells (DC) induces innate immune signals which regulates the differentiation of näive cells into Th1, Th17 and Treg. In this context, we aim to investigate the effect of C5aR and C5L2 in the modulation of the Th1/Th17 profile in the immune response against local strains of Mtb. The expression of both receptors was evaluated ex vivo and in cultured monocytes from healthy donors (N) and patients with active TB. Monocytes were stimulated with 2 local MDR clinical isolates (M or Ra) or H37Rv for 48 hr. The expression of C5aR and C5L2 in CD14$^+$ cells was measured by flow cytometry. To evaluate whether C5aR modulates IFNγ and IL-21 expression in CD4+ T cells, monocytes were cultured with Mtb in the presence of anti-C5aR for 18hs; then, autologue T cells were added and IFNγ and IL-21 expression in CD4$^+$ subset was determined by flow cytometry. Although no differences were found in C5aR expression in CD14$^+$ between patients and N ex vivo, C5L2 was increased in TB patients ($p<0.05$). All Mtb strains diminished C5aR expression in CD14$^+$ while C5L2 was increased ($p<0.05$). Preliminary blockade results suggest that IFNγ and IL-21 expression was differentially modified by C5aR in CD4$^+$. These results suggest that C5a receptors could be modulating T cell responses against Mtb.

132. Role of IL-4 in limiting neuroinflammation in vivo

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Microglial cells (MC) are key immune cells within the central nervous system (CNS). They participate in CNS homeostasis being able to become activated once they contact exogenous and endogenous pro-inflammatory signals. Some CNS injuries are accompanied by cell infiltration and the recruitment of inflammatory cells could contribute to tissue damage and may be critical for inducing persistent activation of MC. Our hypothesis is that anti-inflammatory cytokines, such as IL-4, may regulate microglial cell activation and infiltration and activation of peripheral leucocytes in the CNS. Here, we evaluated the impact of the absence of IL4 on CNS inflammatory responses induced by systemic injection of bacterial lipopolysacharide (LPS) in IL4 KO mice. Our preliminary results indicated that after LPS injection, both IL-4 KO and WT mice increased the numbers of MC and infiltrating cells. Although, the magnitude of the increases in MC were similar in both groups, the number of recruited cells before and after LPS injection seems higher in IL4 KO than in WT mice (statistical significance is currently tested). The results indicated that monocytes/macrophages were the main cellular component of the recruited cells showing phenotypical features of inflammatory monocytes (based on Ly6Chi expression), both in LPS-injected IL-4 KO and WT groups (both $p<0.01$ vs mock-injected). Interestingly, parenchymal MC from LPS-injected IL-4 KO mice showed increased levels of CD80 ($p<0.002$), CD86 ($p<0.05$) and CD11c ($p<0.003$) compared with WT controls. Moreover, after LPS stimulation, MC from IL4 KO mice produced higher levels of TNFα and IL1-β (protein and mRNA) compared to WT mice. The enhanced activation of MC observed in IL4 KO mice suggests that this cytokine may be important in the regulation of CNS inflammatory response in vivo.

133. Modulation of microglial cells survival by IL4: targeting neuroinflammation

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Microglial cells (MC) are the brain-resident immune cells. After acute activation, as a result of neuronal injury, MC proliferates, change their morphology and secrete neurotoxic and neurotrophic factors. The actions of these cells are mostly beneficial, becoming destructive only when they escape from the strict control normally executed by the central nervous system (CNS) environment. Downregulation of inflammatory mediators and removal of activated microglia may be key underlying mechanisms by which brain inflammation is controlled. Furthermore, anti-inflammatory cytokines, such as IL-4 and IL-13, may participate in these regulatory mechanisms present in the CNS. Therefore, we evaluated the effects of IL-4 on MC survival. Here, we show that IL4 induced a significant increase in the death of N9 microglial cells after 48 h of culture (hypodiploid DNA content, $p = 0.0001$). IL4 also increased the percentage of annexin-V single positive and annexin-V/7AAD double positive cells, indicating induction of early and late apoptosis respectively. In addition, we found that IL4 significantly increased the cleavage of caspase 3 ($p <0.01$). Moreover, the IL-4-induced cell death was

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blocked by the presence of the classical pancaspase inhibitor zVAD ($p < 0.05$). We also determined, by detection of cleaved PARP, that caspases were not only cleaved but also activated in IL-4-stimulated cells. PARP is one of the down-stream substrates of caspase 3 and a critical factor involved in DNA repair and cell survival. In another set of experiments we observed that IL-4 was able to reduce the levels of Bcl-xL (western blot) and constitutive authophagy (microscopy, MDC staining), two factors related to cell survival. Our preliminary results suggest that induction of MC death may be an additional mechanism induced by IL4 to control the extent and duration of neuroinflammation.

134. Diminution Natural Killer (NK) cells in common variable immunodeficiency (CVID)

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CVID is a heterogeneous group of antibody deficiency, involving both B-cell and T cell immune function. The predominant manifestations are recurrent bacterial infections other complication are autoimmunity, chronic inflammation and neoplasm. Less was reported about the role of NK cells in these patients. We evaluated NK cell counts in CVID at diagnosis and the follow up and clinical correlation. We performed a retrospective study of a cohort of 45 CVID diagnosed according ESID criteria. Lymphocyte populations was performed by flow cytometry (FACSCalibur, BD®): T cells: CD3, CD4, CD8; B cells: CD19; NK cells: $CD56^{+}CD16^{+}CD3^{-}$. 23 male 22 women, age range 4-71 years. At diagnosis absolute NK (aNK) counts 10/43 (23%) between < 1-2 SD and 9/43 (21%) <2SD. At follow up with substitutive intravenous gammaglobulin (IVIG) 7/30 (23%) < 1-2 SD and 6/30 (20%) <2SD absolute NK. We classify the patients into two groups according to aNK count. Group 1 (G1) 21/45 (46%) normal NK counts, Group 2 (G2) 24/45 (54%): < 1-2 SD or < 2SD NK counts. Clinical manifestation: 93 % recurrent bacterial respiratory airway infections. We associated clinical characteristics of CVID patients and NK counts: chronic inflammation: G1 6/21 (28%) and G2 2/24 (16%); autoimmunity: G1 4/21 (19%) and G2 8/24 (33%). 1/45 poliovirus viral infection < 2SD NK. 7/45 had lymphopenia CD4 (LCD4), 10/45 had malignancies (M). We do not find correlation between LCD4, M and reduced NK cells. Conclusion: 54% of patients with CVID showed diminution of absolute NK counts. We do not observe changes in NK cells with IVIG treatment. Although we do not believe that NK cells are directly involved, we find correlation into NK count and complications (chronic inflammation with normal NK and autoimmunity with reduced NK count). In the patients with malignancies, we should evaluate the phenotype expanded and NK activity.

135. Increased bone marrow B cells export in CTSLnkt/nkt mice

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Cathepsin-L deficient mice (CTSLnkt/nkt) have alterations in lymph node (LN) cellular composition including an increase in B cells number. Besides, the levels of extracellular matrix components are increased in the LN of CTSLnkt/nkt mice, whereas in the bone marrow (BM) they are decreased. We previously demonstrated that neither CTSLnkt/nkt LN nor spleen have alterations in in vivo B cells basal proliferative and apoptosis levels. Furthermore, we showed that although B cell development in CTSLnkt/nkt BM is normal, CTSLnkt/nkt BM is able to produce more B cells progenitors in vitro, being the BM stroma involved in this increase. Taking into account these results, we hypothesized that CTSLnkt/nkt BM might export higher B cells number to the periphery. We measured by FACS the splenic transitional B cells subset (HSAhiB220lo) which represents newly formed BM-derived B cells. We found significant increased levels of transitional B cells in CTSLnkt/nkt spleen (mean % HSAhiB220lo cells ± SD: 11.3 ± 2.2 in CTSLnkt/nkt mice vs 5.1 ± 0.9 in wild type (wt) mice; n=4; $p<0.005$). To confirm if a greater number of BM B cells reach the CTSLnkt/nkt spleen, we injected mice with bromodeoxyuridine (BrdU). Considering that pro-B and pre-B cells are actively cycling cells and that most splenic B cells are quiescent, a brief in vivo labeling pulse of BM precursors allowed us to follow the fate of a cohort of newly generated B cells. Analysis by FACS revealed an increase in the absolute numbers (AN) of BrdU-labeled splenic B cells fractions I, II y III in CTSLnkt/nkt mice (mean AN BrdU+cells $x10^5$ ± SD; n=4; FI: 4.9 ± 1.1 in CTSLnkt/nkt mice vs 2.3 ± 0.9 in wt mice; $p<0.01$; FII: 8.0 ± 1.1 in CTSLnkt/nkt mice vs 3.6 ± 1.2 in wt mice; $p<0.005$; FIII: 25.5 ± 3.9 in CTSLnkt/nkt mice vs 14.8 ± 3.4 in wt mice; $p<0.05$). These results indicate that CTSL deficiency increases both production and output of BM B cells in CTSLnkt/nkt mice, probably as a consequence of an abnormal BM microenvironment.

136. The effect of secretory leukoprotease inhibitor (SLPI) on dendritic cells (DC) phenotype and function

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Dendritic cells (DCs) are the main antigen presenting cells that mediate the link between the innate and adaptive immune response. In the tissues, the immature DCs are exposed to a variety of factors. SLPI is a serine protease inhibitor constitutively expressed in the epithelial mucosal lining cells and its expression increases following an inflammatory stimulus. The aim of the present study was to examine the effect of SLPI on

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immature DCs. For this, DCs were generated from peripheral blood monocytes in the presence of IL-4 and GM-CSF. DCs were incubated 48 h with rhSLPI (4 µg/ml) or control buffer and the expression of surface molecules and apoptosis were analyzed by flow cytometry. Also, lymphocyte proliferation induced by SLPI-treated DCs or untreated DCs was analyzed by ^{3}H-Tymidine incorporation. SLPI-treated cells showed an increase of CD83 (8 ± 2%, $p<0.05$), but not MHC class I and II, CD40, CD80 and CD86. Basal apoptosis of immature DCs was low (12 ± 3%). When DCs were treated with SLPI, early apoptosis was significantly increased (SLPI: 20 ± 4%, $p<0.05$). The effect of SLPI on DCs apoptosis was not observed with heat denatured SLPI. Furthermore, SLPI treated-DCs showed higher lymphocyte allogeneic proliferative ability compared to untreated DCs (Control: 23709 ± 2033 cpm; SLPI: 40726 ± 5733 cpm, $p<0.05$). The effect of SLPI-treated DCs on lymphocyte proliferation was dose dependent and it was not observed with heat denatured SLPI. These results demonstrate that SLPI modify the phenotype and function of immature DCs. Based on the CD83 expression, the increase in apoptosis and the proliferation, it is probably that SLPI promote the maturation of DCs.

137. Plasticity in Melanoma-associated-Antigens in human melanoma clonogenic cells

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Cutaneous melanoma (CM) is the neoplasia with the fastest growing incidence. It expresses immunogenic Melanoma Associated Antigens (MAA) such as MART-1, gp100, and the cancer testis Ag NY-ESO-1. Since CM tends to down-regulate MAA expression, it is important to investigate if such down regulation affects cell proliferation. We studied anchorage-independent growing clonogenic cells (CC) of two human CM cell lines (MEL-XY1 and MEL-XY3) as a model of in vitro proliferation. Two growth stages were analyzed: an early stage, after 7 days, with colonies of about 100 cells, and a late stage, after 14 days, with colonies of several thousand cells, many of them with apoptotic/necrotic cores. We analyzed MART-1, gp100 and NY-ESO-1 Ags and the proliferation marker Ki-67 by IHQ. At 7 days, MEL-XY3 only had MART-1/gp100^{+} colonies, whereas MEL-XY1 colonies were either positive (49%), negative (21%) or mixed (30%). Since every colony was Ki-67^{+}, it is concluded that the expression of MAA does not interfere with the proliferative potential. These observations were in accordance with MAA expression in biopsies and source lines. Lysis by specific CTL clones for MART-1 and gp100 on heterogeneous CC revealed that only positive cells were lysed (0.3% survived). By 14-days, the proportion of positive colonies for MART-1 and gp100 expression decreased, and mixed colonies increased ($p<0.05$). Both cell lines were uniformly NY-ESO-1^{+} in 7 and 14-day colonies. Then, NY-ESO-1 expression appears necessary to CC proliferation. Another objective was to elucidate intrinsic mechanisms governing MAA expression. We studied CpG methylation in MART-1 promoter by MSP, and found that the MEL-XY1 CC promoter was 60 fold more methylated than the MEL-XY3 CC one. Thus, Methylation of MART-1 promoter would be involved in MART-1 heterogeneity. Therefore, melanoma CC showed plasticity for MAA expression in time, and immunotherapeutic strategies should take this into account.

138. Distribution of several activating receptors on natural killer (NK) cells from tumors and peripheral blood in colorectal cancer (CRC) patients

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Despite NK cells being originally identified and named because of their ability to kill tumor cells in vitro, only limited information is available in humans, on NK infiltrating malignant tumors and from peripheral blood. NK cell recognition of target cells is guided by the balance of the activating and inhibitory signals given by different groups of surface receptors. The purpose of the present study was to analyze NK tumor infiltrating cells (CRC-NKi), as well as NK from peripheral blood (CRC-NK-PB) in CRC patients and to identify a potential profile of NK receptors expression. Samples were obtained from 11 healthy volunteers (HV) and 16 patients. Tissues were obtained immediately after surgical resection of their primary tumors, mechanically processed, and mononuclear cells (MNC) were isolated using density gradient. The same procedure was utilized to isolate peripheral blood (PB) MNC (PBMC) from CRC patients and healthy volunteers. Cells were directly analyzed by flow cytometry. We observed that percentage of CRC-NKi identified as CD3^{-}CD56^{+}CD45^{+} was consistently lower (mean value, 4.4±2.8) than CRC-NK-PB counterpart (mean value 18.76 ± 2.1) ($p<0.05$). CCR-NKi displayed a peculiar surface pattern in activating receptors, calculated as percentage of positive cells in CD56^{+}CD3^{-} population. CD16, NKG2D, NKp46, CD94, CD161 and DNAM1 were expressed in a fewer number of cells compared with CRC-NK-PB ($p<0.01$). In a clustering analysis (TMeV software) global comparisons including expression of all receptors between all samples showed that NKG2D is the most significant discriminant receptor ($p<0.05$) between NK-PB-CCR (mean value 88.5±3.5) and NK-PB-HV (97.2± 3.2). The present study of a small group of CRC patients suggest that lower NKG2D expression could be a hallmark in NK-PB-CCR. CRC-NKi cells display particular phenotypic features that could affect their functional abilities, and should be further analyzed in a larger numbers of patients.

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doi: 10:3823/413

139. TLR4-dependent early recruitment of phagocytes to airways is critical for Bordetella pertussis clearance

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TLR4 dependent processes shape adaptative anti-pertussis immunity; however there is scarce knowledge on its contribution to the antimicrobial scenario during the early stage of B. pertussis infection. The aim of this study was to characterize the recruitment and antimicrobial capacity of cell populations from broncoalveolar lavage (BALF) at 2, 6, and 24 h after B. pertussis mice intranasal infection (10^8CFU/40µl). By flow cytometry we observed that the influx of neutrophils (PMN, $CD11c^-$, $CD11b^+$, $Ly6G^+$) into the airways was significant in TLR4 competent mice, rising from less than 10^3/BALF to $7x10^5 \pm 10^5$ cells/BALF at 6 h. For TLR4 deficient mice strain (C3H/HeJ mice strain), recruitment of PMN was evident only at 24h, being in all time points analyzed lower than in TLR4 competent strain. The expression of proinflammatory chemokines such as CXCL1, CXCL2, CXCL5 was higher in TLR4 competent mice than in C3H/HeJ mice. Depletion of PMN by specific monoclonal antibodies, increased bacterial loads from 7.7±0.12 to 8.4±0.53 log CFU/lung at 24 hs (p=0.097) and 7.15 ± 0.21 to 8.36 ± 0.026 log CFU/lung at 48 hs (p=0.0023). Using CFSE labeled bacteria we observed that during the first 24 h of infection bacteria were mainly associated to PMN (27.66±0.66 % vs 11.41± 1.99% at 6h and 16.54±3.02% vs 7.29±6.33% at 24h. This result seems to be independent of TLR4 since there was no difference between TLR4 competent and deficient mice strains. As an effector mechanism we observed that PMN contained higher level of reactive oxygen species (ROS) in comparison with AM cell population PMN ROS+: 33.72±1.50% vs AM ROS+: 9.10±3.89% at 6hs; 57.70±10.18% vs 18.50±5.10% at 24hs), indicated that PMN were the main producer of ROS in the first 24hs of infection. Altogether these results indicate that TLR4 activation at early time points of B. pertussis infection is critical for eliciting anti-pertussis response and that PMNs recruitment participates actively in the bacterial clearance.

140. The inhibitory effect of Fasciola hepatica antigens on induced LPS-DC activation correlates with a decrease in p38 and STAT3 phosphorylation and is independent on NF-κB translocation

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The ability of different Fasciola hepatica (Fh) antigens to diminish the classical TLR ligand dendritic cell (DC) activation has been demonstrated. However, little is know about the signals used for this helminth to bias DC activation toward a tolerogenic profile. The objective of this work was to investigate the signals involved in the modulation of F.h total extract (TE) to decrease the LPS induced DC maturation when these cells are simultaneously exposed to both stimuli (T/L). The T/L treatment of bone marrow derived DC induced a significant increase in IL-10 production (determined by ELISA) (Student T test $p<0.05$) and ERK phosphorylation (determined by western blot) compared to LPS treated DC. Although IL-10 production was dependent on ERK phosphorylation, neither IL-10 blocking nor ERK inhibition restored the IL-12 levels of LPS treated DC. As a measure of NF-κB activation, a crucial factor involved in the IL-12 production, we determined IκB degradation and Rel A translocation. The activation of these factors were not affected when DC were treated with T/L compared to LPS treated DC. However, STAT-3 and p38 phosphorylation levels were lower in DC by T/L treatment than those presented in LPS-treated DC, suggesting that other signaling pathways different from NF-κB could be affected by TE. Besides a Kunitz type molecule (KTM) purified from TE by FPLC, showed identical effects on MAPKs phosphorylation and the NF-κB activation than that exerted by TE. Overall, our data show that the modulatory effect exerted by TE as well as KTM on LPS-activated DC is depending on the regulation of specific intracellular signals such as p38, ERK and STAT-3.

141. Lymphocytic vasculitis involving the central nervous system and lung occurs in a patient with X-linked lymphoproliferative disease (XLP)

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Systemic vasculitis is an uncommon manifestation of XLP, in which there is a selective immune deficiency to Epstein-Barr virus (EBV). XLP is an immunodeficiency caused by defects in the adaptor molecule SAP. We describe a patient who died as a result of chronic systemic vasculitis and fulfilled clinical criteria for the diagnosis of XLP. Sequencing of this patient's SAP gene uncovered a novel point mutation c.28_31delA in SAP gene. The patient presented with severe hypogammglobulinemia and two episodes of pneumonia in 1 year. The immunological evaluation showed normal lymphocyte distribution, save for NK cells, with also normal NK cytotocicity function and proliferative response both to mitogens and antigens. B phenotype: decreased memory $B27^+$ cells. EBV DNA was detected by polymerase chain reaction in periphery blood, cerebral spinal fluid and both lung and

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doi: 10:3823/413

bone marrow biopsy. During treatment the patient develops severe neurological impairment and respiratory failure, which resulted in the disease of the patient. Chest and CNS tomography showed evidence of a hypodense lesion in the posterior limb of the right internal capsule, and nodular bilateral pulmonary infiltration, with extended alveolar damage. The lumbar puncture evidenced CSF with high cell count, predominantly mononuclear cells and elevated protein level. Lung biopsy showed lymphocytic vasculitis, with non malignant $CD3CD8^{+}CD4^{-}$ T cells. CNS MRI and MR-angiogram revealed signs of acute small vessel infracts and biventricular bleeding, with signs of ecstasies and aneurysms. He was treated with metilprednisolone and rituximab pulses, and intrathecal therapy with dexamethasona, of which he received 2 doses, with no response. Conclusion: loss of SAP function can lead to dysregulated immune responses characterized by the uncontrolled expansion and activation of T cells independent of EBV infection. Treatment, as reported, is highly difficult, and it should include intensive immune-suppression since the beginning.

142. Cysteinyl leukotriene C4 trigger an "immature" state on DC able to induce a inflammatory response

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Previously, we proved that cysteinil leukotriene C4 (CysLT) increases dendritic cell (DC) uptake and inhibited IL-12p70 production by mature DC. Here, we focused on the cytokine profile induced by CysLT on immature and mature DC and the mechanism involved in this modulation. For this, DC were obtained from C57BL/6 bone marrow progenitors in the presence of GM-CSF. Then, they were incubated without or with LPS (1µg/ml) for 30 min at 37ºC and treated or untreated with LTC4 (0.01 µM) overnight at 37ºC. Finally, we analyzed in culture supernatants the presence of IL-10, IL-12p40 by ELISA. We showed that CysLT inhibited the production of IL-10 (IL-10: pg/ml mean±SEM; Ct 56±5.7; CysLT: 63±7.1; LPS: 89±7.3; LPS+CysLT: 60±5.2, *p≤0.05. n=6), whereas it stimulated the IL-12p40 secretion by mature DC (IL-12p40: pg/ml mean± SEM; Ct:43.33 ±13.17; CysLT: 32.06±9.4; LPS:93.51±23.31; LPS+CysLT,139.5±22.13, N=8; ***p<0,001). Taking into account that, CysLT activates p38 MAP kinases we decided to evaluate if the modulation of DC by CysLT involves this activation pathway. Immature and mature DC were cultured with SD, a blocker of p38 activation, and after 30 min at 37ºC we added 0.01 µM of CysLT and treated with brefeldin A overnight at 37ºC. We showed that, when we blocked the activation of p38 in mature DC, the production of IL-12p40 was inhibited by treatment with the lipid mediator (IL-12p40: positive cells % mean ±SEM; LPS 19.4±1.2; LPS+CysLT: 35±3.2 vs LPS+SD: 23±2.2; LPS+CysLT+SD: 15±1.2, *p≤0.05, n=3). A similar effect was shown for dextran endocytosis. Finally, we found no variations in the expression of CysLT receptors (CysLT1 and CysLT 2) between immature and mature DC. In conclusion, our results suggest that CysLT via p38 activation, could trigger the differentiation of $CD4^{+}$ T lymphocytes to a Th17 profile.

143. Low NK activity and diminished CD107a expression as markers for evaluating degranulation associated defects in familial hemophagocytic lymphohistiocytosis (FHL)

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Hemophagocytic lymphohistiocytosis (HLH) is a fatal disease of early infancy, characterized by fever, hepatosplenomegaly and pancytopenia. FHL is an autosomal recessive inherited form of HLH. Defects in different proteins cause four clinically indistinguishable types of FHL: Perforin that affects pore formation and Munc13-4, Sintaxin-11 and Munc18-2 that prevent degranulation. NK cytotoxic activity is generally impaired. Degranulation of secretory granules is a necessary step for perforin release and can be measured by CD107a expression on NK cells upon activation. We present NK cytotoxicity and CD107a assays performed in 6 patients suspected of FHL (age x:2.5y; r:1m-8y) in order to evaluate its usefulness in selecting a rationale molecular approach in FLH. NK function was assessed by a standard ^{51}Cr release assay. Perforin expression and up-regulation of CD107a on NK surface after stimulation with K562 plus interleukin-2 were evaluated by flow cytometry. All 6 patients showed NK activity impairment: Pt1 and Pt2 0.4%; Pt3 0.7%; Pt4 18%; Pt5 and Pt6 0% (Normal Values (NV) x: 39%, r:34-50). CD107a expression was markedly reduced: 13%, 4% and 4.9% in Pt1,2 and 3 respectively, while in Pt 4, 5 and 6, it was increased: 70%, 69% and 58% respectively (NV x:30 r:27-37). Perforin was normal in all cases. Among the 3 patients with low CD107a, Pt1 and 2 had mutated Sintaxin-11 gene while Pt3 had not, but a mutation in Munc18-2 gene is suspected. Of the 3 patients with high CD107a expression, Sintaxin-11 gene mutation was ruled out in Pt5 and Pt6 was further diagnosed with NK malignancy (secondary HLH). Low CD107a expression correlates with presence of Sintaxin-11 mutation in Pt1 and 2 while in Pt3 probably other degranulation genetic defects are involved. A normal or abnormally high CD107a would not support analysis of mutations in genes involved in degranulation. Patients with low NK activity and CD107a expression should be tested for Munc13-4, Sintaxin-11 and Munc18-2.

144. Baculovirus Strongly Potentiate Antitumor Immune Responses

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doi: 10:3823/413

Baculoviruses (BVs) are dsDNA viruses that are pathogenic for insects. They infect a broad range of mammalian cell types but do not replicate in these cells. A recombinant BVs was designed which displays OVA on the capsid as a fusion with vp39 gene (BV-OVA). We previously demonstrated that BVs have strong properties as adjuvants and as vectors for MHC I Ag presentation in mice and promoting potent CD4 and CD8 T cell adaptive responses against OVA. BVs also induce in vitro and in vivo maturation of dendritic cells and the production of inflammatory cytokines. Here we assayed the ability of BV-OVA to induce a protective immune response against a tumor cell challenge. We performed a prophylactic and a therapeutic protection protocol. For the prophylactic protocol, mice (n=8) received a single i.v. injection of $5x10^7$ BV-OVA and seven days later (0 dpt) mice received s.c. $1x10^5$ MO5 cells, a melanoma derived tumor cell stably transfected with the OVA gene. For the therapeutic protocol, mice received s.c. $1x10^5$ MO5 cells and then were injected with BV-OVA on days 7 (i.v., $5x10^7$ BV-OVA), 11 and 17 (s.c., $1x10^7$) and 21 (i.t., $1x10^7$). Additional mouse groups (n=8) received similar schemes of immunization with BV-WT or PBS as control. Mice from the prophylactic protocol injected with PBS or BV-WT developed growing tumors (Survival 0 %, day 32 dpt) whereas BV-OVA mice were free of tumor all along the experiment (Survival 100 %, $p<0.001$ for BV-WT or PBS vs BV-OVA). Mice from the therapeutic protocol with PBS or BV-WT developed larger tumors than mice injected with BV-OVA (2.7±1.5 or 3.0±1.4 vs 0.2±0.1 cm^3, $p<0.001$ and $p<0.01$, respectively) at day 25 dpt. Survival was 100 % at day 32 dpt in BV-OVA mice and 0 % in PBS or BV-WT mice ($p<0.05$ for BV-WT or PBS vs BV-OVA). These results demonstrate that the strong CTL and CD4 T cell response induced by recombinant BVs is enough to establish a protective immunity against MO5 challenge, showing the potential of BVs as a new strategy of vaccination.

145. Profiling the repertoire of galectin expression during prostate cancer progression

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As cancer cells grow in a complex microenvironment surrounded by multiple intra- and extra-cellular signals and prostate tumours inevitably progress towards an aggressive phenotype, it is essential to understand how these tumours manage to coordinate the signals which promote dissemination and thwart antigen-specific immune responses. The critical functions of galectins (Gals), evolutionarily conserved beta-galactoside binding lectin, during inflammation, angiogenesis and tumor immune escape prompted us to analyze associations between the expression levels of each member of the galectin family and the progression of prostate cancer. We analyzed the transcriptional pattern of galectin expression in several prostate cancer cell lines characterized by differential biological properties. We found that Gal-8 mRNA was ubiquitously detected in all cell lines whereas Gals-1, -3, -4, -9 and -12 mRNA show differential expression profiles suggesting a fine transcriptional control of galectin members in prostate cancer cell lines. These results induced us to analyze protein levels in patient biopsies by IHC. Gal-1 was the most highly expressed member of this family and was significantly up-regulated during disease progression ($p<0.05$ BHP vs. T1, T2, T3 and T4). On the contrary, Gals-3, -4, -9 and -12 were down-regulated during disease progression ($p<0.05$ by comparing BHP and progressive stages of the disease, respectively). Gal-8, while being expressed by prostate tumours did not experience any statistically significant modulation during the progression of the disease. Prostate cancer progression is associated with a particular galectin signature prompting further investigation of these glycan-binding proteins as selective prognostic biomarkers that could delineate non-progressive from progressive clinical outcomes.

146. NKp46- and IL-10-dependent silencing of NK cell-mediated IFNγ production upon cross talk with tolerogenic dendritic cells

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Cross talk between mature dendritic cells (mDCs) and NK cells through the cell surface receptors NKp30 and DNAM-1 and the cytokines IL-12, IL-15, IL-18 and IFNγ results in a reciprocal activation of both cells. However, the outcome of the cross talk between tolerogenic DCs (tDCs) and NK cells, which may take place within a tumor microenvironment where tDCs are abundant, remains unknown. Previously, we observed that tDCs prevent IFNγ secretion by NK cells in a mechanism involving cell surface receptors and soluble factors. Thus, the objective of this study was to elucidate the mechanisms underlying such NK cell effector function silencing promoted by tDCs. Human tDCs were generated from monocyte-derived DCs treated with LPS+dexamethasone and indentified as CD1a+MHC-II+CD14-$CD86^{low}$ and $IL\text{-}12^{low}IL\text{-}10^{high}$. Upon co-culture with NK cells for 24h, we observed that extensively washed tDCs (but not mDCs) prevented IFNγ secretion by NK cells (202±88pg/ml vs.

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1105±502pg/ml; p<0.001). Inhibition of IFNγ secretion was not due to DC-induced NK cell apoptosis, as total apoptosis of NK cells after co-culture with iDCs, mDCs or tDCs were -3.9±5.3%, 14.4±6.0% and 4.4±5.6%, respectively over basal levels (NK cells alone). To investigate the receptors involved in this response, we performed blocking assays with anti-cytokine and anti-NK cell receptor mAbs. Blockade of IL-10 and, unexpectedly, blockade of the activating receptor NKp46 (but not NKp30, NKp44 or NKG2D) restored the ability of NK cells to secrete IFNγ upon co-culture with tDCs (871.8 ± 343.5pg/ml and 1072±427.1pg/ml vs. 273.7±205.4pg/ml, respectively; p<0.05 in each case). Thus, tDCs preclude NK cell IFNγ secretion through IL-10 production and promote a previously unrecognized inhibitory signal through NKp46. Our findings unravel a novel immunosuppressive mechanism of possible relevance within a tumor milieu and could be relevant as tolerogenic mechanism for NK cells to avoid self attack during autoimmunity.

147. MIB2: a novel BCL10-interacting protein

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Activation through the T-cell receptor (TCR) leads to the initiation of multiple signaling cascades that regulate survival, cytokine production and proliferation of T-cells. A key event upon TCR engagement is the formation of a signaling complex between CARMA1, BCL10 and MALT1, referred to as CBM complex. These three molecules interact physically and functionally. The components of this complex were found to be essential for T-cell activation, in particular by controlling the NF-kB and MAP kinase signal transduction pathways. Even though the role of the CBM complex in T-cell activation is well established, little is known about the signaling events between the CBM complex and downstream effector molecules. Using an affinity purification/ mass spectrometry approach we have identified Mind Bomb-2 (MIB2) as a novel BCL10-interacting protein. MIB2 is constitutively associated with BCL10 at low levels and significantly recruited upon activation. Overexpression of MIB2 leads to a strong increase in transcriptional NF-kB activity, indicating that MIB2 may represent a critical factor for BCL10-dependent NF-kB activation. We have characterized the molecular mechanism of MIB2-dependent NF-kB activation and identified the structural domains of MIB2 that control BCL10 interaction and NF-kB activation. Identification of molecular events downstream the CBM complex will further improve our understanding of T-cell activation, a critical event not only in the defense against pathogens, but also in the initiation and progression of inflammatory diseases such as allergy and asthma or autoimmunity.

148. Primary Immunodeficiencies (PID): report of a reference pediatric center

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Introduction: The prevalence of PIDs in the general population has not been clearly identified. Estimates on the minimum prevalence of PIDs are based on the information compiled from registries. Objective: To report pediatric patients (p) with PIDs diagnosed and followed in our center from 1987 to 2010. Methods: Available data of patients were collected. The categorization was based on the classification defined by the IUIS in 2009. Diagnostic criteria defined by ESID for PIDs have been used. Unclassified PIDs, transient hypogammaglobulinemia and Neutropenias were excluded. Results: 660p have been registered Combined T and B–cell deficiencies were observed in 44p (6.7%), most of them with diagnosis of SCID (39p): γc deficiency (14p) and ADA deficiency (6p) were the most frequent molecular defects identified. Predominantly antibody deficiencies were observed in 273p (41.4%). This included Agammaglobulinemia: 70p (65p Btk deficiency, 1p μ_heavy chain deficiency); CVID: 41p; Hyper IgM syndrome: 12p (9p CD40L deficiency, 3p AID deficiency); Selective IgA deficiency: 135p. Other well defined immunodeficiency syndromes were diagnosed in 225p (34%): DiGeorge anomaly 118p, Ataxia- telangiectasia 42p, Wiskott-Aldrich syndrome 23p, Hyper-IgE Syndromes 28p and Cartilage Hair Hypoplasia 9p. Diseases of immune dysregulation: 43p (6.5%), of which 20p were Immunodeficiencies with hypopigmentation and 9p were definitive ALPS. Congenital defects of phagocyte include 56p (8.5%): LAD-1: 1p, CGD 46p and IL12-IFNγ defects 9p. Complement deficiencies were diagnosed in 17p (2.6%), 8p with C1 inhibitor deficiency. Conclusions: As described in the literature, antibody deficiencies were the most frequent category observed. Selective IgA deficiency is underestimated because of lack of appropriate registry. There are patients reported in all categories, due to the fact that we are a reference center. The availability of specialized immunological studies allows a high rate of definitive diagnosis.

149. Soluble factors released by DU145 human prostate cancer cells stimulated with Poly I:C-liposomes promote the activation of monocyte-derived dendritic cells.

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doi: 10:3823/413

Tumors derived factors significantly inhibit the generation as well as the activation of dendritic cells (DCs). Polyinosinic-polycytidylic acid (Poly I:C, PIC), a dsRNA analog, can trigger the secretion of type I Interferon and induce apoptosis in tumor cells via the innate recognition receptors melanoma-differentiation-associated gene (MDA)-5 and retinod-inducible gene (RIG)-I in the cytosol or via Toll-like receptors 3 in intracellular endosomes. Thus, we hypothesized that prostate tumor cells activated via these receptors could release soluble factors that can restore the suppressive effect of tumor derived factors on DCs. In order to see if factors secreted by PIC-stimulated DU145 cells could somehow overcome this already described inhibitory effect, DU145 cells were cultured with PIC-liposome complex (0.1 µg/ml) for 4 hours, washed three times with PBS, incubated for 20 additional hours and the culture supernatant was then harvested and filtered (CM-PIC). As control, non stimulated DU145 cell culture supernatant was also collected (CM). Human monocyte-derived DCs (MoDCs) cells were matured with LPS in the presence of CM-PIC or CM. MoDCs stimulated with LPS in presence of CM-PIC increased the expression costimulatory molecules compared to MoDC stimulated with LPS in presence of CM (CD86 MFI: 178 vs 97; CD80 MFI: 35 vs 26; CD40, MFI: 23 vs 14). Furthermore, the levels of IL12p70 were significantly increased in LPS-stimulated MoCDs incubated with CM-PIC at 24h (670.1±2.9 vs 90.3±16.3 pg/ml), and at 48h (926.5±14.2 vs 127.5±1.5 pg/ml), $p<0.05$. MoDCs stimulated only with PIC (0.1 µg/ml) did not show any sign of maturation. Together, our results provide new insights in the use of PIC-liposomes as therapeutic agents to enhance the antitumoral immune response.

150. Cryptococcus neoformans-pulsed eosinophils inoculated intraperitoneally migrate to the spleen and mesenteric lymph nodes where stimulate cell proliferation and Th1 cytokine production

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Eosinophils (Eo) have been shown to be components of the inflammatory response to Cryptococcus neoformans (Cn) infection in rat and mouse. We have observed the presence of a large number of Eo in the granulomas surrounding Cn yeasts during disseminated cryptococosis in rats. Moreover, we have demonstrated that rat Eo stimulate in vitro the expansion of Cn-specific CD4+ and CD8+ T cells with a Th1 profile. Therefore, the aim of the present work was to study the role of Eo in the immune response to Cn infections. Cn-pulsed Eo labeled with CFSE were inoculated intraperitoneally (ip) into naive rats to assess their potential to migrate to lymphoid organs (LO). Results indicated that CFSE+ Eo migrate from the peritoneal cavity to the Mesenteric lymph nodes (MLN) and spleen within 3 days. On the other hand, Cn-pulsed Eo were inoculated ip into naive and Cn-infected rats to determine whether Eo could stimulate cell proliferation in vivo. Spleen and MLN cells recovered after 5 days of the inoculation were cultured in the presence of anti-CD3 MAb and it was observed that LO cells from rats inoculated with Cn-pulsed Eo had increased proliferation, compared with LO cells from rats that had received unpulsed Eo ($p<0.01$). Moreover, experiments were performed to analyze cytokines and total immunoglobulin (TIg) production by LO cells recovered from those rats. Spleen and MLN cells were cultured in the presence of heat killed Cn (HKC). The production of IL-12, IFNγ, TNFα and TIg by LO cells isolated from rats inoculated with Cn-pulsed Eo was significantly increased, compared with that in control rats ($p<0.01$). However, the production of IL-4, IL-13 and IL-10 by LO cells was significantly decreased ($p<0.01$). It was therefore concluded that Cn-pulsed Eo are capable of migrating to LO after their inoculation. Finally there, they can stimulate spleen and MLN cells proliferation, the increase of TIg production and the development of an antigen-specific Th1 cytokine response.

151. Effect of Fructooligosaccharides (FOS) on the intestinal mucosal immune system against infection with S.Typhimurium

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The FOS obtained from the roots of Yacón, used as a dietary supplement, can prevent enteric infections when given for a long term administration (30 days). Its effect is mediated by an increase in total secretory-IgA and does not produce increase of T cells. The aim of this study was to establish whether the FOS developes a preventive action against infection with S. Typhimurium by activating other mechanisms mediated by expression of receptors or cytokines produced by cells of the innate immune response. BALB/c mice were divided in 4 groups: normal control (NC), Basal (B– 30 days with FOS), infection control (IC) and treated group (TG–30 days with FOS+ pathogen). At 7 days post challenge we studied: expression of TLR4, CD206, IL6, TNFα, IFNγ and the expression of the chemokine MIP1α (Nº of + cells by IFI) from sections of small intestine, before and post challenge. MIP1α increase in TG with regard to IC group (25±4 vs 17±2), TNFα and IFNγ had no significant difference between TG and IC group (23±3 vs 19±4 and 25±3 vs 19±5 respectively). The expression of IL6 increased in TG over the IC group (34±8

vs 19±2), the same effect was observed for receptors TLR4 and CD206 (24±2 vs 17±2 and 29±3 19±6, respectively). FOS protection against infection with S. Typhimurium would only be mediated by nonspecific immune response, with an increase in the expression of receptors (TLR4 and CD206) and IL6+ and MIP1α+ cells, that would favor immunological barrier mechanisms of innate immune response (phagocytosis and increased total secretory-IgA) against infection.

152. Potentiation of the MVA immunogenicity after the deletion of a viral gene coding for the IL-18 binding protein

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Modified Vaccinia Ankara (MVA) is an attenuated Vaccinia virus strain currently employed in clinical trials against multiple infection diseases such as HIV/AIDS, Malaria and Tuberculosis. Despite its large loss of genomic regions during the attenuation process, MVA still retains viral genes involved in host immune response evasion, raising the possibility to increase its vaccine potential by removing some of these genes. The aim of this work was to evaluate immune responses induced by a MVA bearing an IL-18 binding protein (IL-18bp) gene deletion (MVAd008) previously generated in our laboratory. Balb/c and C57BL/6 female mice were immunized intraperitoneally with 5E+07 pfu of MVAd008 or wild type (wtMVA) and cellular immune responses were evaluated 7 days post-infection (dpi). The proportion of specific IFNγ producing cells was measured by ELISPOT, in Balb/c mice MVAd008 induced a two-fold increase in TCD8+ response to vector epitopes such as E3 peptide compared with wtMVA whereas in C57Bl/6 a three and two-fold increases were detected for the B8R (TCD8+) and E9L (TCD4+) epitopes. The quality of the response was monitored by flow cytometry showing that IL-18bp deletion induced a large proportion of specific IFNγ, TNF and CD107a/b (cytotoxicity parameter) TCD8+ cells. Next, the in vivo protection was evaluated 60 dpi through an intranasal challenge with the replicative WR strain (2E+06 pfu). Mice immunized with 1E+06 pfu of wtMVA lost more weight and started to slowly recover on day 7 whereas those immunized with MVAd008 lost less weight and started to quickly recover at day 6. These results suggest that, during MVA infection, IL-18bp expression contributes to immune response evasion and that its deletion from the viral genome increases specific immune responses against vector epitopes. Further investigation is required to see if this increase in MVA immunogenicity will also augment specific responses against foreign antigens such as HIV proteins.

153. Intranasal co-administration of IL-12 plus CTB in DNA-MVA mucosal schemes enhanced cellular immune responses against the HIV-1 Env antigen, systemically and in mucosal draining lymph nodes

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The major route of HIV transmission is through the exposure of mucosal surfaces to the virus, therefore designing mucosal immunization regimes aimed to induce mucosal immune responses is highly needed. The aim of this study was to analyze the mucosal activity of IL-12 alone or in combination with the subunit B of cholera toxin (CTB), applied in DNA-prime/MVA-boost intranasal immunization schemes. Balb/c mice were immunized by intranasal route with a heterologous DNA prime-MVA boost regimen, in which both vectors express the HIV-1 Env subtype B protein. Two doses of DNA-IL-12 were applied (50ug or 100ug) at priming, in the presence or not of 10 ug of CTB applied at prime and booster doses. Groups receiving CTB, complete cholera toxin (CT) or non-adjuvants were included. Co-inoculated DNAIL-12 with CTB (IL-12+CTB) produced the highest specific TCD8+ immune response (evaluated as IFNγ secreting cells by ELISPOT), detected in the spleen as well as in regional (cervical) and genital (iliac) draining lymph nodes. In general, the co-administration of both adjuvants followed an additive effect in the final response obtained. Both DNA-IL-12 doses generated similar responses, even more the minor IL-12 dose applied plus CTB generated the highest response in the spleen showing a synergistic effect for both adjuvants, indicating that 50 ug is sufficient. Increments in the TCD8+ response detected in IL-12+CTB groups were up to 7 fold in the spleen, and up to four (cervical) and three fold (genital) in mucosal draining lymph nodes. Remarkably, these responses were equivalent or even higher than those detected in the groups with CT. Finally, the highest specific TCD8+ response found in the IL-12+CTB group was accompanied by an increase in the Th1/Th2 specific cytokine secretion against gp120. These results are of importance due to the need to improve mucosal vaccine strategies against HIV.

154. The immunomodulatory activity of chitosan on bovine mammary epithelial cells

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Staphylococcus aureus is an important cause of udder infections in dairy herds, affecting both the quality and quantity of milk production. Bovine mammary epithelial cells (bMEC) contribute to the innate immune response to intramammary infections. An effective strategy to prevent S. aureus infection could be to block

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bacterial adhesion to bMEC and promote the innate immune response by the epithelium. Chitosan (Ch) is an acetylglucosamine polysaccharide obtained from deacetylated chitin and one of the most abundant polymers in nature. It presents antibacterial effects and ability to modulate the immune response. Herein we studied the modulatory activity of the polysaccharide in bMEC. We evaluated the ability to modify the adherence and internalization of V329 S. aureus strain in bMEC, using the MACT cell line. MACT cells were incubated in the presence of increasing concentrations of Ch (0.01 to 100 ug/ml) for 24 h. The adherence of V329 to the MACT decreased by 80% in presence of 10 ug/ml Ch (CFU count, $p<0.05$). The V329-FITC internalization showed a reduced percentage after Ch pre-treatment in a dose dependent manner (34 to 61% of inhibition, flow cytometry (FC), $p<0.05$). Alternatively, we evaluated the effect of Ch in the epithelial innate immune response. We studied the response of V329 infected MACT cells after treatment with 10 ug/ml Ch. We observed an increase in the epithelial MHC-II expression (2.5% vs. 13.6%) and IL-1b production (4.0% vs 15.1%) after stimulation with Ch (FC). In addition, we observed an increase in mRNA expression of IL8, TNFa and CCL2 evaluated by RT-PCR ($p<0.05$). Also, the mRNA of TLR2 and TLR4 receptors was up-regulated. Ch inhibits S. aureus internalization and adherence, and stimulates an inflammatory response by bMEC. These data suggest that Ch might be useful in the control of bovine mastitis and epithelial bacterial infection.

155. Immune mechanisms induced by a probiotic fermented milk administration in an experimental model of allergy

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It was reported the beneficial effect of probiotic in diseases, but the mechanisms involved are not well known. We study if a probiotic fermented milk (PFM) has positive effect on an experimental model of allergy and to determine the immune mechanisms involved. Mice were divided into 5 groups: normal control (NC), Basal (B 5d PFM); Control sensitization (CS), Previous (P 5d PFM+OVA+H2O), Continuous (C 5d PFM+ovo+PFM). CS, P and C groups were sensitized with ovalbumin (OVA) at 1% followed by daily exposures to aerosolized OVA for 10d. Samples were taken at 7 and 15 days post-sensitization (dps). We determined anti-OVA-specific-IgE (aOVAIgE) and IL10 in serum, total IgA, IL10 and IFNγ in intestinal fluid (IF) by ELISA. IL2, IL4 and IL10+ cells were mesured in small intestine (SI) by IFI. In large intestine (LI) we determine total populations of anaerobes, lactobacilli, bifidobacteria and enterobacteria in selective media. At 7 dps, levels of aOVAIgE decreased and IL10 increased significantly in P and C groups (339±72 and 126±38) vs. CS (47±12 pg/ml). IL10+ cells were significantly increased in lamina propria (LP) of SI in P and C groups for 7 dps (20±5 and 17±1) and 15 dps (16±3 and 25±6) vs. CS (7±2), but without release of this cytokine in IF as well as for IFNγ. The Nº of IL2+ cells in all the experimental groups did not change compared with NC. IL4+ cells decreased to 15 dps in groups P and C (10±3 and 15±3) vs CS (21±1). For 7 and 15 dps total IgA levels remain high compared to NC and B. With regard to the intestinal microbiota we determined a reduction of enterobacteria in P and C groups, and increases of bifidobacteria associated with down regulation in B and C. PFM admistration had a beneficial effect in this model evidenced by low leves of aOVAIgE, decrease in the Nº of IL4+ cells in LP and increases of IL10 in serum. High levels of IL10 did not interfer with the total IgA production in gut. Bifidobacteria could be involved in the immune regulation observed.

156. Quantitative alterations in splenic dendritic cell subsets, T regulatory cells and cell proliferation in infected or vaccinated mice with foot-and-mouse disease virus

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Foot-and-mouth disease (FMD) is a highly contagious, acute and feverish viral disease of cloven-hoofed animals. Previously, we have demonstrated that vaccination with inactivated FMDV induces an increase in plasmacytoid dendritic cells, in contrast with the infection that induces a decrease in this subset. Now, we studied the possible alterations in different subsets of dendritic cells (DC) and T lymphocytes between vaccination and infection. For that, we used adult Balb/c mice to infected i.p. with FMDV or vaccinated. Control mice were inoculated with supernatant of uninfected BHK-21 cell cultures. After, 1 or 3 days we obtained the population of splenic DC by using anti-CD11c/anti-mPDCA-1 magnetic beads. We found that the proportion of different DC subsets changes among vaccination and infection. While in vaccination myeloid DC (CD11c+/CD8a-) decreased significantly and lymphoid (CD11c+/CD8a+) DC mantained the same proportion as the control, in case of infected mice both myeloid (% of positive cells mean±SEM, Ct:28.24±3.18; iFMDV:20.96±2.43 ; FMDV:14.41±2.07, *$p<0.05$ iFMDV/FMDV vs Ct, n=12) and lymphoid DC (% of positive cells mean±SEM, Ct:25.41±3.06; iFMDV:28.33±2.67; FMDV:12.21±3.40, *$p<0.01$ FMDV vs Ct, n=12) decrease significantly in proportion as control mice. In addition, only DC from infected mice was able to induce the proliferation of lymphocytes. Finally, when we studied the lymphocyte populations, we noticed a significant increase (% of positive cells mean±SEM, Ct:2.07±0.70; iFMDV:4.57±0.57; FMDV:1.08±0.10, *$p<0.05$ Ct vs iFMDV, n=5) in the proportion of T

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regulatory lymphocytes (Treg) in vaccinated animals. In conclusion our results suggest that infection and vaccination trigger a different short immune response, as indicated by the differential recruitment to the spleen of DC subsets and Treg which may suggest a differential modulation of adaptive immune response.

157. Toxic mediators during systemic treatment with interleukin-12 (IL-12) and IL-18 in cancer gene therapy

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Interleukin-12 is a pro-inflammatory cytokine with several anti-tumoral properties. Recombinant IL-12 is currently used as treatment in different type of cancer. However, its systemic expression presents high toxicity manifested by fever, vomiting, diarrhea and hepatotoxicity. Previously, we found that co-expression of IL-12 and IL-18 significantly attenuate IL-12 toxic effects. Here, we investigated possible mediators involved in the toxicity observed after IL-12 expression. Then, we used hydrodynamic shear of cDNA to achieve systemic production of IL-12+/-IL-18 in C57BL/6 mice (6-12 weeks old). We demonstrated that after cDNAs treatments, high sera levels of IL-12, IFNγ and TNF are detected. Systemic expression of IL-12 alone or IL-12+IL-18 modified the absolute cell number of the splenocyte subsets analyzed (T CD4+, T CD8+, B cells, NK/NKT cells, macrophages and dendritic cells). Moreover, IL-12+/-IL-18 expression induced changes in systemic parameters like a drop in the body weight and elevation of sera levels of the hepatic enzymes ALT and AST. We also observed manifestation of liver toxicity evaluated by hepatic histology. Most of these parameters were significantly attenuated in IL-12+IL-18 mice compared to IL-12 mice. When nitric oxide (NO) was evaluated as possible toxic mediator in this model, we observed that either the expression of iNOS or NO by splenocytes from IL-12+/-IL-18 mice was significantly elevated. However, no improvement in survival was perceived in iNOS KO mice compared to WT mice after IL-12+/-IL-18 treatments. When we investigated the toxic effects of TNFα, we found that abolishment of TNFα signals observed in TNFα or TNFR I KO mice demonstrated a considerable increase in survival together with lower systemic levels of ALT and AST after IL-12 cDNA treatment. These data might contribute to the design of new immunotherapy anti-tumoral strategies to allow the use of IL-12 in cancer patients by minimizing its toxic side effects.

158. Screening for a plasmid DNA construction associated to molecular adjuvants to be employed in a foot and mouth disease virus (FMDV) DNA vaccine

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The aim of this work was to construct eukaryotic plasmids encoding the polyprotein pP12A3C and the cytosolic (pVP1c) and secreted (pVP1s) form of FMDV O1Campos in combination or not with the molecular adjuvants pCD40L and pRANKL and to evaluate the immune efficacy of these DNA vaccinations in a murine model. The plasmids were cloned alone or together with molecular adjuvants, into the expression vector pCineo to be used as DNA vaccines. In vitro expression was evaluated by transfecting BHK cells and ascertained by flow cytometry or Western Blot. To examine their immunogenicity, 5 Balb/c mice per group were inoculated i.d, twice with the different DNA vaccines and a negative control group inoculated with pCineo. On day 7 after the second immunization, total antibodies against VP1 were detected by ELISA. The percentage of animals with a-VP1 titers range from 60 to 40% for the animals primed with the pP12A3C and pVP1s and co-inoculation of molecular adjuvants. Moreover, to evaluate the protective efficacy against viral challenge, the mice were infected with 104.5 TCID50 of O1/Campos FMDV strain on day 7 after the second immunization. We found that mice inoculated with pP12A3C displayed a higher degree of protection when compared with the mice primed with pVP1 (44% versus 19% for pP12A3C and pVP1 respectively; $p<0.027$); whereas negative group were none protected. On other hand, preliminary results indicate that pP12A3C together with molecular adjuvants induce a better protection than pP12A3C alone. This screening work demonstrated that pP12A3C proved to be more effective than pVP1 to protect the mice against a challenge with the FMDV even when both induced similar antibodies titers. Further repeat vaccination, route of inoculation, the combination and different proportions of the vaccines will be tested.

159. Dectin-1 and mannose receptor participate in the interaction of excretory-secretory products from Fasciola hepatica with peritoneal macrophages during early state of the infection

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Fasciolosis is becoming a serious public health problem in humans. Fasciola hepatica releases excretory–secretory products (ESP), and the interaction of these products with peritoneal macrophages (pMΦ) is crucial in the early state of the infection. In previous results we observed that pMΦ from naive Balb/c mice stimulated in vitro with ESP presented increased arginase ac-

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tivity and Arg I expression, high levels of TGF-β and IL-10 production. A partial reversion in these effects was observed when pMΦ were pre-incubated with Mannan (Man), anti-MR, Laminarin (Lam), or anti-Dectin-1, and then stimulated with ESP; as well as in pMΦ stimulated with ESP pre-treated with α-Mannosidase. So, we suggested the participation of at least two C-type lectin receptors (CLRs), MR and Dectin-1, in the interaction of ESP with pMΦ. In this work we evaluated the behavior of pMΦ during the early state of the infection. We observed in pMΦ obtained from mice challenged with F.hepatica metacercariae (48 h after infection) an increased arginase activity and Arginase-I expression ($p<0.018$ and $p<0.05$ respectivelly), as well as high levels in the IL-10 and TGF-β production ($p<0.020$ and $p<0.027$ respectivelly). To evaluate if MR and Dectin-1 participate in the phenotypic changes observed in pMΦ during early state of the infection, we injected mice intraperitoneally with Man or Lam before the infection. We could observe a partial reversion in the increase of the arginase activity ($p<0.05$ to both, Lam and Man), as well as in the high levels of IL-10 ($p<0.05$ to both, Lam and Man) and TGF-β ($p<0.05$ to Lam, $p<0.001$ to Man) production. Therefore, taking into account in vitro and in vivo results, we can conclude that at least two CLRs, MR and Dectin-1, participate in the interaction of F.hepatica products with pMΦ, allowing this parasite to induce immunoregulatory effects on these innate immune cells, which would constitute a crucial event for extend their survival in the host.

160. A differential distribution of CD4/CD8 subpopulations and IFNγ production in children and adults with Nonalcoholic steatohepatitis (NASH)

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Obesity and metabolic syndrome (MS) are risk factors for nonalcoholic steatohepatitis (NASH). Lymphocyte recruitment into adipose and hepatic tissues exacerbates the inflammation. Objective: To examine the distribution of CD4/CD8 subpopulations and to measure its IFNγ production. Pediatric (12 Ped, age < 18y) and adults (12 Ad, age > 18y) with NASH (N), obeses (O) with MS without hepatic damage (15 OPed, 6 OAd) and 30 healthy Ped / Ad controls were studied. Peripheral blood mononuclear cells (PBMC) obtained by Ficoll-Hypaque were co stained with anti-CD4 or -CD8 PerCP and anti-CD45RO or -CD45RA FITC and analyzed by flow cytometry. For intracellular detection, PBMC were stimulated with PMA/Ionomycin/brefeldin and stained with anti-CD4 or -CD8/-IFNg mAbs. Kruskal-Wallis and Mann-Whitney tests were performed. Results: In Ped NASH, total CD4+ subpopulation is decreased ($pc= 0.039$ and 0.012, vs. Co and OPed). Lack of differences for CD8+ subpopulation notwithstanding, naïve ($pc<0.0204$ and $pc<0.05$ vs. Co and OPed) and memory ($pc= pc=0.0168$ and $pc<0.05$ vs. Co and OPed) subpopulations were increased. Only CD8+IFNγ+ subpopulation is larger in NASH ($p=0.034$ vs.OPed). In Ad NASH, total CD4+subpopulation is increased ($pc= 0.006$ and $pc<0.05$, vs. Co and OAd). Memory subpopulation is larger ($pc=0.006$ and $pc<0.05$, vs. Co and OAd) while CD4+CD45RA+ subpopulation is smaller ($pc=0.024$ and $pc<0.05$, vs. Co and OAd). Although total CD8+ subpopulations are similar between groups, NASH patients show larger % of memory cells ($pc=0.03$ and $pc<0.05$, vs. Co and OAd) and smaller % of naïve cells ($pc<0.001$ and $pc<0.05$, vs. Co and OAd). CD4+IFNγ+ subpopulation is larger in NASH ($p=0.031$ and $p<0.05$, vs. Co and OAd whereas CD8+IFNg+ is larger in NASH and OAd($p=0.035$ and $p<0.05$, vs.Co). Conclusions: Although obesity is a common trait between MS and NASH, we demonstrated important differences not only in the distribution of CD4/CD8 subpopulations and IFNγ production, but also with age.

161. Reactive species of oxygen production by monocytes and neutrophils in pediatric patients with nonalcoholic steatohepatitis.

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Nowadays, obesity as a component of the metabolic syndrome (MS) is considered a major worldwide pandemic associated with several metabolic conditions, including nonalcoholic steatohepatitis (NASH). Reactive species of oxygen (ROS) produced by monocytes (Mo) and Neutrophils (Ne), infiltrating the adipose tissue (AT) and the liver, is associated with inflammation and tissue damage. Objective: to evaluate on the effect of fatty acids on oxidative burst in monocytes and neutrophils Methods: 10 patients with NASH (N), 20 obese individuals with MS without hepatic damage (Ob) and 20 healthy controls (Co) from our pediatric population (age < 18 y) were included. Mononuclear cells were obtained from heparinised peripheral blood (PBMC) by Ficoll-Hypaque gradient. Lysis of whole blood was performed for assays with Ne. PBMC and Ne were independently incubated with dichlorofluorescein-diacetate [5 μM], stimulated with Phorbol-Myristate Acetate (PMA, [100 ng/ml]), Palmitic acid [500 μM] and Linoleic acid [200 μM] and analyzed by Flow Cytometry. A Stimulation Index (SI) was defined as: mean fluorescence intensity (MFI) stimulated cells/MFI-unstimulated cells. Statistical analysis was performed by Kruskal-Wallis and Mann-Whitney with Bonferroni correction. Results: A higher SI was found for Ne from Ob ($pc=0.006$) and patients with NASH ($pc=0.006$) compared to Co. Stimulation of Mo with PMA induced higher production of ROS in Op related to Co ($pc=0.002$) and NASH

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(pc<0.05). We found a higher SI for Mo from Op (pc=0.02, vs. Co) and NASH (pc=0.04, vs. Co) in response to Linoleic Acid. No difference was found in SI between the three groups when Mo were stimulated with Palmitic Acid. Conclusions: An increased ROS production by Ne represents a potential risk factor involved in pathogenic damage in AT and liver. Moreover, augmented response to linoleic acid can lead to the establishment of chronic inflammatory damage in AT and liver.

162. Low molecular weight hyaluronan-preconditioning of tumor-pulsed dendritic cells increases their migratory ability and induces immunity against murine colorectal carcinoma

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Colorectal carcinoma (CRC) is one of the leading causes of cancer death worldwide and, unfortunately, there is no curative treatment for patients not amenable to surgical resection. Dendritic cells (DC) are the most potent specialized antigen-presenting cells, ex-vivo generated, tumor-antigen loaded mature DC are currently exploited to immunity against cancer. Hyaluronan (HA) is a glycosaminoglycan found in almost all tissues, its functions are size-dependent and the LMW HA form has been shown to induce the expression of inflammatory genes in many types of cells including DC and was shown to stimulate T cell responses by activating DC. We have recently shown that systemic administration of LMW HA, significantly reduces CRC growth in vivo, response partially mediated by DC. To potentiate the ability of DC loaded with tumor lysate (DC/LT) to induce immunity against CRC in mice we aimed to study the effects of preconditioning DC with LMW HA for therapeutic vaccination. The antitumor effect of LMW HA ex vivo-stimulated DC was evaluated in an in vivo BALB/c CT26 CRC model. When these LMW HA-treated CRC tumor lysate-pulsed DC (DC/TL/LMW-HA) were administered to tumor-bearing mice, a potent antitumor response was observed when compared to DC pulsed with tumor lysate alone, significantly reduced tumor growth ($p<0.05$). We show that splenocytes from animals treated with DC/TL/LMW HA presented a higher proliferative capacity (8585 vs 5700 cpm), increased IFNγ production (368+26pg/ml vs 284+17pg/ml) and secreted lower levels of IL10 (519±124pg/ml vs 863±202pg/ml) respect to DC/LT. Increased specific CTL responses were observed in DC/TL/LMW HA-treated animals ($p<0,05$). Preconditioning of DC/TL with LMW HA increased their ability to migrate in vitro towards CCL19 and CCL21 (cell/field 34.25±1.99 vs 13.64±1.7; 32.8±1.8 vs 13.11±1.4) respect to DC/TL. In addition, LMW HA enhanced the in vivo DC recruitment to tumor-regional lymph nodes as was detected by bioluminescence imaging of life DiR-stained DC. Our results shown the immunostimulatory properties of LMW HA on DC loaded with tumor lysate that will be uses for cancer therapy. We demonstrated that LMW HA could be considered a new adjuvant candidate in the preparation of DC-based anti-cancer vaccines

163. Glucocorticoid-dependent regulation of galectin-3 expression in alveolar and peritoneal macrophages.

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Galectins are expressed in a wide variety of tissues where they can regulate different biological processes. Galectin-3 (Gal-3) modulates innate and adaptive immune responses by controlling cell adhesion, chemotaxis, cytokine secretion and apoptosis. Gal-3 may have positive or negative effects on inflammation depending on its tisular and intracellular localization. Glucocorticoids (GCs) are well known regulators of the inflammatory response, acting through multiple and complex mechanisms. In macrophages, GCs inhibit pro-inflammatory genes, leading to a regulatory pattern. However, the effects of GCs on the expression of Gal-3 have not yet been examined. Here we evaluated the expression of Gal-3 in alveolar (AM) and peritoneal macrophages (PM) of male Wistar rats (n= 9) exposed to fluctuations in GC levels. Rats were subjected to adrenalectomy; after 7 days they were injected sc for 7 days with 2 mg/kg/day dexametasone (ADX-GLU) or its vehicle control (ADX). Sham operated rats were the CONTROL group. Gal-3 expression in AM was evaluated in lung sections by light and electron immunohistochemistry (IQ), and in total homogenates by Western blot (WB). PM were purified from CONTROL and ADX by adherent culture on coverslips for Gal-3 immunofluorescence (IF). AM from ADX showed a decrease in Gal-3 only in cytoplasm at both light and ultrastructural levels ($p<0.01$vs control), whereas ADX-GLU increased cytoplasmic ($p<0,001$vs control) and nuclear Gal-3 ($p<0.001$vs control); changes were verified by WB in total lung. PM isolated from ADX rats also exhibited decreased cytoplasmic Gal-3 by IF. To further address the effects of GCs on Gal-3 expression, we stimulated PM from intact rats in vitro with Dexamethasona (10^{-9}M to 10^{-7}M). Results revealed a dose-dependent increase in PM lysates by WB. Our findings suggest that constitutive expression of galectin-3 in PM and AM is sustained by GCs, and that Gal-3 may be another mediator by which GCs regulate the lung mucosal immunity.

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doi: 10:3823/413

164. Effects of M. vaccae on thyrotropin, freeT4, dehydroepiandrosterone, luteinizing hormone and testosterone, and their relationship with IL10 plasma levels in tuberculosis patients

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We have reported that intradermic (id) and oral (o) M. vaccae therapy added to Direct Observed Therapy (DOTS) immunomodulate several parameters in Tuberculosis patients (TBP). In this study we investigated the effect of M. vaccae on Luteinizing Hormone (LH), Thyrotropin (TSH), FreeT4, Dehydroepiandrosterone (DHEA) and Testosterone, and their relationship with IL10 plasma levels in TBP. After diagnosis, 36TBP (HIV-), both sexes (39±12.5 years) with DOTS therapy were divided in three groups: Placebo (Pl): received 0.1 ml saline solution (id or oral). Intradermic (idMv): were inoculated with M. vaccae (0.1 ml of heat-killed bacterial suspension (NCTC 11659), 10 mg bacillus/ml borate saline buffer) Oral (oMv): same dose of id M.vaccae in capsule. (M vaccae suspension and capsules were provided by Dr. J.Stanford, London University). Next two months, same therapy was supplied. For immune studies S1, S2 and S3 (post-treatment) blood samples were collected. Plasma hormones were evaluated by chemioluminiscence, and IL10 by ELISA (R&D). Results were analyzed using non parametric test. No differences in sex and ages distribution between groups DHEA-S (ug/dl) exhibit significant differences Pl: S0: 30±8.8, S1: 42±9.6, S2: 83.5±6, S3: 84±13; idMv: S0: 42.7±11.7, S1: 67.1±12, S2: 97.1±19.2, S3: 148.3±19.2, S0 vs S2: $p<0.05$, S0 vs S3: $p<0.002$, S1 vs S3: $p<0.01$; oMv: S0: 46.8±10.9, S1: 45.4±7.6, S2: 64.4±17.5, S3: 88.2±14.4. S0 vs S3: $p<0.005$, S1 vs S3: $p<0.01$. At S3: Pl vs idMv: $p<0.04$, idMv vs oMv: $p<0.02$, Pl vs idMv vs oMv: $p<0.04$. IL10 levels were significantly increased in successive samples. Correlation (Pearson r) DHEAS/IL10: r2: oMv: 0.9, idMv: 0.96. No significative differences were shown in LH, FT4, TSH and Testosterone. Our results suggest that M.vaccae (oral or id) enhance IL10 and DHEA-S plasma levels of treated TBP. Since their immunomodulatory effects, the benefits of this therapy could be due to the activation of T reg cells. Further experiments could explain involved mechanisms.

165. The influence of sex hormones on the immunopatogenesis of experimental tuberculosis

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Sex hormones such as androgens, estrogens and progestins can directly interact with lymphocytes and macrophages influencing the development of immune responses. Testosterone impairs macrophage production of TNFα and promotes the production of Th2 cytokines, while estrogens are considered as pro-inflammatory mediators which stimulate the production of TNFα and IFNγ. Thus, gender can affect the outcome of chronic infectious diseases like tuberculosis. To compare the evolution of pulmonary tuberculosis in male and female BALB/c mice, and study the course of the disease in castrated animals.
Methods: Nine weeks old mice of both gender were randomized into two groups: castrated or sham-operated. A week later, animals were infected by the intratracheal route with a high dose of Mycobacterium tuberculosis H37Rv strain. Mice were euthanized at different time points post infection (PI) and their lungs were used to determine bacilli loads (colony-forming units: CFU) and tissue damage (histo-morphometry). The Mann-Whitney test was used to determine statistical significance. Results: During late progressive disease (at day 60 or 120 PI) non-castrated male mice (NCM) showed significant higher lung bacilli burdens than non-castrated females (NCF) ($19 \times 10^6 \pm 2 \times 10^6$ vs $1.5 \times 10^6 \pm 2 \times 10^6$; $p=0.034$, at day 60 PI), while castrated males (CM) exhibited lower bacilli loads than NCM ($7 \times 10^6 \pm 1 \times 10^6$ vs $2 \times 10^6 \pm 1 \times 10^6$, $p=0.034$, at day 120 PI). In agreement with this, there was more tissue damage in NCM than NCF (65%±12% vs 21%±8% of lung surface affected by pneumonia, $p=0.015$). Conclusions: Our preliminary results show that male mice are more susceptible to tuberculosis than females. This higher susceptibility was prevented by castration, suggesting that testosterone is a significant susceptibility factor probably by its antiinflammatory/immunosuppressive effects.

166. Intradermal (id) and oral (o) mycobacterium vaccae (MV) therapy effect on cytokines levels in mononuclear (mn) and polimorphonuclear (PMN) culture supernatant and plasma in tuberculososis patients(tbp)

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We reported that both id and oMv therapy added to DOTS modify clinical and immunological parameters in TBP. After including new cases and several cytokines appraisals in plasma,

id and oMv effect on cytokine levels in MN and PMN culture supernatant (cs) and plasma from 28 untreated TBP (HIV-) were compared in each study (both sexes, age 39±10.5 SD & 38±13.5 SD respectively). In one study 14 TBP received DOTS and 3 doses of idMv, and in the other 10 doses of oMv (each dose 10 mg Mv/ml borato buffer, provided by Dr. J. Stanford, London Univ.). In each research 14 TBP received DOTS and Placebo (Pl: id or capsules with 0.1ml saline). TBP initially received DOTS and the 1st dose of id, oMv or Pl. A blood sample S0 and S1, S2, S3 were obtained in monthly controls for immune exams. MN and PMN were separated (Ficol-Triyosom). 5.10^6cell/ml in RPMI from TBP receptors of idMv or oMv were incubated for 20hs at 37°C, and IFNγ, TNFα e IL10 and IL6 in cs, and plasma were measured (ELISA; R&D Systems). IL10 (pg/ml) (x±SE, Mann Withney, t, Welch test) sc MN: Pl: S0:13.34±0.91, S1: 14.8±0.97, S2: 19.03±1.19, S3:27.57±1.71; idMv: S0:14.88±1.5, S1: 19.98±2.17, S2: 26.72±3.08, S3: 37.14±1.76; oMv: S0: 16±3.04, S1: 21.48±12.61, S2: 32.8±15.13, S3: 51.86±7.88. Pl vs idMv, S3: $p<0.05$, Pl vs oMv: $p<0.01$. sc PMN: Pl: S0: 11.25±0.92, S1: 13.33±0.61, S2: 15.97±1.24, S3: 27.02±2.19; idMv: S0: 14.99±1.4, S1: 19.22±1.08, S2: 24.18±2.02, S3: 45.85±7.34; oMv: S0: 20.85±2.09, S1: 34.7±2.02, S2: 43.81±5.65, S3: 45.85±7.34. Pl vs oMv, S3: $p<0.05$. Plasma: Pl: S0: 455.43±15.25, S1: 530.85±129.82, S2: 492.29±30.39, S3: 663.65±49.83; idMv: S0: 485.78±49.83, S1: 582.19±59.8, S2: 634.9±87.31, S3: 893.74±130.26; oMv: S0: 471.49±28.19, S1: 633.48±48.46, S2: 751.28±115.4, S3: 1785.82±223.61. Pl vs oMv, S3: $p<0.01$.

Cytokine data in cs and plasma from oMv TBP receptors were higher than those of idMv and Pl. Immunomodulatory action on cytokines production of oMv was more effective than that observed in idMv TBP.

167. Spleen dendritic cells from old mice generate a low cytotoxic response against ova in young hosts

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During aging, B and T cells manifest changes that affect their response to antigens (Ag). However, it is practically unknown how dendritic cells (DCs) could participate in these changes. Our previous findings show that in spleen of 18-20 month-old mice (old) there is a lower content of DCs, which have a lower capacity to activate young naïve CD8 T cells than DCs from 3 month-old (young) mice. In this work we analyzed the ability of DCs from old mice to elicit a CTL response in young mice, in order to analyze the ability of DCs independently of the alterations already reported for CD8 T cells during aging. We first analyzed the capacity from old mice to develop a CTL response in vivo. We immunized i.v young and old mice with $2.5x10^9$ latex beads coupled to OVA + PolyU/DOTAP and 7 days later, CTL response was assessed by an in vivo assay. Immunized mice were injected i.v. with equal proportions of CFSE-labeled syngeneic spleen cell populations (a control cell population labeled with 0.5 µM CFSE and an OVA257-264 peptide-loaded cell population labeled with 3µM CFSE). One day after injection cytotoxicity was assessed by FACS analysis on total spleen cells. We found that old mice developed a significant lower CTL response against OVA than young mice (4.7±1.5 vs 84±10%, respectively, $p<0.05$). We also measured IFN-g secretion by ELISA in supernatants of spleen cells in vitro restimulated with OVA257-264 peptide. We found a lower IFN-γ production in splenocytes from old mice compared to the young ones (0.32±0.01 vs. 1.9±1.6 ng/mL, respectively, $p<0.05$). To investigate whether this lower capacity from old mice to develop a CTL response in vivo could be due to a lower capacity of DCs from old mice to prime a CTL response in vivo, we immunized iv young mice with $1x10^6$ DCs purified from the spleen of young and old mice incubated with OVA+PolyU/DOTAP. Seven days later, an in vivo CTL assay by FACS was performed. We found that DCs from old mice have a diminished capacity to prime a CTL response, as we observed a lower percentage of specific lysis in old mice compared to the young ones (41±23 vs. 89±13%, respectively, $p<0.05$). Altogether, the impairment in the generation of effective cytotoxic response against OVA that we observed in aged C57BL/6 is explained, at least in part, by a lower capacity of DCs to induce CTLs after immunization.

168. Effect of controlled ovarian hyperstimulation on endometrial quality

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Assisted Reproduction Technologies have provided considerable insight into the human reproductive process. However, it has been demonstrated that uterine receptivity is diminished during controlled ovarian hyperstimulation (COS), whereas supraphysiological levels of estradiol (E2), in response to gonadotrophins, are deleterious to embryonic implantation. Moreover, clinical evidence suggest that elevated counts of endometrial Natural Killer cells (NK) were related to reproductive failure. The aim of this study was to compare the effect of COS with GnRH agonist in combination with rFSH or hMG on endometrial NK cells of fertile oocyte donors. Material and Methods. Endometrial biopsy and peripheral blood were obtained during the implantation window (5-8 days post-ovulation) of ten fertile women during three consecutives cycles: 1. long GnRH agonist protocol + rFSH, 2. natural cycle (NC) and 3. long GnRH agonist protocol + hMG. Endometrial NK cell subsets (CD9+ CD56+ CD16+/ CD9+ CD56+ CD16-) were determined by flow cytometry. The angiogenic parameters Vascular Index (VI) and Flow Index (FI) were determined by VOCAL-Doppler, whereas VEGF levels were determined by ELISA and RT-qPCR. Results. Both protocols increased the levels of E2 ($p<0.05$), proges-

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terone (P4) ($p<0.05$) and the proportion of cytotoxic NK cells (FSHr: $p<0.05$ and hMG: $p<0.01$) in compare to NC. Moreover, we found a positive correlation between these parameters: the proportion of cytotoxic NK cells has shown a possitive correlation with E2 ($r=0.4047$, $p<0.05$) and P4 ($r=0.4323$, $p<0.05$). On the other hand, angiogenic parameters (endometrial VEGF, VI and FI) were not modified by COS. Conclusions. Ovarian stimulation has a deleterious effect on fertile endometrium by increasing the proportion of cytotoxic NK cells without affecting vascularization parameters (CD9+ CD56+ CD16-, VEGF, VI and FI). This deleterious effect could be related to lower implantation rates of In Vitro Fertilization patients.

169. Endometrial natural killer (NK) cells in implantation failure: characterization and treatment.

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Elevated levels of circulating NK cells have been linked to increased rates of implantation failure. However, the validity of peripheral blood NK cells has been discussed recently. In this sense, endometrial NK subsets are well characterized in recurrent abortion, but they are not enough studied in IVF failure. It has been demonstrated the efficacy of IVIG treatment in IVF failure when NK cell levels are elevated, but this treatment is expensive. On the other hand, heparin is able to modulate implantation, and invasion, having the potential to improve pregnancy rates and outcomes. However, the role of pre-conceptional heparin in modulating NK cell levels is still unknown. The goal of this work is to analyze the ability of NK cells to discriminate fertile and IVF failure patients in peripheral blood vs. endometrium, and also to determine the capacity of pre-conceptional low-molecular weight heparin to modulate NK cell counts.Thirty-six IVF failure patients and 16 fertile women were studied. Peripheral blood and endometrial tissue were obtained during implantation window w/o IVF-ET. Total counts of CD56+CD3-CD16+/- and CD56+CD9+CD16+/- subsets were analyzed by FACS. In the consecutive cycle infertile patients were treated with enoxaparin and NK subset analysis was repeated. Comparison of Medias, Spearman and Pearson correlation tests and ROC curve analysis were performed. Results shown that IVF failure patients has elevated total count of CD56+CD9+CD16+ subset ($p<0.05$). These data did not correlate with peripheral blood values ($p=0.05$).ROC curve analysis shown that total count of CD56+CD9+CD16+ is the parameter that best discriminated between fertile and infertile patients ($p<0.01$). Enoxaparin normalized counts of CD56+CD9+CD16+ ($p<0.05$). We concluded that endometrial CD56+CD16+ counts seem to be a good parameter for immunological testing in IVF failure. Enoxaparin could be a potential immunomodulator in assisted conception of patients with elevated NK cell counts.

170. Myeloid suppressor cells as possible natural regulator of inflammation in experimental Trypanosoma cruzi infection

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Myeloid-derived suppressor cells (MDSCs) accumulate during cancer and infection and have the ability to suppress T-cell response. Their surface markers are CD11b+ Gr1+ and the suppressor mechanisms include production of reactive oxygen species (ROS), the induction of nitric oxide (NO) and/or arginase-1 (Arg-1) expression. Our group previously demonstrated that infection with 1000 trypomastigotes Tulahuen produced a significant increase in hepatic and spleen MDSC of BALB/c vs C57BL/6 (B6) mice. A strong pro-inflammatory profile and severe hepatic damage were observed in B6. Purified MDSC significantly decreased cell proliferation induced by ConA. To gain insight into the MDSC mechanisms, we investigated the production of NO by splenocytes stimulated with TLRs ligands, and purified MDSC from infected BALB/c to analyze the presence of Arg-1. Furthermore, we quantified TLR2 and 4 mRNA in hepatic tissue of infected BALB/c and C57BL/6 mice by RT-qPCR. The NO concentration (by Griess) significantly increased when Pam3Csk4 (TLR2 ligand) or LPS (TLR4 ligand) were added to BALB/c cells ($p<0.001$). The NO produced by MDSC revealed a greater number of fluorescent DAF-2DA positive cells in infected vs uninfected mice ($p<0.05$). In addition, the presence of Arg-1 was detected in purified MDSC from BALB/c by WB, while it was absent in uninfected mice. The study of TLR2 and 4 mRNA comparing infected vs uninfected mice showed an upregulation of TLR2 at 14 and 21 dpi in BALB/c mice ($P<0.001$), whereas a slight increase of TLR2 was observed in B6 at 14 dpi ($p<0.001$) and no change at 21 dpi. TLR4 showed a TLR2 similar kinetic at 14 and at 21 dpi in BALB/c ($p<0.05$ and $p<0.01$ respectively). On the contrary, in B6, TLR4 was downregulated at all times assayed. These results suggest that the MDSC present in infected BALB/c could exert suppression through NO and Arg-1 production. The different modulation of TLR2 and TLR4 mRNA in liver associated with a greater number of MDSC in BALB/c would regulate the hepatic injury in this strain.

171. The protein moiety of the Brucella Omp19 lipoprotein, co-administered as oral adjuvant, induces a Th1 immune response

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doi: 10:3823/413

Previous results demonstrate that the protein moiety of the Brucella Omp19 lipoprotein (U-Omp19) administered by the oral route has self-adjuvant properties. These prompt us to study its mucosal adjuvant capacity using as a model antigen ovalbumin (OVA). For this purpose mice were immunized orally with OVA, U-Omp19+OVA, or choleric toxin (CT)+OVA. By flow cytometry we determined that the percentage of CD4+ and CD8+ T cells expressing a4ß7 integrin increased in those mesenteric lymph nodes from U-Omp19+OVA (CD4+ a4ß7+: 7,7%; CD8+ a4ß7+:12,16%) and CT+OVA (7,15%; 16,46%) in comparison with OVA (0,74%; 1,64%) immunized mice. We also observed a significant delayed type hypersensitivity (DTH) response to OVA injection in U-Omp19+OVA (Δ =0,11mm, P<0.01 vs OVA immunized group) and CT+OVA (Δ=0,07mm P<0.05 vs OVA) while OVA (Δ=0,01mm) group did not. Similarly, splenocytes from mice immunized orally with U-Omp19+OVA secreted significant amounts of IFNg (1045.8 pg/ml, P<0.05 vs OVA immunized group) in response to OVA. In contrast, IFNγ levels secreted by the CT+OVA group (322.8pg/ml) were similar to the OVA (448.5 pg/ml) immunized group. On the other hand, there was no production of Th2 (IL-4) or Th3 (IL-10) cytokines in response to OVA in any immunized mice. We also observed an increase in IL-17 production in response to OVA by the splenocytes of U-Omp19+OVA immunized mice, but this increase was not statistically different from OVA immunized group (P>0.05). By flow cytometry we determined that the percentage of CD4+ and CD8+ T cells producing intracellular IFN-γ -in response to OVA- increased in U-Omp19+OVA (CD4+IFN-γ+ 2.13%; CD8+IFN-γ+ 0.77%) in comparison with CT+OVA (0.45%; 0.55%) and OVA (0.41%; 0.37%) vaccinated mice. In conclusion, U-Omp19 as oral adjuvant induces the migration of CD4+ and CD8+ T cells to gastrointestinal lamina propria, T cells responses in vivo and CD4+ and CD8+ T cells that produce IFNγ in response to OVA.

172. Do HCAbs arise in the context of Th1 or Th2?

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The camelids are susceptible to diseases and infections similar to those who have cattle, sheep and pig. In the latter species the role of cytokines in triggering a Th1 or Th2 response is well studied in order to develop rational vaccination plans. There are three fractions of IgG in camelids namely IgG1, IgG2 and IgG3, of which IgG2 and IgG3 are heavy chain antibodies (HCAbs). HCAbs lack the light chains and the CH1 constant domain. Currently, nothing is known about the behavior of the camelid PBMC in response to the challenge with different Ags. The objectives of this work are: to study the response of different IgG subisotypes against various Ags, and to evaluate the cytokine profile predominating in each case. Llama 415 was immunized with ovoalbumin (OVA), llama 416 with the supernatant of a lysate of T. cruzi (F105) and llama 2025 with both Ags separately. Total IgG was calculated by ELISA and greater OD readings were obtained in the sera of llamas immunized with OVA than with F105. Furthermore, in vitro cultures with the PBMC of immunized llamas were challenged with the Ags used in the immunization plans. After 24 hours of culture, Th1 and Th2 cytokines were evaluated by RT-PCR. There were no differences in relation to Th2 cytokines. In contrast, the challenge with F105 generated an increase of IL-2 and IFNg. After 6 days of culture, the response of the different fractions of IgG was evaluated by ELISA. It was noted that the supernatants of the PBMC cultured with F105 showed significantly higher values of IgG2 compared with controls. Then, we analyzed the relation between HCAbs/IgG1 against F105 in the sera of animals from different places. IgG2/IgG1 ratio was significantly greater in the animals that had higher total IgG titers. From these preliminary results we suggest that IgG2 is related with Th1 responses. These results provide possible design objectives for future vaccines as well as criteria for detecting Th1 and Th2 immune response bias in camelids.

173. In vitro characterization of several bacterial strain properties on human tumor cells

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Our laboratory studies the immunomodulatory effects of the probiotic strain Enterococcus faecalis CECT7121, which shows anti-tumor effects on several models, either by direct inhibition of tumor cell proliferation as well as by stimulating the anti-tumor immune response. The aim of this work was to investigate the in vitro effects of several E. faecalis strains on 2 tumor cell lines: Raji and PL104. Stimuli employed included whole cell bacteria, purified cell walls, soluble lysates, and culture supernatant. Also, on PL104 cells, combinatory assays were performed with CAPE (anti-tumor drug). The tumor cell proliferation was determined by ^{3}H-thymidine uptake assays, on 48 h cultures. Our results show that Raji cell proliferation was inhibited in a dose-dependent manner by both whole cell bacteria and purified cell walls (ANOVA, ***p<0.001). These observations are shared by the different E. faecalis strains tested, but not when a Micrococcus luteus strain was employed as stimulus (different cell wall components). This inhibitory effect could not be achieved employing culture supernatants, indicating that the active compounds are not released to the culture media. In these studies, the strain E. faecalis CECT7121 showed the highest effect, comparing with the other Enterococcus strains (63%; ANOVA, **p<0.01). E. faecalis CECT7121, as well as its purified cell wall and soluble lysate, showed a dose-dependent inhibition on PL104 cell proliferation, specially when employing bacterial

soluble compounds (ANOVA, ***p<0.001). These components, combined with CAPE, achieved a synergic effect on the inhibition of PL104 cells, as determined by the construction of an isobologram. Our results suggest that several bacterial components have an inhibitory effect on different hematological tumor cell lines. Further studies should be performed to identify and isolate the active components involved, and to characterize its behavior in several combinatory treatments.

174. PF-MC: a new human anti-inflammatory fusion protein engineered to target an inflammatory site

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Secretory leukocyte protease inhibitor (SLPI) is a protein with anti-microbial, anti-inflammatory and wound healing activity. In vivo, SLPI is susceptible to proteolytic degradation. In order to improve the therapeutic capacity of SLPI increasing the concentration at the inflammatory site, we constructed a fusion protein comprising an anchoring peptide domain named cementoin and human SLPI. Cementoin is a substrate of the enzyme transglutaminase (TG). TG is upregulated at the inflammatory site, crosslinking proteins on the cell membrane and the extrecellular matrix. PF-MC was cloned and expressed in E. coli. The anti proteinase activity of PF-MC was 35±5 % higher than SLPI (p<0.001). The in vitro binding of PF-MC on TNFalpha treated-A549 cell surface was analyzed by ELISA and fluorescence microscopy. The binding of PF-MC to A549 cells was higher compared to SLPI (p<0.05). In vivo, binding of the PF-MC was examined by using an inflammatory air pouch model on mice. PF-MC or SLPI (52 µg) were inoculated into the air pouch; inflammatory recruited cells were recovered after 2 h of treatment and PF-MC was detected on the inflammatory cell surface by flow cytometry. We observed PF-MC on lymphocytes and monocytes, but not on neutrophils. The binding was higher for PF-MC than SLPI (9±3 vs 4±1%; 31±7 vs 10±4% of lymphocytes and monocytes, respectively). A higher binding of PF-MC on lymphocytes was confirmed by analysing the cells of the popliteal lymph node that were injected with LPS (in the foot pad) and PF-MC (i.p.). The biological activities of PF-MC were similar or improved than SLPI: the wound healing activity was augmented, since the diameter of the wound, measure at day 2 post-injury, was 35±8 % smaller in mice injected with PF-MC vs. SLPI. These results demonstrate that PF-MC binds to inflammatory cells both in vitro and in vivo, retains the biological activity of SLPI and, in some cases, improve it. Therefore, PF-MC may be a new tool to treat inflammatory diseases.

175. Male genital tract infection by Chlamydia trachomatis: special tropism for the prostate gland by and its pathogenic consequences

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Even though the prevalence of Chlamydial infection is similar in male and female, the amount of information currently available about the pathogenesis and immunity to C. trachomatis infection in males is scarce. We have previously demonstrated using an in vivo male genital tract model of Chlamydial infection that the inoculation of Chlamydia muridarum (Cm) within meatus urethra results in an ascending infection with a special tropism for the prostate gland. The presence of the bacteria in the prostate gland was sustained at late time point after inoculation (90dpi) and was accompanied by infiltration of the gland. In the present work we analyze the composition of the infiltrate using immune-histochemical assays. We detected few CD11b+, CD3+, CD4+ and CD8+ cells in prostate glands of control rats. Prostate gland infiltration observed in infected rats was composed mostly by CD3 cells, with high staining for CD8 and CD4 cells. No differences between infected and control glands were observed when CD11b+ cells were analyzed. We also investigated the presence of antibodies against male genital tract antigens in infected and control serum obtained at day 90pi. A high proportion of serum from infected animals showed immune- reactivity against bladder (85%), prostate (100%) and testis (75%) extracts. However, no immune-reactivity was observed when urethra and seminal vesicle extracts were used. Our results prompt us to speculate that during the course of Cm male urogenital tract infection, the special tropism of this bacteria for the prostate gland and its continuous presence together with the production of cytokines and quemokines would induce a chronic inflammation with the release of prostate and other male genital antigens that, in turn, would evolve in the break of tolerance and the onset of an autoimmune process within the male genital tract.

176. Omp19 has the ability to induce the internalization of the co-administered antigen in antigen presenting cells in vitro and triggers cytotoxic T cell responses against the antigen.

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Previously, we have demonstrated that the protein moiety of Omp19 from Brucella spp. (U-Omp19) has adjuvant properties and could be used as a Th1 adjuvant that facilitates antigen (Ag) cross-presentation and induces IFN-γ producing CD8+ T cells. Given the capacity of antigen presenting cells (APCs) to modulate immune responses, in the present study we investigated

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doi: 10:3823/413

if APCs are involved in the ability of U-Omp19 to stimulate a specific CD8 T cell response. To assess this, purified dendritic cells (DCs) from spleen of C57BL/6 mice were treated with ovoalbumin (OVA) alone or plus U-Omp19 or LPS as positive control and then co-cultivated with OTI CD8+ T cells. We evaluated the induction of IFN-_ producing T cells by intracellular IFN-γ staining and degranulation of cytotoxic T cells by CD107a expression on the membrane. A higher percentage of IFN-γ producing T CD8 (32.36%) and cytotoxic T cells (14.06%) was induced when DCs were stimulated with OVA+U-Omp19 compared to T cells co-cultivated with DCs pulsed with OVA alone (23.42% and 8.98% respectively). These results suggested that U-Omp19 can act directly on DCs to enhance T cell responses. Therefore, we evaluated if U-Omp19 has a direct effect on APCs that could enhance the Ag internalization and presentation to T cells. To this end, purified DCs from spleen mice were pulsed in vitro with OVA-FITC alone or OVA-FITC+U-Omp19 or LPS as positive control. DCs pulsed with OVA-FITC+U-Omp19 showed a greatly increase in Ag internalization compared with OVA-FITC alone (MFI 367.88±19.1 vs. 15.29±1.8). We have also found that U-Omp19 increased OVA internalization by J774 macrophage cell line and by A20J B-lymphoma cell line at different times. These results indicate that U-Omp19 increases the Ag capture by immature DCs, macrophages and B lymphocytes. In conclusion, U-Omp19 as adjuvant increases the amount of internalized Ag in APCs. This capacity would lead the improved T CD8 and CTL immune responses observed when U-Omp19 is co-administered with the Ag.

177. Cloning, expression and protective capacity evaluation of two novel B. pertussis vaccine candidates

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Bordetella pertussis is the etiologic agent of whooping cough. Despite the high coverage of vaccination, the disease causes more than 500.000 deaths annually worldwide, which reflect of the low efficiency of current vaccines. The difference between the infecting phenotype and the phenotype of the vaccine strain may contribute to the lack of protection from the vaccines. Thus, the search of new immunogens expressed by the infecting phenotype may contribute to the development of more efficient vaccines. By immune proteomic, we identified two possible antigens in outer membrane subproteome, named AfuA and IRP1-3, which are over-expressed under iron starvation, a condition that an infecting microorganism face in the host. In the present study, we cloned, expressed and purified both recombinant proteins from Escherichia coli. Mice immunization with AfuA or IRP1-3 led to generation of specific antibodies which showed phagocytic activity in a two-color flow cytometric assay. Although immunization of BALB/c mice with the recombinat proteins formulated with Freund's adjuvant elicited a strong IgG1 response asociated with Th2 type immune response, the sera also showed specific IgG2a antibodies suggesting a Th1 type response contribution, known required for protection against B. pertussis. Accordingly, mice immunized with either AfuA or IRP1-3 alone or in combination were significantly ($p<0.01$) protected against intranasal infection of B.pertussis. Importantly, the divalent formulation showed a higher protection level ($p<0.01$), as determined using ANOVA to compare means. Immunoblot analysis demonstrated that both proteins are expressed in clinical isolates grown under iron starvation. Additionally, the presence of antibodies against these proteins in sera from infected individuals, as determined by ELISA, suggests that they are expressed during the infection. Altogether these results point at AfuA and IRP1-3 as promising new candidates to improve the current vaccines.

178. Immunologic profile of a murine lung adenocarcinoma. Effect of Indomethacin.

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LP07 murine lung adenocarcinoma develops in advanced stages a leukocytosis at the expense of polymorphonuclear cells and a high production of inflammatory and soluble factors (PGE2, COX2, IL-6, GM-CSF) that may promote tumor growth. Because the expression of the enzyme COX2 and secretion of these factors by tumor cells can induce immunosuppressive populations, we analyzed the state of immune suppression in BALB/c mice along LP07COX+ (TBM) and LP07 with a shRNA against COX2 (LP07COX-) tumor progression. We previously demonstrated that the in vivo effect of Indomethacin (COX inhibitor), decreased tumor growth. Our aim was to determine 1) MDSC and Tregs in spleen, tumor-draining lymph nodes-TDLN- and tumor by FACS; 2) arginase activity in lung, spleen, and tumor (µg urea/mg protein); 3) specific DTH by foot pad swelling assay (mm); 4) Tumor growth and arginase activity in spleen, lung and tumor of mice bearing LP07COX- tumors. Results: Percentage of MDSC (CD11b+ Gr1+) of total myeloid cells augmented in the spleen in advanced stages of tumor progression (33 days) (Control: 47% TBM: 73%, $p<0.001$), and it was prevented by Indomethacin (56%). Intratumoral and TDLN CD4+CD25+Foxp3+ Tregs did not augment during tumor growth, but interestingly Indomethacin decreased Tregs in TDLN ($p<0.05$). LP07COX- tumors grew less than WT, and INDO treatment diminished tumor size even more. Arginase activity increased in all TBM tissues vs Control (spleen:$p<0.05$; lung:$p<0.05$; tumor:$p<0.01$) and was

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doi: 10:3823/413

prevented by (LP07COX-) and INDO treatment (every $p<0.05$). DTH decreased in late stages, reversed by Indomethacin (TBM: 0.06±0.045; Indo: 0.14±0.038). Conclusion: the increment of spleen MDSC during LP07 progression correlates with specific immune suppression demonstrated by DTH and increased arginase activity. Given that COX2 is known to activate MDSC and differentiate Tregs, we suggest that Indomethacin prevents the immune suppression by tumor COX2 dependent and independent mechanism.

179. Use of a food allergy mouse model to study the in vivo and in vitro cross-reactivity between cow's milk and soybean proteins

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Treatment of food allergy consists in allergen avoidance and food replacement. Cow´s milk (CM) is frequently substituted for soy (S)-containing formulae, however it is not tolerated in some patients even if they were not previously exposed to soy proteins. Our group has identified caseins and soy globulins as the cross-reactive allergens. They were obtained as recombinant proteins and cross-reactive epitopes were mapped.The aim of this work was to employ a validated food allergy mouse model to study the in vivo and in vitro cross-allergenicity between CM and S.BALB/c mice were intragastrically sensitized with CM proteins (CMP) and cholera toxin (CT) as a Th2 mucosal adyuvant. Control group consisted of mice exposed to CMP or CT. An oral challenge was performed with CMP or S, and 15-30 minutes afterwards clinical signs were recorded (clinical score). Cutaneous tests were performed, specific antibody (IgE, IgG1 and IgG2a) and histamine serum levels were quantified, antigen-specific proliferation of splenocytes was analyzed and cytokine production was measured. Sensitized animals showed higher clinical scores than control mice, and both CMP and S induced symptoms. Specific IgE and IgG1 antibodies were increased in sensitized mice, while IgG2a was suppressed. Histamine was increased and splenocytes were stimulated with CMP and S extracts. IL-5 (CMP: 132 ± 64 pg/ml vs. nd, S: 29± 11 vs 7±3 pg/ml, sensitized vs control group resp.) was also increased, while gIFN was basal (CMP: 80±16 vs. 26±13 pg/ml, S: 126±52 vs. 390±170 pg/ml sensitized vs control group resp.). Cutaneous tests were positive for CMP and soy. In conclusion, intragastric sensitization with CMP induced a specific Th2-bias activation of the gut mucosa immune system that also reacted with soy proteins. Cross-allergenicity was also evidenced by elicitation of clinical signs and cutaneous tests. These results may constitute the experimental basis to develop a mucosal vaccine.

180. Brucella abortus induce apoptosis of human t lymphocytes: a potential mechanism for chronic infection

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Despite its ability to generate a vigorous Th1 response, Brucella abortus can persist inside macrophages and establish a chronic infection. We have recently demonstrated that B. abortus inhibits the IFNγ-induced expression of MHC-II and antigen presentation on human monocytes. In this study, we evaluated whether this microorganism can directly affect the response/activation of T lymphocytes (TL). We first investigated if TL could be infected with B. abortus. In order to evaluate this, purified human TL were incubated with B. abortus at different ratios of B. abortus to TL. B. abortus was unable to infect TL, even at the highest ratio used. Next, we evaluated if B. abortus could affect the activation of TL. In this respect, purified TL were stimulated with plate-bound anti-CD3 for 48 h in the presence of B. abortus. IFN-γ and IL-2 production and T-cell proliferation were then measured. B. abortus inhibited significantly ($p<0.05$) the proliferation and the secretion of IFN-γ and IL-2 in a dose-dependent manner. Since a defect on T-cell proliferation after the infection with other pathogens has been reported to be related to T-cell apoptosis, we next investigated whether B. abortus could also induce this phenomenon. Purified TL were incubated with B. abortus for 24 h, 48 h and 5 days. After this, cells were stained with Annexin V/PI and apoptosis was determined by flow cytometry. B. abortus induced increased apoptosis of TL at 48 h and 5 days, in a dose-dependent manner (% Annexin V+ cells at 5-day. Medium 29%; 50:1 52%; 100:1 64%; 1000:1 77%). Furthermore, heat-killed B. abortus (HKBA) also induced elevated T-cell apoptosis indicating that a structural component of the bacterium is implicated in this effect. Together, these results suggest that the diminished activation of T lymphocytes could be related to an increased susceptibility to apoptosis and, that this could be considered as another possible mechanism by which B. abortus evades the immune system.

181. Study of B. pertussis adaptation to intracellular survival using semi-quantitative RT-PCR

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Bordetella pertussis is the etiologic agent of whooping cough. Despite the high coverage of vaccination, the circulation of B. pertussis throughout the world continues largely unabated. The mechanisms that allow this pathogen to evade immune clearance are not yet clear. Our group recently showed that the encounter of B. pertussis with human macrophages in the absence of opsonic antibodies leads to the intracellular survival of a significant number of bacteria which, after a lag period, are able to replicate inside early endosomes. This ability to repli-

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doi: 10:3823/413

cate seems to require bacterial adaptation to the intracellular environment. The goal of this study is to gain a first insight into the adaptive genetic mechanisms utilized by B. pertussis to survive, and eventually grow, inside macrophages. In this work, we report the study of two groups of genes usually implicated in pathogen adaptation to the phagosomal environment: genes involved in bacterial virulence and eventual host cell intoxication, and genes involved in iron homeostasis, one of the main stressors intracellular pathogens have to face. Using semi-quantitative RT-PCR we determined the mRNA expression of selected genes are indicative of iron stress response and bacterial virulence phase.The study of mRNA levels of B. pertussis inside the human macrophage show that genes that are repressed by iron as fumC, irp1-3, and sodA, are 20 to 30 times up regulated compared with infecting bacteria. Regarding virulence genes, cyaA was found down regulated 0.5 times while bipA was up regulated in bacteria inside the cell also 0.5 times, suggesting a possible intermediate virulence state in intracellular bacteria. Accordingly, no vrg-6 expression was observed. These early results suggest that B. pertussis adopts a particular phenotype that allows intracellular life.

182. Tc52 or its N-terminal domain DNA carried by attenuated Salmonella as a DNA delivery system, are able to confer protection against Trypanosoma cruzi infection.

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Tc52 is a T. cruzi protein with glutathione transferase activity, which has immunomodulatory properties. Analyzing candidates for a vaccine against Chagas´ disease we have studied the ability of the DNAs codifying for Tc52 and its amino and carboxi-terminal domains (N-term and C-term) to confer protection against T. cruzi infeccion. As a DNA delivery system we used atenuated Salmonella enterica serovar Typhimurium aro A 7207 (S). Four groups of C3H mice were immunized 4 times with: GI: S (Salmonella carrying an empty pcDNA3.1, as control group), GII: STc52 (S with pcDNA3.1-Tc52), GIII: SN-term (S with pcDNA3.1-N-term), GIV: SC-term (S with pcDNA3.1-C-term). Eighteen days after the last immunization, delayed-type hypersensitivity (DTH) test was performed. Two days later, mice were infected with 1000 bloodstream trypomastigotes (RA strain). Parasitemia was monitored by counting peripheral blood parasites every 2 days. Weight lose was monitored as a measure of physical health. After immunization all test groups developed a cellular immune response specific against the recombinants proteins ($p<0.001$). The GII: STc52 reacted against the two domains ($p<0.05$ for N-term and $p<0.001$ for C-term). GIII and GIV reacted against the whole protein ($p<0.01$ and $p<0.05$ for GIII and GIV, respectively). The area under the curve of parasitemia vs. time pi, for GIV, GII and GIII was respectively, 3.0, 7.0 and 7.2 times lower than the control. At 25 days pi all the test groups showed reduced weight lose compared with control, the difference was more important in the case of GIII ($p<0.05$). We can conclude that the 3 vaccine candidates generated protection against T. cruzi infection. However, the N-terminal domain produces a similar protection than the whole Tc52 but a higher protection than the C-terminal domain, which suggest that the N-term domain of Tc52 would be an excellent candidate for the development of a vaccine against T. cruzi.

183. Immunoprotection against T. cruzi infection elicited by a multicomponent vaccine

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We have previously demonstrated that Salmonella enterica as DNA delivery system for the major cystein proteinase of T. cruzi, cruzipain (SCz), is able to induce immunoprotection against Chagas disease. To improve the performance of this DNA vaccine, we tested the co-administration of SCz and Salmonella (S) carrying genes encoding Tc52 (a thiol-transferase) and Tc24 (a 24 kDa flagellar calcium binding protein). Mice were immunized with: GI- PBS (Control), GII: SCz, GIII: STc52; GIV: STc24; GV: SCz+STc24+STc52. We found that only antibodies generated in GV vaccinated mice were able to increase the complement-mediated killing of T. cruzi ($p<0.005$). On contrary, all immunized mice displayed significant DTH immune response against the antigen they were vaccinated, with $p<0.001$ for GII, $p<0.01$ (GIII) and $p<0.001$ (GIV), compared with controls. Mice vaccinated with the multicomponent vaccine (GV) elicited strong DTH against Cz, Tc52 and Tc24 ($p<0.001$, $p<0.05$ and $p<0.01$, respectively). Trypomastigote challenge rendered a significant decrease in parasitemia levels in GII, GIII and GV, compared with control. When we analyzed the area under the curve of parasitemia, GV presented 6 fold reduction in the number of circulating parasites respect to GI ($p=0.016$). By contrast, mice vaccinated with STc24 were not able to control the infection showing similar parasitemia than control. As indirect parameters of the protection we also analyzed mice weight. While controls presented an important weight lose; GV maintained their weight during the acute phase of the infection ($p=0.0095$). The activity of serum CPK and LDH, as enzyme markers of muscle injury, was significant lower in GV vaccinated mice comparing with controls ($p<0.005$ and $p<0.001$ for CK and LDH, respectively). We conclude that immunization of Salmonella as DNA delivery system for Cz, Tc24 and Tc52 generated an strong immune response able to improved the protection against T. cruzi infection elicited by each antigens.

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doi: 10:3823/413

184. Nanobodies against the M2 protein of Influenza Virus: Preliminar results.

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The Influenza A vaccines currently in used are strain-specific and they need to be updated every year due to antigenic drift/shift. The matrix protein M2 of influenza A is a membrane protein strongly conserved among different virus strains with an extracellular domain of 24 aminoacid residues that plays an important biological function in the virus life cycle. This domain has been the target of antiviral drugs thus we considered that it might be an excellent antigen to develop recombinant monoclonal llama-derived single-chain antibody fragments (VHH) as a pharmaceutical product to prevent influenza A infection. For this, one llama (Lama glama) was immunized with five doses of recombinant protein BLS-4M2 (Inmunova-Algenex(r)) emulsified with Freund adjuvant. Serum and blood samples were taken at days 0, 4 and 7 after each inoculation. The llama antibody response to M2 was monitored by ELISA and the number of specific antibody secreting cells circulating in peripheral blood was followed by ELISPOT. The ELISA antibody titers to BLS-4M2 as well as to M2 peptide were significantly increased reaching the plateau after the third inoculation (1/4096), also a very high number of M2-specific plasmocytes was obtained at the same time point (31 anti-M2 IgG ASC/5.10^5 mononuclear cells). The llama was bled two months after the last inoculation (1000 ml of blood) and the mononuclear cells were extracted from the total volume of blood to generate a phage display library. Total RNA was extracted and the cDNA was synthesized with Random Hexamer Primers. The cDNA encoding VHH was amplified by PCR using the combination of two forward primers VH1, VH6 and two reverse primers Lamb7 and Lamb8. The amplified DNA fragments were loaded in a 1.5% agarose gel and bands of a molecular weight corresponding to VHH were detected. This preliminary result demonstrates that BLS-4M2 was highly immunogenic in llama as an immunization strategy to produce a VHH library directed to M2 protein of influenza A virus.

185. CpG-ODN induces, in vitro and in vivo, myeloid GR1+CD11b+ cells and suppressor functions in young and aged mice

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Besides the ability of CpG-ODN to induce in aged (18 months:18m) and young (3 months:3m) BALB/c mice a powerful Th1 immune response, we showed that in vitro CpG-ODN is able to stimulate both, arginase and inducible nitric oxide synthase (iNOS) in macrophages. In the present study, we investigate the regulatory ability of CpG-ODN in vitro and in vivo in 3m and 18m mice and focused on the role of L-arginine metabolism. Bone marrow (BM) cells from 3m and 18m mice were incubated with GM-CSF (3-4 days), resulting in similar percentage of GR1+CD11b+ myeloid cells (3m:50±4%, 18m:55±5%). The treatment of BM-derived myeloid cells with CpG-ODN plus IFNγ induces arginase activity and nitric oxide (NO) production in both mice ages ($p<0.05$). When myeloid cells from 18m mice were added to young normal mice splenocytes stimulated with Con A, we observed lower suppression of proliferative response than their younger counterparts. In addition, splenic T cells from CpG-ODN injected 3m and 18m mice shown reduced proliferation in response to Con A compared with T cells from non-injected control mice ($p<0.01$). Thus, CpG-ODN induces an increase in Gr1+CD11b+ cell population (3m control: 3.0±0.2% vs CpG:8.2±1.0%; 18m control 3.7±0,1% vs CpG:9.7±1.8%)($p<0.001$) and high arginase activity ($p<0.05$) in the spleen of injected mice compared to their not injected counterparts, but NO production ($p<0.05$) only in 18m mice. Analysis of cytokines shows secretion of IFNγ at both mice age, but IL-10 was significantly higher after treatment with CpG-ODN only in young mice. In conclusion, CpG-ODN is able to induce, in vitro and in vivo, myeloid Gr1+CD11b+ cells and T cell regulatory functions in young and aged mice, likely employing the L-arginine metabolism. The ability of CpG-ODN to induce both stimulatory and regulatory responses offers novel opportunities for the improvement of vaccines applied to elderly.

186. Functional analysis of Peptidoglycan Recognition Proteins (PGRPs). Effects on monocytes activation and bacteria clearance.

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PGRPs are PAMPs that bind or hydrolyze Peptidoglycan (PGN). We previously detected the presence of PGRP-S in serum and PMN and we do not observed bactericidal or bacteriostatic effect of PGRPs.With the aim to study the role of PGRPs in innate immune response, we produced human recombinant PGRPs to analyze their binding to monocytes surface and to determine the effect of those molecules in phagocytosis and proliferation. To study if PGRPs would act as a link between PGN recognition and intracellular signaling we analyzed the binding of PGRPs-FITC to monocytes by flow citometry (FC). We observed an increase of positive cells from 1.4+0.1 to 2.0+0.2 times for different PGRPs at 4º C that disappear after Trypan Blue treatment; and a higher increased (2.3+0.1 to 5.1+0.3 times) when incubated cells at 37°C that still remain positive after Trypan Blue treatment, indicating that binding and endocytosis events occurred, respectively.To analyze the effect of PGRP on phagocytosis, we incubated monocytes with died S. aureus-FITC treated with PGRPs

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observing, by FC, an increase number of cells that phagocyte bacteria of 2.8-3.0 times respect control. When we incubated monocytes with live S. aureus treated with PGRPs, we detected more bacteria inside monocytes in the presence of PGRP-S (10^5 UFC/ml) than others PGRPs or controls (10^4 UFC/ml). Until 72 h of incubation we only detected surviving bacteria inside control cells indicating that PGRPs increase the clearance.Finally, to determine the effect of PGRPs over monocytes proliferation we incubated these cells with PGRPs, observing a decrease proliferation of monocytes respect controls in a concentration dependent way however 1-10 ug/ml of PGRPs reduce the inhibition of proliferation produced by PGN.Our results suggest that PGRPs are recognized by monocytes surface receptor increasing phagocyte capacity and blocking PGN effects at 1-10 ug/ml.

187. Characterization of Foxp3+ regulatory T cells in experimental autoimmune orchitis

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Experimental autoimmune orchitis (EAO) is a useful model to study chronic testicular inflammation and infertility. EAO is characterized by an interstitial lymphomononuclear cell infiltrate and a severe damage of seminiferous tubules (ST) that undergo apoptosis and sloughing. We previously reported an increased number of infiltrating Foxp3+ regulatory T cells (Treg) in the testis of EAO rats. In the present work, we studied the localization and suppressive and proliferative capacity of Foxp3+ Treg in the testis of rats undergoing autoimmune orchitis. EAO was induced by active immunization with testicular homogenate and adjuvants. Control (C) immunized with adjuvants and normal (N), non-treated rats were also used. By immunofluorescence we observed clusters of Foxp3+ cells in the subalbuginea area of the testicular interstitium, in close proximity to damaged ST. For functional analysis, CD4+ CD25high CD27+ T cells containing more than 90% of Foxp3+ cells were isolated from testis draining lymph nodes of N, C and EAO rats by FACS sorting and analyzed for their suppressive activity in vitro. All groups of Treg were found to suppress effector T cell proliferation induced by testicular antigens. However, EAO Treg were more efficient than those of N and C rats (suppressive activity, N Treg: 28%, C Treg: 28%, EAO Treg: 61%, data are representative of three independent experiments). Also, these Treg express TGF-ß1. By bromodeoxiuridine incorporation assay we detected an increased number of proliferating Foxp3+ Treg in the testis of EAO compared to N and C rats (N: 0.09±0.01; C: 0.18±0.01; E: 1.8±0.2 ($x10^5$)*, *p<0,001). Overall results suggest that functional Treg infiltrate EAO testis, locally proliferate and produce TGF-ß1 in the organ. However, as the autoimmune damage occurs we speculate that cytokines present in the inflamed testis render effector T cells resistant to suppressive effect of Treg.

188. Immunization with VirB7 from Brucella abortus, a modulator of macrophage functions, protects mice from virulent challenge

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Brucella survival inside macrophages depends on the type IV secretion system (T4SS) encoded by virB genes. We evaluated in vitro the modulation of macrophage functions by T4SS structural proteins located in Brucella external membrane (VirB7 and VirB9). Monocytic human THP-1 cells were preincubated with recombinant VirB7 or VirB9 proteins and stimulated for 24 h with agonists of TLR4 (LPS), TLR2 (Pam3Cys) or TLR5 (flagellin), and TNF-α and IL-1ß levels were measured in culture supernatants. Only VirB7-pretreated cells showed a significant decrease of LPS- or flagellin-stimulated IL-1ß secretion (>77% and >81%, respectively), and of LPS-, Pam3Cys- or flagellin-stimulated TNF-α secretion (62%, 63% and 90% respectively). In view of these results we decided to evaluate if immunization with VirB proteins awards protection to Brucella challenge. BALB/c mice were immunized twice with either VirB7, VirB9 or PBS mixed with Freund's adjuvant. As positive vaccination control a group received a dose of heat-killed B. melitensis H38. One month after the last dose half of the animals were challenged with B. abortus 544 and the other half was used for immune response studies. One month after challenge animals were sacrificed and spleens were extracted for bacterial counts. Specific anti-VirB7 IgG1 titers were higher than IgG2, and the opposite was true for anti-VirB9. In cellular response tests both proteins specifically stimulated IFN-γ secretion (VirB7, 3223.03 ± 873.39 pg/ml vs. 83.89 ± 80.56 pg/ml in PBS control; VirB9, 477.81 ± 235.42 pg/ml vs. 30.98 ± 34.31 pg/ml). VirB proteins did not stimulate IL-4 secretion. Splenic bacterial loads after B. abortus challenge were significantly lower in mice immunized with VirB proteins than in the PBS group. These results suggest that VirB7 modulates human macrophages functions, contributing to Brucella survival inside these cells. In addition, VirB7 and VirB9 are potentially useful for developing acellular vaccines against Brucella.

189. The potential role of inflammatory immune response in Brucella dissemination from the lung

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Brucella is a human pathogen that can be acquired by inhalation. We investigated potential mechanisms by which Brucella may cross the pulmonary epithelial barrier (transcellular or paracellular passage, and transmigration of infected phagocytes or "Trojan horse mechanism"). We used the normal human bronchial epithelial cell line 16HBE14o- which forms a polarized monolayer when grown on filters. B. abortus 2308 invaded and also replicated in 16HBE14o- polarized monolayers (99.5 ± 22,

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499.5 ± 47.5, 3093.5 ± 12812.5 CFU/well at 2, 24 and 48 h pi, respectively). However, it failed to make either transcellular passage through the monolayer (no bacteria detected in the lower compartments until 48 h p.i) or paracellular passage (no bacteria detected at 4 h after placing B. abortus in the upper compartment). In these experiments, FITC-albumin, added together with the inoculum, did not cross the epithelial barrier indicating preservation of both membrane integrity and intercellular junctions. Since no direct translocation of the epithelial barrier was observed, we decided to evaluate if Brucella induces an epithelial inflammatory response that could induce transepithelial migration of infected phagocytes. Infection with both the smooth strain B. abortus 2308 and the rough strain B. abortus RB51 induced the production of IL-8, GM-CSF but not MCP-1, IL-1 and TNF-α. The specific levels of IL-8 in culture supernatants of infected cells with smooth or rough strains were 3348 ±127.8 pg/ml and 3645.5 ± 74.4 pg/ml, and for GM-CSF were 57.4 ± 15.0 pg/ml and 163.89 ± 20.52 pg/ml respectively at 48 h pi. Neither Brucella LPS nor heat killed bacteria induced these responses. These results indicate that B. abortus does not translocate the lung epithelial barrier by direct passage but is likely to do so by mechanisms related to the inflammatory response triggered by the infection.

190. Brucella abortus infected synoviocytes mediate osteoarticular inflammatory damage through induction of apoptosis and MMPs secretion

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Osteoarticular complications are common in human brucellosis, but the pathogenic mechanisms involved are largely unknown. Matrix metalloproteinases (MMPs) and apoptosis are involved in joint and bone damage in inflammatory and infectious diseases. We investigated the production of MMPs by human synoviocytes (line SW982) upon Brucella abortus infection. Since we had found that Brucella-infected human synoviocytes produce IL-8 we also analyzed the role of neutrophils in MMP production and the effects of supernatants from Brucella-infected neutrophils on synoviocytes and viceversa. The production of MMPs was analyzed by zymography and ELISA. Infection with B. abortus induced MMP-2 secretion by synoviocytes (59.63±2.3 ng/ml) and MMP-9 by neutrophils (54.4±2.9 ng/ml). In both cell types MMPs were induced by L-Omp19 lipoprotein from B. abortus (synoviocytes: 92.2±1.4; neutrophils 61.2±1 ng/ml) and in synoviocytes this induction was mediated by TLR-2.As mentioned, Brucella-infected synoviocytes may attract neutrophils to the site of infection. Thus, we decided to study the effects of cytokines secreted by infected neutrophils on the production of MMPs by synoviocytes and viceversa. Supernatants from Brucella-infected neutrophils induced a significant secretion of MMP-2 (70.1±10.6 ng/ml) by synoviocytes cells as compared with supernatants from uninfected neutrophils. In addition, supernatants from infected synoviocytes induced the production of MMP-9 on uninfected neutrophils. Brucella-infection or L-Omp19 stimulation also induced synoviocyte apoptosis as measured by fluorescence microscopy analysis of TUNEL and Hoechst 33342 reactions (Brucella-infected: 40% and 25% apoptotic cells, respectively; L-Omp19 stimulation: 30% and 18%; untreated: 5% and 3%). These results indicate that host-derived MMPs and apoptosis could contribute to the progressive joint destruction observed in Brucella infection.

191. T cell-mediated regulation of Brucella abortus-induced osteoclastogenesis.

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Osteoarticular complications are common in human brucellosis, but the pathogenic mechanisms involved are largely unknown. We have previously shown that Brucella-infected macrophages not only produce pro-inflammatory cytokines, that may eventually produce bone damage, but they can also differentiate to osteoclasts (cells implicated in bone resorption). T lymphocytes can modulate osteoclastogenesis, inhibiting it or accelerating it, depending on the factors secreted. Thus, we decided to investigate the modulatory role of T lymphocytes in the Brucella-induced differentiation of macrophages into osteoclasts. Supernatants from Brucella-infected murine macrophages were used to stimulate T lymphocytes (LT) pre-activated with anti-CD3e antibodies. After 24 h of incubation, medium was replaced and LT were cultured for another 24 h before harvesting supernatants to determine by ELISA the levels of RANKL and IL-17 (inductors of osteoclastogenesis) and IFNγ (inhibitor of osteoclastogenesis), or were co-cultured with bone marrow cells in the presence of M-CSF to determine their capacity to induce or inhibit osteoclastogenesis. As compared with LT stimulated with supernatants from uninfected macrophages, LT stimulated with supernatants from infected macrophages secreted higher levels of RANKL (2450±122 vs. 144±87 pg/ml) and IL-17 (720±28 vs. 88±12 pg/ml), while IFNγ levels were lower than those secreted by unstimulated cells (560±45 vs. 2100±122 pg/ml). This balance of cytokines resulted in the induction of osteoclastogenesis, as indicated by the formation of tartrate-resistant acid phosphatase (TRAP)-positive multinucleated cells with up-regulated vitronectin receptor and also by the capacity of these cells to re-

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sorb dentine. These results indicate that LT are involved in bone damage in osteoarticular brucellosis through the pathological induction of osteoclastogenesis.

192. Effect of Rab27a on exosome secretion and HIV release in macrophages

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Exosomes are secreted membrane vesicles potentially involved in intercellular communication and in the pathogenesis of infectious diseases. Exosomes are formed as intraluminal vesicles of multivesicular endosomes, and their secretion in HeLa cells is regulated by the GTPase Rab 27a. It has been proposed that HIV can use the exosomal machinery as a dissemination pathway in macrophages. Herein, we determined the involvement of Rab27a in exosome secretion in the model human monocytic cell line THP-1, and its eventual implication in HIV release. The silencing of Rab27a was done with a short hairpin RNA and quantified by quantitative PCR. Exosomes were then purified from cell-culture supernatant by ultracentrifugation. Whereas the protein composition of exosomes secreted by Rab27a-silenced cells was not modified as compared with control cells, their amount was reduced by more than 70%, indicating a secretion defect. HIV production and release was subsequently analyzed. The release and intracellular distribution of HIV exhibited a high variability between experiments, probably reflecting differences in the status of the cells at the moment of infection. The difficulty in interpreting these data led us to study the role of Rab27a in HIV replication in primary monocyte-derived macrophages from Rab27a-deficient patients. Deficiency of Rab27a resulted in the perinuclear accumulation of the HIV-positive compartments observed by confocal microscopy, indicating a defect in the traffic of these organelles. The nature of these compartments and their contribution to the extracellular release of HIV is currently under study. Overall, these data indicate that Rab27a regulates exosome secretion in a cell line broadly used as a macrophage model. Moreover, Rab27a might be involved in the traffic of HIV-positive intracellular compartments in primary macrophages. These finding will allow determining the level of convergence between the exosomal and the HIV-release pathway in macrophages.

193. Role of Ros in apoptosis induced by different MTB strains in Argentina

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One-third of the world's population is infected with Mycobacterium tuberculosis (Mtb). Prevalent Mtb lineages in Argentina are European-Latin American-Mediterranean (LAM) and Haarlem (H), sensible (s) as well as multi-resistance to drugs (r) strains (M and 410). Polymorphonuclear neutrophils (PMN) play a crucial role in immune response against Mtb being the first cells to arrive at sites of infection where once activated limit infection through their antimicrobial functions. We have previously evaluated the immune response generated by clinical isolates compared with the widely described H37Rv strain and we found that LAMs induced higher PMN activation and apoptosis compared with Hs, 410 and specially M strain. Objective: To evaluate weather differences in apoptosis depends on ROS production generated by Mtb strains, and to describe the mechanisms involved. Methods: 3 x 10^6/ml human peripheral PMN were cultured 18 h with or without Mtb strains and thereafter apoptosis (AV binding) (2:1 ratio) were measured using FACScan. Alternatively, Mtb induced apoptosis was evaluated after addition of H2O2 or NADPH oxidase inhibitor (DPI). ROS production (^{123}DHR oxidation) were determined by 1 h culture (1:50 ratio PMN: strain). Optional LPS were used as priming agent 1 h before culture. Results: LAMs induced a higher ROS production compared to H strains ($p<0.05$) that was enhanced by LPS priming ($p<0.05$) while it did not improve other strains response. Apoptosis induced by all strains was prevented by DPI ($p<0.002$) and was restored in H strains by the addition of H2O2 ($p<0.05$). Infection with live bacteria and ROS generated by DHR-labeled strains showed a higher fitness of LAM strains to enter PMN. Conclusion: these differences in Mtb up-take and ROS production among strains may determine the PMN activation/apoptosis fate and explain the characteristic innate immune response of each strain.

194. The CD16+ Monocyte subset gives rise to an altered dendritic cell population in tuberculosis

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During a chronic infection such as tuberculosis (TB), the pool of tissue dendritic cells (DC) must be renewed by recruitment of both circulating DC progenitors and monocytes (Mo). Mo can be classified into at least two subpopulations with distinct phenotypical and functional characteristics: classical CD16– and nonclassical CD16+. We have demonstrated that Mo from TB patients showed an enrichment of the circulating CD16+ subset and presented an altered differentiation process characterized by the generation of CD1alow/CD14+/DC-SIGNlow/CD86high cells which induced low specific T cell proliferation in response to Mycobacterium tuberculosis. Therefore, we wondered if this enlarged CD16+ Mo subset could be responsible for the altered Mo differentiation into DC in TB patients.Mo or isolated

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doi: 10:3823/413

Mo subsets were differentiated in vitro in presence of IL-4 and GM-CSF for 6 days. Afterwards, CD1a, CD14, CD86 and DC-SIGN expression was evaluated by flow cytometry.We found a positive correlation between the percentages of CD16+ Mo and of DC-SIGNlow/CD86high altered DC population ($p<0.05$, $n=18$). We analyzed the acquisition of DC phenotype and observed that CD16+ precursors did express neither CD1a nor DC-SIGN and showed high expression of CD86 at 20h from the onset of the differentiation ($p<0.05$). Besides, the differentiation into DC from the isolated CD16+ subset gave rise to a main population CD1alow/DC-SIGNlow, while the CD16- subset gave rise to a main population CD1a+/DC-SIGNhigh ($p<0.05$). In line with this, the addition of CD16+ cells in CD16depleted-Mo from healthy subjects generated CD1alow/DC-SIGNlow cells ($p<0.05$). Finally, the depletion of CD16+Mo improved DC differentiation in TB patients. These results demonstrate that the CD16+ Mo subset fails to give rise to CD1a+/DC-SIGNhigh cells. Therefore, we were able to link the impairment of DC generation with the expansion of the CD16+ Mo in TB patients.

195. Opsonic antibodies are critical to prevent Bordetella parapertussis survival to PMN phagocytosis. Current whopping cough vaccines do not induce these kinds of antibodies.

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B. pertussis (Bp) and Bordetella parapertussis (Bpp) are the etiologic agents of whooping cough, a human disease that is re-emerging despite high vaccination coverage with Bp vaccine. Bpp showed an increasing impact in the epidemiology of this disease whose start roughly concurs with the introduction of the acellular vaccine against Bp. We recently found that Bp vaccine has a poor efficacy against Bpp. O-antigen of Bpp interfere with Bpp binding Bp–induced antibodies. Importantly, this detrimental effect is particularly drastic in the case of Bp acellular vaccine induced antibodies. In previous studies we showed that the lack of antibody opsonisation of Bp leads to the fail of bacterial killing by immune cells. We hypotheses that Bpp had a similar immune subversion mechanism that, in the absence of proper opsonins, allows it to evade immune clearance by PMN. We used GFP bacteria and fluorescent probes to investigate PMN uptake and intracellular trafficking of B. parapertussis in the presence and the absence of opsonic antibodies by mean of flow citometry and confocal microscopy. Intracellular bacterial survival was evaluated by Polymyxin B protection assays. Means were compared using ANOVA or t-Student ($p<0.05$), depending on the study. In the absence of antibodies Bpp was efficiently phagocytosed by PMN but only 14 ± 4% of the intracellular bacteria were found in acidic vacuoles (lysotracker positive) indicating that most of the bacteria remained in non-killing vacuoles as confirmed by intracellular viable counts. The presence of anti-Bpp but not anti-Bp antibodies increased 2 times PMN bacterial uptake as well as the trafficking of Bpp to lisosomal compartment (26 ± 5% of colocalization) leading to significantly more efficient bacterial killing as confirmed by intracellular viable counts. These data suggest that Bp vaccines induce antibodies that failed to opsonize Bpp leading to a defective immune clearance eventually enabling bacterial survival in the host.

196. Differential cytokines patterns of LPS-stimulated PBMC treated with lactic acid bacteria or its metabolic products: in vitro approach to select strains with specific probiotic properties

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Among the differential immunomodulatory properties observed in Lactic acid bacteria (LAB) are outlined their anti-inflammatory features with potential application in chronic inflammatory diseases. The aim of this work was employing a single in vitro technique to select between LAB with technological interest, those strains with potential anti-inflammatory properties to be further tested in animal models. Human peripheral blood mononuclear cells (PBMC) isolated from healthy donors (24-34 years old), using the Ficoll-Hypaque technique, were stimulated with E. coli O26:B6 LPS (0.5 µg/mL) as a pro-inflammatory challenge and then were incubated in presence of 5 viable LAB strains from CERELA culture collection or with its respective conditioned media (CM), on RPMI medium at 37ºC (5% CO2) at different incubation times. TNF-α and IL-10 were detected in the free cell supernatants by ELISA. Results show that Lactobacillus reuteri CRL 1101 CM reduces release of TNF-α by 46±27% ($p= 0.01$) and 54±17% ($p=0.05$) at 4 and 6 h incubation respectively compared whit control values, whereas increases IL-10 production 2.7±0.7 ($p=0.01$) and 2.9±0.6 ($p=0.05$) folds. CM from Lactobacillus rhamnosus CRL 1505 and Streptococcus thermophilus CRL 1190 strains increase IL-10 release 2.7±0.3 ($p=0.05$) and 1.9±0.3 ($p=0.05$) folds, but CRL 1505 strain CM reduces TNF-α release only by 15±13% ($p=0.05$) at 4 h incubation and CRL 1190 strain CM do not produce significant differences on TNF-α secretion at 4 h incubation (1.24±0.44 folds). Metabolic products present in the CM from L. reuteri CRL 1101 strain has suitable in vitro anti-inflammatory characteristics to be deeper studied and tested as experimental anti-inflammatory therapy on inflammation animal models. This single method could be appropriate to select LAB with potential immunomodulatory properties before animal investigations and could optimize the use of in vivo assays.

197. Study of dendritic cells in cornea of patients with climatic droplet keratopathy using in vivo confocal microscopy

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Vol.1
No. 3:4
doi: 10:3823/413

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Climatic droplet keratopathy (CDK) is an acquired degenerative disease of the human cornea characterized by progressive accumulation of unknown protein globular deposits on the sides of the cornea that spread centrally and change size and colour as the condition worsens. It is clinically divided in 3 stages (I, II, III) depending on the extension and severity of damage.
In contrast to the central portion of the normal corneal epithelium the peripheral area and the limbus contain some dendritic cells (DC). Different types of cornea inflammations are associated with an increased density of DC.We studied the density of epithelial DC in CDK corneas using in vivo confocal microscopy (CFM) and correlated the findings with corneal progressive opacity and nerve abnormalities. In stage I we found reflective punctiform deposits in the basement membrane and Bowman's layer, normal subepithelial nerve plexus, and present of DC in peripheral area (34±4 cells/mm^2) and the limbus (87±7 cells/mm^2). In moderate and advanced stages (II and III) there was increased reflectivity of the surface corneal epithelium and condensation of the punctiform deposits within Bowman's layer and the corneal stroma, abnormal nerves and the DC were confluent at the limbus. In stage II the density of DC in peripheral area and limbus was 77±7 cells/mm^2 and 101±7 cells/mm^2, whereas in stage III the density of DC was 27±5 cells/mm^2 and 237±8 cells/mm^2. We did not observe DC in the central area at any stage of CDK. CFM showed a progression of anterior cornea subepithelial and stromal deposits from early to advanced stages of CDK. Progressive damage of the sub-basal and stromal nerves fibers at stage II and III may lead to changes in corneal sensitivity. Concomitant with these findings we showed an increase number of confluent DC at the limbus.

198. TNFα and a fraction purified from Larrea divaricata Cav: a novel immunomodulatory treatment against Candida albicans infection.

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Larrea divaricata is a burst with several applications in argentinean folk medicine. The aim of this work was test an alternative phytotherapy treatment against Candida albicans infection by enhancing the activity of murine macrophages (MØ). In this work healthy and infected mice were used and the following groups were test in agreement with the treatment: a) PBS (control group); b) fraction obtained from L. divaricata (F1); c) TNFα; d) antibody against dectin-1(15Y9), which is a blocker of β-glucans receptor; e) F1+TNFα; f) F1+15Y9; g) TNFα+15Y9 and h) F1+TNFα+15Y9. Treatments were started 24h after infection and were administered once a day during 3 days. MØ were harvested, and organs (liver, spleen and kidney) and serum were extracted. Phagocytosis activity, superoxide production by NBT test, Nitric oxide (NO) levels, acid phosphatase activity and viability by MTT test were performed to determinate MØ activity. The colony forming units (CFU) were counted in organs homogenates and serum TNFα, IL-10 and NO were determined. Results showed that F1 increase phagocytosis in both: healthy and infected mice ($p<0.05$). The NO was increased in culture supernatants and serum ($p<0.05$) of infected mice treated with (e) procedure. This treatment also showed the highest increase of reduced NBT ($p<0.01$) and acid phosphatase activity ($p<0.05$). Serum from infected mice showed that F1 increased TNFα production ($p<0.05$), but IL-10 was not modified. Infection was eliminated from liver and kidney in mice treated with F1 ($p<0.01$). When treatment (e) was tested the infection of the three study organs was eliminated ($p<0.05$). The mice treated with 15Y9 did not show any signs of MØ activation or clearance of the infection. We suggest that the pathogen is eliminated through the activation of the innate immune system and we could observe a synergistic effect between F1 and TNFα treatment. We propose that the use of F1 and TNFα could be a valuable treatment for C. albicans infection.

199. Cross-reaction between proteins of Larrea divaricata Cav. (jarilla) and several antigens of Pseudomona aeruginosa

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Larrea divaricata Cav. (jarilla) is a plant found in Argentine and used to treat different pathologies. Little is known about its immunological properties. Pseudomonas aeruginosa is a Gram negative bacillus that is considered a nosocomial pathogen. The aim of our work was to study the cross-reactivity between P. aeruginosa's proteins and those from an extract aqueous of jarilla (JPCE). JPCE was partially purified by ultrafiltration. The strain of P. aeruginosa ATCC 27853 was used. Cellular proteins by sonication, total membrane, citoplasmic and extracellular antigens were obtained. Proteins were analized by SDS-PAGE. To evaluate the cross reactivity of anti-JPCE sera an ELISA and Western Blot (WB) tests were used. To confirm the presence of common antigens an ELISA inhibition test was carried out. The similarity between protein profiles on SDS-PAGE and WB was expressed as the Dice correlation coefficient (SD). A high number of bands in the JPCE (18 bands) was identified by SDS-PAGE. The proteic profiles of P. aeruginosa showed 12-18 bands.

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doi: 10:3823/413

The bacterial bands showed a SD greater than 36% in relation to JPCE profile. Several common immunoreactive bands were detected by WB (SD=35% to 85%). No significant differences in IgG titers of anti-JPCE serum against sonicated, membrane and extracellular proteins (>1/900) were found. Significant difference (p<0,006) was observed in heterologous reaction with citoplasmic proteins. The cross reaction observed between JPCE and P. aeruginosa suggest that anti-JPCE recognizes epitopes on bacterial proteins. The binding of antibodies to immobilized JPCE (on the ELISA plaque) was inhibited (15%-40%) by preincubation of the antibodies with the four bacterial antigens. In conclusion, our data demonstrate clearly that bacterial antigenic epitopes were consistently detected by antibodies elicited with L. divaricata proteins. These findings could be relevant in the development of vaccines against infections caused by P. aeruginosa.

200. Induction of CD4+ T and NK cell-mediated antitumoral response by immunization with breast cancer cells transfected with a dominant negative vector of Stat3

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The evidence that disruption of Stat3 signaling in cancer cells can overcome tumor immune evasion leads us to design an immunotherapy based on the administration of irradiated breast cancer cells that express a dominant negative (DN) form of Stat3. We have already shown that this immunization provides protection against the murine progestin-dependent C4HD tumor in a prophylactic protocol. Now, our focus is disclosing the lymphocyte subsets involved in the antitumoral response. To achieve this, immunized BALB/c mice were in vivo depleted of CD4+, CD8+, or NK cells with monoclonal antibodies before tumor challenge. Depletion of CD4+T or NK cells completely abrogated resistance to tumor challenge induced by immunization with DNStat3-transfected C4HD cells (*p<0.01 and **p<0.001, respectively). However, depletion of CD8+ T cells did not affect C4HD tumor rejection. We demonstrated that splenocytes from mice injected with DNStat3-C4HD cells were effective in lysing C4HD in vitro. To determine whether NK cells were responsible for that cytotoxic effect, we isolated them from spleens of immunized mice and performed a 51Cr release assay. NK cells from mice immunized with DNStat3-transfected C4HD cells showed an increased cytotoxicity against YAC-1 cell line and C4HD cells compared to mice immunized with empty vector-transfected C4HD cells (27.5 ± 2.5% vs 5.2 ± 1.9% and 15.7± 2.4 vs 5.3± 2.7, respectively, 5:1 effector-to-target ratio, p<0,05). In addition, IFN-γ production was enhanced in splenocytes from mice immunized with DNStat3-C4HD cells vs control group (249.2± 5,1 vs 143.2± 16.5 pg/ml p<0.05).As we have already demonstrated that immunization with DNStat3-transfected cells in nude mice did not protect against C4HD tumor development, these results support the concept that breast cancer growth can be inhibited through induction of a CD4+ and NK cell-dependent protective immune response in vivo with tumor immunogens derived from DNStat3-transfected breast cancer cells.

201. Cellular cytokine profiles and their relationship with the clinical outcome of Leishmania panamensis infection

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Cutaneous leishmaniasis is endemic at least in 88 countries worldwide, therefore becoming a major public health problem. The outcome of infection depends on the infecting Leishmania species and the host immune response. Contrary to the polarized T cell response in susceptible and resistant mouse models, a mixed Th1/Th2 cytokine response characterizes asymptomatic human infection as well as non-healing disease caused by Leishmania of the Viannia subgenus. The immune mechanisms, the participation of different cell populations in the production of cytokines and their relationship to clinical outcome are still unknown in cutaneous leishmaniasis caused by L. panamensis. Objective: To determine the frequency and the phenotype of cells involved in the pro and anti inflammatory cytokine production and their relationship with the outcome of infection. Materials and Methods: PBMCs of individuals with asymptomatic infection, chronic cutaneous leishmaniasis, recurrent cutaneous leishmaniasis and healthy controls were co-cultured 72 hours with live L. panamensis promastigotes (stationary phase) in the presence or absence of hrIL-2 (10 ng/ml). The cellular phenotype and intracellular cytokine production was assessed by flow cytometry. The level of cytokine secreted was determined by ELISA immunoassay. Results and conclusion: A higher frequency of IFNγ, TNFα, IL-13 producing CD4+ T cells in chronic patients and IL-13 producing CD4 and CD8 T cells in recurrent patients than asymptomatic individuals were observed. Among the lymphocyte subpopulations CD4 was the major producer of these cytokines. The frequency of B cells producing IL-10 was higher in chronic patients compared to asymptomatics (P<0.05). CD14 expression was downregulated in human macrophages exposed to L. panamensis and this population produce TNFα. Differences in pro and anti-inflammatory cytokine production and the frequencies of cells population that produce them were associated with disease outcome.

202. Effect of probiotics on the microbiota and the production of cytokines at the intestinal level using an experimental model of obesity in balb/c mice.

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Vol.1
No. 3:4
doi: 10:3823/413

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Obesity is a chronic disease, multifactorial, characterized by excessive accumulation of body fat, adipose tissue hypertrophy and alterations of the immune system. Dietary factors and intestinal bacteria play an important role in the increased incidence of obesity. The aim of this work was to evaluate the effect of probiotic fermented milk (PFM) or the probiotic bacterium Lactobacillus casei CRL 431 in obese mice. Mice received conventional (G1) or high-fat (G2) diet and were given milk (A), PFM (B), the bacterial suspension (C) or water (D) during 3 months. The samples were taken each month. Body weight, clinical parameters (glucose, TG, LDL, HDL and total cholesterol) in serum, intestinal microbiota, the release of IFN-γ, IL-10 and IL-6 in intestinal fluid and the histological alterations in liver were evaluated. We observed decrease in body weights and in clinical parameters in G2B and G2C compared to G2A and G2D. IL-10 and IFN-γ were higher in G2D (1166±118) and (475±153) respectively than in G1D (824±54) and (363±23) respectively at the 1st month. After 3 months, IL-10 increased in G2B (1196±174) and G2C (1367±118) compared to G2D and IFN-γ increased in G2B (565±30) compared to the others groups. IL-6 increased in G2B after 2 months (575±0) and it was maintained similar to G2C in the 3rd month, being higher than the others groups (G2D:335±109 and G2A:231±188). These immune variations could be related with changes in the intestinal microbiota. Significant increases of Bifidobcterium and Bacteroides were observed in G2B compared to G2A, G2C and G2D after 3 months of obesity. G2B also decreased steatosis in the liver in relation the others groups. PFM administration avoided the clinical and immunological side effects induced by the high fat diet with increases of Bifidobacterium and Bacteroides. The histological recovery of the liver and stimulation the immune response at the intestinal levels was regulated by the increases of IL-10.

203. Immunoregulatory effects of progesterone on peripheral blood-derived human NK cells involve down-regulation of NKp46

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Clinical evidence indicates that progesterone (Pg) and its synthetic analog medroxiprogesterone have pro-tumoral effects through mechanisms that involve a stimulation of tumor growth and suppressive effects on T cells and dendritic cells, a fact that is relevant during pregnancy but that also could be important during the immune response against tumors, in particular some of endocrine origin such as mammary tumors. Pg also affects NK cell responsiveness, inducing apoptosis and suppressing IFN-γ production of CD56dim cells in response to IL-12. Thus, the aim of this work was to explore additional mechanisms by which Pg affects NK cell responsiveness. We assessed the responsiveness of isolated human NK cells stimulated with IL-12, IL-15 and IL-18, a combination of cytokines relevant for NK cell activation. Pg 10^{-6} M, a concentration reached in some microenvironments, inhibited IFN-γ secretion by cytokine-stimulated NK cells in a 19.9±7.8%, as assessed by flow cytometry. Such reduction in IFN-γ+ cells was observed in CD56dim and CD56bright NK cells (9.4±5.6% and 8.2±2.9% reduction of IFN-γ^+ cells, respectively). Pg also inhibited the expression of the NK cell activating receptors NKp30, NKp46 and NKG2D, as assessed by flow cytometry, as incubation of NK cells with Pg 10^{-6} M for 24 h induced a statistically significant down-regulation of NKp46 (SFI without Pg=13.5±4.0 vs. SFI with Pg=10.8±3.4, n=5, p=0.023). For NKp30 and NKG2D, a down-regulatory effect of Pg was also observed although the differences did not reach statistical significance (NKp30: SFI without Pg=7.6±3.2 vs. SFI with Pg=5.9±2.6; NKG2D: SFI without Pg=10.6±1.8 vs. SFI with Pg=8.8±1.4, n=5 for both receptors). Thus, Pg exerts immunosuppressive effects in NK cells that involve a down-regulation of the expression of critical receptors involved in recognition of tumor target cells and the cross-talk with dendritic cells, which may have consequences on their immune surveillance potential.

204. Yersinia pseudotuberculosis derived-mitogen (YpM), increases nitric oxide production from macrophage cell line RAW264.7 and presents different effects on proliferation rates of naïve or activated cells

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Yersinia pseudotuberculosis (Yp) has been suggested as one of the causative agents of Kawasaki syndrome, a vasculitis affecting predominantly infants and young children. Yersinia infection produces suppression of the host defense by interfering with macrophage phagocytosis and oxidative burst, which culminates with the induction of the intrinsic macrophage cell death program. Y.p has been identified as the only gram-negative human pathogen which produces a superantigen (SAg) called YpM. Since SAgs are referred as immune-suppressor factors promoting the spread of bacterial infection, we analyzed the effect of YpM on macrophages. For that purpose, we analyzed the effects of YpM over naive and activated macrophages. Naive RAW

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264.7 murine macrophage cell line (1x10^6/ml) was incubated for 24, 48 and 72h with YpM at concentration ranging from 0.05 to 100 ug/ml. A significant decrease (P<0.05) of tritiated thymidine (3H-TdR) incorporation was observed at all the times analyzed, compared with the basal control (untreated cells). On contrary, incubation with LPS produced a 20% increase in cell proliferation. To determine metabolic state and integrity of the cells after YpM treatment, flow cytometry assay was carried out with previous stain with Resazurin and Sytox Green, showing positive results for both treatments. Taking together, these results suggest YpM induce an inhibition in the proliferation more than a cell death at the time of the assay. In addition, after 72h we observed NO production suggesting an activation of naive cells. On contrary, when RAW 264.7 cells were activated with LPS and then incubated with YpM no inhibition of proliferation occur, but the cells exhibit membrane damage determined by Sytox Green stain. We can conclude that YpM has a differential effect on naive or activated macrophages, modifying the cell morphology and metabolic activity. These results contribute to the explanation of the Yp capacity to evade the immune response

205. Analysis of Draining Leukocytes from the Abdominal Cavity Monitors Immune Events after Intestinal Transplant

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During intestinal transplant (ITx) operation, intestinal lymphatics are not reconstituted. Consequently, migrating immune cells drain freely into the abdominal cavity and could be recovered though a drainage of the intestinal cavity used for clinical postoperative surveillance. Our aim was to evaluate whether leukocytes migrating from the transplanted intestine could be recovered from the abdominal draining fluid and to determine potential applications of the assessment of draining cellular populations to basic and clinical studies. Draining fluid was collected by a peritoneal drainage system in the early postoperative period of 7 ITx patients. Cell composition of the abdominal draining fluid was analyzed by flow cytometry during the first 14 post-ITx days. Cell sorting and molecular fingerprinting by short tandem repeat amplification was performed. The correlation between analyzed parameters and clinical evolution was evaluated. The main populations in the draining fluid were CD3+CD4+CD8-, CD3+CD8+CD4- and CD3-HLA-DR+CD19+ lymphocytes, although several minor populations were identified. Cellular pattern varies along the post-ITx period in non-complicated recipients from a mixed leukocyte pattern to an exclusively lymphocytic pattern. We could associate changes in draining cell patterns to early rejection or infections. Graft derived lymphocytes were recognized by genetic fingerprinting of CD8+ sorted T cells. At days 1-2, donor T cells were detected in the draining fluid (50% of total CD8+ cells) and were mostly replaced by day 11 after ITx (<2%), confirming that cells migrating from the graft can be recovered in the draining fluid. This study demonstrates that cell analysis of the draining fluid from ITx recipients may provide a useful approach for monitoring changes in graft immunobiology during the first 3 weeks post-transplant. Furthermore, this is a unique opportunity to study different immune cell populations migrating from the mucosal intestinal site.

206. Analysis of gene expression in gut mucosa after intestinal transplantation: selection of candidates for diagnosis of graft rejection

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Intestinal transplantation (ITx) is indicated in cases of irreversible intestinal failure and complications associated to total parenteral nutrition. In spite of immunosuppressive treatment, acute cellular rejection (ACR) is a frequent complication and a first cause of graft loss. Graft monitoring by protocol biopsies is usually used for early detection of ACR by histological analysis. So far, no biochemical markers to detect ACR are available. Our aim was to characterize the ACR process by analyzing gene expression in the intestinal mucosa during follow up and to select candidate markers of rejection. Expression of Mx1, IFN-γ, CXCL10, CXCL11, CXCR3, and CCL20 was measured by RT-qPCR in graft biopsies obtained during the follow-up of ITx patients. 42 samples of 9 patients were analyzed, including 10 ACR events, ranging from mild to severe and 2 episodes of enteral viral infection. In samples taken during severe ACR, induction of IFN-γ (fold increase range (FIR) 3 to 7, depending on individual sample), CXCL11 (FIR 8 to 12), CXCL10 (FIR 3 to 18), and CCL20 (FIR 1 to 23) Mx-1 (2 to 24) was observed. Mild rejection episodes were not reflected by significant fold increase of the markers analyzed. Overall induction levels correlate with the severity of the rejection episode and the immunosuppressive management of the patient. Viral enteritis caused increase of the different markers analyzed. Antiviral gene Mx-1 showed no specificity of viral infections. In some cases, CXCL11 expression levels showed a rise before ACR indicating that this gene might be putative predictor of rejection in the clinics. Although a wider set of analysis is necessary, our results demonstrate that graft gene expression analysis reflect that T cell-attracting chemokines are induced during a severe ACR. Therefore, this analysis might be a valuable tool for ACR characterization and eventual selection of candidate markers.

207. Increased tumor growth due to attenuation of the immune response by tumor-induced systemic inflammation

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Although the link between cancer and local inflammation has firmly been established, the relationship between cancer and systemic inflammation (SI) has comparatively been less studied. We know that the subcutaneous (sc) growth of the immunogenic methylchonlanthrene-induced murine fibrosarcoma MCC is accompanied by manifestations of SI when the tumor exceeds 500 mm^3 coincidental with the onset of a state of immunosuppression. The aim of this work was: a) to determine if SI was followed by compensatory manifestations of systemic counter-inflammation (SCI); b) to evaluate the influence of SI/SCI on tumor growth; c) to study if the putative influence of SI and/or SCI on tumor growth could be associated with a deleterious effect on the immune system. Results: a) The anti-inflammatory cytokines TGF-β and IL-10 were increased in serum of tumor-bearing mice that exceeded 500 mm^3 ($p<0.05$; $p<0.01$, respectively). b) When the anti-inflammatory drug indomethacin was inoculated intraperitoneally (ip) every 3 days throughout tumor growth, SI was reduced and (sc) tumor growth was retarded as compared with controls ($p<0.05$). Reciprocally, the (ip) inoculation of the pro-inflamatory thioglicolate (TG) at the time of tumor implant, generated a transient SI accompanied by a faster (sc) tumor growth as compared with controls ($p<0.01$). c) The (ip) inoculation of TG in immunized non-MCC tumor bearing mice and in mice bearing small MCC tumors, sharply reduced both sinecomitant and concomitant anti-tumor immunity ($p<0.01$) and this effect was restored by indomethacin. Taken together, our results suggest that MCC tumor growth is enhanced by SI and that this enhancement would be mediated, at least in part, by a deleterious effect on the immune system.

208. The survival of cardiomyocytes induced by Trypanosoma cruzi experimental infection is dependent on TLR2 signalling, IL6 production and STAT3 activation

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Local innate immunity plays a central role in defense response and homeostasis in the heart. Previously, we have reported that T. cruzi infection protects isolated cardiomyocytes from apoptosis. Our objective was to elucidate cardiomyocyte innate immune response to T. cruzi infection and its possible role in the cytoprotection. We found that TLR2 expression, but not TLR4, was strongly increased by the parasite in BALB/c neonatal cardiomyocyte cultures. Among the cytokines tested (IL6, TNFα, IL1b, IL17, IL10 and IL4), we detected a rapid and sustained production of IL6 ($P<0.02$), and NF-kB-dependent ($P<0.001$), regardless of the cell: parasite ratio used. In order to test the involvement of TLR2 in the inhibition of apoptosis, primary cultures were transfected with a dominant-negative TLR2 (dnTLR2) plasmid or its control vector (CV), followed by incubation with T. cruzi (Tulahuen 1:1) or the TLR2 agonist PAM3CSK4 (PAM3) and then, were subjected to serum starvation for 48h. FACS analysis showed that overexpression of dnTLR2 blocked the parasite- and PAM3-induced cytoprotective effect, whereas in cultures transfected with CV both stimulus decreased the apoptotic rate ($P<0.001$). Besides, infection induced IL6 production in CV-transfected cells ($P<0.05$), but it was blocked in infected cultures transfected with dnTLR2. To investigate whether IL6 released in response to parasite plays a role in the cardiomyocyte survival, this cytokine was depleted using neutralizing antibody. The treatment abolished the parasite-induced cytoprotection ($P<0.02$). In addition, we found that IL6 production strikingly increased the STAT3 phosphorylation in primary cultures incubated with supernatants from infected monolayers. Our results strongly suggest that the triggering of TLR2 signalling, followed by IL6 production and STAT3 activation, play a key role in T. cruzi-induced cardiomyocyte protection.

209. Modulation of osteoclast differentiation by placental cytokines

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During rheumatoid arthritis, macrophages differentiate into osteoclasts and bone destruction occurs. We demonstrated that extracts from mouse placenta (EPs), containing Th1 and Th2 cytokines; regulate the osteoclast differentiation of RAW 264.7 macrophages. Now we analyze, whether IL-10, TGF-beta, as well as the NFATc1 transcription factor are involved in such effect. Placental extracts were obtained at 7 days of pregnancy (EP7). RAW cells were cultured with RANKL and M-CSF (RM) in the presence or absence of EP7, and neutralizing antibodies (NA) against IL-10 and TGF-beta; the differentiation of the cells was determined by assessing multinucleated, tartrate resistent acid phosphatase positive cells (TRAP+), using a commercial kit; and matrix metalloprotease (MMP) activity by zymogram. The effect of EP7 on NFATc1 expression /phosphorylation was assessed by western blot. EP7 inhibited the RM-induced MMP activity and TRAP+ (inhibition vs RM: 68%, $p<0.001$ and 91%, $p<0.001$, respectively). Pre-incubation of RAW cells with a-IL-10 NA reverted the inhibitory effect of EP7 on MMP activity (a-IL10+RM vs a-IL10+RM+EP7, $p>0.05$) while the inhibition of TRAP+ was not affected (a-IL10+RM vs a-IL10+RM+EP7, $p<0.01$). Pre-incubation with a-TGF-beta NA reverted the inhibitory effect of EP7 on both, the MMP

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doi: 10:3823/413

activity and the TRAP+ (a-TGF-beta+RM vs a-TGF-beta+RM+EP7, p>0.05). EP7 inhibited in 79 % the expressión of NFATc1 in cells cultured during 4 days with RM (p<0.001); nevetheless, the NFATc1 dephosphorylation induced by 2 h-treatment of the cells with RM was not affected by EP. We propose that the downregulation of NFATc1 expression by EP7 is involved in the inhibition of the RM-induced osteoclast differentiation of RAW cells. TGF-beta participates in the inhibition of MMP and TRAP+. The inhibitory effect of IL-10 on MMP activity was not enough to inhibit cell differentiation, since a-IL-10 NA was not able to revert the inhibition by EP7 of RM-induced TRAP+.

210. Transcriptional analysis of bone marrow derived dendritic cells from TLR4 deficient mice matured in the presence of soluble factors secreted by LPS-stimulated B16 melanoma cells.

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B16 cells stimulated in vitro with a TLR4-ligand 48h prior to their inoculation into TLR4 deficient mice (TLR4lps-del), induce tumors significantly smaller than controls. Apoptosis-proliferation levels of LPS-stimulated B16 cells are not modified and inhibition of tumor growth was not observed in nude mice. LPS-stimulated B16 cells supernatant (LPS-B16 CM) can significantly improve the maturation and function of TLR4lps-del bone marrow derived dendritic cells (BMDC). Here, we analyze the molecular changes that TLR4lps-delDCs experience when they are incubated with B16-CM or LPS-B16 CM for 20h before inducing their maturation with CpG for 4 hs. Transcriptional analysis was performed by a quantitative PCR array and the results were analysed with the 2-ΔΔCT method. TLR4lps-del BMDCs exposed only to CpG for 4 hs, increase the transcription of IL12a (x 8); Csf2 (x 15); Csf3 (x 16.65); IL1a (x 12); IL1b (x 7); IL6 (x 11) and TNFalpha (x 20) genes. The expression of IL1a and b, TNFalpha and IL6 genes was not altered in TLR4lps-del BMDCs matured with B16-CM or LPS-B16 CM, while Csf2 and Csf3 transcription was extremely down regulated. NF-kB complex expression showed a significant increase (x380 NF-kB1, x468 Rela) only in TLR4lps-del BMDCs matured in the presence of LPS-B16 CM. Interestingly, the expression of IL12a that was inhibited in DCs incubated with B16-CM was partially restored when B16-LPS CM was present at the time of maturation. When a neutralizing antibody against IFNbeta was added to LPS-B16 CM the improvement in the expression of coestimulatory molecules analyzed by flow cytometry was lost. We hypothesize that soluble molecules secreted by LPS-stimulated B16 cells can positively modulate the maturation state of DCs, which are normally inhibited in the presence of tumor derived factors.

211. B16 murine melanoma cells stably expressing shRNA-MyD88 induce reduced tumor growth rate

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Toll-like receptors (TLRs) recognize molecules derived from pathogens (PAMPs) as well as endogenous danger signals possessing similar chemical structures (DAMPs). Upon recognition of their ligands, TLRs transduce signals through two pathways involving distinct adaptors, Toll/IL-1R domain–containing adaptor inducing IFNs (TRIF) and Myeloid differentiation primary response protein (MyD88), which is used by all TLRs except TLR3. Stimulation of TLRs-pathways on antigen presenting cells has always been considered a valuable tool to activate an anticancer immune response. Recently, the presence of functional TLRs in different tumor cell lines and in ex-vivo tumor samples has been demonstrated, raising the question about the role that these TLRs could be playing in sterile environments like tumors. Here, we investigated whether MyD88-dependent signaling modulates tumor development. The expression of MyD88 was stably knocked down in the B16 melanoma cell line (B16-MyD88kd) by using a silencing vector expressing short hairpin RNA (shRNA) targeting mouse MyD88. As control, we generated B16 cells stably transfected with the control vector encoding shRNA targeting luciferase gene (B16-Luc). These vectors encode GFP fusion protein for tracking the transfected cells. The expression of MyD88 in B16-MyD88kd and in B16- Luc was evaluated by qRT-PCR. Approximately, a 65% of inhibition in mRNA expression of MyD88 was observed in B16-MyD88kd. Interestingly, when B16- MyD88kd were inoculated in vivo into syngeneic host, the tumors elicited were bigger than those elicited by B16-Luc (p<0.05). Our preliminary results indicate that in physiological conditions, an endogenous ligand could ordinarily activate this pathway and promoting a reduced tumor growth.

212. Oral treatment with a hybrid protein between E. coli heat labile toxin B subunit and ABC synapsin peptide reduced central nervous system inflammation in experimental autoimmune encephalomyelitis (EAE)

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EAE is an established model for human multiple sclerosis (MS). In both diseases autoreactive T cell clones are peripherally generated and migrate into the central nervous system. Extensive inflammatory reactions mediated by infiltrating mononuclear cells and production of proinflammatory cytokines are responsible for demyelination, axonal disfunction and neurological disability. We have previously shown that oral tolerance induced in rats with LTBABC, a hybrid between the ABC domain of synapsin and the B subunit of Escherichia coli heat-labile enterotoxin (LTB) attenuated EAE clinical signs and diminished cellular

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doi: 10:3823/413

reactivity against myelin basic protein. To further characterize this effect we analyzed histological changes and the presence of CD4+, CD8+, CD45+CD11b+, CD4+ producing INF-γ or IL-17, and CD4+CD25+Foxp3+ cells infiltrating CNS. Rats were orally fed with 6 doses of LTBABC, LTB (0.3 nmol or equivalent amount in biological activity) or vehicle after encephalitogenic challenge. Spinal cords and brains were obtained during the EAE acute stage. Mononuclear cells infiltrating CNS were isolated and analyzed by flow cytometry. Rats treated with LTBABC showed the fewest inflammatory infiltrates in lumbar spinal cord sections stained with H & E ($p<0.01$); and they also exhibited lower percentages of CD4+, CD8+ and CD4+cells producing INF-γ than the ones fed with vehicle or LTB ($p<0.05$). Diminished frequency of CD4+ IL-17+ cells were observed in the LTBABC group respect to control group ($p<0.05$). Frequency of infiltrating macrophages (CD45+CD11b+ cells) did not differ between groups. CD4+CD25+Foxp3+ regulatory T cells were expanded only in CNS of the LTBABC group ($p<0.05$). These results clearly indicate that oral tolerance induced by LTBABC reduced infiltration of T cells and increased the presence of CD4+CD25+Foxp3+ regulatory T cells in CNS, suggesting that synapsin peptides coupled to LTB has a protective effect with therapeutic potential in EAE and MS treatment.

213. Outer membrane vesicles obtained from Bordetella pertussis Tohama expressing the lipid A deacylase PagL as a novel acellular vaccine candidate.

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The use of outer membrane vesicles as antigen for vaccination against Bordetella pertussis seems an attractive option since these vesicles harbor an important number of different bacterial antigens, including all the vaccinal antigens used for acellular commercial vaccines in the membrane context, hence in their native state. In an effort to devise a safer and effective pertussis acelullar vaccine, outer membrane vesicles (OMVs) were engineered to decrease their endotoxicity. The pagL gene from Bordetella bronchiseptica, which encodes a lipid A 3-deacylase, was expressed in B. pertussis strain Tohama I. The resulting OMVs, designated OMVsPagL, contain tetra- instead of penta-acylated LPS, in addition to pertussis surface immunogens such as pertactin and pertussis toxin, as the wild type OMVs. The characterized pertussis OMVsPagL was used in murine B. pertussis intranasal (in) challenge model to examine their protective capacity when delivered by (in) routes. Immunized BALB/c mice were challenged with sublethal doses of B. pertussis. Significant differences between immunized animals and the PBS treated group were observed ($p < 0.001$). Adequate elimination rates ($p < 0.005$) were observed in mice immunized either with OMVsPagL and wild type OMVs. All OMV preparations tested were non toxic according to WHO criteria; however, OMVsPagL displayed almost no weight loss at 3 days post administration, indicating less toxicity when compared with wild type OMVs. Induction of IL6 (177.40 ± 1.09 vs 193.39 ± 1.47fold increase) and IL1 (10.51±4.98 vs 17.70 ± 3.70 fold increase) expression in lung after i.n. delivery showed coincident results, with a lower induction of the proinflammatory cytokines in the case of OMVsPagL compared to wild type OMVs. In view to their lower endotoxic activity and retained protective capacity in the mouse model, OMVsPagL obtained from B pertussis are candidates for novel vaccines against pertussis.

214. Mechanisms of the innate immune response induced by probiotic bacteria in the protection against Salmonella enterica serovar Typhimurium infection

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Some probiotic lactic acid bacteria can be useful for the prevention of enteric infections. Oral administration of the probiotic bacterium L. casei CRL 431 (Lc) decreased the severity of Salmonella enterica serovar Typhimurium (ST) infection in BALB/c mice. The aim of this work was to compare the immune mechanisms exerted by Lc with other lactobacilli L. acidophilus CRL 730 (La) and L. delbrueckii ssp.bulgaricus CRL 423 (Lb) in order to elucidate the role of the innate response in the protection against ST. Mice receiving Lc, La, or Lb during 7 days, were challenged with ST and continued receiving them post ST. Infection control (S) did not receive special feeding. Samples were collected the day of the infection and 7 days post ST. Weight loss, mortality and ST translocation were evaluated. Levels of total and specific s-IgA, IL10, TNFα, IFNγ and IL6 were measured in small intestine (SI) fluids by ELISA, IgA+ cells in histological cuts of SI by IF. Macrophages from peritoneum, spleen and Peyer´s patches were isolated for phagocytosis assay. For mice given Lc, TLRs, macrophages and dendritic cells (DC) markers and CD4+ or CD8+ TL were also analyzed. Mice fed with Lc and Lb decreased mortality compared with S (0% and 20% vs 30%) being Lc the group with less pathogen counts in liver and large intestine. 7 days post ST, Lc increased IL10 (142±9 vs 35±13) and specific s-IgA (0.22±0.11 vs 0.06±0.05) compared to S in SI fluids, and augmented TLR2 (30±6 vs 14±3) TLR5 (20±2 vs 9±2) and TLR9 (17±3 vs 12±3) in SI tissues. IgA+ cells increased in mice given Lc and La compared to S group. Macrophages and DC increased in mice given Lc, TL increases were not observed. Phagocytic activity was higher

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in mice fed with Lc and Lb than in control mice. We demonstrated that Lc exerted its protective effect against ST through the stimulation of the innate immunity, maintained a regulated immune response with increases of IL10. The importance of the specific anti-pathogen s-IgA released to the intestinal fluid was also observed.

215. Improved immune-endocrine profile in tuberculosis patients under specific treatment

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Tuberculosis (TB) remains a mayor cause of morbidity and mortality around the world. The proper mechanisms underlying divergent disease outcome are not fully understood. Earlier studies in TB patients revealed imbalanced immune-endocrine responses with increased and reduced concentrations of Cortisol (Cort) and DHEA, respectively, resulting in a higher Cort/DHEA ratio linked to disease severity. Information about the evolution of the immunoendocrine response during antibiotic therapy of TB is nearly negligible.To proceed with, we have now investigated the levels of both adrenal steroids and pro-inflammatory compounds like IL-1ß and C reactive protein in TB patients (n=10) upon completion of four months of specific treatment. Healthy controls (HCo, n=13) and household contacts (HHC, n=8) were also included for comparison purposes. Adrenal steroids were assayed by electrochemioluminicence (ECH, Roche), whereas IL-1 and CRP were determined by ELISA (Invitrogen) and turbitest (CRP-High sensitivity, Winner Lab). At the time of diagnosis (T0), TB showed higher levels of Cort (median µg/dl, 75%-25%): 29.1 (14.3-43.2), respect HHC 16.7 (12.3-47.0) and HCo 19.2 (12.0-22.8); $p<0.05$. DHEAS concentrations showed no between-group differences. The Cort/DHEAS balance was significantly higher in TB [TB 0.3 (0.15-0.57); HHC 0.11 (0.043-0.35); HCo 0.083 (0.067-0.15); $p<0.01$]. Higher concentrations of IL-1 and CRP were also found in TB cases ($p<0.001$). Paired comparisons between T0 and two months after specific treatment (T2) revealed a decrease of CRP ($p<0.03$), Cort, ($p<0.05$) with a mild increase of DHEAS values that lead to a significant reduction of Cort/DHEAS balance [T0: 0.395 (0.12-0.90); T2: 0.188 (0.12-0.44,) $p<0.03$]. Moreover, there was a correlation between ΔIL-1ß (T0-T2) and ΔCort/DHEAS balance (T0-T2) ($r=0.94$). Such immune-endocrine profile is compatible with the establishment of a better anti-infectious response.

216. Title Cytokine and hormone profile in pulmonary tuberculosis: Assessment of plasma levels of Adiponectin, IL-1β, C reactive protein (CRP), Neuropeptide Y (NPY), Glucagon and Insulin.

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The immune response against Mycobacterium tuberculosis (Mtb) plays a fundamental role in the outcome of the mycobacterial infection. The immune system reacts efficiently in most cases; although in 10 % of Mtb-infected people disease development is likely to occur throughout their lifetime. Communication between the neuroendocrine and immune systems is critical for maintaining physiological homeostasis and an adequate anti-Mtb response. In parallel, the adipose tissue exerts several endocrine functions and releases pro-inflammatory and anti-inflammatory mediators, emerging as an important element in the regulation of many pathological processes. Considering that tuberculosis (TB) coexists with profound metabolic changes, partly reflected in the typical weight loss, we sought to analyze plasma levels of immuno-endocrine mediators playing a role both in disease immunopathology and metabolic changes. As such, 46 newly diagnosed patients with pulmonary TB (9 mild, 19 moderate, 18 severe), 22 household contacts -HHC- and 27healthy controls –HCo- (all of them HIV negative) were assessed for their plasma levels of Adiponectin, IL-1β, CRP, NPY, Glucagon and Insulin. Through a principal component analysis, patients were characterized by greater values of PCR, while Hco and HHC have higher values of BMI and Leptin. TB patients displayed higher levels of Adiponectin ($p<0.05$), CRP ($p<0.0001$)) and IL-1β ($p<0.02$) respect to Hco and HHC. Comparison among TB patients showed an increase in Adiponectin ($p<0.05$), IL-1β ($p<0.05$), CRP ($p<0.001$) and NPY ($p<0.05$) concentrations with rising disease severity. Pairwise correlations showed a positive association between Leptin and Insulin in HCo ($p<0.01$) and HHC ($p <0.001$). Present results are compatible with a deregulated defensive response moving from a Th1 profile to a "less inflammatory" one as TB progresses in presence of an endocrine response inefficient to assure a favourable metabolic balance, altogether favouring disease aggravation.

217. Signaling through SLAM involves p38MAPK activation in patients with active tuberculosis

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The importance of IFN-γ for the protection against M. tuberculosis (Mtb) has been widely recognized. Previously we reported

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doi: 10:3823/413

that signaling lymphocytic activation molecule (SLAM) activation promotes Th1 responses during mycobacterial infections. We also demonstrated that the induction of IFN-γ mediated by SLAM in tuberculosis depends, at least in part, on CREB activation. Moreover, we showed that SLAM signaling induced the activation of the protein kinase Erk. To further analyze the signaling pathways induced by SLAM that contribute to CREB activation and IFN-γ secretion in tuberculosis, we studied the role of p38MAPK. Our results showed that the addition of the p38MAPK inhibitor SB22026 to Mtb stimulated cells from tuberculosis patients and healthy donors inhibited the percentage of IFN-γ+pCREB+ T cells by more than 70%, indicating that p38 would be involved in the activation of CREB. Since, the p38 inhibitor downregulates the expression of SLAM induced by Mtb, we stimulated peripheral blood mononuclear cells from tuberculosis patients and healthy donors for 5 days with the antigen. Cells were then washed and stimulated with SB22026 plus an agonistic α-SLAM mAb. After 48h the production of IFN-γ was significantly decreased by the p38 inhibitor compared with the cells stimulated with Mtb + α-SLAM ($p< 0.05$). Taken together, these results indicate that the production of IFN-γ induced by SLAM signaling is mediated by Erk, p38 and CREB phosphorylation. Then, our data contribute with new information about the molecular basis operating during SLAM ligation that leads to IFN-γ production in human tuberculosis.

218. PD-1 modulates the expansion of Th1/Th17 populations during active tuberculosis.

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An appropriate immune response against M. tuberculosis (Mtb) requires Th1 cytokine responses; in particular, IFN-γ. However, although this cytokine displays a crucial role, its secretion is not enough to achieve a complete protection against the pathogen. Accordingly, it was shown that IL-17 produced by CD4 T cells would be required to eliminate primary infection. Besides the existence of Th1 and Th17 populations, another subset of CD4+IFN-γ. +IL-17+ cells was described. Thus, here we analyzed the role of CD4+ cells during active tuberculosis (TB). Stimulation of PBMC with the specific Ag (Mtb) induced significantly higher levels of IL-17 from TB patients compared to healthy donors (HD) (TB: 414±79pg/ml; HD: 276±45pg/ml; $p<0.01$). Moreover, Mtb induced a striking increment in CD4+IL-17+ cells as well (TB: 9±1%; HD: 5±1%; $p<0.01$). Interestingly, more than 60% of IL-17 producing cells were IFN-γ+ (TB: 62±5%, HD: 64±4%), suggesting the existence of a Th1/Th17 population that secrete IL-17 in response to Mtb. However, the proportions of each population vary significantly, given that in HD to 75% of the cells are IFN-γ+IL-17-, 16% IFN-γ+IL-17+ and 9% IFN-γ-IL-17+, whereas in TB we found, 55%, 29% and 16% respectively. Previously we demonstrated that programmed death 1 protein (PD-1) inhibits Th1 responses in TB. Then, we studied the expression and function of PD-1 on Th17 cells. We found that Mtb stimulation induced PD-1 expression on more than 90% of CD4+IL-17+ cells. Furthermore, by blocking the PD-1 pathway we observed a significant increase in IL-17 production (Mtb: 420±56pg/ml; Mtb+PD-1: 795± 56pg/ml) and in the number of CD4+IL-17+ cells as well (Mtb: 10±1%, Mtb+PD-1: 15±1%). However, PD-1 blockage did not modify IL-17 mean intensity fluorescence, indicating that PD-1 would inhibit the expansion of Th17 cells. Then, our data demonstrated the simultaneous presence of Th1 and Th17 cells to fight Mtb. Furthermore, Th1 and Th17 populations would be modulated by the same pathways during active TB.

219. Quantification of IgM, total IgG, IgG1 and HCAbs in colostrums of llamas (Lama glama) by ELISA

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Camelids possess a particular kind of antibodies devoid of light chains (known as HCAbs). Three IgG isotypes were identified (IgG1, IgG2 and IgG3); IgG2 and IgG3 are HCAbs whereas the IgG1 maintains the conventional structure. Due to the type of placentation in llamas, newborns required the ingestion and absorption of immunoglobulins (Igs) through colostrum to acquire passive immunity. Because of the lack of specific reagents IgM and IgG isotypes had not yet been measured in colostrum. Objective: To determine the quantity of IgM, total IgG, IgG1 and HCAbs (IgG2 and IgG3) in colostrum of llamas. This study represents an important tool for the evaluation of the passive transference in llamas and contributes to the analysis of the biological functions of HCAbs. For this assay we obtained 12 samples of colostrum between the first day post partum. Previously, in our group we developed a specific polyclonal antiserum against IgM, total IgG and IgG isotypes. We used anti-IgM (H), anti-total IgG (H) and anti-IgG1 (CH1) polyclonal antisera in order to design 3 types of ELISAs sandwich (to determine IgM, total IgG and IgG1). HCAbs's concentration was calculated by difference between total IgG and IgG1 (HCAbs = total IgG – IgG1). The mean and the standard deviation (SD) of the results obtained are detailed below: IgM =18.02 mg/ml (SD=10.50), total IgG=42.09mg/ml (SD=30.18), IgG1=24.04mg/ml (SD=15.37) and HCAbs = 18.05 mg/ml (SD=16.73).Concentrations of total IgG are similar to the ones described for species with similar placentation. Besides, if we considered IgG1/HCAbs ratio, it presents an important variation between individuals. However the concentration of IgG1 is higher than HCAbs in 8/12 of the samples. Finally the IgM showed higher concentrations than the rest of mammals

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with similar types of placentation. We are currently developing an anti-IgA serum which enables us to quantify this isotype in colostrum.

220. Endogenous galectin-1 endows dendritic cells with a Th2-polarizing profile during Schistosoma mansoni infection

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Schistosoma mansoni, a parasite trematode that infects 83.3 million people worldwide, promotes a CD4 T cell-mediated response and evokes multiple suppressive mechanisms to thwart host immunity. The clinical outcome relies on the ability of the host to transit from an early pro-inflammatory toward a Th2 chronic response. The mechanisms driving this transition are poorly understood. Our laboratory recently identified a tolerogenic circuit linking galectin-1 (Gal1), IL-27 and IL-10 which instructs dendritic cells (DCs) to become tolerogenic. The present study was conducted to examine whether endogenous Gal1 influences Th2-dependent immunity upon stimulation with S. mansoni egg antigen (SEA). Notably, SEA induced a dose-dependent up-regulation of Gal1 on DCs, as shown by western blot and real time qPCR ($p<0.05$). Analysis of the cytokine profile of wild type (WT) and Lgals1-/- DCs exposed to SEA (DCSEA) revealed no significant differences in IL-23, IL-27 and IL-12p70 secretion, although a statistically-significant decrease was observed in the amounts of IL-10 secreted by Lgals1-/- DCs ($p<0.05$). To investigate the T-cell stimulatory and polarizing capacity of these cells, we studied the cytokine profile in allogeneic co-cultures composed of WT and Lgals1-/- DCSEA from B6 and splenocytes from WT BALBc mice. DCSEA isolated from WT mice primed T cells to produce higher IL-5 and IL-10 levels ($p<0.05$) and lower IFN-γ ($p<0.05$) levels as compared to Lgals1-/- DCSEA. To study the relevance of these findings in vivo, we assessed the presence of Lgals1 mRNA by real time qPCR in liver and lymph nodes of CBA and B6 mice, used as models of high and low S. mansoni pathology. We found a dramatic reduction in Lgals1 mRNA ($p<0.05$) in high pathology CBA as compared to low pathology B6 mice infected with similar doses of this helminth parasite. These results suggest that endogenous Gal-1 plays a key role in S. mansoni -induced TH2 chronic disease by imprinting a DC2-type regulatory signature.

221. Comparison between the local and systemic immune-endocrine response in patients with pleural tuberculosis

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Earlier studies in patients with pulmonary tuberculosis (TB) revealed imbalanced immune-endocrine responses with adrenal steroids (cortisol and DHEA) modifying their specific cell-mediated immune response (IR). To extend these observations, the immuno-endocrine response at the lesional level of tuberculous pleurisy was analyzed. We compared in vitro effects of Cortisol and DHEA on lymphoproliferation by mononuclear cells from pleural fluids (PFC) and the circulating compartment (CC) of patients with pleural TB (PLTB). In parallel, levels of Cortisol, DHEAS (ELISA R&D), IL-1beta (ELISA, Invitrogen) and C reactive protein (PCR; turbitest, Winner Lab) were assessed in pleural fluids (F) and plasma (P) of PLTB patients (n=8), and P of TB cases with no pleural affectation (n=25) and healthy controls (HCo; n=21). In PLTB, Cortisol (10^{-6} M) inhibited lymphoproliferation of PFC and CC ($p<0.05$ and $p<0.01$ respectively, more evident in PFC), whereas DHEA caused no alterations in this regard (10^{-7}, 10^{-8}, 10^{-9}M). Lymphoproliferative capacity of Mtb-stimulated PFC was statistically higher than the CC counterparts and the proliferation yielded by CC cells from TB and HCo ($p<0.01$). PLTB patients had higher Cortisol levels in P respect to F ($p<0.05$), with P of PLTB and TB cases showing higher values than HCo ($p<0.05$). Also, P Cortisol levels in HCo were higher than those seen in F from PLTB ($p<0.05$). Reduced DHEAS levels and increased Cort/DHEAS ratio were seen in all TB samples respect to HCo ($p<0.05$). Pleural fluids had the highest IL-1beta levels respect to P of PLTB or TB and HCo ($p<0.01$). PCR levels were only augmented in P from PLTB ($p<0.05$ vs. P of TBC and HCo). Tuberculous pleurisy coexists with an increased T cell proliferation and production of pro-inflammatory mediators in presence of reduced amounts of Cortisol and DHEAS, all together suggesting a predominant immuno-inflammatory environment.

222. Serotype-Specific antibody response and B cell compartment helping in the therapeutic decision in Transient Hypogammaglobulinemia of Infancy and Specific Polysaccharide Antibody Deficiency

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Primary antibody deficiencies are heterogeneous group of disorders in which the fundamental defect is an inability to produce an effective antibody response to pathogen. Transient hypogammaglobulinemia of infancy (THI) and Specific polysaccharide antibody deficiency (SAD) are the most frequent immunological consult. Aim Correlation between serotype-specific pneumococcal antibody response (SS) and B cell compartment

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(LB) with clinical features in THI and SAD in order to define an adequate treatment. Materials and methods: We reviewed the medical records of 66 patients (Pt) with recurrent infections. 41 were THI (WHO criteria) and 25 were SAD (ESID-LAGID 1997criteria). Immunoglobulin levels were measured by kinetic Nephelometry, Polysaccharide antibody response was measured trough a commercial ELISA (GR) and 10 serotype specific response trough ELISA in house (SS), lymphocyte subsets and LB by flow cytometry. Results: All the patients were refereed to consult with delay. Both disorder had recurrent infection which began during the first year of life. It was predominant in male. Atopic disease and respiratory infections were the most common symptoms. 14% of THI and 45% of SAD had respiratory sequel with bronchiestasis. 73% of HTI recovered normal values of immunoglobulin levels in 2 or 3 years. 11 THI and 25 SAD presented an abnormal SS and LB altered. The most frequent LB alteration was an increase transitional B cells and decrease level of memory B cells. In those patients with respiratory sequel (6/11 THI and all SAD), in which gammaglobulin therapy (IVIG) was indicated, had improved their clinical symptoms. Conclusions: Is important to monitoring symptomatic THI and SAD. Some children spontaneously correct their immunoglobulin levels and functional testing, but other patients need to receive IVIG to improve their quality of life. Assessment of SS and LB was very important to indicate the early therapy in our patients.

223. Application of PCR in the diagnosis of otitis media with effusion: effect of commensal flora on Streptococcus pneumoniae virulence

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Otitis media with effusions (OME) can lead to significant hearing loss in childhood. The aim of the present study was to: a) investigate the presence of Streptococcus pneumoniae (Sp), Moraxella catarrhalis (Mc) and Haemophilus influenzae (Hi) in the clinical materials from OME; b) determine the effect of commensal bacteria isolated from healthy children (Streptococcus salivarius, Streptococcus bovis, Streptococcus mitis, Aerococcus viridians) on the biofilm formed by Sp isolated from OME; c) the effect of commensal bacteria on the necrosis induced by Sp on peripheral blood polymorphonuclear (PMN). Effusion obtained from 38 patients aspirated from the mid-ear were analyzed bacteriologically and also tested with PCR assay. Studies of inhibition of biofilm formation were performed by the technique of crystal violet and the PMN necrosis was measured with IP by flow cytometry. The detection of pathogens was significantly higher by PCR than by bacteriological cultures: Sp (p=0,004), Hi (p=0,002), Mc (p<0,002). Commensal bacteria (p=0.34) or their supernatants (p=0.73) are biofim inhibitory capacity of Sp. The isolated bacteria are more capable of inducing necrosis of PMN that their supernatants (p <0.001). The supernatants of different commensal bacteria differ in the induction of necrosis (p =0.003). Commensal bacteria and their supernatants potentiate the induction of necrosis induced by Sp. Our results indicate the PCR technique is more specific and sensitive in detection of bacteria in middle-ear effusion of OME, compared with conventional methods. We found that commensal bacteria and their supernatants studied not inhibit the Sp biofilm formation in Vitro. We conclude that the bacterial pathogen could promote the action of Sp stimulating the development of OME. To obtain inhibition of patogens like Sp it requires further studies with other bacteria in commensal flora of healthy children.

224. Immune reactivity of glial cells against the opportunistic fungus Candida albicans.

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When C.albicans colonizes the brain the inflammatory reaction is characterized by meningitis, micro and macroabscesses and reactive astrogliosis. Here we explored the In Vitro immune reactivity of glial cells against C.albicans and ligands of innate receptors (IR) involved in the fungal recognition. C57Bl/6 primary cultures from enriched astrocytes (As>96%/microglia M<0,3%, FACS) or Mix cultures (As>80%/M<10%) where exposed to different stimuli: C.albicans(5:1 ratio); Zymozan (Zym), PGN (TLR-2 agonist) and LPS (TLR-4 agonist). After 24h, supernatants were collected to study cytokines and cells for TLR2 and TLR-4 detection. Although the TLR-4 and TLR-2 expression in As is controversial, we detected significant and constitutive expression of both IR in As enriched cultures (IF, FACS). The fungus contact did not modify the TLR-2 expression in As and M. In response to TLR-2 and TLR-4 ligands, As produced significant amounts of TNF-a and IL-6(p<0,05) and undetectable levels of IL-1 and IL-10. Against the same stimuli, Mix cultures released higher concentration of TNF-a, IL-6 and IL-1 (p<0,05). When glial cells were exposed to C.albicans or Zym, a depressed cellular response was observed. Besides the expression of functional IR involved in fungal recognition, the As were unable to release cytokines in response to the fungus or Zym, and Mix cultures only produced low levels of TNF-a and IL-6. To explore if As can be stimulated by M or M-released factors, we exposed Mix cultures to fungus and supernatants (SCa) or cells were collected. SCa was added to As, and Mix cells were depleted in M (CD11b+ population by MACS-beads) to recover As (negative cells). In both conditions As mRNA levels were analyzed. Very low levels of TNF-a and absence of IL-1 were detected. M or SCa were unable to induce the As response. These results suggest that cell wall components or soluble factors could be involved in the modulation of glial reactivity promoting fungal brain infection.

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doi: 10:3823/413

225. Assessment of antibody response to polysaccharide Antigens: Cut-off value for a commercial ELISA.

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The functional study of antibodies (Ab) in response to natural or post vaccination antigenic challenge is a fundamental tool for the diagnosis of immunodeficiency. In children suspected of having immunologic compromise, the failure in the response to the 23 valent polysaccharide vaccine (23Pn), leads to the diagnosis and based on it the therapeutic recommendation. The World Health Organization published a gold standard technique (GS): quantification of serotype-specific pneumococcal (ST) Ab response through an enzymeimmune assay (ELISA). At present, to define the response to polysaccharide antigens, our laboratory performed 2 techniques: a commercial (c) ELISA which used as antigen the total mix of the serotypes present in the 23Pn, and the assessment of specific Ab response to 10 ST (GS). Aim: establish a cut-off point that enables us to improve the commercial ELISA diagnosis capacity, in children with suspected Humoral Immunodeficiency. Materials and methods: we studied 115 patients between 2 and 20 years (y) of age (median:5y), 55 girls and 60 boys, who were immunized to 23Pn. Pneumococcal IgG Ab were measured through a cELISA and specific Ab response against 10 ST, was measured through an ELISA in house. The results were analyzed with ROC curve. Results: based on the ROC curve drawn, we obtained with a positive predictive value (VPP) of 90% and a specificity (E) of 88%, an Ab title of 147 mg/l as cut-off point, that help us to select patients whose response is adequate. Also we defined with a Sensitivity (S) of 99% a cut-off point of 30 mg/l below which there is not response to the vaccine. Conclusions: Based on our results we get 2 cut-off points: one of them to define responder patients with a high VPP (90%), title = 147 mg/l. And the other to define non-responders with high S (99%): title = 30 mg/l. In between these two points, there remains a population of patients on whom the GS should be carried out to confirm the diagnosis.

226. Modulation of intestinal innate immune response by Giardia intestinalis.

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Giardia intestinalis is the etiological agent of giardiasis, one of the most frequent intestinal diseases worldwide that may present from mild symptoms to severe and protracted diarrhea. The specific mechanisms of pathogenicity and the major host defenses against Giardia infection still remain unclear. In the present work, we sought to gain insight in the activation and modulation of chemokine production by intestinal mucosa upon Giardia infection, using in vitro and in vivo models.In order to evaluate the expression of chemokines (CCL20, CXCL10 and CXCL2), cultured enterocyte-like Caco-2 cells were infected with Giardia trophozoites and 4 h later RNA levels were measured by QPCR and normalized to actin. There was a nearly 9-fold (9.18 ± 3.58) increase in CCL20 expression in infected cells. In addition, we assessed the expression in vivo of CCL20 using C57BL/6 infected mice. Giardia trophozoites (strain GS/H7) were administrated, and 2 h or 7 days post-infection duodenum samples were analyzed by QPCR. There was an 11-fold (10.98 ± 3.07) increase in CCL20 expression 2 h post-infection in infected animals compared with uninfected controls. No induction was detected at 7 days post-infection; coincident with no inflammatory alterations in the mucosa.We used a reporter Caco-2 cell line harboring the luciferase gene under the control of the CCL20 promoter to evaluate the modulation of innate response by Giardia infection when co-administered with flagellin, a TLR5 ligand that is an inflammatory stimuli to intestinal epithelial cell. In this format, dose- and strain-dependent inhibition of luciferase production was evidenced. Infection of Giardia 1h previous to flagellin administration showed an even more pronounced inhibition in the reporter activity.In view of these results Giardia intestinalis triggers a transient activation of mucosal innate response but can also modulate the production of proinflammatory chemokines triggered by the activation of innate receptors.

227. Characterization of the tolerogenic potential of T. cruzi-differentiated dendritic cells

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Recently, we have demonstrated the ability of trypomastigotes (Tp) to modulate the differentiation stage and functionality of bone marrow-derived dendritic cells (DC) to a regulatory phenotype in vitro. Tp fail to activate DC and modulate LPS activation. DC display tolerogenic properties: they produce high levels of IL-10, less IL-12 and induce a poor alloresponse. In addition, we have demonstrated that both live and heat-killed Tp (Tphk) induce the regulatory phenotype in DC, characterized by an alternative activation state with enhanced ERK1/2 and STAT3 phosphorylation during LPS-stimulation. MEK inhibition led to a reduced production of IL-10 ($p<0.001$) and a total reversion of the impaired capacity to induce a non-related antigen-specific response ($p<0.01$). In order to characterize other signaling pathways associated with IL-10 induction, we treated DC with specific pharmacologic inhibitors. Inhibition of NF-kappaB abrogated Tp+LPS-induced IL-10 production, but inhibition of STAT3 did not. The modulation of antigen presenting cells functionality as an evasion mechanism is a common strategy displayed by pathogens. To determine the role and the regulatory potential of DC differentiated in the presence of Tphk plus LPS with or

without MEK inhibitor (DCi or DCreg) in vivo, we transferred these cells into syngeneic naïve mice. Five days later, mice were challenged with a lethal dose of Tp. Preliminary results show that both types of DC apparently mediated the induction of a protective anti-parasitic response. Transferred mice exhibit lower counts of bloodstream forms, more pronounced in mice transferred with DCi. In addition, all DC recipient mice survived to the lethal challenge with T. cruzi after 35 days post-infection. In conclusion, these results suggest a possible protective role for regulatory DC during infection in vivo, though further studies are needed in order to unravel the role of immunoregulatory mechanisms during T. cruzi infection.

228. Regulation of microglial cell cytokine production by type 2 cytokines

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Inflammation plays an important role in central nervous system (CNS) infection and the progression of neurodegenerative diseases. Microglial cells are phagocytic myeloid cells located in the nervous parenchyma playing a key role in the initiation, progression and resolution of neuroinflammation. It is well known that microglial cells play a key role in mediating inflammatory processes in the CNS, which are associated with various neurodegenerative diseases. Given the potential beneficial effects of anti-inflammatory drugs and cytokines on the treatment and prevention of neurodegenerative diseases, we investigated the role of IL-4 and IL-13 in the regulation of cytokine production by microglial cells. First of all, we evaluated the effects of stimulation of mouse microglial cell lines with IL-4, IL-13 or INF-gamma, on the production of TNFalpha, IL-10 and IL-27. Both, IL-4 and IL-13 failed to induce the secretion of TNFalpha or IL-27 (p NS). However, IL-4 induced the secretion of the anti-inflammatory cytokine IL-10. In another set of experiments we tested if IL-4 or IL-13 were capable to inhibit the cytokine production of microglial cells activated by pro-inflammatory molecules, such as lipopolysaccharide (LPS) and INFgamma. We found that IL-4 inhibited the production of IL-6 on LPS-stimulated microglial cells ($p<0.01$). The effect of IL-4 was blocked by a monoclonal antibody specific to IL-4 ($p<0.01$). These preliminary results suggest that type 2 cytokines are effective modulators of cytokine production by activated microglial cells, than inducers of cytokine production in resting microglial cells. Interestingly, it seems that IL-4 is able to selectively induce the secretion of IL-10, a molecule important in the resolution of inflammation. Additional experiments are currently performed to evaluate the effects of Type 2 cytokines on the production of neurotrophic factors.

229. Bacteria interactions and virulence expression in cystic fibrosis

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The main cause of morbi-mortality in patients with cystic fibrosis (CF) is chronic lung infection. These infections are polymicrobial with higher incidence of Pseudomonas aeruginosa (PAE) and Burkholderia cepacea Complex (BCC). Commensal bacteria specifically Streptococcus Group millieri (SMG), were involved in the regulation of genes that express virulence factors in these pathogens, exacerbating pulmonary symptoms. The aim was to study the interactions between pathogens and commensal isolated from sputum CF patients on the biofilm formation, production of virulence factors and capacity of necrotizing PMN. Bacteria in sputum samples from CF patients with lung exacerbation were isolated by routine bacteriological methods and confirmed by PCR. The following strains were: BCC, PAE-S (antibiotic-sensible), PAE-R (multi-resistant), SMG. We analyzed the capacity of each bacteria and mixtures to produce: 1) biofilm production by cristal violet technique, 2) elastase by Congo red-elastin technique, 3) pyocyanin by spectrophotometry and 4) rhamnolipids by hemolysis of sheep red blood cells and inhibition of Bacillus subtilis growth (halo measuring) and 5) PMN necrosis by propidium iodide stains and flow cytometry. All strains produced biofilm. Inhibition of biofilm production was observed when mixtures between strain were performed ($p <0.0001$). All strains produced elastase DO540 (SMG: 104 BCC: 145; PAE-S: 197 and SAP-R: 191). Mixtures of strains increased elastase production ($p <0.0001$). Both strains of PAE produced pyocyanin (DO520:17) and rhamnolipids: 9 mm. Mixtures of strains increased production. PMN necrosis with: BCC: 1.9%, SMG: 1.7%, PAE-R: 1.7% and PAE-S: 1.4%. Association among SMG, BCC and PAE enhanced necrosis ($P<0.001$). The combination of pathogenic and "commensal" strains inhibits biofilm formation, but synergizes the virulence of all of them which would promote the pulmonary exacerbation and subsequent tissue destruction.

230. Characterization of the putative binding site of Staphylococcal Enterotoxin R (SER) to the murine TCR Vß8.2.

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Superantigens (SAgs) are bacterial or viral protein characterized by simultaneously binding to MCH-II molecules and TCRs. As a consequence of this interaction they activate a large number of T-cells through interaction with Vß domain of the TCR, triggering the massive release of cytokines that may cause Toxic Shock Syndrome. In addition, SAgs are tightly involved in auto-

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doi: 10:3823/413

immune processes such as diabetes mellitus, rheumatoid arthritis and multiple sclerosis. The potential use of SAgs and TCRs as therapeutics agents for these pathologies makes the biophysical and structural studies of importance. The latest incorporations to SEB subfamily or Group II were the staphylococcal enterotoxins G (SEG) and R (SER), which have amino acid sequence similarity. The Group II is characterized by interacting with the murine Vß8.2 TCR, the principal receptor involved in multiple sclerosis mouse model. Previously we have reported that SER and SEG present a differential behavior with Vß8.2, compared with other members of the group. The aim of the present work was to determine the putative binding site of the complex SER-Vß8.2. On that purpose, a model of SER structure was carried out with the Swiss Model server using SEG as a search model. The refined model was superimposed onto SEG-Vß8.2 structure using the Pymol computational program. Based on structural analysis, we designed SER mutants with point mutations in the Vß8.2 putative binding site. The mutants were expressed in E. coli and purified by exclusion molecular chromatography. The affinity for Vß8.2 was determinated by surface plasmon resonance showing mutants K19A (53 µM) and G20A (34 µM) lower affinity than the wild type (8 µM); or N24D, N24A and F204A mutants no interaction at all. The determined affinities allow us to characterize SER-Vß8.2 binding site and identify N24 and F204 as the residues that make the greatest energetic contribution to stabilizing the interaction.

231. Fcgamma RIIb: differential expression and functions in B cell subsets.

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FcgammaRIIb (FcR) is the only receptor for the IgG Fc portion expressed on B cells. FcR crosslinking induces apoptosis of plasma and splenic B cells. Our aim was to evaluate the expression profile of FcR on different B cell subsets and its relationship with cell survival and the ability to produce antibodies (Abs). Phenotypic analysis showed that among all B cell subsets, peritoneal B1 cells (pB1) expressed the highest levels of FcR. Accordingly, pB1 were much more susceptible to apoptosis induced via FcR than splenic B2 cells (sB2). To address the role of FcR in the homeostasis of the different B cell subsets, we studied FcR knockout (KO) mice. We found higher number of pB1 and similar number of sB2 in comparison with wild type (WT) mice. pB1 from KO mice showed in vivo the same level of BrdU incorporation than pB1 from WT, indicating that the increase in the number of pB1 in KO mice is not a consequence of an accelerated proliferation rate. We also observed that stimuli that induce B cells differentiation to Ab-secreting-cell (ASC), such as LPS or CpG, upregulated FcR expression to levels that were similar in all B cell subsets. Consequently, CpG-stimulated pB1 and sB2 were equally susceptible to FcR-induced apoptosis. To evaluate the association between high levels of FcR expression and the ability of cells to secrete Abs, we determined by ELIspot the frequency of ASC in FcRhigh CD138+ and FcRhigh CD138- cells sorted from CpG-stimulated pB1 and sB2 and FcRlow CD138neg cells sorted from unstimulated pB1 and sB2. We observed similar frequencies of IgM- and IgG- ASC in stimulated pB1 and sB2 cells that expressed high levels of FcR independently on the expression of CD138 while the unstimulated FcRlow cell population contained few or none ASC. This result indicated that the up-regulation of FcR on B cell subsets correlated with the acquisition of the ability to secrete Abs, postulating FcR as a new marker to identify plasmablast. Altogether our findings indicated that FcR is a key receptor controlling pB1 cell survival in steady-state conditions. Under stimulatory conditions, FcR was able to trigger apoptosis in all the B cell subsets in a mechanism highly dependent on the upregulation of this receptor. These results suggested that there may be a threshold in FcR expression beyond which all B cells become equally susceptible to FcR-mediated apoptosis.

232. Targeted disruption of lectin-glycan lattices abrogates hypoxia-driven angiogenesis, restores immune function and promotes remodeling of tumor vascular networks

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Resistance to VEGF-targeted antiangiogenic therapies suggests the contribution of alternative pathways to hypoxia-driven neovascularization. Previously we showed that galectin-1 (Gal1)-glycan lattices couple tumor hypoxia to VEGFR2-mediated angiogenesis through mechanism that are independent of HIF-1alpha and VEGF. The present study was conducted to elucidate whether disruption of Gal1-glycan lattices, using an anti-Gal1 mAb with blocking activity, may contribute to simultaneously remodeling of tumor vascular networks and stimulation of anti-tumor T-cell responses. In a xenograft model of human Kaposi´s sarcoma as well as in syngeneic B16 mouse melanoma, disruption of Gal1-glycan lattices abrogated hypoxia-driven angiogenesis, as shown by reduced frequency of CD34+ endothelial cells (ECs) (3-fold; $p<0.01$), increased association of ECs with mature pericytes (aSMA+, desmin+ and RGS5-) (2-fold; $p<0.01$) and decreased vessel diameter (2.7 fold; $p<0.01$) in tumors treated with anti Gal1mAb (F8.G7) versus those treated with isotype control. Administration of the F8.G7 mAb in the B16 model promoted a significant reduction in tumor growth (5-fold $p<0.01$) and evoked a T-cell specific immune response, as shown by increased T-cell

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proliferation ($p<0.01$) and augmented IFNg ($p<0.05$) and IL-17 ($p<0.05$) production compared to mice receiving control isotope mAb. Moreover, tumor draining LN of F8.G7-treated mice had lower frequency of CD4+CD25+Foxp3+ regulatory T cells ($p<0.05$) and lower IL-10 secretion ($p<0.05$) than mice receiving isotype control. This therapeutic approach was compared to shRNA strategies revealing comparable clinical outcomes. In human biopsies (n=15 KS and n=24 melanoma) expression of Gal-1 and specific glycans delineated the transition toward a malignant pro-angiogenic phenotype. Thus, disruption of lectin-glycan lattices, not only evokes an unleashed anti tumor immune response, but also reduces angiogenesis and favors remodeling of tumor vascular networks.

233. Galectin-1 (Gal1) co-opts VEGFR2 (KDR) signaling pathways through the formation of lectin-glycan lattices on highly branched complex N-glycans

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In previous studies we demonstrated that galectin-1 (Gal1) links tumor hypoxia and VEGF-mediated angiogenesis. The present study was conducted to investigate signaling pathways associated with this pro-angiogenic function and to identify potential glyco-receptors mediating these effects. We first examined the 'glycosylation signature' of ECs in resting conditions or exposed to proliferative, tolerogenic or inflammatory stimuli. In contrast to ECs stimulated with pro-inflammatory (TNF), TH1-(IFN-γ) or TH-17(IL-17) stimuli, ECs exposed to tolerogenic (IL-10 or TGF-β or proliferative (bFGF) signals exhibited a substantial up-regulation of cell surface N- glycans that are critical for galectin-1 signaling, an effect which was consistent with galectin-1 binding to ECs ($p<0.01$). Screening of the phosphorylation status of a spectrum of growth factor receptors using signaling arrays revealed a 2-fold increase in phosphorylation of KDR, Akt and Erk1/2 upon exposure to Gal1, a pattern comparable to that induced by VEGF. Pharmacological inhibition of Akt or Erk1/2 signaling abrogated Gal1-induced EC proliferation ($p<0.01$), migration ($p<0.01$) and angiogenesis ($p<0.05$). siRNA-mediated silencing of KDR completely prevented Akt and ERK1/2 phosphorylation induced by either Gal1 or VEGF-A. Interruption of N-acetylglucosaminyltransferase V (GnT5)-mediated N-glycan branching prevented Gal1 but not VEGF-A signaling. Co-immunoprecipitation revealed specific association of Gal1 with KDR through N-glycan-dependent interactions. Consistently, KDR blockade or interruption of N-glycan elongation prevented Gal1-induced EC migration ($p<0.01$) and morphogenesis ($p<0.05$), whereas blockade of VEGFR1, VEGFR3, NRP-1 or VEGF-A had no effect. Collectively, our results suggest that signaling complexes established between lectins and specific glycans might serve as alternative or compensatory pathways by mimicking 'cognate ligands', thus preserving critical cellular processes such as angiogenesis

234. Trophoblast cells recruit maternal Monocytes and modulate the chemokines and their receptors expression-profile under pathological conditions

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Macrophages constitute 20-30% of decidual immune cells and they coordinate the defense against pathogens, wound healing and tolerance induction against paternal antigens. Here, we study whether the maternal monocytes (MO) are attracting toward the trophoblat cells under physiological and pathological conditions. First, we performed migration assay with 8um transwell system using maternal PBMC in the upper compartment and trophoblat cells (Swan-71 cells line) in the lower compartment. The FACS analysis showed that trophoblast attracts CD14+ cells and this frequency significantly increases in the presence of Poli: IC ($p<0.05$ student T-test). In order to discriminate the chemokines/receptors involved we performed non-migrating 0.4um transwell system seeding maternal MO (purified by CD14-magnetic beads) in the upper compartment and Swan cells in the lower. After 24 hours, both populations were recovered and the expression of MCP-1, IL-8, RANTES and their receptors (CCR1, CCR3 and CCR5) evaluated by RT-PCR. While, MO increased almost 1.5 fold CCR1, CCR3, and CCR5 expression, Swan cells increased MCP-1 and IL-8 expression after the dialogue. When these cultures were performed in the presence of LPS or PGN, MO reduced the expression of all receptors. However, the treatment with Poli:IC, which simulates a viral infection, did not decrease the receptors expression and increased the levels of MCP-1 and RANTES produced by Swan cells. These modulations were not observed in MO without trophoblast dialogue. In conclusion, during the MO-trophobalst dialogue, the MO are more sensitive to chemokines produced by trophoblat cells. In the presence of bacterial antigens, chemokines receptors are reduce making MO less sensitive to the recruitment, however in a viral infection, MO recruitment is potentiated by chemokines produced by trophoblast cells and this effect could be related with the proabortive effect of viral infections.

235. Retrospective analysis of 40 children with the 22q11.2 deletion syndrome suspected: outcome of 17 patients positive by fluorescence in situ hybridization

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doi: 10:3823/413

22q11.2 Deletion syndrome, the most common congenital chromosome deletion syndrome, is associated with developmental abnormalities and defects including cardiac hypoplasia or abnormal migration of the thymus, hypoparathyroidism, and immunodeficiency. Aim: To describe the immunological characteristics of a group of patients suspected of 22q11.2 deletion syndrome and the correlation with clinical manifestations. Material and Methods: Retrospective analysis of medical records of 40 patients, 23 males / 17 females, age range: 0-15 years, 17 with positive cytogenetic study, one with negative cytogenetic study and 22 with results pending. We evaluated the lymphocyte population by flow cytometry, proliferative response, serum immunoglobulins, specific antibodies to tetanus toxoid and pneumococcal. Result: 14 of 17 with positive FISH had congenital heart disease, 6 hypocalcemia, 2 hypoparathyroidism, 11 lymphopenia. 3 of them had extreme lymphopenia (CD4+ <300/mm3) associated with CD31+CD45RA+RO- decreased and only one had low proliferative response. 8/11 showed correlation between lymphopenia and thymus. 11/17 had recurrent infections (otitis media, meningitis, sepsis, pneumonia). Only one patient required immunoglobulin replacement therapy. Conclusions: The most of the patients tested were FISH positive and heart disease cogenital and less frecuently hypocalcemia and hypoparathyroidism. We observed correlation between absent thymus glands, T lymphopenia and recurrent infections.

236. Antibody response to selected Yersinia enterocolitica antigens in synovial fluid of patients with chronic arthropaties

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Yersinia enterocolitica is an important causative agent of enteric infections in humans. Infections have been implicated in the pathogenesis of a number of autoimmune diseases. The mechanisms of Yersinia-triggered arthritis are unknown. We studied the frequency of anti-Yersinia antibodies in patients with rheumatoid arthritis (RA) and spondyloarthropathies (SpA). The IgA against the whole bacteria disrupted by sonication (SO), outer membrane proteins (OMP), cytoplasmic proteins (CIT), lipopolysaccharide (LPS) and culture supernatant proteins (SN) in synovial fluids (SF) were studied by ELISA. We also investigated antibodies (IgM, IgG e IgA) against Yersinia outer proteins (Yops) by western blot. We correlated the response to Yersinia antigens with anti-type I and II collagen antibodies. IFN-γ, IL-17, IL-23(p40/19), IL-1ß and IL-9 were assayed by ELISA in SF. IgA to several antigenic preparations of Yersinia were found in SF. In RA, 11/57 (19%), 4/36 (11%), 11/36 (31%), 10/57 (17%) and 13/57 (23%) SF were positive when SO, OMP, CIT, SN and LPS were used, respectively. In SpA, 1/15 (7%), 1/10 (10%), 1/10 (10%), 0/10 (0%) and 3/15 (20%) SF were positive when SO, OMP, CIT, SN and LPS were used, respectively. After Western blot, the positivity for Yops in patients with AR was 10/57 (17.5%). We found a significant correlation between IgA antibodies against all Yersinia antigens and antibodies to type I collagen ($p<0.05$), and between all IgA antibodies but anti-LPS and type II collagen ($p<0,05$). On the other hand, we found a significant correlation only between SN and IL-1ß ($p<0.05$). We concluded that Yersinia is associated with arthropathies in our population. We found a significant correlation between IgA to Yersinia and antibodies to type I and type II collagen, supporting the relation between mucosal infection and joint disease.We also found significant correlation between response to SN and IL-1ß, a cytokine that amplifies and perpetuates the disease process in RA.

237. Yersinia enterocolitica target Peyer`s patch dendritic cell subpopulations to evade the immune response.

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The gut-associated lymphoid tissue (GALT) comprises the Peyers's patches (PP) and mesenteric lymph nodes (MLN). Dendritic cells (DCs) are thought to be critical in the decision of whether to mount tolerant or protective immune responses. In PP and MLN, DCs consists of CD11c+CD11b+CD8a-, CD11c+CD11b-CD8a+, and CD11c+CD11b-CD8a- subsets (double negative; DN). Yersinia enterocolitica (Ye) is a Gram-negative pathogen that causes gastrointestinal diseases. Ye colonize the PP and may disseminate to the MLN, and subsequently, to spleen, liver, and lung. The interaction between Ye and mucosal DCs have not been previously investigated. The purpose of the present work was investigate the interaction and the role of mucosal DC subpopulations in enteric Ye infection. C57BL/6 mice (female 6 weeks-old) were orally infected with 5 x 108 Ye O:8 pYV+. Phosphate saline buffer (PBS) was orally administrated to a group of mice and used as control mice. After 3 or 5 days, animals were killed, PP were obtained and DCs were isolated. The phenotype of the isolated DCs were analysed by flow cytometry. Ours results indicate that Ye invades PP since colony forming units (CFU) were detected from this organ at days 3 and 5 after infection. A population with low CD11c expression was observed in infected mice, suggesting monocyte migration to PP after Ye infection. On other hand, we observed a significant decrease of CD8+CD11b- and DN cells in infected mice compared with PBS control mice ($p<0.01$). In contrast, CD8-CD11b+ DCs increased in Ye infected mice com-

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pared with control mice (p<0,002). In conclusions, ours results suggested that Ye affect DC subpopulations of PP after oral infection, inducing decrease of CD8+CD11b- and DN DCs and increase of CD8-CD11b+ DCs. Since CD8+CD11b- and DN DCs induce Th1 response which plays a critical role in the immune response to Ye, this result suggests that Ye could target theses DC subpopulations to evade the immune response.

238. Foxp3+ regulatory T cells in regional lymph nodes of arthritic TNFRp55-/- mice after Yersinia enterocolitica infection

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Foxp3+ regulatory T cells (Treg) are CD4+ T cells population that controls immune response in several mouse models. There are some works that demonstrated that selectively altering Treg cell distribution in vivo leads to the development of tissue-specific inflammation. We have demonstrated previously that TNFRp55-/- (KO) mice developed more severe and chronic reactive arthritis (ReA), compared with C57BL/6 wild-type (WT) mice, after an oral Yersinia infection. Also, we found that KO mice exhibited significantly differences in IL-10 levels, compared with WT mice, in regional lymph nodes (RLN) to the joint, and in mesenteric lymph nodes (MLN) at different days after infection. In vivo function of Treg cells in ReA remains unclear. The aim of this work was to determine, by flow cytometry analysis, the relative number of Treg cells in RLN and MLN of KO and WT mice, at days 7, 14 (arthritis onset) and 21 after an oral Y. enterocolitica O:3 infection, and then, to define the in vivo contribution of Treg cells in our mouse model. A significantly decrease in the relative number of Treg cells was detected in KO mice, at day 14 in RLN, compared with WT mice (3.65%±0.13 and 5.12%±0,46, respectively) (p<0,05). At day 21, KO mice showed higher relative number of Treg cells, compared with WT mice (4.36%± 0.26 and 3.39%±0.29 respectively) (p<0,05). However, the total number of Treg cell was unvaried. In addition, we did not find significantly differences in the relative number of Treg cells in MLN from KO and WT mice. We concluded that in contrast to mucosal site, there is a local correlation between the relative number of Treg cells and the IL-10 levels, found in previous studies. The unvaried Treg cell total number could be the result of an influx of other cells. The decreased relative number of Treg cells at arthritis onset could be responsible of a local unregulated response in this animal model.

239. Critical role of endogenous galectin-1 in regulating T helper responses during Yersinia enterocolitica infection

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In spite of considerable advances in elucidating the immunomodulatory functions of galectin-1 in limiting Th1 and Th17 responses in autoimmune settings, the role of this endogenous lectin in microbial infection has not yet been examined. Given that Yersinia enterocolitica, Gram-negative bacteria evokes Th1-producing IFN-γ immune response, we conducted this study to investigate the absence of endogenous galectin-1 in the development of immunity against this pathogen. We used C57BL/6 wild-type (WT) and Gal-1 knockout (Lgals1-/-) mice which were orogastrically infected with Y. enterocolitica O:8. Survival was compared in both infected mouse groups. On days 5, 14 and 21 after infection, mesenteric lymph nodes (MLN), spleen (Sp) and Peyer's patches (PP) were aseptically obtained, and in their homogenates, colony forming units (CFU) were counted. In addition, we determined concentrations of IL-17 and IFN-γ in PP and serum by ELISA. To elucidate their immunoregulatory effects, spleen cells from Lgals1-/- mice were adoptively transferred into WT mice; then, these recipient mice were challenged with Y. enterocolitica and CFU in organs, IL-17 and IFN-? in PP and serum at 5 days after infection were determined. Higher survival rate was observed in Lgals1-/- compared WT mice (63 % vs 37 %). Furthermore, less CFU in PP after 5 and 14 d after infection of Lgals1-/- mice (p <0.05) and adoptived-transferred WT mice (p<0.05) were determined. At day 21, we did not find CFU in PP, though, we found a decreased counting of CFU on Sp of Lgals1-/- mice (p<0.05). Moreover, higher levels of IFN-γ and IL-17 were detected in sera collected from Lgals1-/- mice and also from adoptive-transferred WT mice (p<0.05). We conclude that the absence of Gal-1 enhances Th1 and Th17 responses which can act to maintain protective immune response favouring eradication of Y. enterocolitica. This is the first report demonstrating a role of endogenous galectin-1 in regulating immunity to infection.

240. TCRalfa-beta (+) CD4(-) CD8(-) cells beyond Autoimmune Lymphoproliferative Syndrome

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An expanded (>2%) unusual T-cell subset which express alfa-beta T-cell receptor but neither CD4 nor CD8 (DNs) is pathognomonic for the autoimmune lymphoproliferative syndrome (ALPS). The markers CD28, CD38 and HLA-DR have been found on more than 90% of ALPS DNs, CD45RO on less than 10% and B220 on more than 50%. We have found expanded DNs in three definite conditions unrelated to ALPS, and their immunophenotype was established. Anticoagulated samples were stained using a whole blood lysis method and analyzed by flow cytometry. DNs were evaluated using a plot TCRalfa-beta x CD4+CD8

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(same color) and cell subsets on DNs were identified with third color conjugated monoclonal antibodies. Serum Fas-Ligand (sFasL) was determined by ELISA. Fas induced apoptosis (FIA) was evaluated on activated cells by flow cytometry on the basis of their DNA content. At the moment of DNs evaluation, patients presented as follows. Patient 1 (P1): 8 y-old girl with diagnosis of Systemic Lupus Erythematosus since she was 5 showed anti-dsDNA, anti-Sm, hypergammaglobulinemia, hemolytic anemia and thrombocytopenia without lymphoproliferation. Patient 2 (P2): 4 month-old boy with definitive diagnosis of Wiscott-Aldrich Syndrome, no lymphoproliferation and a history of severe CMV lung infection at 1 month. Patient 3 (P3): 16 month-old boy had lymphadenopathy, persistent hepatosplenomegaly, lymphocytosis, hypergammaglobulinemia, positive autoantibodies to erythrocytes, smooth muscle, and actin. The patient turned out to be HIV (+) by serological test and Western blot. Results: P1: sFasL: N (normal), FIA: N, DNs 6.78% (HLA-DR and CD45RO 52% and 46.7%). P2: DNs 6.3% (CD28 6.9%). P3: sFasL: N, FIA: N, DNs 5.09% (B220 32%). Conclusion: Elevated circulating DNs can be found in conditions others than ALPS. Their immunophenotype showed that they are expanded subsets other than the expected for ALPS. HIV infection represented a difficult differential diagnosis before virological results.

241. Autoimmune Lymphoproliferative Syndrome and lymphocyte apoptosis assay: more than 50% of the cell death should be considered as a normal result?

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Revised diagnostic criteria for autoimmune lymphoproliferative syndrome (ALPS) were recently reported from the 2009 NIH International Workshop. According to it, for a definitive ALPS diagnosis a patient has to meet both required criteria (RC) and one of the two primary accessory criteria (PAC). RC include chronic non-malignant non-infectious lymphadenopathy and/or splenomegaly and elevated TCRalfa-beta (+) CD4(-) CD8(-) cells. Defective lymphocyte apoptosis assay is one of the PAC, and it is considered abnormal if the patient's cells show 50% or less of the cell death observed in the control set up in parallel. Somatic or germline pathogenic mutation in FAS, FASLG or CASP10 is the other PAC. We present the lymphocyte apoptosis assay (LAA) from 4 ALPS-FAS patients (Pt) and 10 mutation-positive relatives (MPRs) with C91Y FAS mutation from 3 unrelated families. Related Pt1 and Pt2 had homozygous FAS mutation and both presented neonatal and severe disease. Pt3 and Pt4 were heterozygous. Fas expression on activated T cells was absent in Pt1 and Pt2 and diminished in Pt3 and Pt4. LAA: Peripheral mononuclear cells were activated for 9 days with PHA and IL-2 and transferred into Apo 1-3 coated culture plates. Apoptotic cells were resuspended in a hypotonic solution containing propidium iodide and apoptotic nuclei were counted as hypodiploid by flow cytometry. Results: Family 1: LAA Pt 1 and Pt 2: 0%; LAA MPRs: mother (m) 73%, father (f) 57%, brother (b) and sisters (s): 66%, 71% and 72% Family 2: LAA Pt 3: 76%; LAA MPRs: 68% (m), 71% (s) Family 3: LAA Pt 4: 55%; LAA MPRs: 59% (m) , 65% (s) 66% (s) It is suitable to stand out that Pt3 was originally subdiagnosed because his LAA was considered as in a normal range (>50%). Conclusion: There are patients with definitive diagnosis of ALPS who shows >50% of LAA. According to that, 50% should not be considered as a threshold for that test. This assumption can induce to ALPS subdiagnosis.

242. TCRalfa-beta (+) CD4(-) CD8(-) cells, B220 and sFasL as markers of Autoimmune Lymphoproliferative Syndrome

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Autoimmune Lymphoproliferative Syndrome (ALPS) is characterized by splenomegaly, lymphadenopaty, hypergamaglobulinemia, accumulation of more than 2% of TCRalfa-beta(+) CD4(-) CD8(-) cells (DNs) and autoimmunity due to lymphocyte apoptosis defects. Besides DNs, soluble Fas ligand (sFasL) and B220 on DNs have been reported to be increased in ALPS patients. We describe the markers found in 11 definitive (d) and 5 probable (p) ALPS patients, 17 mutation-positive relatives (MPRs) and 13 healthy relatives (HRs) studied in our laboratory. Anticoagulated samples were stained using a whole blood lysis method and analized by flow cytometry. DNs cells were evaluated using a plot TCRalfa-beta x CD4+CD8 (same color) and CD45R isoform B220 on DNs were identified with third color rat antimouse RA3-6B2 monoclonal antibody. Serum sFasL was determined by ELISA. B220 on DNs and sFasL were reported to be higher than 50% and 200 pg/ml respectively in ALPS patients. Results: 4% was the lowest value of DNs found either in dALPS or pALPS patients. The highest value (34%) corresponded to a homozygous patient. More than 3% DNs were present in 4/15 MPRs and in 1/8 HRs. More than 50% of B220 on DNs cells was found in 10/11 dALPS, 3/3 pALPS, 6/6 MPRs and 2/4 HRs. B220 more than 50% on non expanded DNs cells was found in 2/6 MPRs and 1/3 HRs. sFas L was increased in 5/5 dALPS, 3/3 p ALPS and 7/8 MPRs. Conclusion: Increased B220 expressed on DNs was not a constant finding in dALPS patients but it was in MPRs, even in those with non expanded DNT. Expanded DNs or increased B220 expressed on DNT in ALPS relatives are not predictive for Fas defect. Further studies must be done in them. Since increased values of sFasL was a constant finding in ALPS patients but it was not found to be increased in all MPRs, sFasL as a marker presents incomplete penetrance. According to the obtained sFasL values, all our tested pALPS patients have to be investigated as possible somatic FAS mutants.

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doi: 10:3823/413

243. Pulmonary tuberculosis infection in autoimmune polyendocrinopathy with candidiasis and ectodermal dystrophy. Acquired susceptibility?

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Auto-antibodies (auto-Abs) have been considered responsible for the multiple endocrinopathies in APECED patients. Besides, reported pathogenic anti-cytokine auto-Abs in these patients has been hypothesized to be involved in their marked susceptibility to Candida albicans. We present a 7-year-old girl with definitive diagnosis of APECED that showed hypoparathyroidism, autoimmune hepatitis, chronic mucocutaneous candidiasis and alopecy, who developed a pulmonary infection by Mycobacterium tuberculosis. We hypothesized that the serum of the patient could have anti-IFNgamma activity as a mechanism responsible for an acquired susceptibility to this pathogenic mycobacterial infection. The presence of a serum factor inhibiting IFNgamma was investigated making patient's serum contact IFNgamma (exogenous recombinant IFNgamma or IFNgamma produced by PHA stimulated PBMCs) and then measuring the remaining IFNgamma by ELISA. The anti-IFNgamma activity was measured by IFNgamma induction of HLA class II expression in control monocytes by flow cytometry in the presence or absence of the patient's serum. Results: Remaining IFNgamma in the patient's serum was 52% (using exogenous recombinant IFNgamma) and 50% (in the presence of stimulated PBMCs) related to the control. Adding patient's serum diminished the HLA class II upregulation by IFNgamma from Mean Channel Fluorescence (MCF): 812 to MCF: 409 Conclusion: The patient's serum showed anti-IFNgamma activity. Acquired susceptibility to mycobacterial infection could stem from the anti-IFNgamma activity. In APECED context, the presence of anti-IFNgamma auto-Abs must be evaluated.

244. Neutrophil function in a severe Leucocyte Adhesion Deficiency Type I (LAD I) patient

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Leukocyte emigration from the bloodstream to sites of inflammation involves multiple steps in an adhesion cascade. Leukocyte interaction with vascular endothelial cells is a pivotal event and is mediated by several families of adhesion molecules. LAD I is an autosomal recessive disorder caused by mutations in the common chain (CD18) of the beta2 integrin family. The absence of pus formation at the sites of infection is one of the hallmarks of LAD I. We present the in vitro studies of a severe LAD I patient that showed purulent material from cutaneous lesions.A 5-month-old boy presented omphalitis with purulent material, delayed separation of the umbilical cord and sepsis by Staphylococcus aureus, perianal and cutaneous abscess, small bowel perforation, neutrophilia, and CD18/CD11b absence. Microscopic observation and colorimetric assay for granulocyte adherence to plastic, aggregation assay and complement opsonized zymosan (OZy) induced respiratory burst (RB) as a measure of ligand binding ability of CD18/CD11b were performed in purified neutrophils. For colorimetric assay, PMA stimulated adherent cells were stained with methylen blue and the dissolved dye was measured at 630 nm. PMA induced aggregation was measured by a standard platelet aggregometer/recorder system. RB triggered by OZy was evaluated by chemiluminiscence in a liquid scintillation counter in the out-of-coincidence mode. In order to test the NADPH oxidase function, Dihydrorhodamine (DHR) 123 assay was done using PMA to bypass beta2-integrins. Results: Neutrophil adhesiveness to substrate and aggregation assays showed a complete functional defect. OZy stimulated RB was completely abolished. PMA stimulated RB showed a normal ability of neutrophil NADPH oxidase to oxygen radical generation. Conclusion: Residual neutrophil functional activity was not found by these in vitro assays. These results do not explain the pus formation in this LAD I patient.

245. Griscelli Syndrome type 2 (GS2): defective lymphocyte cytotoxicity is the only immune deregulation?

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GS2 is a rare autosomal recessive disorder characterized by hypopigmentation of the skin and hair, the presence of large clumps of pigment in hair shafts, and an accumulation of mature melanosomes in melanocytes due to defects in RAB27A gene. The immune deregulation observed in GS2 patients results from the absolute requirement for Rab27a in lymphocyte cytotoxic granule release.

In dendritic cells (DCs) from Rab-27a-deficient ashen mice, delay in the recruitment of a subset of lysosome-related organelles containing the membrane subunits of NOX2 to phagosomes with decreased reactive oxygen species (ROS) production have been demonstrated. We studied neutrophil (N) and monocyte (M) respiratory burst (RB) from 4 GS2 patients free of treatment. All of them showed the specific pigmentary features and hemophagocytic lymphohistiocytic syndrome derived from the critical role of the cytotoxic pathway in lymphocyte homeostasis. Dihydrorhodamine (DHR) 123 test by FACS was used to evaluate RB. Different PMA concentrations and incubation periods were tested. Ms and Ns were selected by their FSC/SSC properties. Intracellular gp91-phox content of saponin permeabilized Ms was determined by FACS using a PE conjugated monoclonal antibody. Results from patients were compared to age-matched normal controls. Results: DHR assay from all of patients showed reduced ROS production by Ms in all tested experimental conditions. Median Fluorescence Intensity from patients vs. controls showed: 44, 35, 24 and 46 vs. 184, 126, 181 and 172 at standard

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assay conditions. ROS production in Ns was normal. Intracellular gp-91 phox content in Ms was comparable to age-matched normal controls. Conclusion: GS2 monocytes showed defective RB like DCs from the mouse model.
Diminished gp-91 phox content was not the cause of defective RB. Results indicate that Rab-27a would be involved in RB depending on the cell type. These findings could widen the spectrum of immune deregulation in GS2 patients.

246. Enhancement of M2 muscarinic receptor-receptor interaction by IgG antibodies in Chagas'disease: Role of epitope specificity and antibody valency

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In previous reports we showed that circulating IgG antibodies (Ab) from Chagas'disease (ChD) patients can enhance M2 muscarinic receptor-receptor interaction. The aim of this study is to assess the role of epitope specificity and Ab valency in this modulatory effect. HEK 293 cells expressing fusion proteins derived from the M2 muscarinic receptor (M2R) (M2R-RLuc and M2R-YFP) were treated with the IgG fraction from ChD patients (ChD IgG), affinity purified Ab against the second extracellular loop (II-ECL) of the human M2R (anti-M2II-ECL IgG), or the Fab fragments from ChD IgG (ChD Fab), at a 50 µM concentration, and M2 receptor-receptor interaction was assessed by bioluminescence resonance energy transfer (BRET). The anti-M2II-ECL Ab fraction induced an increase in BRET (milibrets: mB) (16.2 ± 2.1 mB) which was similar to that promoted by ChD IgG (13.1 ± 3.2 mB) and significantly higher than the activity of ChD IgG depleted from anti-M2II-ECL Ab (0.2 ± 0.6 mB) or IgG from control subjects (0.3 ± 0.3 mB) ($p<0.001$). In BRET assays on membranes at low ionic strength buffer conditions, gallamine (100 µM) (a muscarinic allosteric modulator which binds to the II-ECL of the M2R), but not atropine (10 µM) (a muscarinic antagonist which interacts with the orthosteric binding pocket), impaired the effect of ChD IgG on BRET by 56% ($p<0.05$). Unlike the native ChD IgG, its Fab fragment did not promote an enhancement of BRET on our M2R-RLuc/M2R-YFP cell system (ChD Fab: 1.8 ± 0.56 mB; ChD IgG: 7.5 ± 1.0 mB) ($p<0.05$). However, incubation of cells with ChD Fab in the presence of an anti-human Fab Ab promoted a recovery of the original effect (ChD Fab + anti-human Fab: 8.6 ± 0.5 mB), indicating that the effect of anti-M2R Ab on BRET requires the integrity of the IgG molecule. Taken together, our data suggest that the enhancement of M2 receptor-receptor interaction by ChD-IgG occurs as a result of receptor crosslinking by bivalent antibodies directed against the II-ECL of the M2R.

247. Salmonella Typhi inhibits tumor proliferation and induces cell death in a T-cell lymphoma

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We have previously demonstrated the anticancer properties of an attenuated Salmonella Typhi (S. Typhi) vaccine strain against a murine T-cell lymphoma (EL4). When mice bearing a subcutaneous tumor were immunized twice with Salmonella, by injection into the tumor and in the draining lymph node areas, a significant reduction in tumor size of bacteria-treated mice, as early as 5 days after the first treatment, and a prolonged survival compared to untreated mice, were observed. The aim of present study was to elucidate molecular and immune mechanisms related to the antitumor therapeutic effect mediated by Salmonella. Our results demonstrated that immunizations with bacteria induced T and B lymphocyte expansion in tumor draining lymph nodes, 7 days after the first treatment ($p<0.05$). To investigate the potential of T cell to collaborate in the antitumor therapeutic effect, we performed experiments applying the same immunotherapeutic schedule in EL4 tumor-bearing Nude mice, and evaluated survival time and tumor growth. No differences in survival time were found between control mice and Salmonella-treated animals ($p>0.05$). In contrast, tumor growth was significantly retarded in mice inoculated with these bacteria ($p<0.05$). These findings suggest that T cells could be involved in the increase of survival, whereas the reduction in tumor size seems to be T-cell independent. Our study also demonstrates that EL4 cells fail to proliferate and die ($p<0.01$), in a time and dose-dependent fashion, when cocultured with Salmonella. Acridine orange-ethidium bromide staining of tumor cells suggests that bacteria-induced tumor cell death is mediated by apoptosis. These immune mechanisms could have in vivo relevance and thus contribute to eliciting antitumor immunity in mice treated with Salmonella. Overall, our study illustrates the potential usefulness of S. Typhi in immunotherapies against malignant tumors.

248. A therapeutic monoclonal antibody targeting TfR1 against Junín virus infection

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Junín virus (JUNV) is the etiologic agent of the Argentine Hemorrhagic Fever (AHF) and due to its high fatality rate is classified as NIAID Category A priority pathogen. The most reliable medical treatment in cases of infection with JUNV is passive

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immunotherapy with plasma from convalescent patients, but its availability is limited and poses the risk of transmission of transfusion-associated diseases. The gate of entry of JUNV into human cells is through the binding of its glycoprotein 1 (GP1) to the human transferrin receptor 1 (TfR1). Therefore, it is possible to prevent JUNV infection by blocking its binding to TfR1 with a therapeutic monoclonal antibody (mAb) targeting the same domain. We have developed a recombinant chimeric mAb (ch128.1) that comprises the variable region of the murine mAb 128.1 specific for the extracellular domain of human TfR1 with the constant region of human IgG3. The affinity of ch128.1 for its epitope is high, as determined by surface plasmon resonance using a Langmuir binding model (ka=2.85x10e5 Mse-1; kd=1.61x10e-3 se-1; Kd=5.65x10e-9 M). We observed by flow cytometry that incubation of the human cell line HEK293T with 50nM ch128.1 efficiently blocked nearly 90% of the entry of a retrovirus expressing GFP pseudotyped with the surface glycoprotein of JUNV. Fluorescence microscopy showed that ch128.1 prevented the binding and uptake of an attenuated strain of JUNV by the TRVb-1 cell line expressing human TfR1. In addition, in vitro infectivity assays using TRVb-1 as a host cell, and measured by plaque forming units in monkey Vero cell monolayers, showed that 200nM ch128.1 reduced JUNV infectivity more than 95%. Importantly, the same in vitro infectivity studies in human PBMCs showed similar results. These studies suggest that it is possible to prevent viral infection by means of targeting its gateway of entry into the host cells and that mAbs such as ch128.1 have the potential to be used as therapeutics for AHF.

249. Progesterone-induced immunosuppression in breast cancer is mediated by the coordinated action of different immune escape mechanisms

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Based on the anti-inflammatory and tolerogenic properties of progesterone and its ability to promote breast cancer progression, we sought to examine whether progesterone creates an immunosuppressive tumor microenvironment, either by controlling galectin-glycan interactions, favoring the secretion of inhibitory cytokines such as TGF-ß or inducing the differentiation of regulatory T cells (Tregs). The progesterone analogue medroxiprogesterone acetate (MPA) markedly up-regulated expression of galectin-1 in two hormone-dependent human breast cancer cell lines and in a mouse mammary adenocarcinoma (C4HD) at both protein and mRNA levels (2-3 folds). This effect was abrogated by pre-treatment with the antiprogestin RU486 indicating that the progesterone receptor was involved. In vitro MPA-treatment of mouse splenocytes induced a significant increase in the frequency of Tregs (Ct 5±0.4%, MPA 10^{-7}M 18±1.8%; p<0.01), skewed the balance toward a Th2-type cytokine profile (IFNγ/IL-10 Ct 25±2, MPA 3±0.4; p<0.01) and induced a dose-dependent inhibition of T-cell proliferation (MPA 10^{-7}M 50% inhibition). In vivo MPA-treatment of C4HD tumor-bearing mice increased the frequency of Tregs in spleen (Ct 11±0.4%, MPA 17±2%), tumor-draining lymph nodes (Ct 10±0.4%, MPA 15±1.7%; p<0.05) and tumor microenvironment (Ct 12±4% MPA 25±6%; p<0.05). Augmented frequency of Treg cells induced by MPA was associated with increased synthesis of TGF-ß1 and up-regulated expression of the SMAD4 transcription factor (3-fold) in splenocytes suggesting a progesterone-regulated axis involving galectin-1, Treg cells and the TGF-ß pathway. Finally, progesterone favored the homing of Tregs to tumor sites through induction of tumor-derived CCL22 (2-fold increase). Our results demonstrate that progesterone hierarchically fosters an immunosuppressive tumor microenvironment by co-ordinately regulating galectin-1 expression, stimulating the TGF-ß pathway and augmenting the frequency of Treg cells.

250. Progesterone-induced immunosuppression in breast cancer promotes lung metastasis

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Progesterone is a sexual steroid with immunosuppressive and tolerogenic properties that plays a crucial role in breast cancer progression. Here we investigated whether progesterone promotes tumor progression and metastasis in a hormone-independent breast tumor model by promoting an immunosuppressive tumor microenvironment. We used the invasive 4T1 mammary carcinoma that metastasizes to lymph nodes and lung. We observed that 4T1 cell line expressed high levels of galectin-1 (Gal1) as compared with normal gland tissue and other breast tumor cell lines. Progesterone (medroxiprogesterone acetate=MPA) treatment of 4T1 tumor-bearing mice increased Gal1 expression in the tumor stroma (2-fold) indicating that progesterone induced the recruitment of a Gal1+ population within the tumor. As regulatory T cells (Tregs) express high levels of Gal1, we then studied whether progesterone facilitates Treg cell differentiation and their recruitment to tumor sites. Interestingly, MPA augmented Treg cell frequency in the spleen both in tumor-free and in tumor-bearing mice (Ct 12% MPA14.5% 4T1 12% 4T1+MPA 15%; p<0.05). This increase in Treg cell frequency was accompanied with an induction in Treg cell homing to tumor sites (Ct 40±5% MPA60±8%; p<0.05). Increased Treg cell infiltration induced by MPA was dependent on the expression of tumor-derived Gal1, as 4T1 Gal1 knockdown tumors recruit significantly lower amounts of Treg cells to lymph nodes and tumor sites as compared to Gal1-expressing 4T1 tumors (4T1Scr 60±8% vs 4T1RNAsh3.7 38±2% p<0.05). Finally, in vivo treatment

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of mice with MPA induced lung tumor micrometastasis following inoculation of Gal1-expressing but not Gal1 knockdown 4T1 tumors (4T1Scr 325±20 4T1RNAsh3.7 60±8; p<0.01). These results indicate that progesterone fosters an immunosuppressive tumor microenvironment that facilitates lung metastasis through a pathway involving galectin-1-induced immunoregulation and recruitment of Treg cells to site of tumor growth.

251. Red blood cell ageing markers by flow cytometric analysis

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Red Blood Cells (RBC) senescence is associated mainly with a decrease in cell volume, an increase in cell density, loss of plasma membrane phospholipids asymmetry and by binding of autologous immunoglobulin G (IgG). Analysis of the processes that take place during the ageing of RBC is still very much hindered by the fact that it is very difficult to obtain homogeneous fractions that contain RBC of the same age.The aim of this study was to characterize cells of different ages: Senescent RBC (SeRBC) and Young RBC (YRBC) using light scater measurements, externalization of phosphatidylserine and binding autologous IgG. ACD anticoagulated blood samples were obtained from normal volunteer donors (n=11). RBC were labeled with: 1) PE-annexin-V in calcium buffer for 15 min in the dark, and 2) FITC-conjugated mouse anti-human IgG at 22ºC in the dark for 30 minutes. Flow cytometric analyses were carried out using a FACSAria II and analyzed using FACSDiva and Paint a Gate software. RBC were selected using forward scatter (FSC) and side scatter (SSC) gates and read on a dot plot (FL1 vs FSC and FL2 vs FSC). Thirty thousand cells were analyzed from each sample. Dot-plot analysis based on the FSC (cell size) versus SSC (cell density) parameters shows two RBC populations of different sizes and density. The fraction of annexin-V positive RBC were SeRBC:0.98±0.12% and YRBC:0.15±0.05%, p<0.01. Events that correlated with the IgG binding RBC were analyzed for mean fluorescence intensity (MIF) (SeRBC:748±108, YRBC:47±15; p<0.001). The % of IgG positive cells were SeRBC: 1.5% and YRBC: 0.16%; p<0.01. The MIF of IgG binding and % of IgG positive cells are significantly different in the two regions: low FSC and higher SSC (SeRBC) over the area of highest FSC and lowest SSC (YRBC). These findings indicate that flow cytometry permit differentiating RBC populations of different ages. This methodology could be an alternative tool to study RBC ageing. .

252. Fermented soy product consumption modulates experimental mammary tumor growth

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Cancer is generated by uncontroled cellular proliferation. Activated macrophages polarize and may stimulate a cellular immune response or a humoral immune response. On theory, an effective immunoterapy can modulate differentiation of involved cells. Among immunotherapies, there is functional food consumption. We intended to observe if consumption of soy product fermented by Enterococcus faecium CRL 183 and Lactobacillus helveticus ssp jugurti 416 has the ability to modulate immune system or tumor growth. Balb/c mice received 0.5 mL of saline (SS, control) by gavage, or 0.5 mL of fermented soy product (FSP) daily for 40 days. On 10th day, animals were inoculated subcutaneously with LM3 cells ($1.25x10^4$ cells), a murine mammary adenocarcinoma cell line. Animals were sacrificed and tumor volume was observed and analyses for apoptotic cell presence were done using In Situ Cell Death Detection kit (Roche). Macrophages and lymphocytes were cultivated separatelly with stimuli (LPS and ConA respectivally) for 24hrs. Cytokine measurement on supernatant was done using capture ELISA kits (BD Biosciences). FSP showed a final tumor volume reduced (FSP: 1.45±0.39 cm^3; SS: 3.68±0.76 cm^3; p<0.05). Apoptotic tumor cells were more present on FSP (3.80±0.29 cells/field) than SS (0.79±0.25 cells/field) (p<0.05). FSP showed a higher production of TNF by macrophages (FSP: 132.40±14.25 pg/mL; SS: 77.11±5.10 pg/mL; p<0.001). Interestingly, splenic lymphocytes showed lower production of IFNγ (FSP: 62.61±14.10 pg/mL; SS: 169.40±20.07 pg/mL; p<0.05), lower IL-5 production (FSP: 60.84±6.32 pg/mL; SS: 232.10±38.73 pg/mL; p<0.001), without affecting IL-4 production (FSP: 59.01±7.25 pg/mL; SS: 54.38±5.92 pg/mL; p>0.05). These results indicate that FSP consumption may influence tumor growth favoring the host, but it is not clear if this action may be generated trough immune system modulation.

253. Autoantibodies from breast cancer patients regulate adhesion in MCF-7 human breast adenocarcinoma cells by activation of muscarinic acetylcholine receptors

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Muscarinic acetylcholine receptors (mAChR) are expressed in human breast tumor tissue and in MCF-7 cells, a human mammary adenocarcinoma cell line, while they are absent in normal mammary cells, MCF-10A. We described the presence of anti-mAChR antibodies (Abs) in the sera of breast cancer patients in stage 1 (T1N0Mx: tumor diameter<2cm, without lymph node

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metastasis). We have demonstrated that the muscarinic agonist carbachol (CARB) stimulated tumor cell proliferation and migration. Metastases are the first cause of death in cancer and it occurs when cells lose adhesion and migrate to distant organs. The aim of this work is to investigate the role of mAChR activation by CARB or T1NoMx-Abs on MCF-7 cells adhesion in an in vitro assay. We demonstrated that CARB inhibited tumor cell adhesion in a concentration dependent-manner, being 10^{-10}M the maximal effective concentration (25.6 ± 0.97 %; p<0.001 vs. basal). Pre-incubation of cells with atropine (AT: 10^{-9}M) a non selective muscarinic antagonist, 4-diphenylacetoxy-N-methylpiperidine methiodide (4-DAMP: 10^{-9}M) a selective antagonist for mAChR3 or tropicamide (TROP: 10^{-9}M) a selective antagonist mAChR4, reverted the inhibition produced by CARB. In addition we observed that T1N0Mx-Abs from 5 patients with breast cancer mimicked the action of CARB producing a concentration-dependent inhibition in MCF-7 cells adhesion, being 10^{-8}M the maximal effective concentration. The inhibition produced by T1N0Mx-Abs ranged from 16.71±3.4 % to 40.19±8.8 % (p<0.001 vs. basal). These effects were always reverted with AT and TROP but 4-DAMP was only effective to reduce Abs action in 3 of 5 patientes. CARB or Abs did not modify MCF-10A cells adhesion. IgG from normal patients or with mammary benign fribroadenoma, did not modify MCF-7 cells adhesion. We can conclude that anti-mAChR Abs present in breast cancer patients' sera at early stages by stimulating migration and inhibiting adhesion could be promoting breast cancer metastases.

254. Role of INFγ, IL17 and IL10 producing cells in an experimental model of autoimmune prostatitis.

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Extensive work has been done regarding the development and characterization of rodent models of experimental autoimmune prostatitis (EAP), models that could be considered as appropriate animal models for the human disease named Chronic prostatitis/chronic pelvic pain syndrome. In the present work we analyze the role of INFγ, IL10 and IL17 producing cells in autoimmune prostatitis. Prostate extract plus adjuvant was used to immunize Balb/c, NOD and NOD INFγ deficient (NODINFγ-/-) mice. Control groups, immunized just with adjuvant were also included. Mice were sacrificed at day 24 after immunization and the presence of antigen specific INFγ, IL10 and IL17 producing cells was evaluated by ELISA and FACS. Infiltration of the prostate gland was also analyzed by conventional histology and FACS. No antigen specific INFγ, IL10 and IL17 producing cells were observed in lymph nodes and spleen of control mice of the 3 strains tested. A high secretion of specific IL17 was observed in culture supernatants of Balb/c, NOD and NODINFγ-/- mice (Balb/c: 356±53 pg/ml; NOD: 870±94 pg/ml; NODINFγ-/-: 1678±233 pg/ml). Specific IL10 secretion was mainly observed in culture supernatants of Balb/c and NODINFγ-/- mice (Balb/c: 230±22 pg/ml; NOD: 89±39 pg/ml; NODINFγ-/-: 150±40 pg/ml). A significant elevated secretion of specific INFγ was observed in culture supernatants of NOD mice (Balb/c: 370±25 pg/ml; NOD: 4039±660 pg/ml; NODINFγ-/-: ND). When histological infiltration was analyzed higher severity scores were observed in NOD prostate glands while minor infiltration was detected in Balb/c and NODINF-/-. Prostate gland infiltration observed in NOD mice was composed mostly by CD3 cells with high proportion of CD8 and CD4 cells. These results indicate that the presence of specific INFγ producing cells is highly associated with marked infiltration of the prostate gland, while the only presence of IL-17 producing cells is not enough to generate the disease.

255. Susceptibility of mice deficient for IL17 receptor to the development of Experimental Autoimmune Prostatitis.

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Our laboratory has developed over the past decade a mouse model of experimental autoimmune prostatitis (EAP) that is considered a valid model for the human disease named Chronic prostatitis/chronic pelvic pain syndrome. In the present work we analyze the susceptibility of mice deficient in IL17 receptor IL17 (RIL17-/-) to the development of EAP. Prostate extract plus adjuvant was used to immunize wild type and RIL17-/- C57BL6 mice on days 0 and 15. Control groups immunized only with adjuvant were included. Mice were sacrificed at day 24 after immunization and the presence of antigen specific INFγ, IL10 and IL17 producing cells was evaluated by ELISA and FACS. Infiltration of the prostate gland was also analyzed by conventional histology and FACS. No antigen specific INFγ, IL10 and IL17 producing cells were observed in lymph nodes and spleen of control mice. Lower secretion of specific IL17 and INFγ was observed in culture supernatants of RIL17-/- mice when compared with values observed in wild type mice (IL17= wt 893±140 pg/ml, RIL17-/-: 178±96 pg/ml; INFγ= wt 2431±348 pg/ml, RIL17-/-: 153±140 pg/ml). No significant differences were observed when specific IL10 was analyzed in both groups (IL10= wt 270±70 pg/ml, RIL17-/-: 261±43 pg/ml). When prostate sections were analyzed, mononuclear cell infiltration with epithelial acini atrophy was seen in C57BL6 wild type glands but neither infiltration nor lesions were observed in RIL17-/- mice. These results indicate that the absence of IL17R made mice resistant to EAP, being these mice not able to generate neither Th17 nor Th1cells. Our results argue for an important role of IL17R in EAP and suggest a cooperative relation between Th1 and Th17 cells.

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256. Over expression of CD95 and opportunistic recurrent infection in a man with idiopathic CD4 T lymphocytopenia. Loss of expression of CD95 after immunomodulation by IL2 therapy.

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Idiopathic CD4+T lymphocytopenia (ICL) is a rare disorder defined by CD4+ T cell counts below 300 cells/mm^3 in the absence of HIV infection or other known immune deficiency. Apoptosis provides one possible mechanism involved in peripheral CD4+ T cell homeostasis, Authors reported a possible association between high expression of CD95 (Fas/APO-1) in CD4+ lymphocyte and ICL. Aim: Immunomodulation with recombinant IL2 (rIL2) treatment in a 49 years old with ICL. Case report: heterosexual man with opportunistic infections (Histoplasmosis, Herpes Zoster and Papilloma virus). HIV I/II and HTLVII negative, CD4: 120 - 180 cells/mm^3, CD95 over expression, increase count of CD45R0CD95CD4 and low CD45RACD4 cells, abnormal T cells proliferative response to mitogens, OkT3 and antigens. He had a decrease of memory B cells compartment. He received the rIL2 treatment of 0.4 UI every 12 hours, for 5 days per month, for 12 months. Method: Was conducted clinical monitoring immune. Result: After of rIL2 treatment CD4CD45RA, CD95CD45RO counts and T proliferative response improved. Since two years maintained good levels of CD4 cell proliferative response and disease-free. Conclusion: ICL should be considered as one of the predisposing conditions of opportunistic infections. CD4 T lymphocytopenia may be associated with high levels of CD95. CD4 depletion has an impact on B cells development. rIL2 treatment reduced CD95 expression in CD4Tcells. After 2 year of rIL2 treatment he is in health. rIL2 could be a therapeutic option.

257. Administration of IL-12 and GM-CSF in immunizations schemes delivering CRF12_BF Nef from DNA and MVA vectors enhanced both the magnitude and breadth of the immune response induced

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The Argentine HIV epidemic is characterized by the co-circulation of subtype B and BF variants. During the acute infection the majority of the HIV cellular immune response (CIR) is directed against Nef, placing it as a good candidate vaccine antigen. Here, we used it as a tool to study the impact of HIV-1 BF variants in the design of vaccines, since it is one of the most HIV variable proteins (difference B and BF: 24%). Previously, DNA and MVA vectors expressing NefBF were developed, finding a high specific response but with low cross-reactivity against NefB. The aim of this study was to analyze if the co-administration of molecular adjuvants, IL-12 and GM-CSF, could enhance the induced CIR generating higher levels of cross-reactivity. Mice received a prime, intramuscularly as follows: 3xDNAnefBF (Group I, GI), 3x DNAnefBF+DNA-IL-12 (GII), or DNA-GM-CSF (GIII), or both cytokine vectors (GIV). Afterwards all the groups were boosted with a dose of MVAnefBF intraperitoneally. Nine days after the last immunization, the specific CIR was evaluated in the spleen by ELISPOT using overlapping peptides comprising the full NefBF or B proteins. The highest response was detected in mice with both adjuvants (GIV, $p<0.02$). Cross-reactivity was higher in the groups with adjuvants (GII, GIII and GIV) compared to GI ($p<0.0007$), but there was no difference among them ($p>0.05$). After mapping the response to identify the peptides targeted, we found some differences: all the groups with one or two adyuvants recognized more peptides (mainly two BF peptides from N-terminal region and the loop of the protein) than the group without adjuvants (only recognized the protein loop), increasing the response magnitude and breadth. Here, we found that the use of the molecular adjuvants enhanced the CIR, expanding the breadth and cross-reactivity against subtype B. These results will be of high relevance in the design of future vaccines for our region.

258. Pediatric paroxysmal nocturnal hemoglobinuria. Experience in a single institution.

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Paroxysmal nocturnal hemoglobinuria (PNH) is defined as a complement disorder in the classification of the International Union of Immunological Societies Expert Committee on Primary Immunodeficiencies, although it is a nonmalignant clonal X-linked acquired disorder that results from somatic mutations in PIG-A gene in a hematopoietic stem cell. As a consequence, a partial or complete deficiency of all glycosyl phosphatidylinositol-anchored proteins (GPI-AP) is found in all its progeny. It is very rare in the pediatric population. The lack of one of the GPI-AP complement regulatory proteins, CD59, leads to complement mediated hemolysis that is the primary clinical manifestation of the disease. We present three pediatric patients (9, 15 and 16 years old at onset of symptoms) that showed clinical and laboratory findings indicative of intravascular hemolysis and in 2 of them, thrombotic events. The diagnosis of PNH was based on flow cytometry detection of abnormal GPI-AP expression on peripheral blood cells. PNH was diagnosed in two patients due to complete absence of CD55 and CD59 on neutrophils, monocytes and/or erythrocytes, and in the other one by the detection of an initial clone affecting 40% of neutrophils that evolved to 92% on neutrophils and 20-60% of erythrocytes in a 4 year follow-up period. The three patients had a fatal course, one after receiving a related identical bone marrow transplantation. Based on the expression profile of GPI-AP, evaluated by flow

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doi: 10:3823/413

cytometry on neutrophils (mainly CD58 and CD59), monocytes (CD14, CD55, CD59) and erythrocytes, three pediatric patients were diagnosed with PNH. Considering the fatal outcome of these patients, and the feasibility of the flow cytometric evaluation of the GPI-AP, it´s important to consider this diagnosis in patients presenting clinical symptoms suggestive of PNH.

259. Nanostructure as system for optimizing efficacy of CpG-ODN

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CpG-ODN has successfully used as vaccine adjuvant but unfortunately has a reduced bioavailability. In order to improve their bioavailability we have used CpG-ODN formulated in a nanostructure of 6-O- ascorbyl palmitate (Coa-ASC16), which has the ability to form supramolecular aggregate that are produced by phase cooling below a critical micellar temperature. Previously, we have observed that this strategy increased the bioavailability considering that improved the CpG-ODN adjuvant activity. However, we still ignore the mechanisms by which Coa-ASC16 works. Perhaps more than one factor could be synergistically contributing. Here, we tested whether Coa-ASC16 "per se" is able to stimulate immune system. Mice were i.p. injected with Coa-ASC16 or without Coa-ASC16 (control group). Coa-ASC16 induced a recruitment of neutrophils (Ly6Ghigh, F4/80-, CD11b+, Ly6C+) (23±8 vs control: 0.06±0.09 % after two hours injection $p<0.05$) and inflammatory monocytes (Ly6Chigh, Ly6G-, F4/80-, CD11b+) (25±3 vs control: 3±1 % after six hours injection $p<0.05$) into the peritoneal cavity. We also observed production of IL-6 (ng/ml, 0.8±0.4 vs control: 0.07±0.02 after two hours injection $p<0.05$) in macrophages, neutrophils and inflammatory monocytes. These experiments were also carried out in TLR4-/- animals obtaining the same result. In addition, we evaluated the levels of LDH, ALT and AST enzymes released into the peritoneal lavage to assess cell damage/lysis. Coa-ASC16 induced an increase of levels of these enzymes two hours after injection: LDH (U/l) (7100±1600 vs control: 1400±780 $p<0.001$), AST (U/l) (230±74 vs control: 37±12 $p<0.001$), ALT (U/l) (92±38 vs control: 19±7 $p<0.005$). These observations suggest that one of the factors by Coa-ASC16 may enhance the effect of CpG-ODN is the recruiting of innate immune cells to the site of injection. Coa-ASC16 cause tissue damage at the site of injection and then could act as "endogenous danger signals" which may initiate a sterile inflammatory response.

260. Potential use of immunobiotic lactic acid bacteria for the recovery of innate immune response in a cyclophosphamide-immunosuppression mice model

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Cyclophosphamide (Cy) is a drug commonly used in the cancer treatment because of its high toxicity on tumor cells. However, Cy induces myelosuppression leading to a state of immunosuppression with increased susceptibility to opportunistic infections. Previously, we demonstrated that the preventive treatment with immunobiotic lactic acid bacteria (LAB) improves the recovery of myeloid cells populations in Cy-treated mice. This work evaluated the ability of the preventive treatment with immunobiotic LAB to improve the innate immune response against Candida albicans (Ca) in Cy-treated mice. Different groups of Swiss-albino mice were fed Lactobacillus casei CRL431 (Lc431, 10^9 cells/d/mouse) or L. rhamnosus CRL1506 (Lr06, 10^8 cells/d/mouse) for 2 or 5 consecutive days respectively. After each treatment, these mice and untreated control mice (CG) received an intraperitoneal injection of Cy (150 mg/Kg). Later we analyzed the resistance to systemic challenge with Ca by assessing the survival of infected mice and pathogen count in blood, liver and spleen. In addition, we studied the innate immune response against Ca through the analysis of phagocytic cell counts in blood, and their recruitment to peritoneal cavity (pc), using both hemacytometer methods and flow cytometry. The preventive administration of Lc431 and Lr06 induced: a) The increase of the survival of Cy-treated mice infected with Ca (CG=40; Lc431=70; Lr06=60 %); b) The decrease of the pathogen number in infected tissues and its early elimination from liver and spleen; c) The improvement of leukocytes recruitment into the injury site (pc leukocytes CG=1,7±0,7; Lc431=6,0±0,5; Lr06=6,3±0,1 10^6 cells/L); and d) The increase of Gr-1+ cells in blood and peritoneal fluid (pc Gr1+ cells CG=2,1±0,6; Lc431=37,35±3,0; Lr06=35,0±2,4 10^5 cells/L). Therefore, the influence of preventive administration of LAB on innate immunity would be responsible, at least in part, of the increased resistance to infection by Ca.

261. Effect of immunobiotic Lactobacillus rhamnosus nasal treatment on the recovery of T lymphopoiesis in malnourished mice

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Thymus produces T cells throughout life but it has no self-renewing ability and requires replenishment and recruitment of progenitors derived from the bone marrow (BM). In this work we evaluated the effect of the Lactobacillus rhamnosus CRL1505 nasal administration on recovery of T lymphopoiesis in malnourished mice. Weaned mice were malnourished after consuming

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protein-free diet for 21d. Malnourished mice were repleted for 7d with balanced conventional diet (BD) or BD with nasal administration of Lr05 (10^8 cells/d/mouse) on d 6 and 7 (Lr05). Well-nourished (WC) and malnourished mice (MC) were used as control. At the end of treatments we determined body weight and studied the total cell number and T cells (CD3, CD4, CD8) in BM and thymus. Malnutrition induced a significant decrease in total cells and lymphocytes (FSC vs SSC) number in the BM and thymus ($p<0.05$). Both repletion treatments normalized total cells number in BM, but only Lr05 treatment was able to normalize lymphocytes number (WC=10.5±2.3; MC=5.5±0.4; BD=5.1±0.7; BD+Lr05=11.7±2.4 106 cells/mouse). In addition, repletion with BD or Lr05 induced a significant improvement in the number of these cells in thymus. Malnutrition significantly reduced CD3+CD4+, CD3+CD8+ and CD4+CD8+ cells, while CD4-CD8- cells were not affected in thymus. Both repletion treatments recovered the number of CD3+CD4+ cells, reaching higher values than WC. Lr05 group showed higher CD3+CD8+ cells number than BD group (WC=78.0±14.0; MC=2.1±0.7; BD=22.4±2.0; BD+Lr05=36.9±5.3 106 cells/mouse). In addition, we found that the number of immature T cells (CD4+CD8+ and CD4-CD8-) in BD group was higher than Lr05 group. Malnutrition increased CD3+CD4+ and CD3+CD8+ cells in BM. Repletion treatments significantly decreased these cells that reached lower values than WC. Hence, the nasal treatment with immunobiotic Lr05 is able to induce thymus replenishment and it should be considered in therapeutic approaches in malnutrition.

262. The nasal administration of Lactobacillus rhamnosus promotes the recovery of lung B cells affected by malnutrition

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The nasal administration of Lactobacillus rhamnosus CRL1505 (Lr05) is able to improve the innate and specific immune responses against Streptococcus pneumoniae in malnourished mice. In this work, we analyzed the effect of the nasal administration of Lr05 on the recovery of lung B cells during a repletion treatment. Weaned mice were malnourished after consuming a protein-free diet (PFD) for 21d. Malnourished mice were repleted with a balanced diet (BD) for 7d or BD 7d with Lr05 nasal administration (10^8cells/mouse/d) on d 6 and 7 (BD+Lr05). The malnourished control mice (MC) received PFD while the well-nourished control group (WC) consumed BD. At the end of the treatments, we evaluated: body weight, total number of lung cells and the B cell population of lung by flow cytometry (CD19, B220, HSA, IgM, IgD). The MC mice showed lung cell counts significantly lower than the WC. Both repletion treatments increased lung cells but only the BD+Lr05 mice reached higher values than the WC group ($p<0.05$). Malnutrition decreased lung lymphocytes (FSC vs SSC) and BD+Lr05 tretament was able to normalize this parameter (WC=9.71±1.8; MC=3.81±0.39; BD=6.78±1.6; BD+Lr05=10.9±1.17 105 cells/mouse). The MC group showed a significant decrease of B cells in lung compared to WC mice ($p<0.05$); due to the decrease in the number of mature (B220highHSAlowIgM+IgD+) and immature (B220low HSAhighIgM+IgD-) B cells. The treatment with Lr05 induced an improvement in the number of lung mature B cells (WNC=1.38±0.2; MNC=0.38±0.02; BD=0.89±0.2; BD+Lr05=1.99±0.21 10^5cells/mouse). On the contrary, BD treatment failed to normalize this parameter. In addition, both repletion treatments induced the normalization of immature B cells. This work demonstrates that the nasal administration of Lr05 during the repletion diet promotes the recovery of lung B cells. This effect on B cell would explain the ability of immunobiotic Lr05 to improve the specific immune response against S. pneumoniae in malnourished mice.

263. Spermatozoa modulate the differentiation profile of dendritic cells: a new tolerogenic mechanism?

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Semen is one of the main vectors for the dissemination of a number of infectious agents. It is well known that seminal plasma contains immunomodulatory agents such as TGF-β and PgE_2. Much less is known about the imunomodulatory abilities of spermatozoa (Sp). This study intends to analyze the ability of Sp to modulate the differentiation profile of human dendritic cells (DC).

Semen samples were obtained by masturbation of healthy donors and Sp were purified by swim-up. Cell supernatants (SN) were obtained from Sp ($10x10^6$ Sp/ml) cultured for 24 h at 37°C in RPMI 1640 medium supplemented with 10% FBS. DC were obtained by incubating monocytes (>85% purity) with GM-CSF and IL-4 for 5 days, in the absence or presence of Sp (Sp: monocyte ratio 4:1) or SN, used in a final dilution of 1:15. DC phenotype was analyzed by flow cytometry and the ability of DC to induce the proliferation of allogeneic lymphocytes was assessed using CFSE-labeled lymphocytes. We observed that differentiation of DC performed in the presence of Sp or SN resulted in a marked reduction in the expression of CD1a and an increased expression of CD14. The mean fluorescence intensity (MFI) for CD1a was: 7464 ± 106, 766 ± 35 and 210 ± 25 (n=5-8, $p<0.05$ for controls vs Sp- or SN-treated cells, respectively). We also found that either Sp or SN significantly ($p < 0.05$) increased the expression of HLA-DR, CD86, CD40 and CD80, as well as the production of IL-10 but not IL-12p70. We finally observed that DC differentiated in the presence of Sp or SN showed a lower ability to induce the proliferation of alloreactive lymphocytes

(p<0.05), while Sp showed a higher ability to induce the expansion of CD25+FOXP3+ cells: % of CD25+FOXP3+ cells: 5±1 vs 12 ±4 (p<0.05 control vs Sp-treated DC). Our results support the notion that Sp induce the differentiation of DC into a tolerogenic profile through a mechanism at least partially dependent on the release of immunomodulatory agents.

264. Junin arenavirus infection enhanced by DC-sign C type lectin. Preliminary study

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Cell tropism of enveloped viruses is regulated by binding of viral envelope glycoproteins to specific cell-surface receptors which determines susceptibility at a host cell, tissue or species level. However, a number of additional cell-surface moieties can also bind viral envelope glycoproteins and could act as capture receptors, serving as attachment factors to concentrate virus particles on the cell surface to disseminate the virus infection to target organs or susceptible cells within the host. Junin virus (JUNV) is a pathogenic member of the Arenaviridae family and it has been shown that transferrin receptor function as its cellular receptor specifically binding to the viral glycoprotein. In addition previous experiments using relatively non-permissive mouse 3T3 cells showed that they became significantly more susceptible to JUNV when the cells expressed DC-sign, been blocked by addition of mannan or anti–DC-sign antibodies. We aim to investigate the role of C-lectins on JUNV infection. Transmission mediated by DC-sign expressing cells was studied for HIV and others viruses. In this preliminary report we used Raji or Raji DC-sign expressing cells to explore the ability of these cells to mediate cell-cell JUNV transmission. Incubation of JUNV with Raji cells resulted in a productive infection with maximum titer in supernatants on day 21 post-infection. Conversely cells expressing DC-sign showed similar yields already on day 5. On the other hand, in both Raji and Raji DC-sign cell-associated infectivity was 2 logs higher than in supernatants. In co-culture assays Raji cells were infected and then incubated with Vero cells. We measured infected Vero cells at different times by indirect immunofluorescence with the Raji ability to produce infectious centers on Vero cells was also examined. Our study shows that Raji linfocitic cell line could be infected by JUNV and that the presence of DC-sign lectin notable increases the chance of infection and cell transmission.

265. Evaluation of a parasite-derived mucin as a potential anti-tumor vaccine

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Cancer is one of the main health problems around the world, being the cause of a considerable morbidity and mortality. During the last decade the immunological approach has gained interest since it represents a possible way to target only cancerous cells, without causing damage to healthy tissues. Tumor Associated Carbohydrate Antigens comprise truncated O-glycosylated-molecules specifically expressed by cancer cells. Some examples are Tn, sialyl-Tn and TF antigens, which are mainly expressed on mucins. Mucins have been implied in cancer adhesion, invasion and metastasis. Interestingly, Tn containing mucins have also been found in some parasites. A negative correlation between some helminths parasites and cancer prevalence has been reported, indicating a possible cross-reactivity between antigens coming from helminth parasites and cancer cells. In this context, we propose to evaluate the immunological properties of a parasite-derived mucin (called C317) carrying the Tn antigen and to analyze its potential as anti-tumor vaccine. All studies were performed with C317 protein as well as the immunodominant peptide and a di-glycosylated form carrying the Tn antigen. All molecules were successfully internalized by dendritic cells, as evaluated by flow cytometry or confocal microscopy. When maturated in the presence of C317, dendritic cells showed an increase in MHC class II expression that was correlated with an increased production of IL-12. The study of the T cell response was evaluated by proliferation assays in animals immunized with the protein together with complete Freund´s Adjuvant, showing a production of IFNγ. Preliminary protection assays suggest that the di-glycosylated peptide carrying the Tn antigen might increase survival of tumor-bearing mice. Altogether, this information suggests that the parasite-derived mucin (C317) could constitute a potential cancer vaccine.

266. HLA Class I and II study in a meztizo family from Chaco, Argentina with high incidence of autoimmune disease.

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The major histocompatibility complex has been extensively studied in different populations and some alleles or haplotypes have been reported as associated with autoimmune diseases. The aim of this study was to investigate the genotype HLA Class I and II in a family with high incidence of autoimmune disease to establish if there is any connection with an HLA allele and/or haplotypes or not. From eight members of this family (father, mother and 6 children) who had a genetic background Hispanic Amerindian, four of them had been diagnosed with systemic lupus erythematosus and one with autoimmune hemolytic anemia. These results were established following the immunologic and clinic criteria of the American College of Rheumatology. DNA was extracted from peripheral blood mononuclear and HLA-A*,

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B* DRB1* DQB1* genotyping were performed by mens of PCR followed by sequence-specific oligonucleotide probe reverse hybridization.Four members of the family who were diagnosed with Lupus showed the presence of HLA-A*02, B*40, DRB1*0407 DQB1*0302 haplotypes (from the father). In three healthy individuals and one patient with AHA this haplotype was not observed. The HLA class I and II haplotype observed were compared with the expected under Hardy-Weinberg equilibrium by 2 tests using Yates correction, X2=18.48 and p = 0.0000091. It is important to notice that, the patient with AHA and one of the healthy individuals carried the DRB1*0407- DQB1*0302 Class II haplotype from the mother.Altohugh we may establish that this class I and II haplotype may be one of the factors in the development of lupus and not the a DRB1*0407 DQB1*03 class II haplotype by its own, we may think on the implicance that HLA class I has on development of lupus.

267. The role of DC-SIGN in the balance between Tregs and effector T cells during mycobacterial infection

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Tuberculosis is a chronic infectious disease caused by Mycobacterium tuberculosis (Mtb) that is responsible for almost 2 million deaths per year. Dendritic cells (DC) are the most important mediators between innate and adaptive immune responses and they can also mediate tolerance. Thus, recognition of Mtb by DC in the early phase of the infection may represent a crucial mechanism for disease outcome. Dendritic cell-specific intercellular adhesion molecule-3 grabbing nonintegrin (DC-SIGN: CD209) is a C-type lectin that interacts with Mtb and has been suggested to limit the tissue damaging inflammatory response after Mtb infection. Here we investigated the importance of DC-SIGN for the induction of T cell effector mechanisms versus expansion/induction of Treg cells during mycobacterial infection. Methods: Mice transgenic for human DC-SIGN (termed "hSIGN"), in which DC-SIGN is expressed under the control of the CD11c promoter, were infected with OVA- expressing M. bovis BCG or wild type BCG and the adaptive immune response was evaluated by means of CD4+ and CD8+ cytokine expression pattern and expansion of Foxp3+ T regulatory cells. Results: We have previously shown that hSIGN mice exhibit significantly less structural lung damage during Mtb infection associated with a dampened CD8 T cell response. We are currently analysing the role of Tregs as an underlying mechanism. Conclusions: Our results will provide further insights into the role of DC-SIGN during mycobacterial infection and the importance of Tregs in the control of Tb.

268. New STAT3 mutation in a patient with Hyper IgE Syndrome (HIGES) without criteria in HIES STAT3 score

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Hyper IgE Syndrome is a primary immunodeficiency marked by frequent piogenic skin abscess and respiratory infections, elevated IgE and involvement of the soft and bony tissues. HIES has been associated with heterozygous dominant negative mutations in the signal transducer and activator of transcription 3 (STAT3) and severe reductions of TH17 cells. Objective: To describe a single clinical case with a new STAT3 mutation but without criteria in HIES STAT3 score. Material and methods: We collected clinical data, determined TH17 cell numbers, and sequenced STAT3 in a patient with a strong clinical suspicion of HIES and serum IgE >1000 IU/ml. Case report: Male 15 years, Caucasian from South America. Background of neonatal rash, chronic eczema, piogenic ear infections since first years of life and multiple skin abscess. Trush, failure to shed deciduous teeth, pathologic bone fractures and scoliosis. Severe peritonitis at 12 years, with unremitting intra abdominal collections, and positive cultures for Histoplasma capsulatum. Evidence of bronchiectasia and lung cyst formation, but no characteristic face. Persistent elevated IgE, eosinophilia, absent specific polisacaryde response after pneumococo vaccination, marked low memory CD19+CD27+IgM- B cells and absent Th17 cells. NIH score 67 points, HIES STAT3 score 26.64 points. STAT3 heterozigotic S611I mutation, 1832 G>T substitution. Conclusion: In this case the proposed score did not predicted mutation, but because of the higly sugerent clinics and de Grimbacher Score >40, we solicitated for the secuenciation. Strongly suspicion of HIGES should leave to genetic analysis.

269. Four patients with Hyper-immunoglobulin E syndrome and different clinical presentation

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Hyper-immunoglobulin E syndrome (HIES) is a primary immunodeficiency characterized by a highly elevated serum IgE, recurrent skin abscesses and cyst-forming pneumonia, with milder inflammatory responses, and skeletal abnormalities. Objective: To describe the different clinical presentation in 4 HIES patient with NHI Grimbacher score (GS) > 40 points. Retrospective analysis of 4 HIES patients. P1: Male 14 years, scoliosis, recurrent skin abscesses, middle ear pyogenic infections and pneumonia with pneumatocele formation. Candida albicans invasive infection. Persistent elevated IgE levels and eosinofilia. GS 47 points. P2:

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doi: 10:3823/413

Female 3 years, recurrent scalp abscesses, recurrent pneumonia with pneumatocele formation, chronic osteomielitis, hyperextensibility and facial asymmetry, a broad fleshy nose, fissures of the roof of the mouth and central depressions of the tongue. GS 50 points. P3: Male, 16 years, neonatal onset severe eczema, recurrent middle ear pyogenic infections, chronic persistent Candida infections, facial asymmetry, a broad fleshy nose and elevated roof of the mouth. Elevated IgE >2000UI/ml and eosinofilia. GS 46 points. P4: Male 15 years, neonatal onset chronic eczema, piogenic ear infections and multiple skin abscess. Failure to shed deciduous teeth, pathologic bone fractures and scoliosis. Severe peritonitis at 12 years, with unremitting intra abdominal Histoplasma capsulatum collections. Evidence of bronchiectasia and lung cyst formation. Persistent elevated IgE, eosinophilia and absent of Th17 cells. GS 67 points. The first 3 have ongoing mutation analysis and the last have a confirmed mutation on STAT 3. Conclusion: All of the cases have different clinical manifestations and not only elevated IgE levels. The proposed clinical Score is useful to determinate whether patient requires specific care treatment and immunological follow up. Early diagnosis should give the pacients the opportunity of less severe compromise and appropriate care given.

270. Monitoring of 72 patients with Transient hypogammaglobulinemia of infancy in 4 years. Clinical features and Laboratory results

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Transient hypogammaglobulinemia of infancy (THI), is the most frequent immunological consult. It present between the 6 and 36 months, it could prolong to 96 months. THI is characterized by 1 or more reduced Immunoglobulins (Igs) levels below 2 standard deviation (SD) as Word Health Organization define (WHO). Aim: Determine the clinical manifestation, immunological changes and outcome of infants with THI. Material: Igs were measured by Nephelometry, Polysaccharide antibody response was measured trough a commercial ELISA (GR) and 10 serotype specific response trough ELISA in house (SS), lymphocyte subsets and B lymphocyte (BL) measured by flow cytometry. Results: 2 groups (Gr). Gr1: 41/72 with THI according to WHO criteria, Gr2: 31/72 with one or more Igs between 1 and 2 SD. Clinical features: GR1: 40% bronchospasm, 60%acute otitis, 37%pneumonia, 10%diarrhea, 7%invasive infections. Gr2: 60% bronchospasm, 80% accute otitis, 60% pneumonia, 22% diarrhea, 0,6% invasive infections. Laboratory results: Gr1:29/41 were exposed to pneumococcal polysaccharide vaccine (23Pn).19/29 had good GR but 2 of them had fallen titles at 12 months with inadequate SS. 10/29 present low GR: 5/10 good SS and 5/10 SS altered. Gr2:13/31 had received 23Pn. 6/13 with normal GR and 7/13 inadequate GR, 4 of them with bad SS. BL was done in 30/72: 1 normal, 29/30 increase transitional BL and CD5+ and decrease pre and post switched BL. Follow up for 4 years of 30 Ps: 19/30 asymptomatic with normal Igs. 11/30 continued with symptoms and SS and BL altered. 6/11 received Intravenous Gammaglobulin (IG), 2 antibiotic prophylaxis and 3 without treatment. Conclusion:Gr1 had more severe invasive infections. Gr2 had more upper respiratory infections. The monitoring of 30 patients for 4 years allowed us to define compromise immune in 11 Ps, begin an early treatment and decrease their morbidity.

271. Thymic selection and peptide-MHC topology determine the size of naive peptide-MHC class I-specific CD8 T cell populations in humans.

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T lymphocytes recognize peptidic antigens bound to polymorphic major histocompatibility complex (MHC) molecules. T cell precursors undergo several intrathymic selection processes that allow generation of a functional yet self-tolerant repertoire. Whether such processes bias the T cell repertoire towards recognition of self-MHC molecules, and whether such a bias, if any, is required for generation of efficient peripheral T cell responses remain debated. Here we exploited a technique allowing immunomagnetic enrichment for specific T cells using recombinant multimers of peptide / MHC (pMHC) complexes, to assess the frequency and repertoire features of naive T cells specific for several HLA-A*0201 (referred to as A2)-restricted antigens, before and after thymic selection processes, in individuals expressing or not this MHC allele. While A2-restricted T cells showed similar repertoire features and antigenic avidities in A2+

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and A2- donors, thymic selection by A2 had a heterogeneous impact on the size of A2-restricted populations, depending on their fine specificity. Frequencies of naive A2-restricted T cells varied up to 100 fold from one antigenic specificity to another, and showed a conserved hierarchy irrespective of the maturation stage of the studied subset and the HLA phenotype of the donor (n=5, r2=0.94, p=0.017). This first comparative analysis of the human T cell repertoire before and after thymic selection provides several new insights into the mechanisms regulating its composition and MHC restriction. It indicates that both selections by self-MHC and structural parameters of the pMHC complexes regulate the frequency of naive T cell precursors specific for defined pMHC. Moreover this study also suggests that T cell repertoire shaping by a given self-MHC allele is dispensable for generation of immunodominant T cell responses restricted by this particular allele.