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CELLULAR IMMUNOLOGY

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OPTIC NEURITIS EVALUATION IN EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS INDUCED BY TWO CONCENTRATIONS OF MOG_{35-55}

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Introduction: Optic neuritis (ON) is an acute inflammation in the optic nerve with axonal injury that may cause loss of sight. Experimental autoimmune encephalomyelitis (EAE) is a model to study multiple sclerosis and ON, mediated by autoreactive lymphocytes recognizing myelin self-antigens. Leukocyte infiltration contributes to the pathogenesis of autoimmune inflammation in the optic nerve in ON. The aim was evaluate the ON in the 100 µg or 300 µg of peptide MOG_{35-55} to the induction of C57BL/6 EAE model.
Method and Results: EAE was induced in female C57BL/6 mice by subcutaneous injection of 100 µg or 300 µg $MOG_{35–55}$ peptide in complete Freund's adjuvant containing 4 mg/mL of *Mycobacterium tuberculosis*. Intra-peritoneal injection of 300 ng of pertussis toxin was administered at the time of induction and again 48 hours later. Three groups of mice were studied: 100 µg $MOG_{35–55}$ group, 300 µg $MOG_{35–55}$ group and control group. Clinical signs of EAE were monitored daily until the 58th day post immunization (dpi). The optic nerves were dissected and evaluated at 7, 10, 14, 21 and 58 dpi for the presence of inflammatory cell infiltrates on a scale from 0 to 4. CCL5 production in the optic nerve was measured in the 10th dpi. No differences were observed in the mean clinical score between the immunized groups. On day 10 a discrete inflammatory infiltrate was observed just in the 300µg MOG_{35-55} group (1.13±0.13). The inflammatory infiltrate was higher on day 14 in both groups, but more intense in the 100µg MOG_{35-55} group than 300 µg MOG_{35-55} group (3.25±0.25 and 1.75±0.25). After this point, a gradual reduction in the inflammatory infiltrate was observed in both groups. CCL5 was evaluated on the onset of inflammatory infiltrate, day 10, showing higher production in the 300 µg MOG_{35-55} group (6536±237.6) in relation to 100 µg MOG_{35-55} group (4374±247.1).
Conclusion: The EAE induction with different concentrations of MOG peptide results in distinct clinical forms of optic neuritis in this model.

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V Extra Section of Clinical Immunology - ESCI

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β-HYDROXY-β-METHYLBUTYRATE (HMB) NEGATIVELY MODIFIES PROLIFERATION, VIABILLITY AND PRODUCTION OF INFLAMMATORY MEDIATORS BY RAW 264.7 CELLS *IN VITRO*

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Introduction: The leucine metabolite β-hydroxy-β-methylbutyrate (HMB) has shown to module immune function by increasing the lymphocyte proliferative and cytotoxic capacities, and also the phagocytic capacity and NO production in experiments using primary culture and cell lines. However, there is a lack of information regarding the range of such modulation in other cell types and the parameters influenced by HMB. **Methods and Results:** Raw 264.7 cells were cultivated in presence of increasing amounts of HMB (0;0.1;0.5;1.0 or 10mM) and assayed for proliferation (by flow cytometry) and adhesion (colorimetric assay using Giemsa stain) capacities, viability (exclusion in trypan blue), and lipopolysaccharide (LPS) stimulated production of nitric oxide (NO) (by nitrite measure using Griess reagent), interleukin-1β (IL-1β) and tumor necrosis factor (TNF) by ELISA. At 10mM, HMB was able to promote a delay in the proliferation profile and decrement in viability of Raw 264.7 cells after 72h in culture. LPS stimulated adhesion capacity was not influenced by any HMB concentration tested. As expected after the proliferation and viability results analysis, the amount NO and IL-1β in culture medium supernatants, after 48h of culture, were reduced by about 50% using HMB at 10mM. For IL-1β, values were 166.2±21,62 *vs.* 91±12,60 pg/mL ($p<0.05$), respectively for HMB at 10mM and control situation. In addition, HMB at 0.1 and 1.0mM decreased the amount of IL-1β in about 25% (130.1±19,21 and 137.7±14,11 respectively *vs.* 166,2±21,62 pg/mL in control). When the TNF concentrations in the same samples were analyzed, only HMB at 10mM influenced

the content of the cytokine in culture medium. Surprisingly, the TNF amount was increased in this situation (372.3±20,38 in control *vs.*1029.0±28,06 pg/mL, $p<0.05$). One possible hypothesis to explain such contradictory data could be that the release of cell components caused by cell death in HMB higher concentration caused and additive stimulation of TNF production by the living cells. **Conclusion:** In summary**,** high concentration of HMB showed to negatively modulate all parameters analyzed in Raw 264.7 cells besides TNF production. Additionally, at 0.1 and 1.0mM (concentrations reached in blood stream after ingestion in animal and human studies), the LPS stimulated IL-1β production was reduced without compromising cell viability and proliferation. Further studies should focus in the mechanisms underneath such observation.
1

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INCREASED INTRACELLULAR CALCIUM MOBILIZATION IN LEUCOCYTES FROM TYPE 2 DIABETIC PATIENTS BY PALMITATE

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Introduction: Intracellular calcium (Ca^{2+}) is a key second messenger in the regulation of multiple cell functions, including leukocytes cells, and the role of calcium in NADPH activation is well documented [J. Leukoc. Biol. 84:1223–1237, 2008]. Studies have suggested that FFA are involved in modulation of the immune system, and, as well as high glucose level, may stimulate ROS (reactive oxygen species) production through protein kinase C (PKC)-dependent activation of NADPH oxidase in cultured endothelial cells, possibly through *de novo* synthesis of diacyl glycerol (DAG) [Diabetes, 49:1839-1945, 2000; J. Clin. Invest., 83:31–38, 1991]. In this context, we speculated that FFA - especially palmitate, which is the most abundant satured fatty acid (SFA) in plasma [J Biol Chem, 279:23942–52, 2004] - might affect intracellular calcium mobilization in mononuclear cells from T2DM.

Methods and results: This study was approved by the ethics committee of Hospital Santa Casa Belo Horizonte. Diabetic patients type 2 (T2DM, n=10) and nondiabetic control (ND, n=10) were aged between 40 and 70 years. PBMNC from T2DM and ND were purified using the Ficoll-Hypaque gradient method. Intracellular Ca^{2+} levels were measured by monitoring the intensity of fura-2AM fluorescence. Cellular viability was determined by the trypan blue exclusion test. Statistical analysis was performed by Student *t*-test ($p<0.05$ considered significant). PBMNC ($1x10^6$ cells/ml) were suspended in KRH and incubated for 30 min at 37ºC with Fura-2AM. PBMNC fluorescence were measured at 500nm emission at 340/380nm excitation for 20 minutes without stimulation or in the presence of

Palmitate complexed with BSA (endotoxin free) (Pal-BSA-100uM), BSA (1mg/mL) and PDB ($1x10^{-3}$M). Results, expressed in nM Ca^{2+}±DP, were PBMNC+KRH= 84.9±18.4 and 99.0±14.3; PBMNC+PDB= 122.9±48.7 and 178.9±49.8; PBMNC+Pal-BSA= 115.8±29.3 and 165.3±39.3; PBMNC+BSA= 97.2±21.5 and 56.7±16.3 to ND and T2DM, respectively. Our results showed that Pal-BSA and PDB increases intracellular Ca^{2+} mobilization in PBMNC from both T2DM and ND ($p<0.05$), however it was significantly ($p<0.05$) greater in PBMNC from T2DM than from ND subjects.

Conclusion: Palmitate and PDB induce greater intracellular Ca^{2+} mobilization in PBMNC from T2DM than from ND. These results could generate a better understanding of the complex inflammatory process that occurs in diabetic patients.

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DEVELOPMENT OF A SKIN TEST FOR THE DIAGNOSIS OF CASEOUS LYMPHADENITIS IN SMALL RUMINANTS

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Introduction: Caseous lymphadenitis (CLA) is a disease caused by *Corynebacterium pseudotuberculosis* that affects small ruminants, leading to economic losses in goat and sheep industry. Several methods for the detection of subclinical CLA have been designed but without success. The present study has developed a Skin Test based on secreted proteins of *C. pseudotuberculosis* for subclinical CLA diagnosis in small ruminants.

Methods and Results: The animals used in the assays were divided into two different groups: 10 positive (A) and 10 negative goats (B) for CLA. Secreted proteins were obtained through Three-Phase Partitioning, diluted in PBS solution and then intradermically inoculated in the left cervical region. Control inoculations of PBS solution without proteins were performed on the right side of the same tested animals. Group A showed higher hypersensitivity compared to group B during 6 days of evaluation, with a peak response after 24 hours.
Conclusion: The standardized Skin Test showed promising results and might be applied for controlling CLA in herds.

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INFLUENCE OF G-CSF ON CYTOKINES IFN-γ, IL-4, TNF-α AND IL-10 PRODUCTION IN PRIMARY CULTURE OF BONE MARROW AND PERIPHERAL BLOOD MONONUCLEAR CELLS

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Introduction: Mononuclear cells are modulated by cells soluble factors and they are potentiated by the co-administration *in vivo* factors such as G-CSF, which act in the host immune response, interfering with tissue healing. This study aimed to evaluate the production of IFN-γ, IL-4, TNF-α and IL-10 in the supernatant primary culture of the mononuclear cells collected from bone marrow (BM) and peripheral blood (PB).**Methods and Results:** We used 12 female Swiss mice, 3-4 weeks of age, which received four doses of G-CSF (200µg/kg/day, i.p). The PB was collected by ocular plexus and the BM was obtained from the femur. The mononuclear cells identification and cytokines assay was performed by flow cytometry. A total of $1x10^6$ cells/ml was plated in 5mL of IMDM. The medium exchanges and collection of the supernatant were made every 72 hours to complete 15 days of primary culture. The data are shown as mean fluorescence intensity (MFI). The results showed an increase of IFN-γ (2.44) and IL-4 (4.82) at the beginning of BM culture and decreased in the 12th until the 15th day. The IL-4 profile in PB and BM cultures was similar until 12th day (4.64 MO; 4.97 PB), but did not decrease until the 15th day of the PB culture. On the other hand, the IFN-γ increased progressively over the time of the PB culture. The IL-10 peak was observed on the 9th day (2.59) after the BM culture has begun, although in the PB culture the peak was on the 15th day. The TNF-α level decreased from the 3th day of cultivation (5.70) until the 15th day in BM cultures. However, in PB cultures the TNF-α remained constant. **Conclusion:** The G-CSF appears to modulate or induce the production of inflammatory and anti-inflammatory cytokines at different times of culture. These *in vitro* findings suggest that cytokines may act "starting" mononuclear cells differentiation and immunomodulation, thus inducing a propitious environment for their maintenance.

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October 20 - 24, 2012

EVALUATION OF NOTCH RECEPTORS AND LIGANDS ON DEVELOPING LYMPHOCYTES IN HUMAN THYMUS

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Introduction: The thymus is a primary lymphoid organ responsible of maturing T lymphocytes. Several signaling pathways act during T lymphocytes maturation including Notch signaling pathway which comprises in receptors (Notch1-4) and ligands (DLL1-3-4, Jagged1-2). We sought to verify the expression of Notch receptors and ligands in human developing lymphocytes.
Methods and Results: Total thymocytes from 10 children were sorted into CD4-8-, CD4+8+, CD4+8-, CD4-8+, and total RNA of these populations were isolated and retrotranscribed. Thymus fragments were stained by immunohistochemistry and quantified in total thymus and between three thymic regions: cortex, cortical-medullary and medulla. The CD4+CD8+ lymphocyte subset presented higher percentage among all sorted populations (51,82%), followed by CD4+CD8- (25,7%), CD4-CD8+ (11,52%) and CD4-CD8- (5,55%). All Notch receptors and ligands genes were detected in lymphocytes subsets, except DLL3 gene. Notch1 gene was less expressed in the CD4+CD8- subset when compared to CD4-CD8- cells, Notch2 gene was less expressed in CD4+CD8+ subset compared to CD4+CD8- cells, and JAG2 gene presented lower expression in CD4+CD8- when compared to CD4+CD8+. NOTCH3, DLL1, DLL4 and JAG1 genes showed no difference in expression levels among lymphocytes subsets. In thymic tissue we detected all Notch receptors and ligands by immunohystochemistry assays evaluating the mean of positive cells in the thymus. Notch1 receptor showed the highest expression, followed by ligand DLL4, Notch4 and Notch3 receptors, DLL1 and Jagged2 ligands, Notch2 receptor, and finally Jagged1 ligand. The evaluation of Notch receptors and ligands at the three thymic regions showed no significant difference.

Conclusions: NOTCH1 gene may have a key role in the first stages of T lymphocytes development due to its higher expression in early stages, different of NOTCH2 gene which showed significant expression at later stages of thymic subsets. JAGGED2 gene may represent a consequence of TCD4+ maturation owing to its prior expression at CD4+CD8+ subset. Different from genes analysis, the homogeneous distribution of Notch components in thymic tissue suggests that a new approach should be considered for Notch pathway during human T lymphocytes development and maturation.

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EFFECTS OF THYMOSIN-ALPHA1 AND OF THE STAT3 INHIBITOR, JSI-124 ON MO-DCS PHENOTYPE AND FUNCTION

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Introduction: Efficient initiation of the adaptive immune response, performed by dendritic cells (DCs), is essential for antitumor immunity. Hence, it is not surprising that cancer interferes in the phenotype and function of DCs. One mechanism for this is the hyperactivation of STAT3. Thus, modulation of this phenomenon may be an effective strategy to increase antitumor responses. Since we had data suggesting that the thymic hormone thymosin α-1 (Tα1) is able to decrease PD-L1 expression, which is dependent on STAT3 signaling, the objective of this study was to compare the effects of Tα1 and an inhibitor of STAT3 had similar effects on monocyte-derived dendrític cells (Mo-DCs). **Methods and Results:** Mo-DCs were submitted to a 24 hour-treatment with different concentrations of Tα1 or with the STAT3 inhibitor, JSI-124. Thereafter, the surface phenotype and the allostimulatory ability of these Mo-DCs were evaluated by flow cytometry. STAT3 inhibition increased significantly ($p<0,05$) the percentage of cells expressing CD86 (**0,5nM**: 88,60 ± 8,81; **1,0nM**: 90,87 ± 6,32; **2,0nM**: 89,79 ± 7,15 vs **NT:** 39,70 ± 15,91). It also increased ($p<0,05$) the mean fluorescence intensity of HLA-DR (**0,5nM**: 47,27 ± 15,59; **1,0nM**: 51,23 ± 14,46; vs **NT:** 20,73 ± 8,16) and decreased ($p<0,05$) CD80 expression (**2,0nM**: 21,02 ± 3,14 vs **NT:** 37,30 ± 3,93) and CD11c expression ($p<0,001$) (**0,5nM**:15,82 ± 2,90; **1,0nM**: 13,89 ± 3,02; **2,0nM:** 11,94 ± 2,45 vs **NT**: 47,25 ± 12,04). DCs treated with JSI-124 induced significantly higher ($p<0,05$) $CD8^+$ cell proliferation (**2,0nM**: 11,43 ± 1,86 vs **NT**: 6,38 ± 2,45). Contrastingly, Tα1 did not influence significantly DCs phenotype, although some tendencies to increased expression of HLA-DR, CD86 and PD-L1 occurred. Likewise, DCs treated with Tα1 showed a non-statistically significant increase ability to induce $CD4^+$ and $CD8^+$ cell proliferation. **Conclusion:** Treatment of Mo-DCs with Tα1 and the STAT3 inhibitor JSI-124 have different effects on cell's phenotype and function, thus suggesting that the effects of Tα1 do not depend on STAT3 inhibition. On the other hand, we noticed significant functional alterations on Mo-DCs treated with JSI-124, which are currently under further investigation.

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EVALUATION OF THE NEUTRALIZING ACTIVITY OF THE ANTICROTALIC SERUM PRODUCED AT VITAL BRAZIL INSTITUTE FOR VENOMS OF CROTALUS (RATTLESNAKES) FROM THE STATE OF BAHIA

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Introduction: In Brazil there is a high incidence of accidents caused by venomous animals, paying particular attention to accidents caused by snakes, which have a significant number of sequelae. In Bahia, accidents caused by snakes, from 2000 to 2010, displayed the second highest mortality rate in Brazil, occurring pathological changes, such as renal disease (ESRD) and amputation of limbs. There is little research on the causes of these sequelae in Latin American, what led the team of the Laboratory of Venomous Animals and Herpetology - LAPH/UEFS, to establish the "Institutional Program of Venomous Animals" to study the snakebites in a multidisciplinary way, showing that from 1990 to 1993 a total of 245 severe accidents with rattlesnakes occurred in Bahia, with 09 deaths and 15 ESRD sequelae. The objective of this study was to evaluate the degree of protection conferred by anticrotalic monospecific sera against accidents caused by Crotalus in the state of Bahia.**Methods and Results**: A total of two batches of anticrotalic sera manufactured by Vital Brazil Institute were evaluated. The *Crotalus* venom was from animals in captivity at LAPH. To evaluate the challenge dose (200ul and 400ul) we used 36 Swiss mice divided into 04 groups, namely: (i) and (ii) experimental (n=12 each) inoculated with the dosage of 3DL50% (3µl/venom) mixed with 200µl and 400µl of serum respectively; (iii) positive control (n=6) inoculated with the dosage of 3DL50% in a final volume of 500ul, and (iv) negative control (n=6) inoculated with the dosage of 500µl/PBS. All inoculated intraperitoneally. Group (iii) died and the others were under observation for 96 hours, being observed in groups (i) and (ii) clinical manifestations such as intense itching, restlessness, and exudate in the left lateral region of the abdomen. After this time the groups (i) and (ii) were anesthetized by intravital microscopy technique, and it was observed accelerated blood flow in arteries and venules with disruption of the endothelial wall. The animals were then sacrificed and analyzed histopathologically, where it was observed lungs with accumulation of inflammatory cells, thickening of alveolar septa and disintegration of alveoli. **Conclusion:**

Experimentally, the sera tested do not protect 100% of animals inoculated with the *Crotalus* venom.

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COMPARISON OF PROTOCOLS FOR THE QUANTITATION OF CYTOKINES PRODUCED BY PERIPHERAL BLOOD MONONUCLEAR CELLS AND WHOLE BLOOD UNDER DIFFERENT MITOGENIC STIMULI

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Introduction: Several protocols of stimuli with different cellular populations have been used to assess response and responsiveness immune in various in vitro. The peripheral blood mononuclear cells (PBMC) are described in different studies there decades. In recent years the PMN has been described as important cells in the triggering and modulation of immune response. The LPS mitogen has been widely used in research protocols. The PHA is used into the control of IGRA in the Quantiferon TB kit. The aim of this study was to compare cytokine production by PBMC and whole blood (WB) stimulated with PWM, LPS and PHA. **Methods/Results**: From 16 healthy individuals, without signs or diagnosis of comorbidities it was obtained heparinized blood: a) $1x10^6$ cells/mL of PBMC (Ficoll-Paque PLUS-GE) in RPMI (SIGMA), and b) 1mL Whole Blood (WB). After incubation of the samples with 5µg/mL of LPS, PHA or PWM (SIGMA), during 48h (37°C/5%CO2), the cytokines were measured with the Human Th1/Th2 Cytokines Beads Array (CBA) kit (Bender MedSystems) by cytometry (FACSCalibur, BD Biosciences CellQuest software). Based on the CBA it was observed: Regardless of the protocol of choice for IFN-g, IL-2, IL-5 and IL-10 the best stimulus was PWM followed for LPS. The best protocol for IL-12p70 was PBMC with PWM. For TNF, IL-6 and IL-8 the protocol of choice is the PBMC, regardless of the mitogen. In the other hand for IL-4 and IL-1b the best results were found in WB stimulated with LPS, followed by value with PWM. For most cytokines it was observed best results in PBMC with PHA, probably due to its ability of agglutinate erythrocytes. **Conclusion**: The protocol of choice for in vitro immune response studies should be established depending on the focus of the studied cytokines. In the case of a descriptive pattern of multiple cytokines the protocol PBMC using PWM seems to be the best.

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Inhibition of neutrophil apoptosis with caspases blockers kills *Leishmania chagasi* by inducing an inflammatory response

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Introduction: Neutrophils are among the host's first line of defense against infections and have been implicated in the immunopathogenesis of Leishmaniasis. We have demonstrated that *Leishmania chagasi*, the etiological agent of Visceral Leishmaniasis, causes the induction of neutrophil apoptosis. Because different cell death pathways have been implicated in the pathogen killing or survival by host cells, we tested whether inhibition of neutrophil apoptosis by pre-treatment with zVAD-fmk or zIETD-fmk increases *L. chagasi* killing, since it has been demonstrated that inhibition of caspase 8 reorients the cell death to necroptosis.
Methods and Results: Mouse peritoneal neutrophils obtained by thioglycolate injection were pre-treated with zVAD-fmk (a pan caspase inhibitor) or with appropriated controls and were infected with *L. chagasi*. Neutrophils pre-treated with zVAD presented a significant decrease on viable parasite ($4.68x10^4 \pm 1.59$) compared with controls (cell culture medium: $13.06x10^4 \pm 2.08$; DMSO: $13.81\ x10^4 \pm 1.28$ or zFA-fmk: $16.13\ x10^4 \pm 2.01$). Interestingly, when we use a specific caspase 8 inhibitor, zIETD-fmk, we also observed a significant increase on parasite killing by neutrophils (cell culture medium: $13.06x10^4 \pm 2.08$; DMSO: $13.81\ x10^4 \pm 1.28$; zIETD-fmk: $16.13\ x10^4 \pm 2.01$). The inhibition of caspases increased the activation state of infected neutrophils. The neutrophils pre-treated with zVAD-fmk had higher concentrations of TNF-α (-zVAD 231.8pg/ml ± 88.3; +zVAD 474.5pg/ml ± 198.5) and ROS (MFI: -zVAD 44.95 ± 2.05; +zVAD 64.8 ± 1.27). In another hand, TGF-β release was reduced by this treatment (-zVAD 107ng/ml ± 23.52; +zVAD 57.15ng/ml ± 12.15).
Conclusion: Taken together, our data suggest that inhibition of neutrophil apoptosis by using caspase inhibitors have biological significance in the control of *Leishmania* infection, probably by inducing an inflammatory cell death type.

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IMMUNO 2012
XXXVII Congress of the Brazilian Society of Immunology
V Extra Section of Clinical Immunology - ESCI

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October 20 - 24, 2012

D1-LIKE DOPAMINERGIC RECEPTOR SIGNALING IMPAIRS DENDRITIC CELL MATURATION AND THEIR ABILITY TO INDUCE T CELL PROLIFERATION.

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Introduction: The characterization of the sympathetic innervation in lymphoid tissues and expression of catecholaminergic receptors on cells of the immune system indicate that neurotransmitters may mediate cross talk between the nervous and immune systems. The dopamine is one of the important neurotransmitters released by the sympathetic nerve system. Some cell types of the immune system do express dopaminergic receptors, one of them are the dendritic cells (DCs). These are considered to be the most potent antigen-presenting cell (APC), and are thus specialized in the initiation of adaptive immune responses through activation and differentiation of antigen-specific naive T cells. However very little is known on how dopamine may participate in the regulation of DC function with consequences on the development of adaptive immunity. **Objective:** The aim of the present work is to evaluate the modulation of functional activity of $CD11c^+$ dendritic cells after signaling through D1-like dopaminergic receptors. **Methods and Results:** Bone marrow cells from C57BL/6 mice were cultured in the presence of 20ng/mL granulocyte/macrophage colony stimulating factor (GM-CSF). At day 9 cells were collected and sorted by CD11c positivity using a FACSAriaII. These cells were considered immature DCs (iDCs). iDCs that were activated with 5ug/mL LPS for 18 hours were considered mature DCs (mDCs). Relative gene expression of dopaminergic receptors was studied by qPCR with SybrGreen system and calculated using the $\Delta\Delta$Ct method. At day 9 iDCs were treated with 1 uM SKF-38,393, a selective D1/D5 receptor agonist, and after 1 hour the cells were activated with 5ug/mL LPS and R848 for 18 hours. The expression of MHC II and co-stimulatory (CD86) molecules were evaluated by flow cytometry. Their ability to act as APCs was tested using an assay in which DCs pulsed with OVA peptide were co-cultured with OT-II T cells. The proliferation of Violet Cell Tracer-labeled OT-II T cells was evaluated by flow cytometry. Our results showed that the D1-like dopaminergic receptors are expressed in both iDC and mDC. We also found that treatment of iDCs with the selective D1/D5 receptor agonist diminished the expression of MHCII molecules and CD86 as compared to control. Moreover, DCs treated with the D1/D5 agonist displayed reduced ability to induce antigen-specific T cell proliferation. **Conclusion:** Our results suggest a

modulatory mechanism by which signaling through D1-like dopaminergic receptors impairs DC maturation, here inferred by lower MHC II and CD86 expression and lower ability to induce T cell proliferation.

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XXXVII Congress of the Brazilian Society of Immunology
V Extra Section of Clinical Immunology - ESCI

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October 20 - 24, 2012

Characterization of Cellular Immune Response in Horses Immunized with *Crotalus* Venom

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Introduction: In Brazil, *Crotalus durissus* venom causes the highest annual fatality rate by snakes. Specific antivenom therapy is the only effective treatment. Horses are used for antivenom production by their large size, resistance to toxins and production of large amounts of effective IgG(T) antibodies. Despite the efficacy of the rattlesnake antivenom obtained from immunized horses, there are no data about the equine cellular immune response to the venom. The aim of this study was to compare the specific cellular and humoral immune responses to *C. durissus* venom in the peripheral blood of venom-immunized horses.
Methods and Results: Three groups (n=5 each) of horses were immunized with whole venom according to a standard protocol for anti-crotalic serum production. Blood samples were collected before and at different periods after immunization. Peripheral blood mononuclear cells (PBMC) were separated and stimulated *in vitro* with *C. durissus* venom and proliferation was measured by thymidine [^{3}H] incorporation and cytokines from culture supernatant were measured by sandwich ELISA. Venom specific antibody titers in sera were determined by direct ELISA. The venom-immunized horses presented positive proliferative responses and high titers of specific antibodies against the venom. In addition, there was a variability of the response within each group, along the immunization protocol. There was a positive correlation ($p < 0.05$) between maximum proliferative response and maximum antibody title of each animal. In addition, *C. durissus* venom induced *in vitro* production of IFNg, TNFα, IL-4 and IL-10. The production of these cytokines increased with the number of venom immunizations. Moreover, there was a positive correlation between proliferative response and IFNg, and also between proliferative response and TNFa induced by *C. durissus* venom.
Conclusions: *C. durissus* venom induces cellular as well as the known humoral immune response in immunized equines. Evaluation of specific proliferative response and cytokine production by immunized horses may contribute to improve the immunization protocol for the production of sera with higher antibody titers.

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IMMUNO 2012
XXXVII Congress of the Brazilian Society of Immunology
V Extra Section of Clinical Immunology - ESCI

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Campos do Jordão SP Brazil
October 20 - 24, 2012

PERSISTENT BRAIN INFLAMMATION DURING CHRONIC EAE DESPITE LOCAL ABSENCE OF IL-17 PRODUCTION

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Introduction: Multiple sclerosis (MS) is a progressive inflammatory autoimmune disorder of the central nervous system (CNS) mediated by myelin self-reactive T cells. Experimental autoimmune encephalomyelitis (EAE) is an artificially induced demyelination of the CNS that resembles MS in its clinical, histopathological and immunological features. Activated Th1 and Th17 cells are thought to be the main immunological players during EAE and MS development. In this context, the present study was designed to evaluate the peripheral and local contribution of IL-17 to acute and chronic EAE stages. **Methods and Results**: Female C57BL/6 mice (n=6) were subcutaneously immunized with myelin oligodendrocyte glycoprotein (MOG) emulsified in Complete Freund's adjuvant plus BCG followed by two *Bordetella pertussis* toxin doses. Body weight loss and clinical scores were daily evaluated. IFN-γ and IL-17 production, Foxp3+ Treg cells proportion and intensity of brain inflammation were evaluated during acute and chronic phases. Mice presented an initial acute phase characterized by accentuated weight loss and high clinical score, followed by a partial recovery when they reached normal body weight and smaller clinical scores. Spleen cells stimulated with MOG produced significantly higher levels of IFN-γ (38236 ± 14262 pg/ml) during the

acute period whereas similar IL-17 levels were produced during both disease stages. Brain infiltrating cells stimulated with MOG produced similar amounts of IFN-γ but IL-17 (93 ± 21 pg/ml) was produced only at the acute phase of EAE. The percentage of Foxp3+ Treg cells was elevated, during both phases, at the spleen and brain. The degree of inflammation was similar at both disease stages.

Conclusion: Partial clinical recovery observed during chronic EAE is associated with no production of IL-17 and presence of Foxp3+ Treg cells in the brain.

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IMMUNO 2012
XXXVII Congress of the Brazilian Society of Immunology
V Extra Section of Clinical Immunology - ESCI

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October 20 - 24, 2012

VARIABILITY OF CYTOKINES PRODUCED BY DIFFERENT T CELL SUBSETS DIRECTING IMMUNE RESPONSE IN EXPERIENTAL SPOROTRICHOSIS

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Introduction: The dimorphic fungus *Sporothrix schenckii* is the causative agent of sporotrichosis, a worldwide spread mycosis mostly seen in tropical regions, which causes disease in humans and in a great variety of animals. Nowadays, sporotrichosis greater role lies in its zoonotic potential and opportunistic infection in immunocompromised patients. In this study we analyzed the host resistance to *S. schenckii* regarding the induction of cellular immunity, mainly mediated by effector phagocytes and a variety of T cell subsets, by evaluating the cytokine profile in response to the fungus exoantigen. **Methods and Results**: Six-week old male Swiss mice infected or not with *S. schenckii* were analyzed during 10 weeks of infection. CFU (count of colony forming units grown on Sabouraud agar plates), NO production (Griess reagent), apoptosis (annexinV), spleen cell proliferation ([^{3}H]-thymidine incorporation) and cytokine production (ELISA) were assessed every two weeks throughout all the experiment. At the 4th and 6th weeks post-infection we have found the highest amounts of NO (p<0,01) and TGF-β (p<0,001). At this same period, we detected reduced spleen cell proliferative response (p< 0,001) for both the 4th and 6th weeks) and IL-2 release (p< 0,01 and p<0,001 for the 4th and 6th weeks, respectively), which seems related to the host increased susceptibility, as seen by increased fungal burdens at the time. Also, increased levels of the anti-inflammatory cytokine IL-10 found at the beginning of infection (p<0,001 at the 2nd and 4th weeks) are consistent with the diminished production of the aforementioned mediators at the same time, suggesting a role for IL-10 in the initial establishment of the fungus. Apoptotic cells were detected in larger numbers between the 4th (40%) and 6th (70%) weeks post-infection, along NO and TGF-β responses. From the 6th to the 10th week post-infection there was a marked increase in IL-6, IL-17 and IL-4 production, while IFN-γ production peaked at the 6th, decreasing in the following weeks. This lead us to believe in a conjunct Th1/Th17 inflammation before the onset of a Th2 response, as suggested by a profile increased IL-4 release. **Conclusion:** Together, our results point towards the activation of different T cell effector subsets during experimental murine

sporotrichosis and may contribute to a better understanding of the immune mechanisms triggered by *S. schenckii*.

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IMMUNO 2012
XXXVII Congress of the Brazilian Society of Immunology
V Extra Section of Clinical Immunology - ESCI

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Campos do Jordão SP Brazil
October 20 - 24, 2012

DECREASE OF *LEISHMANIA*-SPECIFIC MEMORY CD4$^+$ T-CELLS IN ACTIVE TEGUMENTARY LEISHMANIASIS IN AIDS PATIENTS

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Introduction *Leishmania* is an opportunistic pathogen in HIV-infected individuals. In Brazil, Tegumentary Leishmaniasis (TL) is the most common presentation of Leishmaniasis in AIDS patients, with, aggressive disease manifestation, including multiple mucocutaneous ulcers, disseminated lesions and relapsing disease. The effects of HIV on immune responses in Leishmaniasis is unclear.

Objective To evaluate the maturation state of T-cell subsets from patients co-infected with HIV and *Leishmania* with active TL.

Methods and Results Five TL/HIV co-infected and 10 HIV-seronegative subjects with TL were enrolled. The proliferative response was evaluated by culturing PBMC previously stained with Carboxyfluorescein succinimidyl ester with *Leishmania* antigens (SLA), by flow cytometry, after 5 days of culture. Cells were stained with anti-CD4 and anti-CD8 monoclonal antibodies. The cell division index was calculated using FlowJo software. Central (CM: CD4+CD45RA-CD62L+CCR7+) and effector memory (EM: CD4+CD45RA-CD62L-CCR7-) T-cells were quantified after 18 hours of culture with SLA, using flow cytometry. The median cell division index for CD4+ (0.06, IQR 0.05-0.1) and CD8+ T-cells (0.05, IQR 0.01-0.08) after stimulation with SLA from co-infected patients was significantly lower than in patients with Leishmaniasis solely (CD4+ T-cell: 0.18, IQR 0.15-0.26; CD8+ T-cell: 0.2,

IQR 0.15-0.26)($p<0.05$). Only cells from one co-infected patient demonstrated any lymphoproliferative response after stimulation. In contrast, both $CD4^+$ and $CD8^+$ T-cells subsets from all patients with Leishmaniasis mono-infection proliferated in response to SLA. The proportions of *Leishmania*-specific central and effector memory CD4+ T-cells were similar between the co-infected patients (CM: 12%, IQR 2-25; EM: 15%, IQR 11-54) and patients with Leishmaniasis mono-infection (CM: 11%, IQR 2-22; EM: 17% 13-22). The median of count of *Leishmania*-specific CM (3 cell/mm^3, IQR 1-13) and EM CD4+ T-cells (5 cell/mm^3, IQR 3-28) from co-infected patients were significantly lower compared to patients infected *Leishmania* solely (CM: 46 cell/mm^3, IQR 20-128; EM: 121 cell/mm^3, IQR 61-139)($p<0.05$).

Conclusion Our results indicate that a decrease in the count of *Leishmania*-specific memory T-cells in peripheral blood results in the reduction of the specific lymphoproliferative response, dissemination of *Leishmania* and severity of lesions presented by HIV/*Leishmania* co-infected patients.

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IMMUNO 2012
XXXVII Congress of the Brazilian Society of Immunology
V Extra Section of Clinical Immunology - ESCI

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Campos do Jordão SP Brazil
October 20 - 24, 2012

CONTRIBUTION OF TOLL-LIKE RECEPTOR 4 TO THE HOST RESPONSE IN EXPERIMENTAL SPOROTRICHOSIS

DANIELLE CARDOSO GERALDO MAIA[1]; AMANDA COSTA GONÇALVES[1]; FABIANA CRISTINA DONOFRIO[1]; LUCAS SOUZA FERREIRA[1]; PÂMELA ALEGRANCI[1]; THAIS DE CÁSSIA NEGRINI[1]; MARISA POLESI PLACERES[1]; IRACILDA ZEPPONE CARLOS[1].

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Introduction: The dimorphic fungus *Sporothrix schenckii* is the causative agent of sporotrichosis, a worldwide spread mycosis mostly seen in tropical regions, which causes disease in humans and in a great variety of animals.Toll-like receptors have been implicated in the recognition of various pathogens, including bacteria, viruses and fungi. However, no information is available about Toll-like receptor4 (TLR4) participation in *S. schenckii* recognition and the consequent triggering of the immune response to this fungal pathogen. Following activation of TLRs by ligands of microbial origin, several responses are provoked, including reactions in immune cells that may lead them to produce signaling factors that trigger inflammation. In this study were available the role of Toll-like receptor-4 in the experimental sporotrichosis. **Methods and Results:** TLR4-deficient (C3H/HeJ) and control mice (C3H/HePas) were infected with *S. schenckii* yeast cells and immune response was assessed over 8 weeks. NO production and NO inhibition (Griess reagent), phagocytosis (microscopy analysis), fungal invasion (histopathology analysis) were realized in all studied period. The presence immune response observed in *S. schenckii*-infected C3H/HePas (TLR4 normal) mice was stronger than that observed in C3H/HeJ (TLR4 defective) mice. We have observed an inhibition of phagocytic activity of macrophages of TLR4-deficient animals when these cells were treated with the fungal lipid extract. Similarly, NO production was inhibited in same period. We observed that lipid extract inhibited NO production in C3H/HeJ mice in all analyzed period. In C3H/HePas mice the highest amounts of NO were observed in 4^{th} (24,28±3,25) and 6^{th}(23,47±2,30) weeks of infection. In the same period was observed highest percentual of inhibition of NO in C3H/HeJ mice 4^{th} (75%) and 6^{th} (88%) weeks of infection. To better characterize the severity of *S. schenckii* infection, histopathological examination of spleens was done at week 8 of infection. In C3H/HeJ mice was seen severe infection with intense

inflammatory infiltrate and the higher level of yeast cells when compared with TLR4 normal. **Conclusion:** The TLR4 receptor participated in host defense against *S. schenckii*, and the lipid extract showed an important virulence factor on the progression of experimental sporotrichosis.

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IMMUNO 2012
XXXVII Congress of the Brazilian Society of Immunology
V Extra Section of Clinical Immunology - ESCI

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Campos do Jordão SP Brazil
October 20 - 24, 2012

INFECTION INDUCED BY FUNGUS SPOROTHRIX SCHENCKII: ROLE OF TLR-2 RECEPTOR IN THE IMMUNE RESPONSE

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Introduction: Sporotrichosis is a mycosis resulting from *Sporothrix schenckii* infection, which gains entry to the host through minor trauma, causing cutaneous and subcutaneous lesions that may spread to other tissues. The known immunological mechanisms involved in the sporotrichosis prevention and control suggest an important role of the cellular immunity in protecting the host against *S. schenckii*. The Toll-like receptors (TLR), expressed in innate immune system cells, stand out for their central role in pathogen binding and immune response triggering. Binding of these receptors by PAMPs results in phagocytosis and release of cytokines and other chemical mediators which drive the following immune response. The present study aimed to characterize the *in vitro* role of TLR-2 in the production of cytokines by mice peritoneal macrophages lacking this receptor (from TLR-2$^{-/-}$ C57BL/6 mice). **Methods and Results:** Macrophages from both the knockout (TLR-2$^{-/-}$) and wild type strain were individually cultured in RPMI-1640C medium and exposed to the fungus for 2 hours. The absence of TLR-2 led to lower *in vitro* production of the cytokines TNF-α, IL-1β, IL-12 and IL-10 by

macrophages from TLR-2$^{-/-}$ mice when cultured with *S. schenckii* live yeast. The results suggest the important role of TLR-2 in the induction of proinflammatory mediators by macrophages exposed to the *S. schenckii*, contributing to the elucidation of the recognition mechanisms taking part in host response to sporotrichosis. **Conclusion:** TLR-2 receptor is important in the recognize of the fungus *S. schenckii*.

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IMMUNO 2012
XXXVII Congress of the Brazilian Society of Immunology
V Extra Section of Clinical Immunology - ESCI

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Campos do Jordão SP Brazil
October 20 - 24, 2012

PURIFICATION AND ANALYSIS OF THE CYTOTOXICITY OF THE METALLOPROTEASE FROM *Bothrops moojeni* SNAKE VENOM.

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Introduction: Snake venom glands are a rich source of bioactive molecules such as peptides, proteins and enzymes that show different biological actions. Many of the components of bothropic venom are metalloproteases. These enzymes are responsible for interfering with the homeostatic system of the prey due to its proteolytic activity. In the present work**,** we isolated the metalloproteinase from *Bothrops moojeni* snake venom and analyze its cytotoxicity effects using HeLa cells *in vitro*. **Methods and results:** BmooMPα-I was isolated from crude venom of *B. moojeni* (200 mg), by DEAE-Sephacel, Sephadex 75 and Benzamidine-Sepharose column chromatography and represented 8.7% (w/w) of the initial desiccated venom. The 2D electrophoresis showed purity, molecular mass (Mr) and isoelectric point (pI) of a proteic trimer under reducing conditions (~23.6 kDa, ~ 21.2 kDa and ~18.7 kDa, pI ranging from 7.24 to 7.33) and for no reducing conditions only one spot protein can be demonstrated (~23.0 kDa; pI 6.82). Cytotoxic activity of the enzyme BmooMPα-I determined *in vitro* by MTT assays. The Hela cells were cultured for 24 h in the presence of twofold serial dilutions of the BmooMPα-I (50 µg/mL to 0,01 µg/mL).The enzyme BmooMPα-I induced cell death around 81% in HeLa cells for the concentration of 50 µg/mL and its median toxic concentration (TC50) was established in 30 µg/mL. In the concentration below of the 30 µg/mL HeLa cells showed cellular viability above of 100%. **Conclusion:** The metalloprotease BmooMPα-I caused cytotoxic effects in the HeLa cells in a dose-dependent manner.

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IMMUNO 2012
XXXVII Congress of the Brazilian Society of Immunology
V Extra Section of Clinical Immunology - ESCI

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October 20 - 24, 2012

DISTRIBUTION OF TH1, TH2, TH17 AND NAÏVE VERSUS MEMORY T CELLS IN B-CLL IS RELATED TO THE PROGNOSIS OF DISEASE

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B-CLL is characterized by the progressive accumulation of small B lymphocytes that do not undergo apoptosis due an underlying defect. One potential mechanism of defective apoptosis may be irregular cytokine production by T cells. Our hypothesis is that patient CD4+ T cells may participate in the course of this hematological malignancy and contribute to the pathophysiology and evolution of the disease. In this context, we aimed to determine the cytokine profile in the serum of B-CLL patients, as well as the relative frequency of TH1, TH2, TH17 and the size of the CD4+ and CD8+ T central memory (T_{CM}), effector memory (T_{EM}) and terminal effector memory (T_{EMRA}) compartments. **Patients and Methods:** We performed flow cytometry analysis of peripheral blood mononuclear cell samples from 20 healthy donors and 10 chronic lymphocytic leukemia (B-CLL) patients. Naïve and memory CD4+ and CD8+ T cells were characterized based on differential expression of CD45RA and CD62L. TH1, TH2, TH17 cells were determined by the expression of INF-γ, IL-4 and IL-17, respectively. In addition, ZAP-70, an important tyrosine-kinase protein associated with a more severe disease, was also measured in B and T lymphocytes from B-CLL patients. **Results:** We observed an increased frequency of TH1 cells in B-CLL patients compared to healthy donors, particularly in patients positive for ZAP-70 (poorer prognosis). Also, we observed a bias of CD4+ T cell population towards a memory pool, especially T_{CM} and T_{EM} cells, which was again, much more pronounced in patients with ZAP-70+ B-CLL. **Conclusion:** These results suggested that IFN-γ-producing TH1 cells and CD4+ memory T-cells could play an important role in the outcome of B-CLL. **Financial support:** FAPESP, CNPq and Instituto Israelita de Ensino e Pesquisa Albert Einstein - IIEPAE.

IMMUNO 2012
XXXVII Congress of the Brazilian Society of Immunology
V Extra Section of Clinical Immunology - ESCI

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October 20 - 24, 2012

ROLE OF B CELLS AND IL-10 IN BCG IMMUNIZATION AND SUBSEQUENT CHALLENGE WITH *MYCOBACTERIUM TUBERCULOSIS*

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Introduction: Tuberculosis is the second cause of death by infectious disease in the world. The unique vaccine certified nowadays is BCG. But its efficacy differs between 0 to 80%. However, the role of B cells and IL-10 in BCG immunization and tuberculosis infection is poorly understood yet. **Objective:** Investigate the role of B cells and IL-10 in T cell memory formation and lungs inflammation after *Mycobacterium tuberculosis* infection. **Methods:** C57BL/6, Bko and IL-10Ko mice were immunized intraperitoneally with BCG (1x 10^5 bacili) and 30 days after immunization, mice were infected with 1x 10^5 bacili with *Mtb* H37Rv strain via intranasal. After 30 days post infection, lungs were harvested and the percentage of T memory cells ($CD4^+$ or $CD8^+/CD44^{high}/CD62L^{low}$), B regulatory cells and neutrophils infiltrate were determined by flow citometry. In parallel the bacilli charge were determined in the lungs by CFU assay. **Results:** Our results showed that in lack of B cells or IL-10, the percentage of memory CD4 and CD8 T cells were significant decreased in lungs of infected mice. BKo mice immunized with BCG failed to promote a significant control of CFU when compared to WT mice. But animals IL-10Ko immunized were able to promote the reduction of CFU in lungs. Furthermore, the absence of B cells induced an increase of neutrophils into the lungs of infected animals. **Conclusion:** B cells and IL-10 cytokine are important for the generation of memory T cells after immunization with BCG and further infection and to control the bacterial growth in the lungs of infected animals with *Mtb*.

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IMMUNO 2012
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ORAL INFECTION MODEL FOR THE ANALYSIS OF THE IMMUNE RESPONSE IN *Neospora caninum* INFECTION

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Introduction: *Neospora caninum* is a an intracellular protozoan parasite with worldwide distribution, which has been associated to abortions since the early 1990's, and is estimated to cause major economic impact in cattle raising farms. The mechanisms underlying host resistance against this pathogen remains unclear and has been the subject of study by our group. Experimental models that assess host responses against the parasite are usually based on parenteral infections. Standardization of a model of oral infection would facilitate the evaluation of immune response against this pathogen. In that sense, this work aimed to establish an infection protocol using oral inoculation of *N. caninum* tissue cysts, simulating natural condition to evaluation the immune response. **Methods and results:** stage interconversion of tachyzoites to bradyzoites was induced *in vitro*, through continuous selective pressure by a nitric oxide donor compound (Sodium Nitroprusside - SNP). Stage interconversion was confirmed by RT-PCR, where it could be observed that parasites submitted to culture in the presence of SNP decreased mRNA expression of tachyzoite related SAG1 gene, opposed to an increment of bradyzoite specific SAG4 gene expression. Additionally, cell cultures containing tissue cyst like forms were positively stained with parasite specific antibodies and Dolichos biflorus agglutinin, a lectin with affinity to carbohydrates contained in tissue cyst walls. Different groups of mice were inoculated orally with in vitro induced tissue cysts, tachyzoites after pre-blockage of gastric pH, along with intraperitoneal controls. As observed by IgG seroconversion, mice infected with tissue cysts produced similar responses to animals infected by intraperitoneal tachyzoites. **Conclusion:** More experiments are underway in order to confirm the

success of oral infection protocol using in vitro induced bradyzoites. If confirmed, this protocol will be a promising tool to be applied in studies involving host-parasite relationship of *N. caninum* infection.

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IMMUNO 2012
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V Extra Section of Clinical Immunology - ESCI

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October 20 - 24, 2012

LAMININ POLYMER MODULATES SPLEEN-DERIVED DENDRITIC CELLS

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Introduction: Dendritic cells (DCs) are professional antigen-presenting cells (APCs), playing important roles during immune responses. For APC function, DCs have to display foreign antigens by the major histocompatibility complex (MHC), express Toll-like receptors and co-stimulatory molecules on their surface and release cytokines. While DCs migrate through different tissues, they crossing various proteins of the extracellular matrix (ECM). Laminin (LN) is a major ECM protein, which plays a role in the cell differentiation, migration, adhesion and survival of several cell types. LN can self-polymerize in vitro in basic buffer and acid buffer. It's known that a6B1 integrin is the receptor mediating adhesion of DCs to LN, but the effect of LN on DCs signaling is poorly understood. Our objective is to characterize the effect of a LN-polymer in modulating spleen-derived DCs.

Methods and Results: The LN was diluted in two different buffers, in sodium acetate pH 4,0 or Tris pH 7,0 both with additional calcium chloride. We used BSA for the protein control. DCs were obtained from spleen and subjected to an EasySep CD11c-PE positive selection according to the manufacturer's instructions. After incubation between DCs and LN-polymer, these DCs were recovered to analyze: pathways signaling that LN-polymers can activate by western blotting, cytokines release that LN-polymers can stimulate the produce by CBA, surface molecules that LN-polymers can activate the expression by FACS and NFkB translocation that LN-polymers can activate by EMSA. We did not observe any difference at cytokines release and in expression of costimulatory molecules in DCs incubated with LN-polymer. We observed an increase at p38 and ERK phosphorylation in DCs incubated with LN for 18h, but not for 1h of incubation. DCs incubated for 2h with LN-polymer had more activated NFkB.

Conclusion - These results suggest that LN-polymer activates long-term phosphorylation of signaling protein. Also, LN-polymer is able to enhance NFkB activation. Other DCs features must be analyzed in order to understand the effects of NFkB activation observed after LN-polymer stimulation. NFkB activation can modulate the T cells proliferation, antigen uptake or DCs migration.

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IMMUNO 2012
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October 20 - 24, 2012

MODULATION OF MTOR BY RESPIRATORY SYNCYTIAL VIRUS AND ITS CONTRIBUTION TO CD8 T CELLS MEMORY DIFFERENTIATION

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Introduction: RSV is the most common cause of serious lower respiratory tract disease of infants worldwide and is a high priority for vaccine development. Despite the generation of RSV-specific adaptive immune responses, RSV does not confer protective immunity and recurrent infections throughout life are common. The mammalian target of rapamycin (mTOR) exhibits immunostimulatory effects on memory CD8 T cell differentiation. Our objective was to evaluate if RSV can modulate mTOR T cells and contribute to a novel viral mechanism to induce immune regulation. **Methods and Results:** To evaluate the mTOR phosphorilation induced by RSV human peripheral blood mononuclear cells (PBMC) ($4x10^5$/well) were culture in RPMI 2% of fetal bovine serum for 30 min with different concentration of RSV, 500ηg of LPS, protein F and 100ηM of phormol miristate acetate (PMA) as positive control and only media as a negative control. Alternatively, the cells were previously incubated with 20ηg/ml of rapamicyn (Cell Signaling) or 50µM of PI3K inhibitor LY294002 for 2hs before the stimulation. After that, the cells were stained with anti-CD8 FITC and than fixed with Citofix buffer (BD Bioscience), permeabilized with Perm III (BD bioscience) and stained with rabbit anti-pmTOR ser2448 (Millipore) and anti-rabbit IgG Cy3 conjugated (Chemicon International). Cells were acquired at BD FACS Canto II flow cytometer and the data were analyzed using FlowJo software (TreeStar). We found that mTOR stimulation by RSV was completed inhibited using rapamycin, since the

percentage of the CD8+ T cells pmTOR+ decreased 5 fold in the presence of rapamycin. However, the mTOR phosphorilation induced by RSV on T CD8+ cells was partially depend on PI3K activation, given that the mean of CD8+ pmTOR+ T cells were 23.56 ± 15.52 when incubated with the virus and we observed a slight reduction to 17.81 ± 4.2 when PI3K pathway is inhibited. **Conclusion:** The data suggested that RSV induced mTOR in CD8 T cells; however it is not depend on PI3K activation. These results are preliminary and we still need to investigate the role of mTOR activation by RSV on memory T cells differentiation.

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IMMUNO 2012
XXXVII Congress of the Brazilian Society of Immunology
V Extra Section of Clinical Immunology - ESCI

SBI Sociedade Brasileira de Imunologia
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Campos do Jordão SP Brazil
October 20 - 24, 2012

THE INVOLVEMENT OF SIALYLATION MEDIATED BY ST3GAL-I OR ST3GAL-IV IN PATHOGENESIS OF POLYMICROBIAL INFECTION

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Introduction: Reduction of neutrophil migration to the focus of infection is a central event that correlates with mortality in sepsis. Neutrophil migration is mediated by the interaction of a variety of sialylated glycoproteins, especially by glycoconjugates that contains SLeX oligossacharide, such as alpha-1 acid glycoprotein (AGP). These sialoglycoconjugates can attach to adhesion molecules and block adhesion of neutrophil to endothelial cells, therefore decreasing neutrophil migration.

Methods and Results: Our group has demonstrated that the AGP, inhibits neutrophil migration to the focus of infection during mice sepsis. Thus, understanding the mechanisms that control the synthesis of sialylated glycoconjugates and/or soluble glycoproteins could be of very impact in sepsis and other systemic inflammatory conditions. The objective of this study was to investigate the role of alpha2,3 sialyltransferase-I and -IV in severe sepsis induced by cecal ligation and puncture model. Our results showed that ST3Gal-I- or ST3Gal-IV-deficient mice exhibited higher survival rate and higher neutrophil migration to the focus of infection when compared to their wild-type littermates. We do not observe differences in the levels of pro-inflammatory cytokines such as TNF-α, CXCL2 and CXCL1in the local of infection or in freshly blood as well. Corroborating with these results, bacterial content in these compartments were similar in both mice strains.

Conclusions: Interestingly, we observed a high percent of neutrophils expressing the AGP receptor, Siglec-F, in ST3Gal-I deficient mice when compared to the wild type, suggesting a compensatory mechanism to control neutrophil migration in the absence of ST3Gal-1.However, the mechanisms involved in the survival rate improvement during sepsis of the ST3Gal-I- or ST3Gal-IV-deficient mice are under investigation.

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IMMUNO 2012
XXXVII Congress of the Brazilian Society of Immunology
V Extra Section of Clinical Immunology - ESCI

SBI Sociedade Brasileira de Imunologia
www.sbicongressos.com
Campos do Jordão SP Brazil
October 20 - 24, 2012

13693 **INTERACTION BETWEEN RAGE AND TLR4 IN THE MODULATION OF PROLIFERATION AND CELL DEATH AND REGULATION OF THE EXPRESSION OF CYTOKINES IN PBMC OF DIABETIC AND NON DIABETIC PATIENTS.**

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Introduction: The process of apoptosis or programmed cell death is essential for homeostasis of living beings, acting as part of a mechanism of "quality control" and repair, compensating stochastic genetic errors or errors of development. In vitro, studies have associated the induction of apoptosis to hyperglycemia in diabetes mellitus. This study aimed to evaluate the possible synergism between RAGE and TLR4 in the processes of cell proliferation, death and expression of inflammatory cytokines on peripheral blood mononuclear cells (PBMC).

Methods and Results: Peripheral blood mononuclear cells (PBMC) were collected from seven poorly-controlled type II diabetics and six non-diabetic individuals and stimulated in vitro with bacterial LPS (1µg/ml) and BSA-AGE (200µg/ml), both independently and associated. This stimulation was done for 6 hours, both in the presence and absence of inhibitors of TLR4 (R. sphaeroides LPS, 20 µg/ml) and RAGE (blocking monoclonal antibody). Cell proliferation was studied by direct counting of viable cells on a trypan blue dye exclusion assay. Apoptosis at early and late stages was assessed by Annexin-V/PI staining using flow cytometry. Regulation of TNF-α and IL-10 gene expression was determined by RT-qPCR. PBMCs from diabetes patients tended to be more resistant to apoptosis (approximately 30-40% less cell death) and RAGE and TLR4 signaling had no significant effect on cell proliferation and death. There were no synergistic or antagonistic effects associated with simultaneous activation of TLR4 and RAGE in PBMCs from either diabetics or non-diabetics. TLR4 activation is more relevant for

the induction of TNF-α (20 fold in cells from non-diabetic patients and 10 fold in cells from diabetics) and IL-10 (6 fold induction in cells from diabetics and non-diabetics). Simultaneous activation of RAGE signaling inhibited LPS-induced expression of TNF-α.

Conclusion: Activation of TLR4 and RAGE do not have relevant roles in the induction of apoptosis in PBMC cells. RAGE signaling had a negative regulatory effect on LPS-induced TNF-α.

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IMMUNO 2012
XXXVII Congress of the Brazilian Society of Immunology
V Extra Section of Clinical Immunology - ESCI

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Campos do Jordão SP Brazil
October 20 - 24, 2012

T cell apoptosis in thymus from alloxan-induced diabetic mice

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Background: Thymus is a target organ in metabolic diseases. Hormones alterations provoke deeply alterations in thymocytes which can differentiate in regulatory cells or be induced to death. Thymus from diabetic mice presents leptin and ghrelin alterations (previous results presented in SBBC Congress) and both hormones control the lifetime and development of thymocytes.

Objectives: Here in, we aimed analyze cell death in thymocytes ($CD3^+$population) as expression of some regulatory proteins involved in death by apoptosis process.

Methods and Results: Cell death was detected by Tunel technique and death in thymocytes subpopulation was determined by flow cytometry for Annexin-V. Expression of proteins involved in apoptosis pathway was determined by real time PCR using TaqMan probes (Invitrogen).By immunohistochemical stain we evaluated Bcl-2, Bax and caspase-3 expression in thymus tissue. In thymus from diabetic mouse is possible observe an increase in gene expression of TNFR1, TNFR5, Gzmab, PGPR, SGLP and GADD45 proteins that are involved in apoptosis. Indeed, in situ stain show high levels of Tunel positive cells as deposition of Bax and caspase in all thymus tissue accompanied by less Bcl-2 stain. Cell death in subpopulation analyzes revealed that immature thymocyte population (DP and DN) is more sensible to apoptosis in atrophy thymus.

Conclusions: Atrophic thymus from diabetic mouse presents a deep commitment of all humoral as cellular components. Alterations demonstrated in this work, together with others previous results of our lab disclose that thymus is a target organ in leptin/ghrelin serum levels deregulation. This can be evidenced by high levels of thymocyte apoptosis.

Ethical approval: All experiments were approved by the internal ethical committee (CEUA #2312-1).

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XXXVII Congress of the Brazilian Society of Immunology
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SBI Sociedade Brasileira de Imunologia
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Campos do Jordão SP Brazil
October 20 - 24, 2012

INFLAMMATORY MARKERS IN GRANULOMAS FROM TUBERCULOSIS-ASSOCIATED IMMUNE RECONSTITUTION INFLAMMATORY SYNDROME

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Text: Immune reconstitution inflammatory syndrome (IRIS) results from the rapid restoration of pathogen-specific responses to opportunistic infections after initiation of highly active antiretroviral therapy (HAART). Exacerbation of tuberculous lymphadenitis, with a marked inflammatory component, is the most common clinical manifestation in patients with TB-IRIS. Transcription factor forkhead box P3 ($FoxP3^+$) regulatory T (Treg) cells, which suppress the effector T cell function, are a key mechanism to limit the inappropriate or excessive responses by producing inhibitory cytokines like IL-10 and TGF-β. However, while Treg cells are crucial for inhibiting tissue inflammation, Th17 cells, that produce IL17 cytokine, are highly pathogenic during the inflammatory process. Our **objective** was to analyze the *in situ* inflammatory immune response in HIV infected patients whom presented TB lymphadenitis associated to IRIS. **Methods:** We evaluated lymph node (LN) biopsy samples from TB-IRIS, Tuberculous Lymphadenitis (TBL), and Reactive Lymphadenitis (LR) patients. The study was performed by immunohistochemistry with monoclonal antibodies to CD3, CD4, CD8, CD68, CD25, IFN-γ, TNF-α, IL-10, TGF-β, IL17 and FoxP3. **Results and Conclusions:** TB-IRIS and TBL lymph nodes presented high numbers of $FoxP3^+$ cells. However, only TB-IRIS patients

showed an increased IL10 and TGF-β expression as well as decreased numbers of IFN-γ^+ cells in the granulomatous lesions (supposed Treg cells). Moreover, our preliminary data showed many IL17$^+$ cells in such lesions. Although Th17 cells also appear to play an important role in the mycobacteria infection, a dysregulated Th17 response can induce severe tissue inflammation. The understanding of the reciprocal relationship between Th17 and Treg cells may provide important targets for treatment of multiple inflammatory conditions.

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IMMUNO 2012
XXXVII Congress of the Brazilian Society of Immunology
V Extra Section of Clinical Immunology - ESCI

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Campos do Jordão SP Brazil
October 20 - 24, 2012

Influence of Nod proteins on periodontal disease-related signaling pathways in vitro

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Introduction: Nucleotide-oligomerization domain (Nod) proteins play an important role on recognition of bacterial invasion during innate response; however little is known about their role in Gram-positive and Gram-negative mixed infections such as periodontal diseases. **Objective**: The aim of this study was to evaluate the involvement of Nod on the cytokine networks as well as on activation of the signaling pathways induced by Gram-positive and Gram-negative bacterial challenge. **Method**: bone marrow-derived macrophages were obtained from WT C57/Bl6 mice as well as knockout mice for Nod1, Nod2 and Rip2. These cells were stimulated for 24h with 10^6 CFU/mL of heat-killed Gram-negative *Aggregatibacter actinomycetemcomitans* (Aa) associated or not with 10^6 CFU/mL of Gram-positive *Lactobacillus fermentans* (Lf). Total RNA from cell lysates was harvested and modulation of 84 TLR signaling-linked genes was assessed by RT-qPCR array. Activation of the signaling pathways linked to the expression of inflammatory mediators was performed in total protein samples of macrophages collected after 10, 30 and 60 minutes of stimulation was assessed by multi-ligand ELISA assays, determining the activation status of NF-kB (p65), p38, JNK e STAT3. **Results**: Nod2 and Rip2 attenuated the response of macrophages to Gram-negative bacteria as only 37 of the 84 genes evaluated were upregulated (> 2 fold) in comparison to 51 and 60 genes in Nod1 KO and WT cells. However, expression of IL-1a, IL-1b and IL-6 by stimulated macrophages was markedly enhanced in Nod1 KO cells. Deletion of Nod1, Nod2 and Rip2 decreased microbial-induced activation of NF-kB, p38 MAPK and STAT3 on macrophages. There was a delayed activation of JNK, starting at 30 min after stimulation, which as attenuated mainly in Nod1 KO cells. Moreover, there was a similar effect of the different stimuli (Aa or Aa with Lf) on the signaling proteins analyzed. **Conclusion**: This study showed a differential profile of the signaling pathways activated by microbial stimuli on macrophages with a common requirement of Nod1, Nod2 and Rip2.
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IMMUNO 2012
XXXVII Congress of the Brazilian Society of Immunology
V Extra Section of Clinical Immunology - ESCI

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Campos do Jordão SP Brazil
October 20 - 24, 2012

EFFECT OF PRO-INFLAMMATORY CYTOKINES IN PLATELET CONCENTRATE PRODUCED DURING THE PERIOD OF STORAGE IN HEMOAM FOUNDATION.

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Introduction: Transfusion of Platelet Concentrates (PCs) is used for the treatment of patients with quantitative or qualitative platelet disorders. The PCs stored produce several cytokines during storage and it has been demonstrated an immunomodulatory effect. This effect may be associated with the occurrence of febrile non-hemolytic transfusion reaction (FNHTR). In hemotherapy the irradiation of blood and yours components often have as a $_{137}$Cs radioactive source (Césio137) or $_{60}$Co (Cobalto60). The dose of 25 Gy may be able to inactivate lymphocytes and maintain the biological integrity of blood components this study aimed to analyze the pro-inflammatory cytokines of platelet concentrates during storage in HEMOAM Foundation and compare the pro-inflammatory cytokines levels found in platelet concentrates irradiated (PCI) and non-irradiated (PCNI). **Methods and Results :** In the period from February 2011 to June 2012 were collected 111 bags of PCs of blood donors on days 1, 3 and 5 of storage totaling 333 samples, distributed into two groups, irradiated (I) and non-irradiated (NI). The cytokines levels (IL-1β, IL-6, IL-8 and TNF-α) were determinate for Enzyme-Linked Immunosorbent Assays (ELISA). We results showed statistically significant difference to IL-6 and TNF-α, when compared both groups, PCs I and NI. The IL-6 showed a higher concentration in PCNI ($p<0,001$) while TNF-α decreased in this group. We When we analysed the differents days of the stored, we observed a higher concentration of IL1-β increased on day 5 and IL-6 in all days of the stored, in CPNI when compared with CPI group ($p <0.001$). However, TNF-α increased on days 1 and 3 in CPI ($p <0.05$) when compared CPNI. **Conclusion:** in this study we found that gamma irradiation appears to inhibit the synthesis of IL-6. Although not accompanied by the occurrence of adverse reactions, we support the hypothesis that transfusion reactions are not always the result of antigen-antibody interaction, but that may be caused by the administration of blood products containing large amounts of cytokines.

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IMMUNO 2012
XXXVII Congress of the Brazilian Society of Immunology
V Extra Section of Clinical Immunology - ESCI

SBI Sociedade Brasileira de Imunologia
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Campos do Jordão SP Brazil
October 20 - 24, 2012

TREATMENT WITH LIVE *LACTOBACILLUS DELBRUECKII* UFV- H2B20 PROTECT AGAINST ALLERGIC RESPONSE IN A MURINE MODEL OF ASTHMA

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Introduction and Objectives: Probiotics are formulations containing viable microorganisms that have beneficial influence on the maintenance of a balanced intestinal microbiota and on the resistance to infections. Several studies have shown the ability of probiotics to prevent and alleviate allergic diseases by modulating the immune system. We have previously reported that *Lactobacillus delbrueckii* UFV-H2b20, a probiotic candidate, is able to modulate the immune response of the host and therefore protect against infectious diseases. Here, we investigated the effect of *L. delbrueckii* UFV-H2b20 on the allergic response in a murine model of asthma. **Methods and Results:** Balb/c mice (n=6) received 10^9 viable *L. delbrueckii* by oral route starting ten days before sensitization and continuing until the euthanasia day. Control group received saline. For sensitization mice were inoculated subcutaneously with OVA and alumen. Two weeks later mice received a second inoculation. Experimental asthma was induced by exposing ovalbumin-sensitized Balb/c mice to OVA aerosol for six consecutive days. Mice were euthanized 24 hours after the last challenge with OVA. Our results showed that the oral treatment with *L. delbrueckii* significantly attenuated the influx of cells to the airway lumen. The numbers of eosinophils, lymphocytes and monocytes in the ***bronchio***-alveolar lavage were reduced in *L. delbrueckii*-treated mice when comparing to mice that received saline. Also we found higher levels of MPO and EPO in the lungs of probiotic-treated. The levels of IgE anti-OVA in serum were not significant different between saline and *L. delbrueckii*-treated mice. Chemokine levels were increased after treatment with *L. delbrueckii.* **Conclusion:** The oral treatment with *L. delbrueckii* UFV-H2b20 inhibited the airway inflammatory response in Balb/c mice. This effect does not seem to be

related to a diminished Th2 response, since the secretion of IgE was not different between the asthmatic groups.

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IMMUNO 2012
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V Extra Section of Clinical Immunology - ESCI

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Campos do Jordão SP Brazil
October 20 - 24, 2012

PRODUCTION OF IFN-γ and IL-10 BY MURINE MACROPHAGES IN RESPONSE TO INFECTION BY *CORYNEBACTERIUM PSEUDOTUBERCULOSIS*

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Introduction: C*orynebacterium pseudotuberculosis* is a facultative intracellular bacteria of macrophages. This pathogen causes caseous lymphadenitis (CLA) in goats and sheep. The CLA is a chronic disease characterized by the formation of abscesses in the lymph nodes and viscera. The spread of the CLA in herds progresses with significant economic losses (Microbes and infection 7: 1352-1363, 2005). It is particularly important to study the interaction between *C. pseudotuberculosis* and their host cell, since macrophages play an important role in defense against intracellular bacteria, being essential for the control and elimination of the parasite. This work intends to evaluate the production of cytokines IFN-γ and IL-10 in infected macrophages by wild strain C57 or by attenuated strain T1 from *C. pseudotuberculosis*. **Methods and Results:** Macrophages were obtained from the induction of inflammation with the injection of thioglycolate 4% (3ml) in the peritoneal cavity of five CBA mouse. The peritoneal cells were harvested and seeded in 24 well plates at 37 °C in a CO_2 incubator for 24 hours. After this period the adherent macrophages were infected by strain C57 or by strain T1 from *C. pseudotuberculosis* at a multiplicity of 10 bacteria per cell and incubated at 37°C in a CO_2 incubator for 1 hour. The supernatant was collected and then the Flow Cytometry test was performed to measure the production of cytokines IFN-γ and IL-10. There was a greater production of both IL-10 and IFN-γ by macrophages infected by the attenuated strain T1 than by macrophages infected by the wild strain C57. Uninfected macrophages (control) showed a less production of this cytokine compared to macrophages infected with both strains. This results showed a statistically significant difference ($p<0,05$). **Conclusion:** The strain T1 stimulated a greater production of IFN-g and IL-10 by macrophages, suggesting be favorable for generating a protective immune response Th1 type and regulation of this response respectively, without induce the formation of granuloma characteristic of

the disease since this strain is attenuated.

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XXXVII Congress of the Brazilian Society of Immunology
V Extra Section of Clinical Immunology - ESCI

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October 20 - 24, 2012

OPTIMIZATION OF MONOCLONAL ANTIBODY PRODUCTION IN CHO CELLS UNDER T-REX™ OVEREXPRESSING XBP(S) SYSTEM.

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Introduction: Optimization of the protein production, which by now has been under consideration for several decades, is still a challenging problem in pharmaceutical biotechnology. Different techniques of transcription and translation engineering have been used to improve protein secretion, but there still remain many open problems in post-translation modifications of the secreted protein and cell line stability. Overexpression of x-box binding protein (spliced form) (xbp(s)) has been shown to increase production of several proteins by the expansion of size of endoplasmic reticulum (ER) and enhancing of the total protein synthesis, including some immunoglobulins. In our work, we study the production of immunoglobulin G (IgG) under different conditions and under overexpression of xbp(s) in mammalian cells.

Methods and Results: To analyze the production of IgG, we used enzyme-linked immunosorbent assay (ELISA) and the obtained results show the increase of production of IgG under induction of 1µg/mL of deoxycycline in chinese hamster ovary cells (CHO) comparing to the control. Also, viability tests and qRT-PCR demonstrate the enforcing effect of overexpression of apoptotic xbp(s) by toxicity of higher concentration deoxycycline on cell viability.

Conclusion: The obtained data demonstrates the potential of T-Rex™ overexpressing xbp(s) system to improve CHO cell culture protein production. As the next step, we are planning to study the possibility of using of the system under consideration as “on-off switch” in IgG production.

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IMMUNO 2012
XXXVII Congress of the Brazilian Society of Immunology
V Extra Section of Clinical Immunology - ESCI

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October 20 - 24, 2012

PATTERN OF CYTOKINES PRODUCED IN SUB-POPULATIONS OF PERIPHERAL BLOOD LEUKOCYTES OF PATIENTS WITH LEPROSY AND HOUSEHOLD CONTACTS RESIDENT IN THE MUNICIPALITY OF GOVERNADOR VALADARES, MG.

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Introduction: Leprosy is a chronic infectious disease that primarily affects the skin and peripheral nerves, with clinical manifestations that depends on the host immune response to *Mycobacterium leprae*.

Methods and Results: The pattern of cytokine IFN-γ, IL-4, IL-10 intracytoplasmic by Flow Cytometry and in culture supernatants by ELISA assay was analyzed in a peripheral blood leukocytes population following specific antigen stimulation. Patients classified as tuberculoid showed higher frequency of IL-10 high producers after challenge with the *M.leprae* crude antigen. However, it was observed a much greater frequency of IFN-γ high producers in this group of patients, when compared to the frequency of IFN-γ high producers in groups of lepromatous and borderlines patients. The individuals of these two groups (lepromatous and borderline) showed a higher frequency of IL-4 and IL-10 high producers than tuberculoid individuals. A comparison between the household contacts (paucibacillary patients contacts and multibacillary patients contacts), showed a higher frequency of IFN-γ high producers in the paucibacillary patients contacts group, indicating a profile of possible resistance against the *M. leprae* infection. In

contrast, the multibacillary patients contacts group showed a higher frequency of IL-4 high producers.

Conclusion: It is known that multibacillary patients contacts, are exposed to high bacterial load, which indicates the possibility of an increased risk of disease in this group. Furthermore, due to their relationship to index cases classified as multibacillary, we suggest that there may be a genetic predisposition of these individuals to develop in the future leprosy in a more severe clinical form.

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XXXVII Congress of the Brazilian Society of Immunology
V Extra Section of Clinical Immunology - ESCI

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October 20 - 24, 2012

FERRIC AEROBACTIN RECEPTOR FROM *ESCHERICHIA COLI* – IutA: A NEW TYPE 1 T-INDEPENDENT ANTIGEN

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Introduction: Among the different indigenous bacterial species in the gut, *Escherichia coli* is one with potential to cause infections. Certain strains of *E. coli* have a plasmid (pColV), which encode ferric aerobactin outer membrane receptor, IutA, often associated with urinary tract infection. Our group has recently described IutA as an inducer of B cell proliferation. Here, we identify the molecules and mechanisms that modulate the proliferation of B lymphocytes induced by rIutA from *E. coli*.
Methods and Results: To determine whether the B cell proliferation induced by rIutA is dependent on other cell, we carried out assays with CFSE-labeled B cells either cocultured separately with macrophages or dendritic cells stimulated with rIutA using transwell membranes or cultured with conditioned medium from these accessory cells. The results showed that rIutA induced indirectly the proliferation of B cells, dependent on molecules released from accessory cells. When we analyzed the ability of rIutA in inducing proliferation of cells from mice deficient in adapter molecule MyD88, we determined that MyD88 is crucial for signaling induced by rIutA in B cells, but not in accessory cells. A similar analysis with cells from mice deficient in TLR4, TLR2 or IL-33R revealed that these receptors were not required for rIutA signaling. Conversely, the pretreatment of the B cells with IL-1R antagonist significantly decreased the proliferation of these cells in response to conditioned medium from cultures of IutA-stimulated macrophages. Moreover, we determined that IutA induced the expression of IL-1 in macrophages and dendritic cells stimulated with IutA.
Conclusion: Altogether, our results suggest that IutA from *E. coli* induces polyclonal B-cell proliferation independently of T cells in a mechanism mediated by accessory cells such as macrophages and dendritic cells. These results open perspectives for studying IutA as a molecule that stimulates the mucosa-associated lymphoid tissue and as an immune-evasion molecule from pathogenic *E. coli*.

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V Extra Section of Clinical Immunology - ESCI

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October 20 - 24, 2012

ALLORESPONSE SIMULATION "IN VITRO" TO EVALUATE MESENCHYMAL STEM CELL INTERFERENCE ON LYMPHOCYTE PROFILE IN THIS SCENARIO.

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Graft vs host disease (GVHD) it is a disease that occur after allogeneic hematopoietic stem cell transplantation (HSCT). GVHD is mediated by donor T cells immune response and it is responsible for 15-30% of deaths. Mesenchymal stem cells (MSC) represent an adult population of non hematopoietic stem cells, which can self-renew and differentiate into mesoderm lineage cells. They display immunomodulatory properties that have driven their use to the inhibition of immune inflammatory responses. Therefore, this study evaluates phenotypic changes on lymphocytes using as stimuli allogeneic dendritic cells, and co-culturing these cells in presence or absence of MSC.

Human MSCs (hMSC) were obtained from dischargeable bone marrow collection filters (Einstein Ethical Committee Approval-10/1412); which were washed with cell culture medium (DMEM-LG). hMSC were characterized by flow cytometry and differentiated according to International Society for Cell Therapy criteria. Peripheral blood was obtained form health volunteers after informed consented and performed density gradient (Ficoll-Hypaque) to obtain PBMC. Dendritic cells were derived from CD14+ cells isolated by affinity column. The allorecognition model was designed by culturing lymphocytes with non-HLA-matching dendritic cells. These cells were co-cultured in hMSC's absence (LD) or presence (MLD). After 72 hours of co-culture the lymphocytes from LD or MLD (n=3) were analyzed by flow cytometry for CD3, CD4, CD25, CD69, CCR5, CCR6 CD45RO, T-bet and RORγt.

We observed an increase of CD69 on MLD (9% ± 8.4) compared to LD (2% ± 2.4), CD45RO on MLD (42.15% ± 0.3) to LD (39.70% ± 2.4), CCR6 on MLD (39% ± 22.4) to LD (33% ± 22), and CCR5 MLD (28.5% ± 14.7) compare to LD (25% ± 17.5); down regulation of CD25 on MLD (6.89% ± 1.5) compare to LD (8.89% ± 0.3), and the same for T-bet on MLD (4.65% ± 6.4) to LD (6.98% ± 2.7) RORγt on MLD (1.52% ± 2.1) to LD (1.64% ± 1.3).

Despite CD3+CD4+ lymphocytes co-cultured with hMSC presented up regulation on CD69 and CD45RO, activation and memory markers, they presented a down

regulation on Tbet and RORγt that are transcription factors related to Th1 and Th17, the main effectors of acute GVHD indicating a possible pathway involved in immune-modulation played by hMSC in this scenario. There is also up regulation of CCR5 and CCR6 which can indicate a homing capacity to inflammation sites. However, it is necessary to perform further experiments to confirm these data and hypothesis.

IMMUNO 2012
XXXVII Congress of the Brazilian Society of Immunology
V Extra Section of Clinical Immunology - ESCI

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Campos do Jordão SP Brazil
October 20 - 24, 2012

IMMUNOSENESCENCE AND DENDRITIC CELLS: ROLE OF TLR2 AND TLR4 IN PHAGOCYTOSIS AND NO PRODUCTION AGAINST *CANDIDA ALBICANS*

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Introduction: Dendritic cells (DC) are professional antigen presenting cells, and play a key role in the induction of both innate and adaptive immunity. Aging is associated with progressive decline in immune function, resulting in an increased susceptibility to infection, taking into account the specific changes in T cells, macrophages, neutrophils and DC, usually referred to as immunosenescence. The nitric oxide (NO) is involved in the pathogenesis and control of infectious diseases, tumors, autoimmune processes and chronic degenerative diseases. Among microorganisms, the fungus *Candida albicans* (*C. albicans*) is an important pathogen for the development of invasive infections, especially in immunocompromised individuals and elderly persons. In addition, functional alterations in DC with aging, including their role in *C. albicans* infections are poorly understood. Objectives: To study the phagocytic capacity and NO production from mature DC against *C. albicans* in aged patients. The involvement of TLR2 and TLR4 receptors was also evaluated.

Methods and results: Peripheral blood monocytes were isolated from healthy elderly donors (n=18) and young donors (n=20), and were cultured for seven days to differentiate into DC under the influence of IL-4 and GM-CSF. Participation of receptors type TLR2 and TLR4 was assessed in vitro by using specific blocking antibodies. The phagocytosis assays were realized for 1 hour and analyzed by confocal laser scanning microscopy and NO formation was determined by using a Griess method. The data was statistically analyzed and $p<0.05$ was considered significant. The elderly presented more DC with internalized *C.albicans* than young individuals (89.7 % and 63.3%, respectively). There was no difference in NO production between the groups regardless of the stimulus. The TLR2 or TLR4 blocking did not affect these two events.

Conclusions: These data suggest the DC from elderly internalize more *C. albicans* than young subjects. However, the fungus did not increase the NO production in any group, suggesting that during the course of infection, *C. albicans* could deregulate the immune system and inhibit NO production by DC. So, more internalized fungi associated with faulty production of NO could suggest that elderly are more susceptible to develop this infection. Beside, the TLR2 and TLR4 receptors do not appear be involved in this mechanism.

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XXXVII Congress of the Brazilian Society of Immunology
V Extra Section of Clinical Immunology - ESCI

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October 20 - 24, 2012

IL-13 AND COLLAGEN PORCENTAGE IN THE PERI-IMPLANT MUCOSA OF PATIENTS WITH PERI-IMPLANTITIS

GABRIELA PEGORARI DA SILVA (IC)[1]; MÁRCIA FERNANDES DE ARAÚJO (PG)[2]; DENISE BERTULUCCI ROCHA RODRIGUES[1,2]; MARCELO LUIZ RIBEIRO DE MELO[1]; MARCELO HENRIQUE NAPIMOGA[3]; POLLYANA MIRANDA ALVES[2]; SANIVIA APARECIDA DE LIMA PEREIRA[1,2]

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Introduction: It is known that the inflammatory response causes periimplant tissue changes with release of several cytokines. However there are few studies that demonstrate the histopathological and immunohistochemical changes in the tissues with peri-implantitis. It is also known that IL-13 tiggers deposition of collagen in different areas of the body. However, until now there were no studies linking IL-13 with collagen deposition in periimplant tissues.

Methods and Results: We selected 18 patients in a dental practice in private in Uberaba, Minas Gerais, Brazil: nine patients with peri-implantitis and nine control patients. After approval by the Ethics Committee at the Federal University of Minas Gerais (UFTM) (n^0 1657) were collected fragments of the marginal gingiva in the palatal region measuring approximately 2x1mm. Each fragment was divided into two fragments. One fragment was processed histologically and the slides stained with picrossirius for morphometric analysis of collagen. The other fragment was frozen for later determination of IL-13 by ELISA using monoclonal antibodies (eBioscience, Vienna, Austria). The determination of the percentage of collagen was performed using the software Axiovision (Zeiss, Germany) in all fields of the fragments. In patients with peri-implantitis the percentage of collagen was lower (18.92 ± 11.69) compared to control patients (26.96 ± 14.24) with significant difference ($p < 0.0001$). The concentration of IL-13 was lower in patients with peri-

implantitis (5.13 ± 4.57) compared to control patients (8.9 ± 4.2), also with a significant difference ($p < 0.04$). **Conclusion:** In patients with peri-implantitis, the lowest percentage of collagen found in periimplant tissues could be caused not only by the action of collagenases, as already shown in other studies, but also due to lower concentration of IL-13 in this region, first demonstrated in this study. Thus, the decrease of collagen in periimplant tissues could contribute to the reduction of support and consequent loss of the dental implant in peri-implantitis.

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IMMUNO 2012
XXXVII Congress of the Brazilian Society of Immunology
V Extra Section of Clinical Immunology - ESCI

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Campos do Jordão SP Brazil
October 20 - 24, 2012

THE BIFLAVONOID MORELLOFLAVONE INDUCES THE HLA-G EXPRESSION

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Introduction: The morelloflavone is a biflavonoid extracted from the *Garcinia xanthochymus,* and recently recognized for its properties antiangiogenic and antitumorigenic. HLA-G is an immunomodulatory molecule selectively expressed on cytotrophoblasts at the feto–maternal interface, where it play an important role in maternal tolerance of the fetus. Its exhibits alternative transcription of spliced mRNAs that encode different HLA-G isoforms, namely the membrane-bound (HLA-G1) and solubles (HLA-G5, *shed*HLA-G1) proteins. Thus, the aim of this study was evaluating the morelloflavone effect in modulating the expression of HLA-G (human leukocyte antigen- G). **Methods and Results**: To evaluate the potential ability of morelloflavone to induce HLA-G (human leukocyte antigen- G) *in vitro*, we performed Flow cytometry to observe the HLA-G1 expression with antigen-specific antibody phycoerythrin (PE)–conjugated (MEM-G/9-PE). Moreover, was realized the Enzyme-linked immunosorbent assay- ELISA for HLA-G5 plus sHLA-G1 using antigen-specific antibody (MEM-G/9). *HLA-G* expression was analyzed by Real-time PCR and the data was normalized to *GAPDH*. The results have shown an increased expression of HLA-G1 in melanoma cell line (FON+), cervical choriocarcinoma (JEG-3) and in cells transfected with HLA-G1cDNA (M8-HLA-G1). Also, we observed a remarkable induction of HLA-G1 shedding in M8-HLA-G1. Moreover, the quantitative PCR showed that the gene expression of HLA-G was also induced by morelloflavone in melanoma cells M8 wild type, which do not express HLA-G constitutively. **Conclusion**: These results suggest that morelloflavone could induce the production of HLA-G and also increasing the expression of the molecule. The induction of shedding HLA-G suggests that morelloflavona could act at the systemic level helping therapies against autoimmune diseases and transplantation.

Key words: HLA-G, morelloflavone, Flow cytometry, Real Time PCR, ELISA

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IMMUNO 2012
XXXVII Congress of the Brazilian Society of Immunology
V Extra Section of Clinical Immunology - ESCI

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October 20 - 24, 2012

CHARACTERIZATION OF THE IMMUNOPHARMACOLOGICAL ACTIVITY OF *Aedes aegypti* SALIVARY SERPIN AET-7393

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Introduction: To overcome the barriers imposed by vertebrate hosts and be able to feed, hematophagous mosquitoes possess salivary components capable of regulating hemostasis, inhibit the inflammation and modulate the host immunity. However, the characterization of the biological activities of the salivary molecules in the host still needs further studies. We intend to characterize the biochemical and immunopharmacological activities of the transcript AET-7393 product, present in the *Aedes aegypti* saliva, classified as a serpin.

Methods and Results: The gene sequence of the serpin AET-7393, corresponding to the mature peptide, was synthesized and cloned into the expression vector VR2001 by Biobasic. Recombinant AET-7393 was expressed in HEK293 cells and purified by affinity chromatography followed by size exclusion chromatography. The plasmid only (VR2001-Naked) and the serpin-cloned plasmid (VR2001-Serpin) were used for the *in vivo* bleeding assays. BALB/c female mice were injected with the plasmids (40 μg/mouse, i.m.) and after 1, 3, 6 and 10 days a small incision in mice tail was performed. Blood was collected in a tube with distilled water maintained at 37°C for 30 min and then the optical density (O.D.) determined by a spectrophotometer at λ 540 nm. We have observed an increased bleeding in the VR2001-Serpin group after 3 days of plasmid injection, compared to the VR2001-Naked group (O.D.: 0.658 ± 0.086 *versus* 0.306 ± 0.052, respectively; n=8). We also performed the tail bleeding assay with the recombinant protein and observed an increased bleeding in animals injected with 2 mg/Kg (O.D.: 0.537 ± 0.081 *versus* 0.271 ± 0.071 from control group; n=10). In addition, mice immunized with VR2001-Serpin, but not VR2001-Naked, presented detectable concentrations of IgG1 (14.60 ± 5.737 μg/mL; n=10) and IgG2a (50.88 ± 17.20 μg/mL; n=10) specific for the recombinant protein as quantified by ELISA. Using the AET-7393 anti-serum, we were able to detect a positive reaction in

salivary gland extract (SGE) from *A. aegypti*, and found that adult mosquitoes contain approximately 10 ng of this salivary serpin per μg of SGE (1% of total protein). Our work also showed that the AET-7393 treatment had no effect in paw edema induced by carrageenan or nitric oxide produced by peritoneal macrophages.

Conclusion: The serpin AET-7393 seems to work as an anticoagulant in *A. aegypti* saliva, but it does not affect the inflammatory parameters studied so far.

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XXXVII Congress of the Brazilian Society of Immunology
V Extra Section of Clinical Immunology - ESCI

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October 20 - 24, 2012

DEVELOPMENT OF A MURINE MODEL OF ALLERGIC INFLAMMATION BY EXPOSURE TO SALIVARY COMPONENTS OF *Aedes aegypti* MOSQUITO

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Introduction: During blood feeding, *Aedes aegypti* female mosquitoes inoculate saliva into the skin of their vertebrate hosts, modulating their immune functions and facilitating disease transmission. Some studies have shown that salivary secretion is responsible for the sensitization to mosquito bites and some of the components present in saliva are allergenic. The aim of this project was to develop a model to evaluate the profile of the systemic immune response developed by mice naturally exposed to *Ae. aegypti* mosquito bite and compare it with the classical OVA-induced allergy model.

Methods and Results: BALB/c female mice were sensitized by exposure to *Ae aegypti* mosquito bites and i.n. challenged with PBS or mosquito salivary gland extract (SGE). Another group was sensitized with OVA adsorbed in alum and challenged i.n. with OVA. Sensitization and challenge with salivary components induced lung inflammation when compared to the control group. We have observed increased numbers of eosinophils (28,27 ± 4,13 *versus* 0,03 ± 0,03 x 10^4, n= 8), T $CD4^+$ (6,62 ± 0,68 *versus* 0,27 ± 0,06 x 10^4, n= 8) and $CD19^+$ cells (7,25 ± 0,53 *versus* 2,52 ± 0,27 x 10^4, n= 8) in bronchoalveolar lavage (BAL). The challenge with SGE also induced an increase in the population of T $CD4^+$ cells producing IL-4 (6.6 ± 2.1 *versus* 0.2 ± 0.2 x 10^3, n= 5-6) and IL-5 (95 ± 9.2 *versus* 26 ± 2.4 x 10^3, n= 5-6) in lung, as well as an intense mucus production and little deposition of collagen around the bronchioles, as determined by PAS and Masson trichrome staining, respectively. These features were very similar to the OVA-induced model. However, while both *Ae. aegypti*- and OVA-induced models induced levels of total IgE and specific IgG1 in serum, only the group sensitized by mosquito bites produced significant amounts of specific IgG2a. Moreover, unlike the OVA-induced

model, the group exposed to mosquitoes showed no change in breathing pattern or reactivity of the trachea in response to methacholine.

Conclusions: Sensitization with *Ae. aegypti* saliva and challenge with EGS was able to induce a response that resembles allergic airway inflammation. However, it promotes a mixed response with production antibodies of both Th1 and Th2 profiles with no changes in breathing pattern and no hiperreactivity of the trachea that differs of classical allergic models.

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IMMUNO 2012
XXXVII Congress of the Brazilian Society of Immunology
V Extra Section of Clinical Immunology - ESCI

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October 20 - 24, 2012

A ROLE FOR THE MAST CELL/KALLIKREIN-KININ PATHWAY IN THE EXTRAVASCULAR GENERATION OF BRADYKININ, AN ENDOGENOUS STIMULATOR OF THE IMMUNE SYSTEM

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Introduction: Initiated by contact with negatively charged molecules displayed by pathogens or secreted by cells, the contact system of coagulation, also referred as Kallikrein-Kinin System (KKS), is integrated by serine protease zymogens and the non-enzymatic high molecular weight kininogen (HK). After reciprocal activation between FXII and Plasma Kallikrein (PK), HK is cleaved by PKa, releasing bradykinin (BK), a proinflammatory nonapeptide. Pertinent to immunity, we have recently demonstrated that BK activates dendritic cells (DCs) and upregulates TH1 responses through bradykinin B2 receptors (BK2R). Awareness that the KKS is activated by soluble heparin released from mast cells (MC) granules prompted the undertaking of the present study, whose principal aim is the investigation of the functional interplay between MCs and the KKS.
Methods and Results: A digital camera coupled with an intravital microscope was used to measure plasma leakage with FITC-dextran (Relative Fluorescence Units, RFU) in hamster cheek pouch preparations (HCPs, n = 30). HCPs of anesthetized hamsters were subjected to topical applications of the contact system activator dextran sulfate (DXS) alone or in the presence of the ACE-inhibitor captopril, BK2R-antagonist HOE-140 and cromolyn (a stabilizer of MC granules). DXS failed to induce plasma leakage until 30 min; at this timepoint, we observed an intense plasma leakage at postcapillary venules (10265 ± 1650 RFU). This reaction was blunted by HOE-140 and by cromolyn, respectively evidencing BK and MC involvement. The leakage response started in a few postcapillary venules, suggesting that a discrete increase in permeability was required for contact system (DXS)-dependent intensification of plasma leakage. Strikingly, we found that low doses of histamine, which are capable of inducing subtle increase in plasma leakage (950 ± 490 RFU), strongly enhanced the response of DXS (3907 ± 533 RFU). Furthermore, these effects were potentiated by Captopril (11340 ± 733 RFU). Conversely, the leakage reaction induced by histamine combined to DXS was almost completely blocked by HOE-140 or by cromolyn. Our data strongly suggest that a minute plasma leakage such as that induced by MC-derived

histamine may intensify inflammatory edema through contact-system dependent activation of the KKS.

Conclusions: Our findings provide a mechanistic framework to investigate the impact of MC-driven activation of the KKS on DC function in the draining lymph node.

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IMMUNO 2012
XXXVII Congress of the Brazilian Society of Immunology
V Extra Section of Clinical Immunology - ESCI

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October 20 - 24, 2012

IMPACT OF SEA IN CHRONIC OSTEOMYELITIS CAUSED BY *STAPHYLOCOCCUS AUREUS: CHEMOKINES SECRETION*

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Introduction: Osteomyelitis is a difficult-to-treat bone infection that is characterized by progressive inflammatory destruction of the infected one and new apposition of bone at the site of infection. *Staphylococcus aureus* is the most frequent microorganism isolated in both posttraumatic and hematogenous osteomyelitis. The virulence of this bacterium is associated with its ability to penetrate the bone tissue and to secrete exotoxins, like the superantigen Staphylococcal enterotoxin A (SEA**). Methods and Results**: Twenty individuals suffering from chronic staphylococcal osteomyelitis were selected amongst patients attending at the Municipal Hospital, Governador Valadares, State of Minas Gerais. The inclusion criteria included evidence of *S. aureus* infection, presence of fistulas over a period ≤ 1 year and bone sequestrum. The selected staphylococcal chronic group was comprised mainly of males and had an average age of 35 years. In nearly all cases (19/20), bone infection was caused by trauma and the most affected bones were the tibia and femur. The average duration of hospital confinement was 25 days, and the average time of the last acute recurrence was 5.2 months, as assessed by the manifestation of fever, intense bone pain, presence of fistulas and purulent secretion. The control group comprised 20 health employs from the Universidade Vale do Rio Doce. Blood samples were collected from all participants and PBMC were isolated for determination of chemokines by flow cytometry. The results showed higher ability to secret MIP-1β, MIP-1α and RANTES chemokines upon in vitro recall with SEA by the cells from the patients than by the health individuals. Distinct chemokine profiles with dichotomic MIP-1beta, MIP-1α and RANTES dynamic were observed during early and late

osteomyelitis episodes. The analyses of low and high chemokine producers further support the establishment of dichotomic chemokine during osteomyelitis episodes.**Conclusion:** Increased frequency of high MIP-1β chemokine producers is observed at the late stage of chronic osteomyelitis. At early stage of disease, it was observed high MIP-1α chemokine producers. Our data showed a prominent involvement of SEA-stimulated PBMC for chemokines secretion in osteomyelitis patients.

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IMMUNO 2012
XXXVII Congress of the Brazilian Society of Immunology
V Extra Section of Clinical Immunology - ESCI

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Campos do Jordão SP Brazil
October 20 - 24, 2012

ANALYSIS OF THE RESPONSE PROFILE OF CYTOKINES TNF-A, IL-10 AND TGFB OF MACROPHAGES CO-CULTURE WITH MUTANT VACCINE OF *CORYNEBACTERIUM PSEUDOTUBERCULOSIS*

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The caseous lymphadenitis (CLA), a disease caused by the bacterial *Corynebacterium pseudotuberculosis*, is characterized by the formation of abscesses in superficial and visceral lymph nodules. This disease mainly affects sheep and goats (Dorella *et al*., 2006). During the infection with this bacterial the innate immune system is responsible for the elimination of *C. pseudotuberculosis* through the mononuclear phagocytic cells. These phagocytic cells, taking the example of macrophages, are responsible for producing molecules, depending on the response profile of these cells, can kill bacteria or be permissive for maintenance in the host micro-organisms (Mosser & Edwards, 2008). The development of a mutant strain of *C. pseudotuberculosis* (CP13) by our research group, was used as a vaccine against LCA, and generated 81% protection in Balb / c (Dorella, 2009). This raised the question of mechanisms as to how the host can obtain this protection by the vaccine CP13 strain. The objective of this research was to evaluate the expression profile of cytokines TNF-α, IL-10 and TGF-β in co-culture of macrophages with the mutant CP13. Macrophages were co-incubated with the mutant CP13 strain and its T1 parental. After the 24 hour period of co-incubation the supernatant was collected and evaluated for the production of cytokines TNF-α, IL-10 and TGF-β by ELISA. We observed an increased production of pro-inflammatory cytokine TNF-α by strain T1, when compared with CP13 strain ($p < 0.05$) and increased production of anti-inflammatory cytokines IL-10 and TGF-β by CP13 strain compared with the T1 strain ($p < 0.05$) (Student T test).

We can conclude that, in agreement with our results CP13 mutant strain can generate a profile of resistance to septic shock, for the highest induction of anti-inflammatory cytokines in a subsequent contact with virulent wild strains of *C. pseudotuberculosis.* This may account for the of 81% protection generated by the CP13 strain. However, further studies are needed to explain this mechanism.

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IMMUNO 2012
XXXVII Congress of the Brazilian Society of Immunology
V Extra Section of Clinical Immunology - ESCI

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Campos do Jordão SP Brazil
October 20 - 24, 2012

IMMUNOMODULATORY ACTION OF GEOPROPOLIS PRODUCED BY *MELIPONA FASCICULATA* SMITH ON HUMAN MONOCYTES

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Introduction: Geopropolis is produced by indigenous stingless bees from resinous materials of plants, adding salivary secretions, wax, land or clay. Since there are few works regarding the immunomodulatory action of geopropolis, the objective of this study was to investigate a possible cytotoxic action of geopropolis on human monocytes and to analyze its effect on cytokines (TNF-α and IL-10) production.
Methods and Results: Monocytes (1 x 10^6 cells / mL) were obtained from peripheral blood of healthy individuals and incubated for 18h with geopropolis (5, 10, 25, 50 and 100 µg) or lipopolysaccharide (LPS - 10 μg / mL). Control cells were incubated with medium alone. Supernatants were collected and subjected to enzyme linked immunosorbent assay (ELISA) for cytokines measurement. Cell viability was assessed using the MTT test. Data revealed that only the highest concentration of geopropolis (100 µg) exerted a cytotoxic action on monocytes, higher concentrations (10, 25 and 50 µg) stimulated significantly TNF-α production. IL-10 production was also increased significantly using the highest concentrations showing a similar profile to that of LPS.
Conclusion: Geopropolis stimulated the production of TNF-α and IL-10 in non-cytotoxic concentrations, suggesting its ability to upregulate human monocytes activity.

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IMMUNO 2012
XXXVII Congress of the Brazilian Society of Immunology
V Extra Section of Clinical Immunology - ESCI

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Campos do Jordão SP Brazil
October 20 - 24, 2012

CD8$^+$ T CELL MIGRATION TO THE MYOCARDIUM IS ENHANCED IN MYD88-DEFICIENT MICE INFECTED WITH *TRYPANOSOMA CRUZI*.

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Introduction: During acute infection with *Trypanosoma cruzi*, both CD4+ and CD8+ T cell play a crucial role in the control of the parasite burden. On the other hand, lymphocyte accumulation in the myocardium during chronic infection may cause tissue injury leading to chronic chagasic cardiomyopathy. It is known that MyD88-deficient mice are particularly susceptible to infection with *T. cruzi*, although the pathogenesis of myocarditis during *T. cruzi* infection in this strain of mice was not evaluated until now. Also, the role of MyD88 expression in T lymphocytes, during the infection, has not been established yet. Here, we first investigated whether MyD88 expression in CD4+ and CD8+ T cells is necessary for the migration of T lymphocytes to the myocardium.
Methods and Results: To answer this question, the heart of mixed (WT + MyD88KO→WT) bone marrow chimeric mice infected with the Y strain were analyzed by cytometry. Our data show that at day 14 pi, the ratio of CD4$^+$MyD88KO (CD45.2) to CD4$^+$WT(CD45.1) T cells among total CD4$^+$ T lymphocytes that are infiltrating the myocardium is the same found in the spleen of these chimeric mice. The same picture was found for CD8+ T cells. We also investigated CD4+ and CD8+ T cell migration to the heart during acute *T. cruzi* infection in the following mouse strains: MyD88KO, IL1RKO, IL-18RKO and compared these to C57BL/6 WT mice. We found that effector CD4+ T cells are diminished in the heart of MyD88-KO, compared to WT mice, reflecting their lower numbers in MyD88KO spleens. On the other hand, CD8+ T cells are significantly augmented in the MyD88-deficient myocardium, although equal numbers are found in the spleen of WT and KO mice.
Conclusions: First, these data show that MyD88-deficient CD4+ and CD8+ T cells have no intrinsic defect for tissue migration. Moreover, in a MyD88-deficient environment, the presence of CD8+ T lymphocytes in the heart is enhanced when compared to WT mice. Chemokines and NO levels in the myocardium of WT and KO mice are currently been investigated.

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IMMUNO 2012
XXXVII Congress of the Brazilian Society of Immunology
V Extra Section of Clinical Immunology - ESCI

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Campos do Jordão SP Brazil
October 20 - 24, 2012

LEPTIN ACTIVATES LIPID BODY-DRIVEN LEUKOTRIENE C_4 SYNTHESIZING MACHINERY WITHIN EOSINOPHILS.

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Introduction: Eosinophils are effector cells classically involved with allergic inflammation, although immunomodulatory roles have been also described to eosinophils. For instance in adipose tissue, eosinophils were shown able to modulate macrophage phenotype and interfere with metabolic homeostasis. Leptin is an adipocytokine involved not only in body weight regulation but also in neuro-immuno-endocrine modulation. It is well established that leptin activates immune cells and promotes eosinophil survival. In this study, using *in vivo* and *in vitro* approaches, we evaluated the means by which leptin acts on eosinophils to affect migration and capacity to activate release of leukotriene C_4 (LTC_4).
Methods and Results: In BALB/c sensitized mice, intrapleural injection of leptin (1 mg/Kg) induced, within 24 h, eosinophil influx which parallels with increased pleural amounts of LTC_4 (n=6, 3 different experiments). Leptin was also able to directly activate eosinophils *in vitro* (n=3). In *in vitro* stimulated human eosinophils, leptin dose-dependently (0.5-50 nM) elicited lipid body biogenesis and enhanced synthesis of LTC_4, but not of prostaglandin E_2. Intracellular signaling involved in leptin-induced eosinophil activation involves PI3K, since eosinophil migration was inhibited in $PI3K^{-/-}$ mice *in vivo*, while *in vitro* two inhibitors of PI3K activation (wortmanin and LY294002) blocked both leptin-triggered lipid body biogenesis and LTC_4 synthesis. Although leptin receptors are not G-protein coupled, pertussis toxin inhibited leptin-induced eosinophil activation, an effect found to be mediated by an autocrine activity by endogenous RANTES, since: (i) increased levels of RANTES are found in leptin-elicited inflammatory site; (ii) leptin induced RANTES secretion by murine eosinophils *in vitro*; (iii) *in vivo* treatment with anti-RANTES,

while failed to inhibit leptin-induced eosinophil influx, blocked eosinophil activation; and (iv) *in vitro* stimulatory effects of leptin were blocked by neutralizing anti-RANTES and anti-CCR3.

Conclusions: Our findings show that leptin-induced eosinophil activation is a highly regulated process and unveil a new mechanism by which leptin may control eosinophilic inflammatory disorders, reinforcing the connection between obesity and allergy-related pathologies.

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IMMUNO 2012
XXXVII Congress of the Brazilian Society of Immunology
V Extra Section of Clinical Immunology - ESCI

SBI Sociedade Brasileira de Imunologia
www.sbicongressos.com
Campos do Jordão SP Brazil
October 20 - 24, 2012

MECHANISMS OF VITAMIN-A MODULATION OF DSS-INDUCED COLITIS IN MICE.

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Introduction: Vitamin A has been reported to present regulatory effects on cell proliferation, antioxidant activity and ability to differentiate naïve T cells into regulatory T cells (Tregs). In previous experiments, we found that dietary supplementation with vitamin A resulted in reduction of gut inflammation in DSS-induced colitis in mice.

Objectives: Our aim in this study is to evaluate specific mechanisms driven by vitamin A that helped to recover the intestinal homeostasis.

Methods and Results: C57BL/6 mice were fed *ad libitum* four different diets: normal diet containing 4000 UI vitamin A, a vitamin A-free diet, and vitamin A-supplemented diets (containing either 10000 UI or 50000 UI vitamin A). After 7 weeks, colitis was induced by oral administration of 2% dextran sodium sulfate (DSS) for 6 days, and immunological parameters were evaluated. We had also fed normal diet to a control group in which colitis was not induced. Supplementation with both doses of vitamin A led to an increase in the frequency of CD4+Foxp3+ Tregs in spleen, and mucosal lymphoid sites, whereas vitamin A deficiency decreased these cells in spleen. On the other hand, the number of CD8+FoxP3+ Treg cells decreased in spleen of supplemented animals when compared to control

group. The number of CD103+ dendritic cells also decreased in mice that were fed normal and supplemented diets both in spleen and cecal lymph nodes.

Conclusion: CD4+Foxp3+Treg cells seem to be important for homeostasis when intestinal inflammation occurs, but the role of CD8+Foxp3+Treg cells and CD103+ dendritic cells was not clear in our system. We will evaluate other lymphoid sites, including colonic lamina propria, the affected site in colitis, to further understand the role of different cells in the modulatory effect of vitamin A in colitis.

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IMMUNO 2012
XXXVII Congress of the Brazilian Society of Immunology
V Extra Section of Clinical Immunology - ESCI

SBI Sociedade Brasileira de Imunologia
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Campos do Jordão SP Brazil
October 20 - 24, 2012

THE IMPACT OF BET A 2 ADRENERGIC RECEPTOR SIGNALING IN THE RECIPROCAL DIFFERENTIATION OF TH17 AND FOXP3+ TREG CELLS

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Introduction: The concept that the nervous and the immune systems interact is already well established. One way by which this interaction occurs is through the sympathetic nervous system (SNS). It has been already described that lymphoid organs such as spleen and lymph nodes are richly innervated by sympathetic fibers, which exert their actions by releasing catecholamines. CD4+T cells express adrenergic receptors, mainly β2 adrenergic receptor (β2AR). The signaling of β2AR on CD4+ T cells seems to impair Th1 differentiation and function. Thus, it was already demonstrated that increased intracellular cAMP following β2AR signaling leads to inhibition of cell proliferation and decreased production of IL-2 and IFN-γ in anti-CD3 stimulated T cells. Nevertheless, very little is known about the effect of β2AR signaling on Th17 differentiation.

Objective: The aim of this study was to investigate the impact of β2AR signaling in the reciprocal differentiation of Th17 and Foxp3+ Treg cells.

Methods: Spleen cells from $Foxp3^{gfp}$ KI mice were sorted (FACS Aria II) based on the expression of $CD4^{+}CD62L^{+}Foxp3^{-}$. Naïve $CD4^{+}$T cells were stimulated with plate bound anti-CD3 and cultured in the presence of IL-6, TGF-β, IL-23, IL-1β, TNF-α, anti-IL-4 and anti-IFN for 3 or 5 days. The specific β2AR antagonist (ICI 118 551) was administered in the culture. After 3 and 5 days, the expression of IL-17A was determined by intracellular cytokine staining (FACS Canto II) and enzyme-linked immunosorbent assay (ELISA).

Results: We observed that in control cultures about 12-16% of the naïve $CD4^{+}$ T cells differentiated into IL-17-producing cells. The treatment with the specific β2AR

antagonist did not change the percentage of the IL-17-producing CD4+ T cells as compared to control. However, the expression of IL-17 (MFI) was significantly reduced in cells from the cultures treated with the β2AR antagonist. Confirming this result, we also found lower IL-17 levels (ELISA) in the supernatants from cultures treated with the β2AR antagonist as compared to those observed in control cultures. Following β2AR blockade, the lower IL-17 production by anti-CD3 activated CD4+ T cells cultured under Th17 skewing conditions was associated with higher frequency of CD4+ T cells expressing Foxp3 (3 day).

Conclusion: Our data suggest that the endogenous production of catecholamines can impact the differentiation of Treg cells and the production of IL-17 by CD4+ T cells via β2 adrenergic receptor signaling.

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IMMUNO 2012
XXXVII Congress of the Brazilian Society of Immunology
V Extra Section of Clinical Immunology - ESCI

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October 20 - 24, 2012

NITRIC OXIDE, ARGINASE I AND REACTIVE OXYGEN SPECIES PRODUCTION IN CO-CULTURE WITH MACROPHAGES AND MUTANT CP13

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Corynebacterium pseudotuberculosis is an actinomyces bacterial with a great infectious potential and is the etiological agent of caseous lymphadenitis (CLA) in sheep and goats. Currently, there is no effective treatment or vaccine against this disease (Dorella *et al*., 2006). The cells of the immune defense are essential to contain its spread in the host and macrophages play a key role in eliminating this bacteria. However, this action depends on how these macrophages are activated according to the external stimulus that they suffer, resulting in profiles of classical or alternative activation (Mosser & Edwards, 2008). The development of a mutant strain of *C. pseudotuberculosis* (CP13) by our research group obtained 81% protection against LCA *in vivo*, but still have no information on how this protection occurs (Dorella, 2009). The objective of this study was to evaluate the immunological profile of macrophages in co-culture with the CP13 mutant in the parameters of nitric oxide (NO), reactive oxygen species (ROS) and the enzyme arginase I. We used primary cultures of bone marrow macrophages differentiated in co-culture with CP13 strain mutant and its parental T1. The results showed increased activity of the enzyme arginase I and nitric oxide production ($p < 0.05$) when compared with control. The reactive oxygen species production was lower compared with our control ($p < 0.05$) (Student T test).It can be concluded that the activation profile of macrophages was similar for both CP13 and T1, suggesting that the profile of mutant CP13 should induce a protection that is independent of the activation profile of macrophages.

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IMMUNO 2012
XXXVII Congress of the Brazilian Society of Immunology
V Extra Section of Clinical Immunology - ESCI

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Campos do Jordão SP Brazil
October 20 - 24, 2012

PARTICIPATION OF NEUTROPHIL AND THE CYTOKINE TNF-α IN PSORIASIFORM SKIN INFLAMMATION IN MICE

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Introduction: Psoriasis is a chronic autoimmune skin disease of unknown cause that involves dysregulated interplay between immune cells and keratinocytes. Topical application of imiquimod (IMQ), a Toll like receptor 7 and 8 ligand can induce psoriasis by stimulating production of inflammatory cytokines and chemokines. In this study, we investigated the alterations involved in psoriasis-like skin inflammation in mice induced by IMQ, focusing on the characterization of immune cells and inflammatory mediators involved in this condition.
Methods and Results: BALB/c mice were shaved on the back skin and received daily, for 5 consecutive days (days 1- 5), topical applications of imiquimod (3.125 mg) from a commercially available cream (5%) (Modik; EMS). Control mice were treated with vaseline cream. On day 6, mice were euthanized and spleen, inguinal lymph node and back skin fragments were collected. The spleen mass was evaluated. The number of B cells and neutrophils both in skin and inguinal lymph node were investigated by flow cytometry and the levels of MPO were measured in skin. In addition, TNF-α and CXCL1 levels were measured in the skin by ELISA. Daily application of IMQ on mice skin induced a significant spleen enlargement with an increase in weight of approximately 2 fold. The treatment also induced an increase in the number of activated B cell (control: $3.5 \times 10^6 \pm 1.4 \times 10^6$; IMQ treated: $26.6 \times 10^6 \pm 10 \times 10^6$) and neutrophils (control: $0.8 \times 10^4 \pm 0.6 \times 10^4$; IMQ treated: $5.2 \times 10^4 \pm 1.1 \times 10^4$) in the inguinal lymph node. Moreover, we found increased levels of MPO (2.2 fold increase), CXCL1 (control: 480.7 ± 49.9 pg/mL; IMQ treated: 786.9 ± 59 pg/mL) and TNF-α (control: 130.5 ± 25.2 pg/mL; IMQ treated: 765 ± 121.3 pg/mL) in the skin of IMQ treated mice.
Conclusion: Our results indicate that immune cells such as B cells and neutrophils may have a role in the psoriasiform skin inflammation by a mechanism involving the production of inflammatory mediators such as TNF-α.

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IMMUNO 2012
XXXVII Congress of the Brazilian Society of Immunology
V Extra Section of Clinical Immunology - ESCI

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Campos do Jordão SP Brazil
October 20 - 24, 2012

IN VITRO ASSAY DEVELOPMENT TO REPLACE ANIMAL TESTING IN ALLERGENIC POTENTIAL EVALUATION OF CHEMICAL AGENTS

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Modifications in the regulation for cosmetics in Europe prohibiting animal tests for evaluating the sensitization potential of chemicals and new ethical standards of society led to the necessity of development of new methods to evaluate the allergenic potential of chemicals. The European Union-funded program on the development of novel testing Strategies for In Vitro Assessment of Allergens (SENS-IT-IV project) has already shown that myelomonocytic cell lines are activated by the presence of some chemicals, working as antigen presenting cells in *in vitro* assays. In the present work, a human acute leukemia cell line THP-1 has been incubated with sensitizers and non-sensitizers chemicals. For each chemical, the effective concentration 50 (EC50) was determined as well as the concentration of cell viability (CV) 75%. The CV75 was employed to analyze IL-8 secretion and the patterns of CD86 expression after 24 or 48h-incubation of THP-1 cells with each chemical. The mRNA IL-8 expression analyses performed after 3h-incubation of THP-1 cells with selected chemicals were performed to confirm the results. In our conditions, the analyses of CD86 expression on THP-1 after chemical exposure allowed differentiating sensitizer categories from non-sensitizer chemicals. The analyses of IL-8 release in the THP-1 culture supernatants by enzyme immunoassay showed that it is not ever possible to differentiate sensitizers from non-sensitizers in contrast to the IL-8 mRNA expression analyses that allowed to distinguish them (CEP0250/09).

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IMMUNO 2012
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October 20 - 24, 2012

ANALYSIS OF THE ROLES OF MEMBRANE CD83 MOLECULE IN CALCIUM SIGNALING IN T-LYMPHOCYTES STIMULATED BY DENDRITIC CELLS

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Introduction: CD83 is a key marker present on the surface of mature dendritic cells (mDCs) and is directly related to the linfostimulation ability of these cells. Several functions are associated to the CD83 molecule which apparently has a central role in the regulation of the immune system. However, even today, very little is known about the mechanism of action of CD83. The aim of this study was to show the influence of this molecule, present in DCs, in the modulation of the calcium signaling in T lymphocytes during the immunological synapse made between these two cell types. **Methods and Results**: Monocytes, isolated from peripheral blood of healthy donor, were differentiated into mDCs by culture, for seven days, in the presence of GM-CSF, IL-4, and, in the last two days, TNF-α. The knockdown of CD83 was made four hours before the activation by lipofection with iMAX and a specific CD83 siRNA ($siRNA_{CD83}$) or, as a control, a siRNA without known specificity ($siRNA_{scr}$). The percentage of $CD83^+$ cells was 84.8 ± 5.8 in the $siRNA_{scr}$ group and 68.2 ± 10.6 in the $siRNA_{CD83}$ group (n=5; $p<0,05$). Moreover, mRNA for CD83 was also reduced with the knockdown. Purified T lymphocytes were incubated with the calcium indicator Fluo-4AM and, then, analyzed by flow cytometry in the presence of allogeneic mDCs. $siRNA_{CD83}$-treated mDCs induced, in T lymphocytes, a relative calcium signal amplitude of 34.4 ± 7.5 compared to 45.3 ± 5.5 induced by mDCs treated with $siRNA_{scr}$ (n=5). Another approach used to identify the role of CD83 in calcium signaling, that is less harmful to DCs, was to block this membrane molecule with a specific antibody. Dendritic cells that were treated with those antibodies induced a relative signal amplitude of 48.4 ± 12.0 compared to 88.7 ± 22.5 induced by mDCs from an untreated group (n=5; $p<0,05$). Furthermore, the maximum fluorescence observed by fluorescent microscopy in T lymphocytes stimulated by anti-CD83 treated mDCs was significant lower (58.2 ± 3.6) when compared to the untreated group (80.5 ± 4.3)

(n=41; p<0.0001). Similar results were obtained with confocal microscopy. The presence of antibodies also reduced the proliferation of lymphocytes in the presence of mDCs (n=3). **Conclusion**: These data suggest that CD83, expressed in mDCs, plays an important role in calcium signaling in T lymphocytes stimulated by these cells, which is in agreement with the observed role of this molecule in the linfostimulation ability of mDCs.

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XXXVII Congress of the Brazilian Society of Immunology
V Extra Section of Clinical Immunology - ESCI

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October 20 - 24, 2012

IDENTIFICATION OF THE ANTIGEN RECOGNIZED BY ANTI-ANTI-ID MAB 5.G8 ON HUMAN MELANOMA CELL LINE.

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Introduction: Studies showed that the expression pattern of cell surface ganglioside changes during the melanocyte malignant transformation and, as a consequence, the ganglioside(GD)3 becomes hyper-expressed on melanoma cells, becoming a good target for therapy. An anti-GD3 monoclonal antibody (mAb), mAb R24, is commercially available. Although the use of mAb R24 promotes regression of metastatic melanoma in patients, its therapeutic application is limited because of its toxicity. In an attempt to obtain a new anti-GD3 mAb, we did anti-anti-Idiotypic (Id) mAbs. The anti-anti-Id mAb 5.G8, *in vitro*, recognizes melanoma cell lines, promotes complement fixation and antibody-dependent cell cytotoxicity. *In vivo*, mAb 5.G8 is able to inhibit tumor growth. This work seeks to identify the antigen recognized by anti-anti-Id mAb 5.G8.

Methods and Results: ST8D4 cell line, engineered to express the GD3 synthase enzyme, was treated with saponin, trypsin or GD3 synthase inhibitor before being incubated with 5.G8, R24 or irrelevant antibodies. The analysis by flow cytometry showed that the treatments with saponin or trypsin did not modify the recognition by R24 nor 5.G8. However, cells treated with GD3 synthase inhibitor were recognized by mAb 5.G8 but not by R24. In parallel, 5.G8 and R24 mAbs were coupled to CNBr-activated sepharose and SKMel-28 melanoma cell line extract was applied to both columns. The eluted materials were analyzed by SDS-PAGE and western blotting.

Conclusions: Preliminary results suggest that the anti-anti-Id mAb 5.G8 recognizes a mimotope.

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V Extra Section of Clinical Immunology - ESCI

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October 20 - 24, 2012

PROTEIN GALECTIN-1: PROTECTIVE EFFECT ON THE PROCESS OF EXPERIMENTAL UVEITIS INDUCED BY LIPOPOLYSACCHARIDE

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Introduction: Galectins are a family of carbohydrate-binding proteins with an affinity for β-galactosides. Galectin-1 (Gal-1) is differentially expressed by various normal and pathological tissues and appears to be functionally polyvalent, with a wide range of biological activity. In the present study we monitored the expression and the mechanism of action of endogenous Gal-1 in rat ocular tissues after 24 and 48 hours using a model of endotoxin-induced-uveitis (EIU)**.** We also analyzed the possible anti-inflammatory effect of the administration of recombinant Gal-1 (rGal-1) in control and experimental conditions. **Methods:** *Rattus norvegicus* were induced to uveitis (lipopolysaccharide - 1 mg/kg) and divided into experimental groups (n=10/group): EIU untreated for 24 and 48h, EIU treated intraperitoneally with rGal-1 (3 µg / animal) and were sacrificed after 24 hours. Enucleated eyes were processed to histopathological and immunohistochemistry, and drawing samples of aqueous humor (AH) to quantification of inflammatory cells. **Results**: After 24h of EIU, ocular tissues presented inflammatory response characterized by the intense recruitment of neutrophils (EIU 1203 ± 392.9 and control 17.72 ± 15.47 cells/mm^2), especially in the anterior eye segment. LPS also provokes a significant influx of neutrophils (54.06 ± 15.38 x 10^5 cells/mL) and mononuclear phagocytic cells (6.375 ± 2.742 x 10^5) in the AH compared to control group (4.44 ± 4.19 x 10^5; 0.6250 ± 0.629 x10^5, respectively). The effect of rGal treatment after EIU 24 hours was associated with decreased numbers of neutrophils both in the ocular tissues and AH (294.6 ± 221; 24.33 ± 27.73 cells/mm^2, respectively) and significant influx of phagocytic mononuclear cells in AH (20.53 ± 3.72x10^5 cells/mL). The expression of Gal-1 was detected in the anterior and posterior eye segment. Densitometric

analysis of ocular tissues showed a significant upregulation of Gal-1 expression in the anterior segment of eyes compared to control (174.7 ± 8.371; 152.9 ± 12.65 a.u., respectively), and a significant downregulation compared to treated group with rGal-1 (148.3 ± 13.91). **Conclusion**: These data provides not only further insight into the protective effect of Gal-1 by inhibition of inflammatory cells, but also provides a rationale for a clinical use for drugs developed from this line of research.

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IMMUNO 2012
XXXVII Congress of the Brazilian Society of Immunology
V Extra Section of Clinical Immunology - ESCI

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October 20 - 24, 2012

IL-10 MEDIATES CROSS TALK BETWEEN PERITONEAL MACROPHAGE SUBPOPULATIONS

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Introduction: Mononuclear phagocytes play an important role in innate immunity, such as clearance of cellular debris, pathogens and antigen presentation, being responsible for shaping the adaptive immune response. The peritoneal cavity (PerC) is a singular compartment where many cell populations reside and interact. We recently demonstrated that there are two different subpopulations of macrophages in the PerC, named large peritoneal macrophages (LPM) and small peritoneal macrophages (SPM) and that they display particular expression of a variety of surface markers and distinct responses *in vivo* and *in vitro.* Despite the widely adopted experimental approach of intraperitoneal (i.p.) inoculation, little is known about the behavior of the different cell populations within the PerC. **Methods and results:** To evaluate the dynamics of SPM and LPM, we analyzed the *in vivo* response of these cells to zymosan. There was a significant increase in the frequency of SPM 30 minutes after i.p. inoculation of zymosan, which correlated with the production of IL-10 by LPM. Interestingly, the inoculation of zymosan did not induce migration of SPM in mice deficient in IL-10, suggesting a role for this cytokine in the recruitment of SPM to the PerC during infection stimuli. In order to determine the mechanism by which IL-10 mediate SPM recruitment, we analyzed the production of MCP-1, an important chemotactic factor for macrophages in the infection site. A high production of MCP-1 was observed in LPM, which correlated with the production of IL-10 and recruitment of SPM. Surprisingly, there was no production of MCP-1 in mice knockout for IL-10, which provides more evidences for the role of IL-10 in the recruitment of cells to the PerC. **Conclusion:** Taken together, these data suggest that LPM is involved in the recruitment of SPM to infection sites through a mechanism that depends on IL-10/MCP-1 axis.

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IMMUNO 2012
XXXVII Congress of the Brazilian Society of Immunology
V Extra Section of Clinical Immunology - ESCI

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October 20 - 24, 2012

EFFECTS OF TREATMENT WITH THE ANTIMONY ASSOCIATED TO PENTOXIFYLLINE IN MODULATION OF THE IMMUNE RESPONSE IN SITU OF PATIENTS WITH MUCOSAL LEISHMANIASIS

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Introduction: Human infection with *Leishmania braziliensis* can lead to cutaneous leishmaniasis (CL) or mucosal leishmaniasis (ML). ML is associated with exacerbation of inflammatory immune response, causing extensive damage tissue. Treatment of leishmaniasis is still based on the use of pentavalent antimony (Sb^V). However, the failure of this treatment is common and the resistant parasites have been detected. Due to the problems resulting from Sb^V treatment a wide variety of alternative therapies has been evaluated for leishmaniasis treatment. The aim this study was to investigate the effect of antimony associated or not to pentoxifylline (Ptx) in the modulation of the *in situ* immune response of ML patients. **Methods:** We evaluated the frequency of T $CD4^+$, $CD8^+$ cells and $CD68^+$ macrophages, as well as the expression of cytokines IFN-γ^+, TNF-α^+ and IL-10^+ and cytotoxic molecules granzyme A^+ and the correlation between these immunological parameters in lesions from ML patients before and after Sb^V and Sb^V associated with Ptx treatment, by confocal microscopy. **Results**: We did not observe differences in the numbers of the cell populations or cytokines and granzyme A, comparing lesions from patients before and after treatment with Sb^V. Lesions from ML patients after Ptx associated with Sb^V treatment had lower frequency of macrophages, as compared to lesions before treatment. There was no change in expression of IFN-γ, IL-10 and granzyme A in lesions from patients after Sb^V +

Ptx treatment. However, Ptx associated with Sb^V treatment leads to a reduction in the number of $CD68^+TNF\text{-}\alpha^+$ cells. There were no differences in the frequencies of cell populations and cytokines comparing between lesions from patients submitted to both treatments. The Ptx associated with Sb^V treatment induced loss of positive correlation between the inflammatory infiltrate and the number of TNF-α^+ cells, as well as the number of $CD8^+$ T cells and granzyme A^+ cells.

Conclusion: Ptx associated with Sb^V treatment may result in a modulation of the immune response more efficient and consequently an accelerated healing.

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XXXVII Congress of the Brazilian Society of Immunology
V Extra Section of Clinical Immunology - ESCI

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October 20 - 24, 2012

ROLE OF PGD_2 ON EOSINOPHIL ACTIVATION INDUCED BY LEPTIN

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Introduction: Eosinophils are granulocytes classically associated with allergic diseases and helminth infections. Recently it has been also described an immunomodulatory role of eosinophils, such as the regulatory role of resident eosinophils found in adipose tissue, capable of regulating macrophage functions and tissue hemostasis. Leptin, a hormone/cytokine produced by adipocytes, is a survival factor for eosinophils which are known to express the leptin receptor Ob-Rb. Inasmuch as we just had identified eosinophils as producers of prostaglandin D_2 (PGD_2) which function in an autocrine/paracrine fashion regulating eosinophil activation (J Immunol. 187(12):6518-26, 2011), the aim of this study was to evaluate the role of eosinophil-derived PGD_2 in leptin-induced eosinophil activation.

Methods and Results: For *in vivo* assays, eosinophilic pleurisy was triggered by intrapleural injection of leptin (1 mg/Kg) in Balb/c mice. After 24 h of leptin *in vivo* stimulation, in parallel to eosinophil influx and activation (characterized by lipid body biogenesis in infiltrating eosinophils), we found increased levels of PGD_2 in the pleural cavity compared to saline-stimulated animals ($n = 6$; $p<0.05$). Accordinlgy, *in vitro*, using bone marrow-differentiated mouse eosinophils, leptin (50 nM) was also able to trigger PGD_2 synthesis as detected in eosinophil supernatants within 1 h of stimulation. Similarly, using human eosinophils purified from healthy donors as cell model, leptin again appears to activates PGD_2 synthesis, since the pre-treatment with HQL-79 (10 µM), an inhibitor of hematopoetic PGD synthase, inhibited both lipid bdoy biogenesis and leukotriene C_4 synthesis elicited by *in vitro* leptin stimulation (50 nM).

Conlclusion: Our data demonstrated clearly the ability of leptin to activate PGD_2 synthesizing machinery in either mouse or human eosinophils. Moreover, our results with human cells unveil an autocrine/paracrine regulatory activity by endogenous PGD_2 on eosinophil activation stimulated by leptin. Inasmuch as PGD_2 is now emerging as a immunomodulatory molecule, its role in leptin-driven effects may indicate potential functions on obesity and other inflammatory disorders.

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IMMUNO 2012
XXXVII Congress of the Brazilian Society of Immunology
V Extra Section of Clinical Immunology - ESCI

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Campos do Jordão SP Brazil
October 20 - 24, 2012

SMOKING INFLUENCES THE LEVELS OF INTERFERON GAMMA (IFN-γ) IN HUMAN COLOSTRUM?

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Introduction: Breast milk plays a protective role to the newborn. Breastfeeding represents the continued exposure of the child to the maternal immune environment. There are numerous immune-protective components in human milk, such as cells and molecules, including interferons. Interferon gamma (IFN-γ) is a cytokine secreted by activated Th1 cells and natural killer cells. IFN-γ may have multiple mechanisms for protecting the neonate. Smoke impairs the production of IFN-γ by peripheral blood immune cells. The aim was to assess the impact of cigarette smoke on the levels of IFN-γ in human colostrum.
Methods and Results: In this work, the IFN-γ levels were detected by Elisa method (Biolegend, San Diego, CA) in human colostrum collected in the morning of mother's smokers and nonsmokers. The results were analyzed using the Unpaired T test. We detected an IFN-γ average concentration of the 38.2 ± 8.63 pg/ml and 55.4 ± 9.75 pg/ml in colostrum of women smokers (n = 7) and nonsmokers (n = 7), respectively. Smoking tends to reduce the levels of IFN-γ in human colostrum, even though the difference was not statistically significant ($P>0.05$).
Conclusion: Our results indicate that cigarette smoke exposure in pregnancy seems to impair the levels of IFN-γ during lactation.

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XXXVII Congress of the Brazilian Society of Immunology
V Extra Section of Clinical Immunology - ESCI

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Campos do Jordão SP Brazil
October 20 - 24, 2012

V BETA 12 CD4 AND CD8 CELLS OF PATIENTS WITH CUTANEOUS LEISHMANIASIS SHOW PREDOMINANT PHENOTYPIC CENTRAL MEMORY CELLS.

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Introduction: Leishmaniasis are parasitic diseases caused by protozoa of the genus *Leishmania*. They are transmitted to the vertebrate host by the bite of the insect vector and present different clinical forms. Cutaneous leishmaniasis (CL) patients develop cellular immunity against the parasite, a response crucial for protection. Previously, we have demonstrated that T cells ($CD4^+$ and $CD8^+$) repertories in individuals with LC, showed modulation in the expression of genes coding for the chain V beta TCR, an expansion marked cell type V beta 12. This study aims to characterize the phenotype V beta 12 CD4 and CD8.
Methods and Results: T lymphocytes from CL patients were purified from whole blood by Ficoll and analyzed by flow cytometry following the evaluating the expression of surface molecules which characterize the effector type, the level of activation, the costimulation and adhesion. V beta 12 CD4 and CD8 T cells exhibited a phenotype of central memory cells, $CCR7^+/CD45RA^-$ (79.4% in $CD4^+$ and 93.5% in $CD8^+$) and $CCR7^-/CD45RA^-$ memory effector (18, 8% in $CD4^+$ and 3.3% in $CD8^+$). Presented high expression of CD28, HLA-DR, LFA-1, VLA-4 and low expression of CD62L. markers
Conclusion: V beta 12 CD4 and CD8 T cells in patients with cutaneous leishmaniasis showed a mixed phenotype of memory cells with predominance of central memory with high expression of activation, adhesion and costimulation markers.

IMMUNO 2012
XXXVII Congress of the Brazilian Society of Immunology
V Extra Section of Clinical Immunology - ESCI

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October 20 - 24, 2012

DETERMINATION OF IMMUNOLOGICAL PROFILE OF OVERWEIGHT INDIVIDUALS ACCORDING TO THE OBESITY DEGREE

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Introduction: The obesity is related to many comorbidities. Among the main complications, stand out the chronic state of low-grade inflammation and alterations in the immune system, represented by altered levels of inflammatory cytokines and cell surface markers. For this reason, this study aimed to evaluate the immunological profile of overweight individuals according to the obesity degree.

Methods and Results: Volunteers over the age of 18 years old, with Body Mass Index (BMI) ≥25 kg/m² and a sedentary lifestyle were recruited to participate of the study. After anthropometric measurements, the subjects were classified, according to the Latin American Consensus on Obesity, which defines four categories of obesity degrees: overweight (OW), grade 1 (G1), grade 2 (G2) and grade 3 (G3). Samples were collected from whole blood in heparinized tubes and flow cytometry (FACSCalibur BD) was used for measuring the amount of immune cells for each cell surface marker (CD3/4, CD3/8, CD28 and HLA-DR). Statistical analysis was performed using SPSS version 17.0. One-way ANOVA with Tukey post-hoc was used to compare the results between the groups ($p<0.05$ was considered significant). Results are expressed as mean±standard deviation. This study was approved by the Ethics Committee of Centro Universitário Metodista, do IPA under protocol number 48/12. The sample consisted of 38 individuals, 28 women and 10 men. The results are stratified: Age (years): OW 54±13.2, G1 48.6±7.8, G2 45±10.9, G3 40±11.6; BMI (kg/m²): OW 28.9±1.1, G1 32.7±1.4, G2 37.7±1, G3 44.1±3.1; CD3/4 (%): OW 29.5±5.1, G1 32.3±11.1, G2 39±6.4, G3 34.6±6.5; CD3/8 (%): OW 15.9±4.4, G1 15.6±6.6, G2 14.1±5.1, G3 18.3±4.2; CD28 (%): OW

39.7±17.8, G1 38.9±16.4, G2 48.4±7.9, G3 50.9±8.3; HLA-DR lymphocyte (%): OW 5.2±2.7, G1 11.7±8.1, G2 8.6±3.1, G3 8±3.3; HLA-DR monocyte (%): OW 35±17, G1 36±19.3, G2 37.2±7, G3 45±12. There was a statistical difference in OW, G1 and G2 to the group G3 for BMI. There was no significant difference between the obesity degrees in relation to age and frequency of the markers CD3/4, CD3/8, CD28 and HLA-DR.

Conclusion: With the findings, we can conclude that possibly the immunological profile involving the analyzed markers do not modify according to the obesity degree. However more studies are needed to confirm this study, using a larger sample size and correlating the variables with the senescence level.

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XXXVII Congress of the Brazilian Society of Immunology
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October 20 - 24, 2012

ROLE OF CHEMOKINE RECEPTOR CCR4 AND REGULATORY T CELLS IN WOUND HEALING IN DIABETIC MICE

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Introduction: Wounds of difficult healing affect 2% of population in developed countries (e.g. Am J Surg. 187:38S, 2004). Despite of its clinical relevance, in Brazil there are not statistical data yet. The literature is replete with studies describing a new subtype of T cells, these cells were called regulatory T cells (Tregs) and its main function is to modulate the immune system. Treg cells are found in the skin of humans and mice (e.g. J. Exp. Med. 205:1559, 2008); however the role of regulatory T cells in the healing process has not been described yet. Assuming that Treg cells modulate the immune system and that is fundamental to the healing process, we believe that such cells play an important role in the healing of skin lesions. Our objective was to describe the role of regulatory T cells in cutaneous wound healing in diabetic mice.

Methods and Results: Diabetes was induced by a single dose of alloxan (65 mg/kg), intravenous in C57BL/6 (WT) and $CCR4^{-/-}$ (KO) mice. The full-thickness wounds were induced surgically using $1cm^2$ punch biopsy and followed for 14 days. We observed a faster healing process in KO (9.701 ± 1.819 N=7) compared to WT (90.59 ± 36.47 N=5) mice. Histological analysis performed at day 7 showed that WT mice has a greater number of leucocytes infiltrate and numerous new blood vessels compared to KO mice. However, no difference was observed in collagen deposition. Cytokines TNF-a (WT: 4955 ± 2383 N=4 and KO: 1692 ± 257.4 N=5), IL-6 (WT: 8535 ± 2996 N=3 and KO: 3107 ± 687.6 N=4) and IL-12p70 (WT: 247.9 ± 63.25 N=3 and KO: 41.77 ± 12.12 N=3) were quantified by CBA-kit. We found lesser amounts of these cytokines in KO than WT mice on 2^{nd} day. Neutrophils, macrophages and Tregs were quantified by flow cytometer and we observed that KO mice have an increase in neutrophils (WT: 44.40 ± 14.16 N=4 and KO: 63.48 ± 2.701 N=4) and in macrophages (WT: 2.965 ± 0.2350 N=2 and KO: 6.950 ± 2.430 N=2) numbers and a decrease in Tregs (WT: 6.02 N=1 and KO: 2.04 N=1) numbers compared to WT mice.

Conclusion: So far, KO mice heal faster, suggesting the involvement of CCR4 receptor on recruitment of Tregs to the injury site indicating that this receptor could affect negatively the dermal wound healing in diabetic mice.

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IMMUNO 2012
XXXVII Congress of the Brazilian Society of Immunology
V Extra Section of Clinical Immunology - ESCI

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October 20 - 24, 2012

CHARACTERIZATION OF MYELOID-DERIVED SUPPRESSOR CELLS AND OF A SUBSET OF NATURAL KILLER CELLS IN PERIPHERAL BLOOD AND PERITONEAL FLUID OF ENDOMETRIOSIS PATIENTS

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Introduction: Endometriosis is a gynecological disease that is highly associated with pain and infertility. Cellular function in natural killer cells, $CD8^+$ T lymphocytes and macrophages are decreased in patients with endometriosis. The microenvironment in the uterus, primarily during regulatory cell recruitment, may be responsible for the suppression of immune cell function and the development of the disease. Myeloid-derived suppressor cells (MDSC) are a unique subset of cells involved in the suppression of the immune response in cancer and infectious and autoimmune diseases. Despite its importance, MDSC have not yet been investigated in patients with endometriosis. Another cell subset related with tolerance is a natural killer cell subset, first described as uterine natural killer cells (uNK). These cells are the majority of NK cells in the uterus, and differ from the classical NK type mainly in phenotype, $CD16^-CD56^+$, and in their ability to produce cytokines and regulate immune responses. Given that endometriosis patients present reduced immune cell function, it is worthy to investigate regulatory cells that may be creating a suppressive microenvironment in the uterus, thus allowing the progression of the disease. The aim of this study is to identify and characterize MDSC and uNK in the peripheral blood and peritoneal fluid of patients with endometriosis and controls through flow cytometric assays.

Material and results: Peripheral blood and peritoneal fluid were obtained from a patient with endometriosis (Ethics Committee #11/1711). Red blood cells were eliminated by adding lysing buffer. Samples were then washed and incubated with

specific antibodies. After incubation, cells were acquired and analyzed by FACSARIA flow cytometry and FACSDIVA software. At least 1,000,000 events were acquired and about 0.021% of the cells in peripheral blood and 0.035% of cells in the peritoneal fluid were $CD33^{+}CD34^{+}CD11b^{+}HLA\text{-}DR^{-}Lin1^{-}$ MDSC. 4.0% of the cells in peripheral blood and 1.2% in the peritoneal fluid were CD45+CD3-$CD16^{-}CD56^{+}$ NK cells.

Conclusion: We were able to conclude that it is possible to characterize myeloid-derived suppressor cells and $CD16^{-}CD56^{+}$ natural killer cells in patients with endometriosis. Further analysis will be needed in order to fully characterize these populations and understand their involvement in the development of endometriosis.

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IMMUNO 2012
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October 20 - 24, 2012

DISTINCT CHEMICAL FORMS OF THE LEUCINE METABOLITE β-HYDROXY-β-METHYLBUTYRATE (HMB) DIFFERENTIALLY MODULATE HUMAN BLOOD MONONUCLEAR CELLS CYTOKINE PRODUCTION AND PROLIFERATION IN VITRO.

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Introduction: β-hydroxy-β-methylbutyrate (HMB) is endogeneously synthesized through oxidation of leucine. Animal studies have shown that HMB supplementation modifies immune function, suggesting that this substance may modulate cell immunity. However, there are few studies evaluating HMB influence on function and behavior of human immune cells. The aim of this study is to evaluate the influences of different forms and concentrations of HMB on cytokine production and proliferation profile of human mononuclear cells *in vitro*. **Methods and Results:** Mononuclear cells were collected from venous blood of 10 volunteers (n=10) according to HC/UFPR Human Research Ethics Committee protocols. Human PBMC, stimulated with Concanavalin A (Con A) 5 µg/mL, were

cultivated for 96h in presence of different concentrations (0; 0,1; 1 mM) and forms of HMB, 3-hidroxi-3-metil butyric acid 98% and HMB as calcium salt (CaHMB). Cells were stained with 5-Carboxyfluorescein diacetate (CFDA) to determine mononuclear cell proliferation profile and percentage of parental cell proliferation. CFDA fluorescence was analyzed by flow cytometry. IFN-γ, IL-4, IL-10 and TNF were quantified by flow cytometry. Only acid HMB at 1mM, altered ConA stimulated mononuclear cells proliferation profile. Cells that proliferated until generation 6, progress 13.54%±5.29 less to the following generations compared to ConA alone. In comparison to the acid form, CaHMB reduced by 15.17%±4.19 and 15.08%±4.17 the number cells undergoing proliferation at 0.1mM and at 1mM respectively ($p<0.05$). ConA plus acid HMB at 1mM significantly altered cells to a Th2 type cytokine response (0.61±0.05 IFN-γ/IL-4 ratio) compared to Con A (1.01±0.03) and ConA plus CaHMB at 1mM (0.89±0.07) ($p<0.05$). CaHMB induced a pro-inflammatory cytokine profile, compared to acid HMB, because it reduced IL-10 in 14.89%±3.95 ($p<0.05$), while TNF remained the same. **Conclusion:** HMB seems not to influence in a significant manner mononuclear cell proliferation rate. However, the proliferation profile seems to be slightly altered. New studies need to focus on HMB modulatory effects on cell cytokine production. These effects appear at concentrations of 0.1 to 1mM, independently of HMB form.

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IMMUNO 2012
XXXVII Congress of the Brazilian Society of Immunology
V Extra Section of Clinical Immunology - ESCI

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October 20 - 24, 2012

NOD-LIKE RECEPTOR ACTIVATION STIMULATES OSTEOCLASTS ACTIVITY

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Introduction: The NOD-like receptors (NLRs) are cytoplasmic proteins that sense microbial byproducts released by invasive bacteria. It has been shown that osteoblasts express NOD1 and NOD2 and the activation of NOD2 in this cell can induce RANKL expression in osteoblasts which can stimulate osteoclastogenesis.

Objectives: The purpose of this study is to determine the direct effect of NOD2 receptors in osteoclasts differentiation and activation.

Methods: Bone marrow cells from C57/BL6 and NOD2$^{-/-}$ mice were flushed from long bones and cultured for 3 days with MCSF (30ng/ml). Adherent cells (bone marrow macrophages - BMM) was cultured for 4 more days with MCSF (30ng/ml) and RANKL (50ng/ml) and bacterial peptidoglycan-derived muramyl dipeptide MDP (0,01; 0,1; 1,0 ug/ml). Osteoclasts was stained to identify tartrate-resistant acid phosphatase (TRAP)-positive multinucleated cells and counted. The differentiation and activation markers were evaluated 48h after MCSF/RANKL stimulation by Real time PCR.

Results: Our data showed that expression of mRNA encoding Nod2 in murine BMM was increased (2 fold) evaluated 48h after MCSF/RANKL stimulation. NOD2$^{-/-}$ derived RANKL/MCSF-stimulated BMM presented less multinuclear TRAP+ cells (~334 cells/well) than WT derived BMM (~402 cell/well). MDP did not alter the number of multinuclear TRAP+ cells. MCSF/RANKL/MDP stimulated BMM exhibited 25 fold increases in the expression of TRAP (osteoclastogenesis marker) and 13 fold increase in the expression of RANK (receptor activator of nuclear factor

κB) evaluated 48 hours after MCSF/RANKL/MDP stimulation. The expression of cathepsin K (osteoclast-specific enzyme) was also increased (10 fold) in BMM stimulated with MCSF/RANKL/MDP.

Conclusion: These results confirm that NOD2 activation is important to osteoclastogenesis. NOD2 activation by MDP increase catepsin K expression, suggeting that it increases the osteclasts activity.

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IMMUNO 2012
XXXVII Congress of the Brazilian Society of Immunology
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October 20 - 24, 2012

Th17/Th1 AXIS POLARIZATION IS MEDIATED BY IL-1 RECEPTOR IN TYPE 1 DIABETES MURINE MODEL

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Introduction: Autoimmune diseases including type 1 diabetes (T1D) are thought to have a Th1/Th17 bias, but the underlying mechanisms driving the differentiation and activation of these pathogenic T cells has been investigated. Recently, the IL-1beta has been shown to regulate Th17 cell differentiation through of the expression of the transcription factor RORγt. For this reason, we addressed the respective IL-1 receptor (IL-1R) role in the TD1 pathogenesis. **Methods and Results:** IL-1R deficient mice and their wild-type (C57BL/6) were inoculated intraperitoneally with streptozotocin (STZ/40mg/Kg) for 5 consecutive days. Blood glucose levels and body weight were monitored weekly. The pancreatic lymph nodes (PLN) were removed to assess the Th17, Th1 and regulatory T cell (Treg) number by flow cytometry. The pro and anti-inflammatory cytokine levels were determined in pancreatic tissue homogenates by ELISA assay. Our initial results demonstrate a correlation between IL-β, IL-18, RORγt, T-bet expression in the PLN by RT-PCR and Th1/Th17 cytokine production in the pancreatic tissue of diabetic mice. Diabetic IL-1R deficient mice developed lower hyperglycemia with only 50% of mice becoming diabetic. In agreement, mice lacking IL-1R have reduced inflammatory infiltrate (insulitis) and augmented insulin content into pancreatic islets by second week. In addition, the IL-1R deficiency caused a decrease in IL-17

and IFN-γ-producing $CD4^+$ T cell population without interferes in regulatory T cell (Treg) number in pancreatic lymph nodes (PLN). The IL-17, IL-6, TNF-α and IFN-γ levels were not altered, but IL-10 and IL-4 levels were higher in pancreatic tissue of diabetic IL-1R deficient mice. **Conclusion:** These results suggest that IL-1receptor promotes the Th17/Th1 skewing in the PLN and possibly contributes to pancreatic islet damage during the T1D onset. An intimate comprehension of the mechanism molecular behind the reciprocal Th17/Th1 axis is essential to manipulate pathogenic T cell subsets and control the autoimmune disease progression.

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V Extra Section of Clinical Immunology - ESCI

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NOREPHINEPHRINE MODULATES VIA β2 ADRENERGIC RECEPTOR SIGNALING THE PRODUCTION OF IL12-FAMILY CYTOKINES AT THE TRANSCRIPTIONAL LEVEL IN LPS-STIMULATED DENDRITIC CELLS

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Introduction and objective: Production of different cytokines by immune cells upon several stimuli is a central feature in determining the generation of distinct types of immune response. Usually, LPS- activated dendritic cells (DC) produce higher levels of IL12p70 than of IL23. However, some endogenous molecules, such as norepinephrine (NA), may modulate the profile of cytokines produced by LPS stimulated DC. Some data from our group have demonstrated that NA, signaling through β_2AR, shifts the IL12p70/ IL23 ratio in LPS-activated DC. Thus, in this study we proposed to investigate whether the alterations in the production of IL12-family cytokines following β_2AR signaling in LPS-stimulated DC occur at the transcriptional level. **Methods and results:** DC were generated from C57BL/6 bone marrow cells cultivated in DMEM and 20 ng/mL GM-CSF. On 9th day, the cultures were treated or not with NA or fenoterol (β_2AR specific agonist) and activated with LPS 5 µg/mL for 18 hours. In some experiments, cells were pre-treated with ICI 118,551 (β_2AR specific antagonist) and then stimulated as mentioned above. The supernatants were collected and the production of IL12p70 and IL23 were analyzed by ELISA. To quantify the transcripts of IL12 (p35), IL23 (p19) and IL12/23 (p40) by real-time RT-PCR the cultures were stimulated with LPS for various periods of time in the presence or not of the β_2AR agonist. The results showed that the IL12p70 production was approximately 2 fold higher than IL23 release in LPS-activated DC. However, the exposure to NA during DC LPS-activation did invert the IL12p70/IL23 ratio that was restored when DC were pre-treated with a β_2AR specific antagonist. Likewise the gene expression analysis showed that the β_2AR signaling in LPS-activated DC induced up-regulation of IL23p19 and down-regulation of IL12p35 expression when compared with LPS-activated DC. **Conclusion:** These results indicate that β_2AR signaling during LPS-

activation of DC alters the gene transcription of IL12-family cytokines, favoring IL23 production.

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IMMUNO 2012
XXXVII Congress of the Brazilian Society of Immunology
V Extra Section of Clinical Immunology - ESCI

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October 20 - 24, 2012

β_2 ADRENERGIC RECEPTOR SIGNALING IN LPS-ACTIVATED DC LEADS TO IL12p70 SUPPRESSION AND IL10 UP-REGULATION: SEARCHING THE MECHANISM

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Introduction and objective: The immune response may be modulated by the sympathetic nervous system through release of neurotransmitters such as norepinephrine (NA). Many studies including data from our group show that NA via β_2 adrenergic receptor (β_2AR) inhibits the IL12p70 and enhances the IL10 production, while does not alter IL23 production, in LPS-activated dendritic cells (DCs). However the downstream mechanisms underlying these β_2AR effects remain elusive. Thus, the aim of this study is to investigate the signaling pathways involved in the β2AR-mediated modulation of IL12p70 production in LPS-activated DCs. **Methods and results:** DCs were generated from C57BL/6 WT or IL10-/- bone marrow cells. On 9th day, the cultures were treated or not with fenoterol (specific β_2AR agonist) for 1h and then activated with LPS 5μg/mL for 18h. In some experiments, cells were pre-treated with H89 (PKA inhibitor) or ICI 118,551 (β_2-AR specific antagonist) for 15 min and then stimulated as mentioned above. The supernatants were collected and cytokine levels (IL12p70, IL23, IL10) were analyzed by ELISA. Following β_2AR signaling, we also evaluated intracellular cAMP levels and PKA-induced CREB phosphorylation (Western Blot) in sorted CD11c+ immature DCs (iDCs). β_2AR agonist treatment inhibited IL12p70 and enhanced IL10 production while did not alter IL23 production in LPS-activated DCs. These effects were restored when LPS-activated DCs were pre-treated with a specific β_2AR antagonist. We also found that in iDCs β_2AR signaling led to increased intracellular cAMP levels and PKA-mediated CREB phosphorylation. Although H89 treatment did prevent CREB phosphorylation after β_2AR signaling in iDCs, PKA inhibition did not prevent β_2AR-mediated alterations in cytokine production by LPS-activated DCs. These results suggest that β_2AR signaling

during LPS activation of DCs decreases IL12p70 and enhances the IL10 production via a PKA-independent signaling pathway. Finally, we asked if β_2AR-mediated increase in IL10 release could be the reason by which IL12p70 production is inhibited in LPS-activated DCs. To tackle this question we used iDCs generated from IL10-/- mice. Just as observed in WT mice, β_2AR signaling inhibited LPS-induced IL-12 production in IL-10 deficient DCs. **Conclusion:** Altogether, these results suggest that in LPS-activated DCs, β_2AR-mediated suppression of IL12p70 production is IL10-independent and also PKA-independent.

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V Extra Section of Clinical Immunology - ESCI

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October 20 - 24, 2012

REPEATED EXPOSURE TO INHALED *Blomia tropicalis* EXTRACT RESULTS IN PERSISTENT ALLERGIC AIRWAY INFLAMMATION

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Introduction: Acute exposure to inhaled ovalbumin (OVA) in sensitized mice elicits allergic airway inflammation and elevated serum levels of IgE. In contrast, chronic exposure to OVA results in resolution of airway inflammation but not IgE production, a phenomenon known as local inhalation tolerance. *Blomia tropicalis* (*Bt*), the most prevalent house dust mite in tropical countries, has been shown to induce allergic airway inflammation in mice after acute intranasal challenges. However, the effect of *Bt* on allergic inflammation upon chronic exposure is still unknown. **Objective**: In this study we investigated the impact of chronic intranasal *Bt* challenges on allergic airway inflammation and IgE production. **Methods**: BALB/c mice were sensitized with OVA or *Bt* extract *adsorbed* onto alum on days 0 and 7 and from day 14 on, mice received five weekly intranasal challenges with 10µg of OVA or *Bt* (chronic exposure). Acute exposure consisted of two challenges with OVA or *Bt* starting at day 35. Experiments were performed one day after the last antigen challenge. **Results**: Acute exposure of sensitized mice to OVA or *Bt* resulted in eosinophilic inflammation of the airways and elevated production of IgE. Chronic exposure to OVA drastically reduced the number of eosinophils in the airways but not inhibited IgE. Interestingly, sensitized mice chronically exposure to *Bt* did not reduce eosinophilia nor the levels of IgE in the serum. **Conclusions**: Repeated exposure to inhaled *Bt*, but not OVA results in a chronic allergic disease of the airways, a feature that resemble asthma phenotype.

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IMMUNO 2012
XXXVII Congress of the Brazilian Society of Immunology
V Extra Section of Clinical Immunology - ESCI

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October 20 - 24, 2012

MULTI-WALLED (MWCTNs) CARBON NANOTUBES INTERVENTION IN THE DEVELOPMENT OF AUTOIMMUNE DIABETES IN NON OBESE DIABETIC (NOD) MICE

DANIELA SILVA CAMILO(1,2); WALKYRIA MARA GONÇALVES VOLPINI(1); ROSEMEIRE FLORENÇA OLIVEIRA DE PAULA(1); ADRIEL SANTOS MORAES(1); FERNANDO PRADELLA(1); MARIANA PERES ALMEIDA DOS SANTOS(1); GUILHERME ANTONIO DUTRA MORAES(1); ANA LEDA LONGHINI(1); ALESSANDRO FARIAS(1); ELAINE CONCEIÇÃO DE OLIVEIRA(1); CONCEIÇÃO APARECIDA VILELLA(3); RICARDO DE LIMA ZOLLNER(3); HELDER CERAGIOLI(2); LEONILDA MARIA BARBOSA DOS SANTOS(1) AND VITOR BARANAUSKAS(2)

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Introduction: Carbon nanotubes (CNTs) have been widely evaluated by their structure and properties as a tool for biomedical application. Our group has focused the effect of nonfuncionalized carbon nanotubes on immune response. It was demonstrated that the systemic administration of CNTs induces inflammatory response, stimulating macrophages and activating cellular and humoral immune responses (Nanotechnology 22:265103, 2011). The present study aimed to determine the effect of CTNs administration during the evolution of spontaneous diabetes presented by NOD mice and its inflammatory response.

Methods and Results: Nonfuncionalized MWCTNs (Helix, USA; length: 0,5–40 µm, diameter: 10-30 nm, purity >95%) were suspended in 0,1% F68 Pluronic® solution. Experimental design consisted in two groups of female NOD mice 6 weeks old: Treated (n=46) by ip administration of CNTs (100 µg/animal) and Control (n=45), both with planned follow-up till age of 30 weeks. Time intervals for sacrifice (5 animals/group) are: 6, 10, 14, 20 and 30 weeks of age. The evolution of blood glucose fluctuation of NOD mice reaching 20 weeks showed that CNTs treated have mean glycaemias significantly increased at 12 weeks ($p<0,003$, Mann-Whitney) and tendency to higher values during all the period, as well as to anticipate clinical diabetes onset (75% of treated animals diagnosed before 16 weeks *vs* 25% of controls). Preliminary assays demonstrated a

tendency to increased expression of mRNA of the pro-inflammatory cytokines IL6 and IL-12 in PLNs of CTNs treated animals at the age of 14 weeks. Histologic examination (hematoxylin-eosin) of NOD pancreas and PLNs (serial cryostat sections of snap-frozen organs) showed the persistent presence of black-material (CNTs) surrounded by macrophages mainly in PLNs (confocal microscopy: great concentration of CD11b+cells) in all time intervals studied as far as 20 weeks. The presence of CNTs *in situ* was characterized in treated NOD pancreata by typical Raman spectrum.

Conclusion: This ongoing study demonstrates that MWCTNs were absorbed from the peritoneal cavity of NOD treated mice, impacting lymph nodes as PLNs and causing a strong inflammatory reaction. Clusters of CNTs stimulated the formation of granuloma, seen until the age of 20 weeks. This inflammatory process may be chronic and associated with an anticipation of diabetes onset in NOD mice. If confirmed, the systemic administration of CTNs for medical application must be regarded with caution.

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XXXVII Congress of the Brazilian Society of Immunology
V Extra Section of Clinical Immunology - ESCI

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October 20 - 24, 2012

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V Extra Section of Clinical Immunology - ESCI

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October 20 - 24, 2012

INFLAMMATION IN BIPOLAR DISORDER IS ASSOCIATED WITH REDUCED REGULATORY T CELLS AND ACTIVATED MAPK

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Introduction: Bipolar Disorder (BD) has been associated with an immunologic imbalance shown by increased peripheral inflammatory markers. The underlying mechanisms of this phenomenon may include changes in circulating cells, such as regulatory and activated T cell, as well as differential activation of mitogen-activated protein kinases (MAPKs). By analyzing different immune cell subpopulations, plasmatic cytokines and molecular pathways we aim to verify the immune profile from BD patients. **Method and results:** Twenty-seven euthymic female subjects with BD type I and 24 age- and sex-matched controls were recruited in this study. Lymphocytes were isolated by Ficoll-paque gradient and stimulated *in vitro* to assess Th1/Th17/Th2 cytokines (IL-2, IL-4, IL-6, IL-10, IL-17, IFN-γ and TNF-α) and MAPK phosphorylation. The expression of phospho-MAPKs, a large panel of lymphocyte subsets and cytokines were assessed by multi-color flow cytometry. BD patients had reduced proportions of natural T regulatory cells (CD4+CD25+FoxP3+) ($p<0.01$) in parallel to higher cytokine production (all $p<0.01$) than healthy controls. In particular, BD was associated with a strong bias to Th1 rather than Th2 profile. There was an expansion of senescence-associated cells (CD8+CD28-) in BD ($p<0.0001$). T cells of BD patients showed an increased p-ERK signaling ($p<0.0001$), indicating lymphocyte activation. **Conclusion:** Our data suggest that multiple molecular and cellular mechanisms may contribute to the immunologic imbalance observed in BD. In addition, our data concur to an early senescence process in these patients.

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PATIENTS WITH BIPOLAR DISORDER HAVE PBMCs WITH SHORTENED TELOMERES

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Introduction: Peripheral inflammatory markers have been associated with Bipolar Disorder (BD), suggesting that the immune system may be involved in the pathophysiology of BD. Mood disorders have been associated with accelerated immunosenescence. We aimed to investigate the telomere length in mononuclear cells of BD and its correlation with age-related lymphocyte subsets.
Methods and Results: 24 euthymic BD type I patients and 20 sex- and age-matched controls were recruited. Peripheral blood was collected, PBMCs were isolated and immunophenotyped for memory T cells (CD8+CD45RO+), early activated cells (CD69), NK (CD3-CD56+), NK T (CD3+CD56+) and senescence-associated cells (CD8+CD28-). The relative telomeric length was measured by real time PCR, estimated by the ratio of telomere (T) repeat copy number to single copy gene (S). The T/S ratio is proportional to the average telomere length. PBMCs of BD patients had shortened telomeres (M=0.763 ± 0.051) than cells of healthy controls (M=0.908 ± 0.044)(p=0,038). There was a significant correlation between T/S and age in the BD group only ($r=-0.44$, $p<0.05$). There was also a strong positive correlation between T/S and the percentage of CD8+CD45RO+ cells in BD ($r=0.75$, $p=0.001$). T/S ratio was not correlated to clinical severity (years of illness).
Conclusion: Our results corroborate to previous studies suggesting an accelerated cellular senescence in mood disorders. Future studies will address whether this early senescence is due to the increased low-grade inflammation observed in BD.

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EVIDENCES THAT TLRS DO NOT CONTRIBUTE TO SELECTION OF REPERTOIRE IN NORMAL B CELL DEVELOPMENT

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Introduction: We previous showed that TLR4 triggering promotes B cell maturation *in vitro* from mouse B cell precursors (Hayashi *et al*.,2005;2010). LPS increased generation of functional $CD23^{+}$ B cells in a TLR4/NF-kB dependent way in a manner equivalent but independent to the well-known B cell maturation factor BAFF. LPS injected mice showed a rapid increase of mature B cells in the bone marrow (BM), raising the possibility that TLR4 signaling may effectively stimulate B cell maturation *in vivo*, acting as an accessory stimulus in B cell development (Hayashi *et al.*, 2010). Similar results are observed for TLR9 *in vitro* stimulus (unpublished). **Methods:** To address the question if TLRs play a physiological role during B lymphopoiesis, we analyzed chimeric mice transplanted with WT and TLRs signaling pathway deficient (*myd88*$^{-/-}$) BM cells. To better understand the impact of TLR deficiency on B cell central tolerance, we construct a mice model carrying SP6 BCR transgene and the endogenous κ light chain bearing a human domain in the constant region. Taking advantage of this model, we investigated if TLR9 signals modify the immature B cell pool, modulating compartment size or BCR editing to non-transgenic light chain. **Results:** In the competitive mixed BM chimeras, frequencies of WT and *myd88*$^{-/-}$ in BM and spleen compartments were comparable. Analysis of BrdU incorporation of WT and *myd88*$^{-/-}$ cells showed no differences in the kinetic of B cell maturation in the BM. Using the transgenic model, we observed that $TLR9^{-/-}$ SP6 transgenic mice IgM have an increased fraction of α-DNA antibodies, suggesting a role of TLR9 on BCR editing. However, preliminary results showed no differences in the frequencies of edited light chain in BM, spleen and peritoneal B cell compartments. **Conclusion:** These results suggest that physiological TLR signaling is unlikely to contribute to the regulation of B cell maturation in the BM, acting mainly in the selection of peripheral compartments. Further studies are necessary to explore if TLRs are able to modulate B cell development or tolerance in an infectious disease scenario.

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LTB_4 AS CHEMOATTRACTANT FACTOR IN THE REGULATORY T CELLS MIGRATION

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Introduction: Leukotrienes B_4 (LTB_4) plays a critical role in leukocyte recruitment and activation. It exerts its actions through two receptors, BLT1 and BLT2. LTB_4 chemoattractant function has been shown for $CD4^+$, $CD8^+$ and gd T cells. Regulatory T cells (Tregs) play a key role in regulating the immune response and are responsible for the control of various physiological and pathological conditions. The participation of Tregs in controlling many diseases has been widely studied. Thus, the aim of this work was to evaluate the involvement of LTB_4 in the chemotaxis and activation of Tregs.

Methods: Splenic cells from C57Bl/6 were subjected to a Treg cell isolation Kit (Miltenyi Biotec, USA). BLT1 expression in Treg was assessed by flow cytometer. Also, BLT1 expression was evaluated in Treg activated with anti-CD3/CD28 alone or with LPS (100 ng/mL) overnight. Chemotaxis assay was conducted in a Boyden chamber with LTB_4 concentrations range from 0,3 to 10 nM. Cells were incubated for 1 h in a 5% CO_2 and 37°C. Actin polimerization was assessed by faloidin-FITC staining on purified Treg stimulated with LTB_4 0,3 nM. The specific markers of Treg, Foxp3 and GITR were evaluated in LTB_4-stimulated Treg by flow cytometer. Using the intra-vital microscopy technique the liver of Foxp3-GFP animals was stimulated with LTB_4 and Treg adherence was analyzed. Ethical approval: DFBCICB028

Results and discussion: It was observed that Tregs express BLT1 and that this expression is dependent on the Treg activation state. The functionality of this receptor was assessed in a chemotaxis assay where Tregs responded to LTB_4 in a dose and time dependent manner (1.103 ± 0.2844 and 2.575 ± 0.2994 N=6); LTB_4 was also able to induce the actin polymerization in Treg (0.8300 ± 0.02309 and 1.347 ± 0.09262 N=3). However, LTB_4 induced no changes in Treg markers. LTB_4-induced Treg migration *in vivo* to the liver was also demonstrated (1.333 ± 0.3333 and 5.000 ± 1.000 N=3).

Conclusion: LTB_4 did not change the phenotype of Treg; however, Treg cells respond to chemotactic effects of LTB_4 through the expression of BLT1 receptor on its surfaces.

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ROLE FOR PGE_2 AS A REGULATOR OF EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS (EAE)

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Introduction: CD4+ T lymphocytes (LTCD4+) play a central role in the adaptive immunity, driving the activation of other immune cells, such as macrophages, B cells and CTLs. A normal adaptive immune response involves the activation, differentiation and expansion of specific LTCD4+ cells, followed by a paralleled clonal retraction aimed to re-establish homeostasis. To great extent, the death of LTCD4+ is achieved by either AICD (activated-induced cell death), which depends on FAS/FASL interaction, or ACAD (activated cell autonomous death), which proceeds via activation of the BH3-only protein BIM. Particularly to our interest, defects in proteins that control AICD results in uncontrolled lymphoproliferation, most often associated with autoimmune diseases. Previously results obtained in our lab (Weinlich et al., 2008) demonstrated that PGE_2 is able to modulate the LTCD4+ survival, by preventing TcR/CD3-mediated FASL upregulation and consequent AICD. Because autoimmune diseases, such as EAE (a murine model of multiple sclerosis), involves uncontrolled LTCD4+ proliferation we sought to investigate whether physiological levels of PGE_2 achieved during the initiation of specific autoimmune LTCD4+ expansion could play a role in the establishment and severity of EAE. **Methods and Results:** To test this hypothesis, we induced EAE in wild-type female C57BL/6 mice and treated the experimental group with indomethacin, an inhibitor of cycloxigenase and consequent PGE_2 production. While the control, vehicle-treated mice developed EAE, indomethacin-treated mice showed a significant reduction of disease incidence and severity, as well as a delayed onset of symptoms. **Conclusion and Perspectives:** Our results suggest that endogenous levels of PGE_2 produced during the initiation of the autoimmune response modulates the subsequent onset and the level of the disease. Further

experiments aimed to estimate the frequency of specific autoimmune LTCD4+ cells produced in the presence or absence of indomethacin, and to determine the relative contribution of FAS/FASL to EAE are under way in our lab.

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V Extra Section of Clinical Immunology - ESCI

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CCR4$^+$ cells regulate the pancreatitis induced by Coxsakievirus B5

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Introduction: Coxsackieviruses belong to enterovirus genus of the *Picornaviridae* family. The virus causes both acute and chronic pancreatitis, which is characterized by intense inflammation and edema of the exocrine pancreas. The resistance to injury is associated with the migration of regulatory T cells in the lesion site that are able to control the inflammation. The role of chemokines and their respective receptors in the recruitment of these specific cells to the inflammatory site during the virus infection is still unclear. So the aim of this study was to investigate the role of CCR4, CCR5 e CCR2 in pancreatitis induced by CVB5. **Methods and Results:** C57BL/6, CCR5 KO, CCR2 KO and CCR4 KO mice were infected with 10^7 $TCID_{50}$ of CVB5 and after 3 and 7 days of infection, their serum were collected to verify the presence of pancreatic enzymes. Pancreas damage and inflammation was evaluated by histology and viral titer was determinate by $TCID_{50}$. In addition, the cells recruited after infection were evaluated in the pancreatic lymph node (PLN) by cytometry. Our data show that CCR4 KO mice presented 2-fold more amylase in the serum than C57BL/6 mice 3 days post-infection, whereas CCR2 KO and CCR5 KO presented unchanged levels of these enzymes. Accordingly, CCL17 (CCR4 ligand) is expressed in the pancreas of C57BL/6 mice after CVB5 infection. This data indicate that CCR4$^+$ cells are important to contain the pancreas lesion. The pancreas of CCR4 KO mice exhibited an increase in viral titer and in the inflammatory infiltrate, suggesting that CCR4+ cells are important to regulate the inflammation and promote viral clearance. In the absence of CCR4, PLN present less T regulatory cells and more CD4$^+$IFN-γ^+ T cells on day 7 post-infection, indicating that T regulatory cells regulate the immune mediated tissue injury**. Conclusion:** The recruitment of CCR4$^+$ cells to the lesion site by CCL17 secretion is crucial to control the pancreatitis induced by the virus. These data collaborate to elucidate the unknown mechanisms involved on the pathogenesis of CVB5 infection.

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ORAL TOLERANCE TO OVALBUMIN INFLUENCES THE DEVELOPMENT OF ALLERGIC LUNG DISEASE INDUCED BY *Blomia tropicalis*

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Introduction: The prevalence and severity of asthma has been increasing worldwide. In tropical and subtropical countries, *Blomia tropicalis* (Bt) is one of the most common and prevalent mite associated with allergy and asthma. Murine models have been developed to study allergic lung disease and ovalbumin (OVA) is the most commonly used antigen. It is well established that previous administration of oral OVA and subsequent sensitization prevents development of OVA-induced airway allergic disease. Recently a murine model of allergic lung inflammation with Bt extract was described. Experimental data indicates that tolerance towards an antigen can promote tolerance to another not related antigen, a phenomenon known as bystander suppression.
Objective: Here we used OVA for tolerance induction and Bt for allergen sensitization and examined whether tolerance to OVA could influence allergic lung disease induced by Bt extract.
Methods and results: To induce oral tolerance female C57BL/6 mice received 1% OVA solution *ad libitum* in sterile drinking water for 5 consecutive days. Two days later, mice were subcutaneously sensitized with 5 µg of Bt extract and 4 µg of OVA co-adsorbed onto alum on days 0 and 7 and challenged intranasally with 10 µg of Bt extract on days 14 and 21. Allergic group consisted of non-tolerized mice treated as described above. Control group received two Bt extract challenges. The development of allergic lung disease was analyzed on day 22. We found that OVA-tolerized mice showed decreased total cell migration to the airways, when compared with the allergic group ($14.4x10^5$±2.66 versus $75.6x10^5$±19.4, respectively). Airway inflammatory infiltrating cells in allergic group were mainly constituted of eosinophils (46.2%), followed by mononuclear cells (36,4%), but minimally by neutrophils (17,4%). The inflammatory cells in the airways of OVA-tolerized mice were constituted mainly with mononuclear cells, similar to control group (80,8% and 83%, respectively). Accordingly, sensitization and challenge with Bt extract increased total serum IgE (7.6±2.2µg/mL), which was prevented in tolerized group (2.5±0.2µg/mL). Data are representative of one of two experiments, n=5 per group, $p<0.05$ (one-way analysis of variance and Tukey's post test).
Conclusion: Induction of oral tolerance to OVA induces bystander suppression to Bt-specific Th2 mediated allergic responses.

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LPS STIMULATED-DC PRODUCE PGE_2 *IN VITRO* AND *IN VIVO* AND INCREASE CD4 T CELL SURVIVAL DURING ANTIGEN PRESENTATION

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Introduction: In peripheral lymphoid organs, the clonal activation, proliferation and differentiation into effector cells only occur when CD4+ T lymphocytes recognize antigenic peptides presented by antigen presenting cells (APC), in particular, dendritic cells (DCs). The full maturation of DCs occurs through the stimulation of Toll like receptors (TLRs), which increases the capacity of DCs to present antigens, to express co-stimulatory molecules and to produce certain soluble mediators. During a normal adaptive immune response, CD4+ T cell activation, differentiation and expansion are accompanied by a phase of contraction of the no longer needed, specific T cell population. This contraction occurs by either activation-induced cell death (AICD) or activation cell autonomous death (ACAD). AICD depends on FAS/FASL interaction and ACAD depends on the activation of BIM, a BH3-only member of the BCL-2 family. Our group demonstrated *in vitro* that LPS stimulated-DCs secrete prostaglandin E_2 (PGE_2), which, in turn, protects T cells from AICD by preventing TcR/CD3–mediated upregulation of FASL. Based on this result, we hypothesize that antigen presentation in the context of infection (TLR stimulation) impacts on FASL expression and survival of cognate CD4+ T cells. **Methods and Results:** To approach our hypothesis, we co-culture DO11.10 CD4+ T lymphocytes (specific for Ovalbumin – OVA) with bone marrow derived dendritic cells (BMDCs) pulsed or not with pOVA, in the presence or absence of LPS, a well-known TLR4 agonist. In parallel, we use an *in vivo* model of adoptive cell transfer in which purified DO11.10 CD4+ T cells were labeled with CFSE and transfer to wild-type BALB/c syngeneic mice. After that, mice were inoculated with OVA in the presence or absence of LPS. In both systems, we analyzed cell death, proliferation, activation and the differential expression of FAS and FASL DO11.10 CD4+ T cells by flow cytometry. **Conclusions:** The addition of LPS during pOVA presentation increases $CD4^+$ T cells activation and proliferation. Pretreatment of

cultures or mice with indomethacin reduces OVA-specific CD4+ T cell accumulation, but does not interfere with its proliferation or activation, suggesting that endogenous PGE2 produced in response to LPS modulates the number of specific T cells at the level of AICD.

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CHEMOKINES AND FORMYL-PEPTIDES COLLABORATE IN NEUTROPHIL-MEDIATED HEPATOCYTE DAMAGE DURING ACETAMINOPHEN OVERDOSE

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Introduction: Acute liver failure (ALF) is a severe disorder in which necrotic hepatocytes induce neutrophil recruitment and activation, and could lead to additional hepatocyte damage. It has been described that mitochondrial formyl peptides (FP) and chemokines can both attract and activate neutrophil in sites of necrosis. However, the mechanisms involved in this process are not well established. Here we aimed to investigate in a *in vitro* model of acetaminophen (APAP)-induced cell necrosis if the blockage of FPR1 (FP receptor 1) and/or CXCR2 (CXC chemokine receptor 2) could reduce the APAP-mediated cell death.

Methods and results: The assays were performed using purified human neutrophils and HepG2 cells plated in the ratios of 1:1 or 1:10 (neutrophils:HepG2 cells, HepG2 cells in a density of 10^5 cells/well). Firstly, we observed that the neutrophil cytotoxic ability was completely dependent on neutrophil-HepG2 contact, since their separation by a transwell system reverted the cell death (1:1 = 20%, 1:10 = 87% $p < 0,05$), as observed by the MTT assay of cell viability. In a separated set of experiments, the HepG2 supernatant was replaced by APAP (5mM) -containing RPMI 1640 medium in the presence (APAP+N) or absence of the neutrophils (APAP) and/ or the treatments. Addition of neutrophils to APAP-incubated HepG2 in the ratio of 1:10 caused significant supplementary HepG2 death (cellular viability in APAP = 58%, APAP+N = 40%). The blockage of FPR1 and/or CXCR2 by BOC-1 and DF2156a (BOC, DF and BOC+DF $p < 0,05$), respectively, reverted partially the cell death induced by the neutrophils (N) (HepG2 viability 1:1: N = 20%, BOC = 25%, DF = 30%, BOC+DF =50%; 1:10: N =

62%, BOC = 90%, DF = 125%, BOC+DF = 120%, $p < 0,05$). Furthermore, the incubation with supernatant from APAP-treated (AT) HepG2 induced significant more ROS production in comparison to the supernatant of non-treated cells (NT) (AT = 22, NT = 15, in fluorescence units x 10^5 $p < 0,05$).

Conclusion: We conclude that blockage of the CXCR2-FPR1 activation pathway reduces neutrophil-mediated cell death, consisting in a novel alternative treatment for ALF induced by APAP overdose.

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XXXVII Congress of the Brazilian Society of Immunology
V Extra Section of Clinical Immunology - ESCI

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October 20 - 24, 2012

Evaluation of NKT cells population in patients with atopic and non-atopic asthma

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Introduction: Asthma is characterized as a respiratory disease which causes obstruction and airway hyperresponsiveness. Th2 cells have an important role in the pathophysiology of asthma, as well as IL-4, IL-5 and IL-13 produced by these cells. NKT cells are classified as a subgroup of lymphocytes with characteristics of cells T and NK cells that can activate or suppress the immune response, protecting in some disease like cancer, but can also operating a pathogenic action in lung diseases like asthma. The objective of this work is characterize and quantify iNKT cells in atopic and non-atopic asthma patients and controls in a population of school children from Porto Alegre-RS, and correlate with disease severity. **Methods and Results:** Study participants were 76 children aged 10-14 years old from private and public school of Porto Alegre. The children were included in the study if they were diagnosed with asthma without cold in the last 12 months or use of asthma drugs or without symptoms as a control group. This study was approved by ethics committee of PUCRS (Protocol nº10/05084) and written informed consent were obtained from children's parents. Peripheral venous blood of patients was collected and the peripheral blood mononuclear cells (PBMC) were purified using a Histopaque-1077 gradient. PBMC were stained with anti-CD3 APC and anti-iNKT (Vα24Jα18) FITC. The cells were acquired on a FACS Canto II flow cytometer (BD Biosciences). The cytometric data were analyzed using FlowJo software (TreeStar). We found that there was no difference between the percentage of iNKT cells in PBMC of atopic and non-atopic asthmatic children. However when we compare with the control group we found that the asthmatic children have a significant higher percentage of iNKT (mean 1,058±1,12 asthmatics vs. mean 0,373±0,21 control) **Conclusion:** This results suggested that iNKT might have a role in development of the disease. Although this data are preliminary and we will perform experiment to analyze the function of this cells in vitro. Also, we will correlate the data with the severity of the disease.

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V Extra Section of Clinical Immunology - ESCI

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ROLE OF MELATONIN ON CD4+ T CELL SURVIVAL AND ASSOCIATED IMMUNE RESPONSE

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Introduction: CD4+ T lymphocytes (TCD4) are crucial to the adaptive immunity, since they regulate other cell types. Importantly, at the end of the immune response, specific TCD4 clones are eliminated, mainly by AICD (activated-induced cell death), which depends on FAS/FASL interaction. Previous results from our lab showed that melatonin (MLT) is able to prevent FASL expression after TCR re-stimulation and consequent AICD. However, the physiological role of MLT *in vivo* on the expression of FASL in TCD4 remains elusive. **Objective:** The aim of this study is to evaluate the physiological and pharmacological effect of melatonin on TCD4 activation, proliferation and survival. **Methods & Results:** First, we confirmed the effect of melatonin on the expression of FASL in human CD4+ T cells, expanding our results with murine cells. Second, using a competitive ELISA, we investigated the production of melatonin in draining lymph nodes (DLN) isolated and macerated from four groups of DO11.10 TCR transgenic mice: immunized with a) CFA+OVA; b) OVA; c) CFA or d) saline. We observed the presence of melatonin only in DLN obtained from mice inoculated with CFA+OVA or OVA, suggesting extrapineal production of melatonin during the initiation of a specific adaptive immune response. Third, we tested the pharmacological effect of melatonin on the development of Experimental Autoimmune Encephalomyelitis (EAE), a murine model of multiple sclerosis dependent on TCD4. Groups of C57BL/6 mice (n=10) were treated with vehicle or with 3mg/Kg melatonin i.p. Our data showed that inoculation of melatonin during immunization resulted in impaired remission. **Conclusion/Discussion:** Preliminary data support our hypothesis of extrapineal production of melatonin during the initiation of an immune response. Also, melatonin can interfere with the outcome of EAE, possibly by interfering with FASL expression and survival of autoimmune CD4 T cells. This hypothesis is under investigation in our lab at the moment.

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V Extra Section of Clinical Immunology - ESCI

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INGESTION OF *LACTOCOCCUS LACTIS* ATTENUATES ALLERGIC RESPONSES AND MODULATES BEHAVIOR IN MICE.

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Introduction: Genetically modified *Lactococcus lactis* expressing IL-10 has been used for treatment of human inflammatory disorders such as Crohn's disease. It has been reported that Heat Shock Protein (HSP)-65 from *Mycobacteria leprae* has immunomodulatory effects on inflammatory processes. However, its effect on allergic lung inflammation has not been addressed. We have previously shown that respiratory allergy induces behavioral changes. Here we evaluated the effect of the ingestion of *L. lactis* expressing or not HSP-65 on the allergic experimental asthma and modulation of behavior in mice.

Methods and results: *L. lactis* expressing or not HSP-65 were offered to young adult C57BL/6 female mice for 4 days. One week later mice were sensitized with OVA (4ug) and Alum (1.6mg) subcutaneously, on days 0 and 7, and challenged with OVA (10ug) intra-nasally, on days 14 and 21. Anxiety levels were measured on elevated plus maze test 1h after second challenge. Serum and broncho-alveolar lavage were collected on day 22. The group that received non-expressing HSP-65 *L. lactis* presented a reduction in the number of total cells and eosinophils in the BAL, and lower levels of total and OVA-specific IgE when compared to the group that did not ingest *L. lactis*. Levels of anxiety were reduced in animals that ingested *L. lactis*. Notably, no significant differences were observed between the group that received *L. lactis* expressing HSP-65 with the group that only received *L. lactis*.

Conclusion: Ingestion of *Lactococcus lactis* attenuated airway allergic inflammation, reduced titers of Th-2 type antibodies and reduced the levels of anxiety of OVA-allergic mice. HSP-65 has no further effect on allergy.

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October 20 - 24, 2012

CHARACTERIZATION OF SUBPOPULATIONS OF DENDRITIC CELLS IN THE LUNG OF GENETICALLY DISTINTC HOSTS INFECTED BY *Mycobacterium tuberculosis*

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Introduction: Our group showed that the different magnitude of cellular immune response characteristic of BALB/c and C57BL/6 mice infected with *Mycobacterium tuberculosis* is associated with regulatory T cells. Because dendritic cells (DC) play an essential role in the activation and differentiation of $CD4^+$ lymphocytes, and different populations of DC participate in the induction or regulation of the immune response in diverse diseases, we aimed to quantify the different populations of DC in the lung of mice genetically distinct and infected by *M. tuberculosis*. **Methods and Results:** C57BL/6, BALB/c and DBA/J2 mice were infected with 1 x 10^5 bacilli by intra-tracheal route. Thirty days after infection, the number of DC populations $CD11c^+CD11b^+CD103^+$, $CD11c^+CD11b^+CD103^-$ and $CD11c^+CD11b^-CD103^+$, and IFN-gamma production were evaluated in the lungs. $CD11c^+CD11b^-CD103^+$ DC were found in greater numbers in the lung of infected C57BL/6 mice ($p<0.05$) compared to infected BALB/c and DBAJ2 mice, while the number of $CD11c^+CD11b^+CD103^-$ cells was higher in BALB/c mice compared the other two strains. The IFN-gamma production in the lung of C57BL/6 mice ($p<0.05$) was higher compared to those detected in the lung of BALB/c and DBA/J2 mice. **Conclusion:** Further experiments are undergoing to evaluate the role of different populations of DC in the induction of $CD4^+$ cell differentiation obtained from

different infected strains. These data may be useful to define the immunological parameters which govern the control or susceptibility to the infection and may be helpful for the design of immunotherapy for tuberculosis.

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THE ROLE OF APRIL IN ANTIGEN-INDUCED ARTHRITIS IN MICE

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Introduction: APRIL (A Proliferation-Inducing Ligand) acts as a secreted factor and plays important roles in B cell biology. The reported capacity of APRIL to stimulate B cells *in vitro* raised the hypothesis that APRIL may be a disease promoter in autoimmune diseases such as rheumatoid arthritis. In contrast with the evidences that B Lymphocyte Stimulator Factor (BAFF) can break B cell tolerance in mice and humans, the role of APRIL in autoimmunity remains elusive. This study was undertaken to examine the ability of APRIL to regulate the B cell-mediated inflammatory response in a model of antigen-induced arthritis (AIA) in mice and to influence cell migration in synovial tissue.

Methods and Results: AIA was induced by administration of antigen (mBSA) into the knee joints of previously immunized APRIL-transgenic (APRIL-Tg) mice and their littermates. Flow cytometry was used to analyze the different inflammatory cell populations in spleen and draining lymph nodes in acute and chronic disease, whereas the cytokine levels in joint tissues were evaluated by ELISA. In the acute stage of disease APRIL-Tg mice displayed a decrease in inflammatory response parameters, i.e., joint swelling, disease score and inflammatory infiltrate in articular tissues, as compared with their littermates. In addition, we observed a decrease of cellularity in draining lymph nodes of APRIL-Tg mice. These results were correlated with a milder chronic disease. FACS analysis revealed an increase of IgM+CD5+ cell population in draining lymph nodes in APRIL-Tg mice and increased IL-10 production *in vitro* at the acute phase of the disease. Our results suggest that APRIL and IL-10 producing B cells could reach the inflammed synovia and build an anti-inflammatory environment in arthritis. However this issue remains to be further elucidated.

Conclusion: Our data show that the ectopic APRIL expression can be correlated with higher resistance to arthritis and suggest that this regulatory axis should be investigated in human rheumatoid arthritis.

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XXXVII Congress of the Brazilian Society of Immunology
V Extra Section of Clinical Immunology - ESCI

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October 20 - 24, 2012

INVOLVEMENT OF KINASES AND INTRACELLULAR PHOSPHOLIPASES IN MAST CELL (MC) DEGRANULATION INDUCED BY A SNAKE VENOM PHOSPHOLIPASE A_2

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Introduction: Mast cells are central elements of innate immune response. Upon stimulation these cells release a vast array of inflammatory mediators through degranulation process. Several snake venom phospholipases A_2 (PLA_2) have been shown to induce *in vitro* MCs degranulation. However, the mechanisms involved in this effect are still unknown. This study aimed to evaluate the ability of MT-I, a GIIA-PLA_2 isolated from *Bothrops asper* snake venom to degranulate MCs and the involvement of both kinases (p38MAPK, PI3K and ERK1/2) and intracellular phospholipases (calcium-independent and citosolic PLA2, PLC and PLD) in this effect. **Methods and Results:** RBL-2H3 mast cell lineage was used. Citotoxicity of MT-I (0.7 – 15 µM) on RBL-2H3 cells ($6x10^4$) was evaluated by MTT and LDH assays after selected periods of incubation (30 min - 2 h). MC degranulation was determined by measuring β-hexosaminidase release. Involvement of kinases and intracellular phospholipases in MT-I-induced MCs degranulation was evaluated by pharmacological interferences with specific inhibitors. Results showed that MT-I was cytotoxic to MCs at concentrations higher than 8.8 µM. Stimulation of MCs with MT-I (3.5 µM) resulted in increased MC degranulation by 62.6%, 103% and 95% at 0.5, 1 and 2 h, respectively, in comparison with control MCs (3.96 ± 0.53%, 3.72 ± 0.3% and 3.60 ± 0.24% at 0.5, 1 and 2 h, respectively). Pre-treatment of cells with PD98059 or SB202190, inhibitors of ERK1/2 and p38MAPK, respectively, did not affect MT-I-induced MC degranulation. However, pre-treatment with wortmannin, inhibitior of PI3K, significantly reduced MT-I-induced

MC degranulation by 53.7% at 30 min as compared with controls. Inhibition of iPLA2 by Bel compound did not modify degranulation induced by MT-I, but inhibition of cPLA2, PLC or PLD by treating cells with compounds Pyr-2 or U-73122 or FIPI, respectively, reduced MT-I-induced MC degranulation (29%, 25.2% and 25.4%, respectively). **Conclusion:** MT-I is able to induce a rapid and sustained degranulation of MCs *in vitro*. This effect is dependent on PI3K, iPLA2, PLC and PLD, but not ERK1/2, p38MAPK and iPLA2.

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XXXVII Congress of the Brazilian Society of Immunology
V Extra Section of Clinical Immunology - ESCI

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October 20 - 24, 2012

ADJUVANT EFFECT OF THE *PROPIONIBACTERIUM ACNES* ON ABSOLUTE NUMBER AND ACTIVATION MARKERS IN BOTH iNKT AND B-1 CELLS

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Introduction: The invariant natural killer T cells (iNKT) and B-1 cells participate in the early immune response in inflammatory focus, influencing adaptive immune response. iNKT cells represent a subpopulation of T-lymphocyte which has an invariant TCR, secretes a variety of cytokines after stimulation, mainly when recognizes glycosphingolipid presented via CD1d molecules expressed in antigen presenting cells. B-1 cells are predominantly located in the peritoneal and pleural cavities. These cells are different from conventional B (B2) by surface phenotype ($CD19^+$, IgM^{high}, IgD^{low}, $B220^+$, $CD23^-$) and are divided into B-1a ($CD11b^+CD5^+$) and B-1b ($CD11b^+CD5^-$). Although the interaction between the B-1 cells and iNKT is poorly studied, our group has demonstrated that in addition to macrophages, the suspension of heat killed-*Propionibacterium acnes (P.acnes*) and its polysaccharide component (PS) used as adjuvant when are administered intraperitoneally (i.p.), were able to increase the absolute number of $CD3^+NK1.1^+$ and B-1 cells in C57BL/6 mice peritoneal cavity. Thus, the aim of this study was to verify the effect of *P. acnes* and PS in B-1 and iNKT cells activation. **Methods and Results:** Peritoneal cells from C57Bl/6 wild type untreated or treated with saline (controls groups) and treated with i.p. unique dose of either heat-killed *P. acnes* (140µg), PS (25µg) were obtained after 24 hours and analyzed by flow cytometry. The absolute number of iNKT cells was determined based on $CD3^+$/CD1d αGalCer dimer markers. The activation molecules were analyzed on iNKT (CD44+/CD69) and B-1 (CD1d) cells. After bacterial stimulus, we observed in the absolute number 3-fold higher of iNKT cells and an overexpression of CD44 and CD69 molecules than in control groups. Moreover, *P. acnes* but not PS induced an increase of CD1d expression on B-1a and B-1b cells when compared with control groups. **Conclusion**: The overexpression of the CD1d molecule on B-1 cells by *P.acnes* strongly suggests the interaction between B-1 and iNKT early in the immune response.

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IMMUNO 2012
XXXVII Congress of the Brazilian Society of Immunology
V Extra Section of Clinical Immunology - ESCI

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October 20 - 24, 2012

EFFECTS OF *Aedes aegypti* SALIVARY GLAND EXTRACT ON T CELL BIOLOGY

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Introduction: *Aedes aegypti* is an important mosquito species and transmits diseases such as yellow fever, Chikungunya fever and dengue fever. In order to successfully nourish themselves, these hematophagous mosquitoes find the immunological system of their hosts as a major challenge. During the evolution process, these insects developed a salivary cocktail with an arsenal of molecules presenting several immunomodulatory properties. In the present work, we investigated the effects of *Ae. aegypti* salivary gland extract (SGE) on T cell proliferation as well as the identification of the molecule responsible for the observed activity and its mechanism of action.

Methods and Results: Our data reveals that *Ae. aegypti* SGE impairs antigen-specific and mitogen-induced T cell proliferative responses in a concentration dependent manner, reaching maximum effect (100% inhibition) at 10 μg/mL. This is a unique activity among SGE from other diptera studied (*Phlebotomus duboscqui* and *Anopheles aquasalis* – 0% inhibition). Fractionation of SGE of this vector by FPLC showed one major active fraction with apparent molecular mass of >400 kDa. The mass spectrometry analysis of the fractions revealed a single candidate in which the amount detected correlated with the observed activity. We also observed that the mechanism of action of SGE involves death of naïve T cell by apoptosis. Both $CD4^+$ and $CD8^+$ naïve T cells were affected by SGE in a 3 day-culture (~72% and ~71% of $annexin^+$ cells, respectively), while memory cells were resistant to this effect. Incubations with 8 and 24 hours were also analyzed and presented similar results.

Conclusion: *Ae. aegypti* saliva possesses a potent inhibitor of T cell proliferation with >400 kDa and able to induced apoptosis in naïve, but not memory T cells. Further studies will be required to better understand the mechanisms underlying such effects.

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XXXVII Congress of the Brazilian Society of Immunology
V Extra Section of Clinical Immunology - ESCI

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October 20 - 24, 2012

INNATE AND ADAPTIVE IMMUNE RESPONSES IN PREMATURELY BORN INFANTS EVALUATING MEMORY T CELLS AND TOLL-LIKE RECEPTORS EXPRESSION

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Introduction: Preterm infants are at increased risk of being re-hospitalized during the first few months of life with severe respiratory syncytial virus (RSV) lower respiratory tract infection (LRTI). Two major factors involved in higher morbidity in early life are deficient lung maturation and immature immune responses. It is not well established if the patterns of immune responses differ between term and preterm infants beyond the first year of life. The aim of this study is to evaluate innate and adaptive immune responses at age 3 years in prematurely born infants when compared to term born infants. **Methods and Results:** This is cross-sectional study with data collected at a mean age of 3 years. Blood (5 mL) was collected from preterm (n=15) and term children (n=2); peripheral blood mononuclear cells (PBMC) were isolated and stimulated with TLR ligands for 24hs (LPS, PGN, CPG, Poly I:C). Afterwards, cells were stained with anti- TLR4, -TLR2, -TLR9, -HLADR, and -CD11c antibodies. T cell memory was evaluated by staining the cells with anti-CD3, -CD8, -CD4, -CD45RO and -CD27 antibodies. At age 3 years children born before term presented higher percentage of memory CD3+CD8+CD45RO+ T cells (12.07% ± 5,8) and CD3+CD8+CD27+ (2.15% ± 0.1) compared to those born at term (6.65 ± 2.38) and (0.95% ± 0,99), respectively. Preterms showed a 2-fold increase in the percentage of memory CD4 T cells compared to term children. Interestingly, preterm-born children showed a decreased frequency of dendritic cells CD11c+HLADR+ in peripheral blood (15.83% ± 7,8), compared to terms (26,05% ± 7,6). Conversely, dendritic cells from preterm-born children expressed higher amounts of Toll-like receptors (TLRs) such as, TLR2, TLR4 and TLR9. The most significant difference between these two groups was a 4-fold increase in the expression of TLR2, where the frequency of CD11c+HLADR+CD86+TLR2+ was 20.73% ± 11.28 compared to 5% ± 1.28. The higher expression of TLRs on dendritic cells of the preterms persist after

stimulation with TLR ligands (LPS, CPG, PGN, Poly I:C). **Conclusion:** These data is a preliminary report suggesting that an altered pattern of immune responses persist for prematurely born children, well beyond the first months of life, which may explain the greater clinical morbidity these children present.

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October 20 - 24, 2012

PATIENTS WITH PULMONARY TUBERCULOSIS PRESENT TCD8 CELLS WITH REGULATORY FUNCTIONS

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Introduction: *Mycobacterium tuberculosis* infection is responsible for about 8.8 million new cases of Tuberculosis (TB), causing over 1.5 million deaths each year. The immune response against *M. tuberculosis* is related to many pro-inflammatory and anti-inflammatory cytokines and protective cellular immune response against TB is mediated by TCD4+ together with TCD8+ cytotoxic and cytokine producing cells. The role of TCD4+ cells is well characterized. The function of TCD8+ cells however has not been clarified. In the absence of TCD4+ cells, TCD8+ cells increase their production different cytokines and their cytotoxic function in order to respond to mycobacterial infection. **Objective**: To evaluate peripheral blood TCD8+ cell subsets: Tc1 (CD8+IFN-γ+), Tc17 (CD8+IL-17+) and TCD8+IDO+ within patients with active pulmonary tuberculosis (TB) and compare their profile with healthy controls (TST-).

Methods and Results: PBMC from 10 patients with active pulmonary TB (mean age = 39.4), 10 healthy controls TST- matched by sex and age to TB patients were cultivated with *M. tuberculosis* recombinant antigen (GLcB). T cell subsets were analyzed by flow cytometry. Tc1 cells from patients with tuberculosis express the transcription factor T-bet, a member of the T-box family of transcription factors that appears to regulate lineage commitment in TCD4+ cells in part by activating the hallmark Th1 cytokine, interferon-γ. *M. tuberculosis* specific Tc1 cells responses (TCD8+T-bet+IFN-γ+) were observed among TB patients (media=2.4±1.2; PHA=13.1±1.9; GLcB=3.9±1.3) compared with TST- (media=1.3±0.4; PHA=12.8±1.5; GLcB=1.1±0.5) healthy controls. TB patients presented specific Tc17 cells (TCD8+IL-23R+IL-17+) (media=1.9±1.1; PHA=14.6±2.7; GLcB=3.1±1.2)

but not healthy controls TST- (media=0.8±0.7; PHA=11.9±2.3; GLcB=0.9±0.6). The tryptophan metabolism enzyme indoleamine 2,3-dioxygenase (IDO) has not been established, but under certain conditions the IDO activation may be related to activation or immunomodulation of the immune response. The healthy controls TST- present more specific TCD8+IDO+ cells (media=5.0±1.6; PHA=12.3±3.2; GLcB=6.9±1.5) when compared with TB patients (media=3.2±0.8; PHA=14.3±1.5; GLcB=3.3±1.7).

Conclusions: Patients with TB had specific Tc1 and Tc17 responses, whereas present lower response of TCD8+IDO+ cells, that was observed in healthy controls TST-. These results suggest the importance of TCD8 cells in the tuberculosis disease evolution.

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IMMUNO 2012
XXXVII Congress of the Brazilian Society of Immunology
V Extra Section of Clinical Immunology - ESCI

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October 20 - 24, 2012

MYD88 AND STING SIGNALING PATHWAYS ARE REQUIRED FOR IRF3-MEDIATED IFN-B INDUCTION IN RESPONSE TO *Brucella abortus* INFECTION

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Introduction: Type I interferons (IFNs) are cytokines that orchestrate diverse immune responses to viral and bacterial infections. Although typically considered to be most important molecules in response to viruses, type I IFNs are also induced by most, if not all, bacterial pathogens. In this study, we addressed the role of type I IFN signaling during *Brucella abortus* infection, a facultative intracellular bacterial pathogen that causes abortion in domestic animals and undulant fever in humans. **Methods and Results:** Herein, we have shown that *B. abortus* induced IFN-β in macrophages and splenocytes. Further, IFN-β induction by *Brucella* was mediated by IRF3 signaling pathway and activates IFN-stimulated genes via STAT1 phosphorylation. In addition, IFN-β expression induced by *Brucella* is independent of TLRs and TRIF signaling but MyD88-dependent. Furthermore, we have identified *Brucella* DNA as the major bacterial component to induce IFN-β and our study revealed that this molecule operates through a mechanism dependent on RNA polymerase III to be sensed probably by an unknown receptor via the adaptor molecule STING. Finally, we have demonstrated that IFN-αβR KO mice are more resistant to infection suggesting that type I IFN signaling is detrimental to host control of *Brucella*. This resistance phenotype is accompanied by increased IFN-γ and NO production by IFN-αβR KO spleen cells and reduced apoptosis. In summary, our study demonstrated that *B. abortus* induces IFN-β through IRF3 signaling pathway and activates IFN-stimulated genes via STAT1 phosphorylation. Additionally, this *IFN-β* induction is independent of TLRs but MyD88-dependent, pathway not yet described for Gram-negative bacteria. Further, being *Brucella* DNA a major bacterial component to induce IFN-β, our study suggests that this molecule is transcribed by RNA polymerase III to be sensed by an unknown receptor that signals via STING. **Conclusion:** Taken together, our results suggest that, to the best of our knowledge, this is the first report demonstrating that

bacterial DNA can be sensed by RNA polymerase III/STING pathway coordinating type I IFN production.

IMMUNO 2012
XXXVII Congress of the Brazilian Society of Immunology
V Extra Section of Clinical Immunology - ESCI

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October 20 - 24, 2012

DECREASE OF BACILLUS GROWTH IN *Mycobacterium tuberculosis*-INFECTED AND ALLERGIC MICE DEPENDS ON THE MOUSE STRAIN AND BACTERIAL DOSE

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Introduction: The incidence of asthma is lower in tuberculosis skin test positive individuals. However, there are no evidences about the progression of tuberculosis (TB) during allergic responses. We have used a model of *M. tuberculosis* infection followed by induction of asthma (TB/OVA), in order to evaluate the modulation of immune response in these diseases. Our previous results showed that TB/OVA mice exhibited a lower lung CFU (Colony-Forming Units) number independent on IFN-gamma production compared to infected mice (TB group). We also observed increased levels of leukotrienes B_4 (LTB_4) and higher number of $CD8^+$ cells in TB/OVA mice. In order to evaluate the mechanisms involved in the decrease of lung CFU counts in TB/OVA mice, we used two different strategies: 1- To assess the role of LTB_4, we used mice deficient for 5-lipoxygenase (5-LO), enzyme that catalyze leukotrienes production and 129Sv Wild Type (WT) mice; 2- To evaluate the role of CD8, we used mice deficient for CD8 (CD8-KO). **Methods and results:** 129Sv mice were infected with *M. tuberculosis* and subsequently, were submitted to the asthma protocol and treated with MK-886, an inhibitor of 5-LO. Opposite to what we had previously observed for BALB/c mice, TB/OVA 129Sv mice had a higher CFU counts ($p<0.05$) compared to TB mice. There was no difference between treated TB/OVA and TB mice. In attempt to evaluate the role of $CD8^+$ cells, WT and CD8-KO mice were submitted to the TB/OVA protocol. We observed that TB/OVA CD8-KO were more susceptible than TB CD8-KO mice. Moreover, TB/OVA WT mice had a higher CFU counts ($p<0.05$) compared to TB WT group. Since TB/OVA WT were more susceptible than TB mice, confirmed that the mouse strain or the susceptibility of the host, is an important issue to be considered in the modulation of the immune response in TB/OVA mice. Because the modulation of the immune response in TB/OVA mice depends on mouse strain, we next evaluated whether the infection route and number of bacilli would affect the lung CFU counts. BALB/c mice were infected with $1x10^2$ or $1x10^5$ bacilli by intratracheal

(IT) route or with $1x10^5$ by intranasal (IN) route. We found a decrease in CFU number in the TB/OVA (IT-10^2) mice compared to TB group ($p<0.05$). On the contrary, TB/OVA (IT-10^5) mice showed an increase in the bacillus growth ($p>0.05$). **Conclusion:** These preliminary results suggest that decrease in the lung CFU number in TB/OVA mice depends on the strain, bacterial dose and infection route.

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IMMUNO 2012
XXXVII Congress of the Brazilian Society of Immunology
V Extra Section of Clinical Immunology - ESCI

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October 20 - 24, 2012

EFFEROCYTOSIS BY ALVEOLAR MACROPHAGES SUPRESS THE MICROBICIDAL ACTIVITY AGAINST *Streptococcus pneumonia*

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INTRODUCTION *Streptococcus pneumoniae* is one of infections responsible for deaths in children or adults suffering from chronic lung diseases. Individuals affected by chronic lung diseases have an intense accumulation of apoptotic cells (ACs) and they can be more susceptible to bacterial lung infections such as *S. pneumonia.* In the lung, the scavenger receptor (SR) express in alveolar macrophages (MA) play a critical role in phagocytosis and microbicidal activity of *S. pneumonia*. The goal of our study is to investigate if ACs can suppress the microbicidal activity of AMs against *S. pneumonia* via SR. **METHODOLOGY AND RESULTS:** AMs were pre-treated or not with SR antagonist (fucoidan and Poly:I) for 30 min in presence or not with AC for 1 h, and then challenged with *S. pneumonia*. CFU counts in lysed macrophages were determined 4h and 12h after phagocytosis and the cytokines were quantified in supernatant. The results indicate that efferocytosis by AMs has an immunosuppressive effect on the microbicidal activity against *S. pneumoniae* (CFU/ml - 4h: AMs+SP= $0,5x10^6$ *vs* AMs+AC+Sp= $280x10^6$; 12h: AMs+Sp= $36x10^6$ *vs* AMs+ACs+Sp=$280x10^6$). This suppressive effect mediated by eferocytosis was exacerbated in the presence of SR-A antagonist after 12h (12 h: AMs+AC+Sp=$280x10^6$ *vs* AMs+fucoidan+AC+Sp= $300x10^6$ and AMs+poly:I+AC+Sp= $310x10^{6)}$. Unexpected, when AMs were pre-treated with ACs and challenge with *S. pneumonia* there was elevated levels of TNF-α and IL1-α by AMs when compared to AMs without ACs (TNF-α (pg/ml): - 4h: AMs+SP= undetectable *vs* AMs+AC+Sp= 285; 12h: AMs+Sp= undetectable *vs* AMs+ACs+Sp=780 and IL-1α (pg/ml) - 12h: AMs+Sp= undetectable *vs* AMs+ACs+Sp=39). **CONCLUSION:** In summary, our results demonstrate that: 1) the efferocytosis by AMs impair the microbicidal activity against *S. pneumoniae*; 2) uncontrolled *S. pneumoniae* proliferation mediated by efferocytosis induced intense cytokines production by AMs; 3) the presence of SR-A antagonist increase the suppressive effect induced by eferocytosis.

IMMUNO 2012
XXXVII Congress of the Brazilian Society of Immunology
V Extra Section of Clinical Immunology - ESCI

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IL-1β PRODUCTION BY PHAGOCYTOSIS OF INFECTED APOPTOTIC CELLS: A TRIGGER TO TH17 DIFFERENTIATION?

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Introduction.The phagocytosis of apoptotic cells is a process for tissue homeostasis after injury. We and other groups have previously shown that phagocytosis of apoptotic cells promotes the synthesis of anti-inflammatory mediators such as PGE_2, TGF-β and IL-10 that may result in the suppression of host defense. Recently a study showed that infected apoptotic cells phagocytosis by dendritic cells promotes the production of anti-inflammatory cytokines such as TGF-β and pro-inflammatory cytokines, IL-6 and IL-23. Moreover, these mediators were able to induce an immunostimulatory effect, the Th17 cells differentiation. Some reports have implicated that IL1-β may be important in Th17 development. Our aim here was to evaluate the IL-1β production from dendritic cells when co-cultured with infected apoptotic cells. **Methods and Results**. Bone marrow derived dendritic cells (BMDC) were cultured at ratio of infected apoptotic neutrophils (1:3) during 18 hours. Infected apoptotic neutrophils were prepared as follows: C57BL/6J mice were injected intraperitoneally with thioglicollate with $1x10^5$ or 3 $x10^6$ live *Escherichia coli*, and then after 13h, the peritoneal exudate was collected. Apoptotic neutrophils were generated by UV irradiation (350mJ) and confirmed by Annexin-V/PI detection. Supernatant from co-culture was collected at 18h to detect IL-1β levels by ELISA assay. Our results show that IL1-β production by phagocytosis of infected apoptotic cells depends of the bacterial load, thus neutrophils infected with high *E. coli* load and treated to apoptosis as a source of infected apoptotic cells, induce the production of high levels of IL-1β (Mean/SD=1021±137 pg/mL) by DCs, whereas less bacterial load results in fewer IL-1β production (58±5.8 pg/mL). However, we found the same levels of TGF-β production when DCs were incubated with different source of infected apoptotic neutrophils. **Conclusion.** Here we demonstrate that IL-1β can be produced during paghocytosis of infected apoptotic cells. Our results also suggest that this process depends of infected apoptotic cells with high bacterial load and in the context of phagocytosis of infected apoptotic cells IL-1β may work synergistically with TGF- β, IL-6 and IL-23 in the process of Th17 cell differentiation.

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PHAGOYTOSIS OF APOPTOTIC CELLS BY ALVEOLAR MACROPHAGES: INHIBITION OF PHAGOCYTOSIS OF *Streptococcus pneumoniae*

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Introduction: The scavenger receptors (SR) are the major receptors involved in the *Streptococcus pneumonia* phagocytosis by alveolar macrophages (AMs) that act as the first line of defense in the lung. The increase of susceptibility to bacterial infections has been demonstrated in chronic inflammatory pulmonary disease in which there is an intense accumulation of apoptotic cells (ACs).Our hypothesis is that the uptake of ACs by macrophages could suppress immune responses by releasing anti-inflammatory mediators, such as TGF-β and PGE2. However, the way in which PGE2 suppress the effector mechanisms against *Streptococcus pneumoniae* by SR in alveolar macrophages is unclear.

Methods and Reults: Rat AMs were pre-treated for 30 min with pharmacological compounds and then incubated with ACs (3:1) for 1h, followed by FITC-labeled *S. pneumoniae (*fluorometric assay for phagocytosis) or *S. pneumonia* (killing assay by MTT). We found that the pre-incubation with AC inhibited the ingestion of *S. pneumoniae* by AMs (AMs+AC:Sp = ~ 39%, *, $P < 0.05$ versus AM:Sp). The inhibition of *S. pneumoniae* phagocytosis by efferocytosis was partially reverted when endogenous PGE2 production was repressed with a COX inhibitor (AMs+AC:Sp= ~ 10%, #, $P < 0.05$ versus AM+Sp) and likewise with adenylate cyclase inhibitor (AMs:AC:Sp = ~ 13%, versus AM+Sp). Moreover, we demonstrated that the inhibition of *S. pneumonia* phagocytosis by efferocytosis was more pronounced when SR-AI/II were blocked with antagonists, fucoidan and Poly:I.

Conclusion: In the present study, we demonstrated that efferocytosis by AMs through the PGE2/adenylate cyclase/cAMP pathway inhibits *S. pneumoniae* phagocytosis. We also demonstrated that SR-B are more sensitive to the suppressive effects of efferocytosis by AMs in phagocytosis of *S. pneumoniae*.

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PHAGOCYTOSIS OF INFECTED APOPTOTIC CELLS BY DENDRITC CELLS DEPICTS A ROLE FOR PGE_2 AND IL-β IN TH17 DIFFERENTIATION

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Introduction: Phagocytosis of apoptotic cells (ACs) promotes the synthesis of anti-inflammatory mediators such as PGE_2, TGF-β and IL-10 by macrophages and dendritic cells (DCs) that may result in the suppression of host innate immune defense and Treg cells differentiation. However, a study using infected apoptotic cells showed that phagocytosis of these cells by DC promotes the production of anti-inflammatory cytokines such as TGF-β but also pro-inflammatory cytokines as IL-6 and IL-23 resulting in an immunostimulatory effect, the differentiation of Th17 cells. Our aim was to evaluate the production of PGE_2 and IL-1β, besides TGF-β and IL-6, produced by DC when co-cultured with infected apoptotic cells. **Methods and Results**: As a source of infected apoptotic neutrophils C57BL/6J mice were intraperitoneally injected with 3 mL thioglicollate with 3 $x10^6$ live *Escherichia coli*, after 13 h, the cells were collected from peritoneal cavity lavage. Apoptotic neutrophils were generated by UV irradiation (350mJ) and confirmed by Annexin-V/PI detection. Using this protocol we found ~70% of early infected ACs with minimal late infected ACs. BMDC were co-cultured at 1:3 ratio with infected apoptotic neutrophils during 18 h. Supernatant from co-culture was collected and TGF-β, IL-1β, IL-6 and PGE_2 production was determined by ELISA. Our results show that phagocytosis of infected apoptotic cells induces the production of high levels of PGE_2 (4790 ± 295 pg/mL), IL-1β (1021 ± 137 pg/mL), TGF-β (705 ± 43 pg/mL) and IL-6 (28.9 ± 0.45 ng/mL). Our results show a high production of IL-1β and PGE_2, besides the TGF-β and IL-6 previously shown by others. The high levels of PGE_2 suggest that this lipid mediator might be involved in Th17 differentiation related to infected apoptotic cells phagocytosis. Also, some reports have implicated that IL1-β may be important in Th17 development and it should be true also in this context. **Conclusion:** Therefore, we suggest that this lipid mediator together with IL-1β might be involved in Th17 differentiation regarding phagocytosis of infected apoptotic cells. However, future studies will be important to characterize how PGE_2 and IL-1β can work synergistically with TGF-β and IL-6 in the Th17 cell differentiation.

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MULTIPLE MYELOMA: LYMPHOCYTE STIMULATION BY DENDRITIC CELLS FUSED WITH PLASMA CELLS

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Introduction: Dendritic cell-based immunotherapy is one of most recently alternative to treat multiple myeloma. Fusion of dendritic cells with plasma cells appears to be promising, because its allows the presentation of complete tumor antigen content to T lymphocytes, generating specific cytotoxic antitumor response. Although this treatment is being applied in humans, few studies clarify the lymphocyte response to dendritic cells fused. Our objective was to study the proliferative response of lymphocytes in mixed leukocyte reaction induced by dendritic cells fused with plasma cells from patients with multiple myeloma.
Methodology: Peripheral blood mononuclear cells were obtained from *buffy-coat* of healthy donors and patients with MM and separated by Ficoll density gradient centrifugation. DCs were generated by monocytes cultured with RPMI media with 10% FCS and the cytokines IL-4 and GM-CSF for 5 days, later, the maturation were induced by addition of IFN-α e TNF-α for 2 days. Allogeneic T cells were obtained from PBMC of healthy donor by nylon wool separation. The MM cells were obtained by magnetic microbeads CD138+ separation from bone marrow aspirate. DCs fusion with $CD138^{+}$ cells was performed with polyethylene glycol (PEG). T cell proliferation induced by dendritic cells (DC), dendritic cells and plasma cells (MIX) or fused cells (FUS) was estimated by CFSE labeling. T cell subsets were evaluated by flow cytometry on proliferative cells (CFSElow).
Results: T cell proliferation induced by co-culture with DC was 17,7%(5,8-23,5%), MIX - 17,3% (11,5-29,1%), FUS - 1,7%(0,8-14,3%) and the control of lymphocytes without stimuli - 0,5%(0-1,7%). The rate of CD4+CD8- Tcells stimulated with DC - 43,7±6,2, MIX - 41,8±5,4 and FUS - 52,9±1,0, and the CD4+CD8+ Tcells stimulated with DC - 29,9±6,6, MIX - 28±6 and FUS - 33,1±2,3. The mean fluorescence intensity analysis (MFI) of markers showed that cells that proliferate (CFSElow) when stimulated with DC, MIX or FUS presents higher rates of CD4 and CD4+CD8+ Tcells were CD8low.
Conclusion: Fused cells induced lower rate of T cell proliferation than dendritic cells in co-culture with plasma cells. However, the proportion of CD4+CD8- or CD4+CD8+ cells was maintained. The rate of CD8 (MFI) on CD4+CD8+ Tcells was

lower than on CD4-CD8+ while the rate of CD4(MFI) was increased in all proliferative Tcells.

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