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XXXVII Congress of the Brazilian Society of Immunology
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MOLECULAR IMMUNOLOGY

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Thymic stromal lymphopoietin enhances Th2/Th22 and reduces IL-17A in protease allergen induced airways lung inflammation

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Background: Thymic stromal lymphopoietin (TSLP) is induced in allergic skin and lung inflammation in man and mice.

Methods: Allergic lung inflammation induced by two proteases allergens HDM and papain and a classical allergen ovalbumin was evaluated *in vivo* in mice deficient for TSLPR. Eosinophil recruitment, Th2 and Th17 cytokine and chemokine levels were determined in bronchoalveolar lavage fluid, lung homogenates and lung mononuclear cells ex vivo.

Results: Here we report that mice challenged with house dust mite extract or papain in the absence of TLSPR have a drastic reduction of allergic inflammation with diminished eosinophil recruitment in BAL and lung and reduced mucus overproduction. TLSPR deficient DCs displayed diminished OVA antigen uptake and reduced capacity to activate antigen specific T cells. TLSPR deficient mice had diminished proinflammatory IL-1b, IL-13 and IL-33 and chemokines production. Together with impaired Th2 cytokines, IL-17A expressing TCRb+ T cells were increased, while IL-22 expressing CD4+ T cells were diminished in the lung.

Conclusion: Therefore, TSLPR signaling is required for the development of both Th2 and Th22 responses and to restrain IL-17A. TSLP may mediate its effects in part by increasing allergen uptake and processing by DCs resulting in an exacerbated asthma.

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EFFECTS OF GH OVEREXPRESSION ON IMMUNE SYSTEM OF TRANSGENIC ZEBRAFISH

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Introduction: The immune system plays an important role in the defense of organisms against infectious agents. Therefore, it counts on organs, cells and specialized molecules to carry out such function. Some studies have shown that pituitary hormones, such as growth hormone (GH), may interact with cells and immune organs ergo keeping this system's homeostasis. Based on this, the present study aimed to evaluate GH overexpression influence on immune system of transgenic zebrafish (*Danio rerio*).
Methods and Results: We have evaluated, by qPCR, the expression of genes related with cellular immunity: CD4, CD247, IL-1β and IFN-1α, which codes for proteins involved in humoral immunity, and development-related genes, IKAROS and RAG-1, in 30 days old zebrafish (n=7, 3 animals/sample). Besides, aiming to verify the size of thymus and cephalic kidney, which are important immune organs of teleost fish, histological and morphometric analyses were made. The lymphocyte level from thymus and cephalic kidney was also analyzed by anti-CD3 and anti-CD4 immunohistochemistry. Concerning gene expression, five from the six analyzed genes presented lower expression in transgenic juveniles (± 30 days) in relation to non-transgenic control group (NT)($p<0,05$). CD4 and RAG-1 presented an expression decrease of 60% while CD247, IKAROS and IL1-β have shown a diminishing of 40%, 50% and 55% in its expression, respectively. IFN-1α did not vary. The software Rest was used for statistical analyses. Morphometry of thymus (NT:159.2 ± 0.1504; TG: 123.5 ± 0.2635) and cephalic kidney (NT: 411.9 ± 0.2040; TG: 208.9 ± 0.1909) from transgenic zebrafish revealed a reduction of area in these structures ($p<0,01$ n=10) when compared to non-transgenic individuals. Concerning the lymphocyte level of CD3 (NT: 41.40 ± 1.046; TG: 25.50 ± 1.078) and CD4 (NT: 27.20 ± 0.8273; TG: 14.40 ± 0.8844), there was also observed a significant decrease ($p<0,01$ n= 10). These data were given in mean ± standard error.
Conclusion: Based on these data, we have concluded that a supraphysiological level of GH impairs the development and immunological functions of teleost fish. Thus, these data evince that the maintenance of GH normal levels is important to physiological processes functioning, including the immune system.

Financial support: CNPq

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IDENTIFICATION OF NOVEL MICRORNAS AND MRNA TARGETS IN T CELLS DURING COLLAGEN INDUCED ARTHRITIS (CIA) DEVELOPMENT IN MICE

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Introduction: MicroRNAs (miRNAs) represent a class of endogenous 19-25-nucleotide long, non-coding RNA molecules that regulate a wide variety of other genes by either preventing the translation or inducing the cleavage of their targeted mRNAs. The miRNAs mediate diverse aspects of development and physiology in mammals and their roles in diseases have been just recently determinate. It is become clear that proper miRNA regulation is critical for normal immune functions and for the prevention of autoimmunity. Recent studies have start to demonstrate the importance of specif miRNAs in rheumatoid arthritis (RA), which is characterized by a chronic and systemic inflammation that affects the joins, and which a hallmark pathological feature is the infiltration and accumulation of T cells in the synovium. Our objective is to determine the miRNAs expression profile and reconstruct probabilistic network interactions between miRNAS and their predicted mRNA targets in T cells of DBA-1/J mouse strain during the development of CIA.

Methods and results: DBA-1/J male mice were immunized with collagen and total CD3+ T cells from spleen and lymph nodes were isolated by magnetic beads and used for total RNA extraction. Control mice were immunized without the collagen. These samples were then separately hybridized with whole genome Agilent microarrays containing 44,000 and 15,000 oligos representing the complete set of known murine mRNA and miRNA. The microarray data were analyzed using bioinformatics programs (Agilent Genespring software). The GenMir program was used to reconstruct the miRNA-mRNA interactions network. During emergence of CIA nine “new” miRNAs were differentially expressed, three of them (miR-467a*, miR-721 and miR-500) up regulated and

six (miR-29a*, miR125b-5p, miR15b*, miR-let-7d*, miR-211 and miR-714) down regulated compared to control mice. Network analysis showed miRNA interactions with key target mRNAs involved in important processes as immune response, inflammatory process and T cell activation/ proliferation. The transcriptional factors Rorc and Eomes related to inflammatory response are strongly regulated by the majority of miRNAs in control mice. On the other hand the Stat5 related with the inhibition of Th17 and the positive regulation of Tregs is regulated during CIA.

Conclusions: Novel deregulated miRNAs were identified in the T cells during the CIA induction and control important targets. This deregulation might contribute to the pathogenesis of the disease.

Financial support: CAPES, Fapesp.

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RELATION OF SERUM LEVELS OF HIGH-SENSITIVE C-REACTIVE PROTEIN WITH POLYMORPHISM +1444 C>T OF *CRP* GENE IN OVERWEIGHT ADULTS

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Introduction:The C-reactive protein (CRP) is a marker of cardiovascular disease risk, it is generally high in individuals with obesity and its serum levels have genetic influences. Considering that obesity is a healthy public problem and there are some polymorphisms associated to the inflammatory levels, the objective of this study was to investigate serum levels of high-sensitive CRP (hs-CRP) related to the genotypes of the polymorphism +1444 C>T (rs1130864) of the *CRP* gene in sedentary overweight adults.

Methods and Results: Individuals from Porto Alegre/RS, over 18 years old, sedentary and with Body Mass Index (BMI) ≥25 kg/m² were recruited to participated of the study. Blood samples were collected for determination of serum hs-CRP by turbidimetric method. Also oral mucosa cells were collected to DNA extraction, genotyping (Polymerase Chain Reaction) and fragment digestion with the restriction enzyme HpyCH4III (cleavage site ACN/GT). This study was approved by the Ethics Committee of Centro Universitário Metodista, do IPA, under protocol number 58/2010. Data are presented as mean±standard deviation (SD). Statistical analysis was performed using SPSS (Statistical Package for Social Sciences) version 17.0. Kolmogorov-Smirnov test was used to verify the normality and Kruskal-Wallis test to compare means between the hs-CRP levels and its genotypes, considering significant p value <0.05. The sample consisted of 52 individuals, 42 women and 10 men. The mean±SD age was 48.9±11.6 years old and BMI 34.7±4.9 Kg/m^2. The serum high-sensitive CRP levels according to the genotypes were: CC (n=25) 0.28±0.21 mg/dL; CT (n=22) 0.25±0.19 mg/dL and TT (n=5) 0.16±0.12 mg/dL. There were no

significant differences in relation to CRP levels according to genotypes (p = 0.64).

Conclusion: No relationship was found between serum hs-CRP levels with the investigated polymorphism in these overweight individuals. This result may be due to the limited number of subjects. However, more studies are needed to verify the importance of these gene variations in this parameter.

Financial support: CAPES e Centro Universitário Metodista, do IPA.

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DISTRIBUTION OF TCR-Vβ REPERTOIRE IN PATIENTS WITH CHAGAS DISEASE

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Introduction: Chagas disease, also known as American trypanosomiasis, is a chronic infection caused by *Trypanosoma cruzi*, a protozoan parasite. The disease affects an estimated 8 to 10 million people in the Americas, where 20-30% of patients develop myocardiopathy in a chronic phase of the disease. Cell-mediated immunity plays an important role in the reduction of parasite load, but may also contribute to the development of lesions observed during the chronic phase of the disease. However, the mechanisms involved in the clinical evolution of the disease are not well understood. We investigate the frequency of TCR-Vβ members in $CD4^+$ and $CD8^+$ T cell subpopulations from chronically *T. cruzi* infected patients. **Methods and Results:** We included seventy-nine patients followed for at least seven year and presenting chronic assymptomatic (n=27) or several degree of cardiac involvement, based on Brazilian Consensus of Chagas Disease classification. Flow cytometry TCR-Vβ analysis was simultaneously evaluated with immunophenotyping for CD4 and CD8 T cells subpopulations. Our results showed that no differences were observed in any TCR family members between indeterminate (IND) patients and those with discrete cardiac alterations (A group). However, higher frequency of TCR Vβ-3, Vβ-7.1 and Vβ-9 members were identified on CD8 T cells in patients presenting altered echocardiogram without heart failure (B group, p=0.042, p=0.012 and p=0.003) compared with IND group. Interestingly, TCR Vβ-8 were more frequently expressed on patients with advanced altered echocardiogram with heart failure (C+D groups, p=0,000) myocardiopathy when compared with IND patients. Different TCR-Vβ family members were expressed on CD4 T cells. In those cells TCR Vβ-20 were less expressed in IND patients than those from group B (p=0.024) and TCR Vβ-11 less expressed in IND patients than those from groups C/D (p=0.028). **Conclusion:** These results point to an important role in the expression of some TCR-Vβ family members

meanly on CD8T cells on the cardiac involvement of Chagas disease and its frequencies chance during more severe cardiac alterations.

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IDENTIFICATION BY BIOINFORMATIC AND EXPERIMENTAL APPROACHES OF A CONFORMATIONAL EPITOPE ON TOXIN TS3 FROM TITYUS SERRULATUS SCORPION VENOM

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Introduction: The scorpion *Tityus serrulatus* (Ts) is one of the most dangerous species in Brazil, accounting for more than 50.000 accidents per year. Toxin Ts3 represents only a small percentage of the Ts venom protein content, yet it is responsible for a great part of its toxicity. Immunization of mice with this toxin can raise antibodies with potential to neutralize the toxic and lethal effects of the crude Ts venom. The identification of dominant discontinuous epitopes in toxins is a difficult but necessary task, since it contributes for a better understanding of venom immunochemistry and can help the development of new immunogens for the production of improved antivenoms.
Methods and Results: To predict the localization of potential discontinuous epitopes on the Ts3 surface, we runned PEPOP, a dedicated bioinformatic tool, on the modeled 3D structure of the toxin. A set of 260 peptides were predicted as putative discontinuous epitopes and were synthesized using the SPOT technique. From this set, only a few peptides, encompassing two distinct, surface-exposed segments of the toxin (^{17}WNDNY^{21} and ^{39}YWVHI^{43}), were found to react with the mouse neutralizing serum. These two regions apparently are related with the bioactive surface determined for the α-toxin family, which interacts with the voltage gated sodium channel site 3, causing the toxic effects observed in scorpion envenoming. The interaction of the anti-Ts3 antibodies with this region of the toxin might block the binding to the ion channel, explaining the neutralizing properties of the tested serum. None of the 260 peptides reacted with non-immune serum, ascertaining the specificity of the binding. Other bioinformatics methods for discontinuous epitope prediction were also run with Ts3 modeled structure, but only a few were able to detect the found epitope, stressing the importance of experimental validation of *in silico* epitope predictions.
Conclusion: These results identify a possible discontinuous epitope on Ts3 or a peptide mimic of the true epitope. The chemical synthesis of this identified region will allow further validation and characterization of this possible neutralizing epitope on Ts3.

Financial support: CAPES, CNPq, Fapemig, INCTTox

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IDENTIFICATION OF B CELLS EXPRESSING ANTI-TETANUS TOXOID IgG IN HUMAN PERIPHERAL BLOOD BY FLOW CYTOMETRY

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Introduction: The interest in monoclonal antibodies (mAbs) is increasingly observed and many researches are devoted to obtain "fully human" mAbs for therapeutic purposes. Recently, a technique was developed for the production of recombinant human mAbs (J. Immunol. Meth. 329:112-124, 2008). It is based on the single cell sorting of peripheral B lymphocytes using a biotinylated protein recognized by their surface IgG receptor. The sorted cell mRNA corresponding to the variable regions of the heavy and light chains can be amplified and used to transfect host cells that will produce the mAbs for screening of the desired properties. The success of this protocol depends on the collection of memory B lymphocytes, which may be present in the blood, or in secondary lymphoid organs. In this work we tested four potential candidates aiming to determine the presence of B cells that produce anti-tetanus toxoid (TT) antibodies and thus select the more suitable candidate for blood donation for subsequent obtainment of human monoclonal anti-tetanus toxoid IgGs.

Methods and Results: Peripheral blood mononuclear cells (PBMC) were isolated by Ficoll-Hypaque density gradient centrifugation from blood collected by venipuncture from four donors after signature of informed consent in accordance to the Ethics Commission of the Biomedical Sciences Institute, USP. These donors were selected based on the elevated titers of anti-tetanus antibodies. TT obtained from the Butantan Institute was biotinylated by chemical conjugation. ELISA was performed to evaluate the influence of biotinylation on the binding of anti-TT antibodies to the antigen. Analysis of the population of anti-tetanus toxoid IgG producing B lymphocytes was performed by flow cytometry after incubation of PBMC with biotinylated tetanus toxoid and staining with anti-CD19 FITC, anti-sIgG APC, and streptavidin PercP-Cy5.5. The results,

expressed in number of B lymphocytes expressing IgG anti-tetanus toxoid/10^6 PBMC, were: (1) 16.13; (2) 36.18; (3) 11.15; and (4) 2.82. The control group (no booster immunization after the infant vaccination) showed no detectable cells with this triple staining.

Conclusion: Considering the major probability of finding memory B lymphocytes in secondary lymphoid organs, the detection of this population in the blood of the three selected donors in this prospective screening qualifies them as candidates for further development of anti-tetanus human mAbs.

Financial support: CAPES, FAPESP, CNPq.

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COMPARISON OF MRNA LEVELS OF PRO AND ANTI-INFLAMMATORY CYTOKINES IN PBMC OF BIPOLAR PATIENTS AFTER LITHIUM CARBONATE TREATMENT

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Introduction: Bipolar disorder (BD) is a condition characterized by mood changes that manifests as depressive episodes alternated with enhanced mood episodes. Various aspects of the pathophysiology of BD are not thoroughly understood. Cytokines can act as potential mediators of the interaction between the immune and neuroendocrine systems, and increased plasma levels of pro-inflammatory cytokines may be associated with BD. We sought to evaluate whether there are differences in mRNA expression between pro and anti-inflammatory cytokine expression between BD patients and healthy control subjects and whether oral administration of lithium carbonate can modulate cytokine expression in peripheral blood mononuclear cells (PBMC) in BD subjects. **Methods and results:** We analyzed mRNA expression of pro-inflammatory cytokines such as TNF-α, IFN-γ and IL-8 and also anti-inflammatory cytokines such as IL-10 and IL-4 by Real-Time RT-PCR in PBMC of seven bipolar patients and eleven healthy control subjects. From the BD patients we obtained baseline samples, as well as samples collected after an eight-week lithium carbonate treatment. Real-Time RT-PCR data were calculated using the $2^{-\Delta Ct}$ method and the Kruskal-Wallis test with Dunn's post-test were used for statistical analysis. IL-10 mRNA levels were significantly lower in BD patients when compared to healthy controls, regardless of treatment. TNF-α was significantly lower in untreated BD patients, while IFN-γ was significantly lower in treated BD patients when compared to healthy controls. We failed to find differences between treated and untreated BD patients. **Conclusion:** Bipolar disorder was associated to an overall reduced cytokine mRNA expression, independently of lithium treatment. Our results suggest that the increased pro-inflamatory cytokine levels observed in the plasma of these patients is most likely not directly associated to PBMC or that protein and mRNA levels are in disagreement due to post transcriptional

modifications. The cytokine profile BD patients is extremely relevant for the understanding of its pathogenesis.

Financial support: CNPQ, FAPESP and Fundap.

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TNF regulates dual death pathways in mice at E10.5

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Caspase-8, the initiator caspase of the death receptor pathway of apoptosis, and its catalytically inactive homologue FLIP are both essential for embryonic development as these knockout animals die at E10.5 due to a failure of yolk sac vascularization. Recent studies show that ablation of RIPK3, a kinase that promotes a form of programmed, necrotic cell death, rescues development in caspase-8-deficient mice. In contrast, mice lacking both FLIP and RIPK3 still die at E10.5, but via apoptosis rather than necrosis, suggesting that inhibition of both death pathways is necessary for successful development. We investigated whether TNF triggered these dual death pathways at E10.5 via ablation of the death receptor Tnfr1 in both Casp8-/- and FLIP-/-RIPK3-/- mice. Both Casp8-/-Tnfr1-/- and FLIP-/-RIPK3-/-Tnfr1-/- mice survived past E10.5 until E15.5, implicating TNF as the regulator of E10.5 lethality and suggesting that others signals regulate both apoptosis and RIPK3-mediated necrosis later in development.

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GENERATION OF A NEUTRALIZING MONOCLONAL ANTIBODY MAPPING A CONTINUOUS AND CONSERVED EPITOPE IN SPHINGOMYELINASES D FROM *LOXOSCELES* SPIDER VENOMS

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Introduction: The spiders *Loxosceles intermedia*, *L. laeta* and *L. gaucho* are a group of arachnids known as “brown spider” with medical importance in Brazil. The intravenous administration of horse-derived serum is the mainstay treatment in human envenomations by *Loxosceles* spider bite. However, there are remaining doubts about its use, because a considerable risk of serum sickness exists and it is thought that a large delay between a spider's bite and the immunotherapy treatment would lead to an inefficient administration of the antivenom. For this reason there is a growing interest for the use of alternative approaches in the production of safer, controlled and reproducible antivenoms. **Methods and results:** We report the production of a neutralizing monoclonal antibody able to recognize the venoms of three major medically important species of *Loxosceles* spiders in Brazil. The mAb was produced by immunization of mice with a toxic recombinant *L. intermedia* sphingomyelinase D (rLiD1) and screened by enzyme-linked immunosorbent assay (ELISA) using *L. intermedia, L. laeta* and *L. gaucho* venoms as antigens. One clone (LiD1mAb16) out of seventeen anti-rLiD1 hybridomas was cross-reactive with the three whole *Loxosceles* venoms. 2D Western blot analysis indicated that LiD1mAb16 was capable of interacting with 34 proteins of 29-36 kDa in *L. intermedia*, 33 in *L. gaucho* and 27 in *L. laeta* venoms. The results of immunoassays with cellulose-bound peptides revealed that the

LiD1mAb16 recognize a highly conserved linear epitope localized in the catalytic region of SMases D toxins. The selected mAb displayed *in vivo* protective activity in rabbits after challenge with rLiD1. **Conclusions:** These results show the potential usefulness of LiD1mAb16 for future therapeutic approaches and also open the perspective of utilization of this or similar antibodies in assays for loxoscelism diagnosis.

Financial support: CAPES, CNPq, FAPEMIG, CNRS.

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VITAMINA D IS UNRELATED TO THE PRODUCTION OF BLOOD PLASMA CATHELICIDINS DURING SEPTIC SHOCK

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Introduction: Antimicrobial peptides are ancient weapons of the innate immunity. Cathelicidin antimicrobial peptides (AMPs) support the epithelial barrier against infection and are constitutively secreted in the bloodstream by immune cells. Most of the studies regarding the effects of Cathelicidins during infection, however, focused on models of local infection. Here, we focus on the neutrophil production of human Cathelicidin, during septic shock and how it correlates with the vitamin D pathways, since the former induces potent Cathelicidin transcription and the later is an useful marker of NFκB activation. **Methods and Results:** The current study was conducted in one of the Hospital das Clinicas Intensive Care Units. After blood collection, samples were treated with Ficoll-Hipaque to separate plasma and the different cell populations. Cathelicidins protein levels were measured in the plasma and vitamin D measurements were obtained at the Hospital das Clinicas Central Laboratory. Continuous variables were analyzed by using Student´s t test or analysis of variance (ANOVA), as appropriated. We could not detect any difference in VDR (vitamin D receptor) gene expression (p=0.959) or vitamin D plasma values (p=0.175) when performing multivariate analysis of the study groups. **Conclusion:** Here, we show that human cathelicidins are not related to vitamin D serum levels, a potent inducer cathelicidin gene expression, pointing out that the mechanisms that control antimicrobial peptides activity cannot be simplified.

Financial support: FAPESP 2009/17731-2

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PHOSPHATIDYLINOSITOL 3-KINASE GAMMA (PI3Kγ) SIGNALING IS IMPORTANT TO NITRIC OXIDE PRODUCTION BY MACROPHAGE AND ENDOTOXIC SHOCK

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Introduction and aim: Macrophages participate in the first line of defense of the immune system and are responsible for control and elimination of pathogens through phagocytosis and production of reactive oxygen and nitrogen species. These cells show pattern recognition receptors on their surface and in the cytosol, which are activated by constituents present on pathogens, triggering the early innate immune response and activation of distinct intracellular signaling pathways. Among these different signal transduction pathways, those that are dependent on the enzyme phosphatidylinositol 3-kinase regulate several cell functions, including survival, proliferation, apoptosis, differentiation and cell migration. In addition, several studies report that the class IB PI3K, represented by PI3Kγ, exerts a strong influence on the production of reactive oxygen species and hence the microbicidal activity of macrophages. However, the mechanisms involved in this activity have not yet been elucidated. Taking into account these information, the aim of this study was investigate the importance of PI3Kγ pathway on thein vitro macrophages activation . **Methods**: After i.p. injection of 3 ml of 3% thioglycolate, peritoneal macrophages were recovered from C57BL/6 (wild type) and PI3K$\gamma^{-/-}$ mice, and cultured in the presence or not of LPS (1, 3, 10, 30, 100 or 300ng/ml for 48 hours. After the incubation, the level of nitric oxide (NO) was measured from the supernatant with Griess reagent. Endotoxic shock was induced in WT and PI3K$\gamma^{-/-}$ mice by intravenous administration of LPS at 15 mg/kg and the survival rate was monitored for at least 72 hours. The results are expressed as means ± standard error of mean. **Results**: It was found that macrophages from PI3K$\gamma^{-/-}$ mice (41.09 ± 0.1189; n=4) produced less NO when compared with macrophages from WT mice (51.50 ± 0.4131; n=4). Moreover, during LPS shock, PI3K$\gamma^{-/-}$ mice had a delay death compared with WT mice. **Conclusion**: These results indicate that PI3Kγ is critical for complete nitric oxide production of by macrophages and is consequently involved in the physiopathology of LPS shock. Therefore, it is possible to suggest that inhibition of PI3Kγ might be a target to control septic shock.

Financial Support: CNPq

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REGULATION OF IFN-γ EXPRESSION IN T LYMPHOCYTE SUBSETS BY CHROMATIN MODIFICATIONS IN THE *IFNG* GENE

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Introduction: Interferon (IFN)-γ is an essential cytokine for immunity against intracellular pathogens and cancer. While primary CD8 T cells rapidly produce IFN-γ upon activation, a significant increase in IFN-γ expression by CD4 T cells is observed only after Th1 differentiation. This differential production is reflected at the transcriptional level and epigenetic changes that occur during Th1 differentiation at the *Ifng* locus are required for expression of this cytokine. **Methods and Results:** To evaluate whether the differential expression of IFN-γ in CD4 and CD8 T cells is also dependent on chromatin changes, we investigated the chromatin accessibility in *Ifng* locus by DNase I hypersensitivity assay in naïve CD4 and CD8 cells, Th1 and Th2 cells. We identified a region localized at *Ifng* promoter (HSI-4) that was more sensitive at Th1 and CD8 cells, which associates chromatin accessibility to IFN-γ production. We also analyzed the pattern of CpG methylation at regulatory regions of *Ifng* in naïve CD4 and CD8 T cells, Th1 and Th2 cells at 0, 3, and 72 hours after TCR stimulation. At one distal regulatory element (CNS1), previously described as an enhancer of *Ifng* expression, all CpG sites are found consistently methylated in all T cell subsets. The promoter is essentially hypomethylated in all cell types, however we have identified discrete differences at individual sites. The CpG sites +12 and +114 are less methylated in activated CD8 cells. The CpG -58, an important CREB/ATF2-binding site localized at HSI-4, becomes methylated during helper T-cell polarization. However 72h after TCR stimulus, this site is no longer methylated in Th1 cells, as well as in the whole kinetics of CD4 and CD8 naive cells. At last, we showed by transactivation assay that a sequence contained in the HSI-4 region plays a positive role in regulation of the *Ifng* gene and *in silico* analysis indicated that several transcription factors relevant to *Ifng* expression have binding sites in this region. **Conclusion:** Taken together our data suggest that the differential expression of IFN-γ by CD4 and CD8 cells may involve the chromatin accessibility to the region HSI-4, which positively regulates *Ifng* gene. This differential expression does not correlate with the

methylation status of CpG sites in the promoter of *Ifng* gene, neither in the region CNS1.

Financial support: CNPq, FAPERJ, CAPES, ICGEB.

IMMUNO 2012
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IDENTIFICATION OF POLIMORPHISM IN *TNFR1 +36A/G* IN CERVICAL LESIONS FROM HPV-INFECTED WOMEN

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Introduction: HPV infection is the major risk factor for cervical cancer development. Moreover, most women remained asymptomatic, developing low and high intraepithelial lesions (LSIL and HSIL, respectively). Studies have shown that polymorphism in promoter regions of cytokines are associated with many infections diseases severity. Among them, we can cite a proinflammatory cytokine TNF-α. However, the modulation of cellular activies is modulated by interaction between the cytokine and its receptors (TNFR1 e R2) having an important rule in apoptosis induction. Thus, our goal is to evaluate the polymorphisms of *TNFR1 +36A>G*, in order to identify potential markers that may be associated with an increased number of malignant lesions cases that can be used in the Brazilian population as prognostic markers. Besides, to evaluate the phenotypic expression in cervical lesions, aiming to show that HPV can be modulating *in vivo* expression of antiapoptotic proteins, and so, blocking the cellular death induction. **Methods and Results:** PCR and RLPF techniques were performed to identify the single polymorphism (SNP) of *TNFR1 +36A>G*. In this study, 335 women were included, 170 patients and 165 controls. There is no statistical significant difference between genotypes and alleles frequency in this polymporphism in LSIL women when compared with control group. Similarly, we don't find any statistical difference in genotype and allele frequency in LSIL and HSIL women when compared between them and with control group. **Conclusion:** Therefore, we conclude that *TNFR1 +36A>G* polymorphism is not associated with appearance and/or progression to HPV-induced cervical lesions.

Financial support: IPEC/Fiocruz

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POLYMORPHISMS IN GENES ENCODING IL-17 AND IL-10 CYTOKINES AND THEIR ASSOCIATIONS WITH THE DIFFERENTIAL CLINICAL OUTCOME OF CHAGAS DISEASE

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Introduction: In the chronic phase of Chagas disease (CD), caused by *Trypanosoma cruzi*, most of the patients do not present any clinical signs or symptoms being clinically classified as indeterminate (IND). On the other hand, about 30% of patients develop the symptomatic clinical forms: cardiac and/or digestive. The heterogeneous clinical course of CD suggests that genetic background of human host may contribute to high predisposition/resistance to the development of chronic chagasic cardiomyopathy (CCC). Studies of genetic polymorphisms have consolidated a new approach in the analysis of the susceptibility of the human host to CD. IL-17 is considered a pro-inflammatory cytokine and IL-10 is an important immunorregulatory cytokine that plays an important role in balancing the immune response. The aim of this study was to investigate single nucleotide polymorphisms in genes encoding IL-17A, IL-17F and IL-10 and their possible associations with the development of the CCC in Brazilian subjects from Bahia and Minas Gerais. **Methods and Results:** The genomic DNA was obtained from oral mucosal cells and the genotyping of *IL17A* (-197A>G), *IL17F* (+7488T>C) and *IL10* (-1082G>A) genes were performed in a sample of 135 CCC and 59 IND patients by real time PCR. Patients carrying the ancestral allele (A) for *IL17A* have two times more chance of developing the IND form as compared to cardiac form (OR=2.15, p=0.017). A similar result was observed for the allelic frequency (OR=1.81, p=0.023). For *IL17F*, no significant association was found. Patients carrying the variant G for *IL10* have two times more chance of developing the IND form as compared to cardiac form (OR=2.35, p=0.011). A similar result was found for the allelic frequency (OR=1.84, p=0.007). In addition, a combining analysis was made between *IL17A* and *IL10* gene polymorphism. Patients carrying the genotype A+G+ (versus A-G-) have 5 times more chance of developing de IND form as compared to cardiac form (OR=5.24, p=0.0005). **Conclusion:** Our results showed that polymorphisms in IL-17A and IL-10 are associated with the differential clinical outcome of CD. This study is first one to analyze IL-17 polymorphisms. The association of IL-17 and IL-10 polymorphisms in CD has

opened up new perspectives in the comprehension of the host immunogenetic involved in the differential clinical outcomes of this pathology.

Financial support: CNPq; Ministério da Saúde–Doenças Negligenciadas; INCT-DT; FAPEMIG.

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IMMUNOLOCALIZATION OF PULP STEM CELLS IN HUMAN PERMANET TEETH.

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Introduction: Pulp stem cells can be isolated from primary and permanent teeth and exhibit the essential characteristics of a stem cell, like the capacity for self-renewal and differentiation into multiple cell lineages. However, little is known about the location of these cells in the dental pulp tissue as well as the possible interactions between the stem cells and the surrounding microenvironment. Therefore, the aim of this work was to study the distribution of stem cells in the pulp tissue of human permanent teeth.
Methods and Results: For the immunohistochemistry assay, pulp tissue was removed from nine permanent teeth, fixed in paraformaldehyde paraffin embedded and processed for immunohistochemistry. Antibodies used were raised against STRO-1, CD44 and CD90, which are mesenchymal stem cell markers commonly used and ALDH, a marker of normal and cancer stem cells. The same antibodies were used in the western blot assay, employing the total protein extracted from the pulp tissue of five third molars. CD90 and ALDH were localized mainly around blood vessels, but were expressed near the nerves also. STRO-1 and CD44 did not show immunoreactivity in the dental pulp tissue. Western blot analysis showed positive expression for STRO-1, CD44 and ALDH, but not for CD90.
Conclusion: This study found that the majority of the ALDH and CD90 positive cells were found to be located around blood vessels by immunohistochemical staining, suggesting that this location could be a possible niche of dental pulp stem cells. This study also suggests that ALDH, a recognized marker of cancer stem cells, could be a surface marker of mesenchymal stem cells from human dental pulp.

Financial support: The authors thank FAPESB for supporting this work.

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Construction of humanized chimeric antibody from the monoclonal antibody against the protein of 70 kDa Sporotrix schenckii (P6E7).

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Introduction: The fungus *Sporothrix shenckii* is the etiological agent of sporotrichosis, wich is a chronic mycosis and with worldwide distribuition. We observed the involvement of a 70 kDa of a proteic antigenic component, secreted by yeast cells of *S. schenckiii* in regulating the immune response in sporotrichosis. Subsequently, was produced in our laboratory, a monoclonal antibody against this protein, termed P6E7 mAb. This antibody was able to modify the course of experimental infection with *S. schenckii*, with prophylactic and therapeutic activity in experimental sporotrichosis. Despite promising results in the use of monoclonal murine experimental P6E7 in sporotrichosis, there are some limitations in the therapeutic use of this molecules in humans. Considering these limitations our objective is humanize the mAb P6E7. **Methods and results:** The RNA from the hybridoma producer of the mAb P6E7 was extracted and was determined the nucleotide sequences of the genes encoding the variable light chain (VL) and heavy chain (VH). From a library of VL and VH primers, DNA fragments of interest were amplified by PCR and cloned in pGEM T-easy vector. Sequencing of the variable light and heavy chains was done. Specific primers for the VH and VL of murine mAb P6E7 was constrected, including restriction sites of interest for cloning. The sequences of VH and VL were determined and the specific inserts were cloned into pGEM T-easy. Subsequently, this mateiral was transformed into Escherichia coli strain DH5-α, and the positive clones were analyzed using restriction enzymes. We purified the VH and VL fragments from pGEM T-easy and constructed the scFv in pIg16 intermediate vector by inserting fragments of light chain and heavy chain. Using specific restriction enzymes to the scFv fragment the pIg16 vector was released and cloned in vector pMIRES wich has human Fc fragment. Construction of vector containing pMIRES chimeric anti-P6E7 profile was confirmed by restriction enzyme present in our sequence. **Conclusion:** In this

way, we constructed a chimeric antibody that have the variable regions murine light and heavy connected via a flexible peptide to an FC portion human FvFc, this was made to reduce the immunogenicity of murine antibody and enable the use in the treatment of human sporotrichosis

Supported by FAPESP.

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ASSOCIATION POLYMORPHISMS OF THE *IL12B* GENE IN SUSCEPTIBILITY TO EVOLUTION CHAGAS' DISEASE CARDIOMYOPATHY

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Introduction: The most important clinical consequence of Chagas disease is chronic Chagas cardiomyopathy (CCC) while the remaining are asymptomatic (ASY). Clinical symptoms of Chagas' disease occur in 30% of the individuals infected with *Trypanosoma cruzi* and are characterized by ventricular dysfunction and arrythmia. Heart damage is a consequence of by the host immune response against infection. **Interleukin-12 (IL-12)** plays a major role in establishing the type 1(Th1) cytokine response that leads to both protection and tissue damage. Few data exist about functional polymorphisms in the *IL12* and the susceptibility to CCC, and little is known about its importance in Th1 phenotype definition, important in the pathogenesis of the disease.

Methods and Results: Samples from 350 CCC and 156 ASY patients were genotyped with TaqMan SNP genotyping assays. Statistical analysis was performed by SPSS (univariate and multivariate analysis). In this work we studied 9 polymorphisms in the *IL12*. We found four to be associated with disease susceptibility. The polymorphisms that showed significant results were rs2546893(A/G)(p=0,004;OR=0,67;95%CI=0,51-0,88) rs1003199(A/G) (p=0,016;OR=0,70;95%CI=0,52-0,94) rs11574790(C/T) (p=0,032;OR=0,75;95%CI=0,57-0,97) and rs919766(A/T)(p=0,012;OR=1,43; 95%CI =1,08-1,88).In a multivariate analysis, including the gender as covariate, on CCC patients versus ASY patients all associations continue significant. **Conclusion:** We identified four polymorphisms within *IL12* that are associated to risk of development of CCC. It is possible that the identified polymorphisms affect IL12B expression and thereby disease pathogenesis. Studies on this subject are under way.

Keyword: *IL12*; Chagas disease; Genetic polymorphism.

Financial support: FAPESP; iii;InCor; INSERM UMR-France.

IMMUNO 2012
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RECONSTITUTION OF THE TCR REPERTOIRE IN TYPE 1 DIABETES PATIENTS AFTER AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Introduction: Type I diabetes mellitus (T1D) is a polygenic autoimmune disease characterized by insulin deficiency resulting from the autoimmune destruction of insulin-secreting β-cells. High dose immunosuppression followed by autologous hematopoietic stem cell transplantation (HDI/AHSCT) has emerged in last years as a therapeutic alternative for recent-diagnosed T1D patients (Couri CE *et al*., JAMA 2009). The rationale of this therapy is based on immune ablation and regeneration of a new immune system. After transplantation, C-peptide levels increased significantly and the majority of patients achieved insulin independence with good glycemic control, but the immune mechanism of action of the AHSCT has not yet been totally elucidated. To address the immune mechanisms by which HDI/AHSCT induces remission in T1D patients, we evaluated the reconstitution of T cell repertoire diversity by the TCRBV CDR3 Spectratyping method in T1D patients treated with HDI/AHSCT. **Methods and Results:** Peripheral blood mononuclear cells (PBMCs) from five T1D patients (pre-transplantation, and D+540 and D+720 days post-transplantation periods) and from five healthy donors were isolated by Ficoll-Hypaque density gradient centrifugation. Total RNA was isolated and used to analyze the T cell receptor (TCR) Vβ repertoire by the TCRBV CDR3 Spectratyping method. There were changes in the composition of the T cell repertoire after transplantation, evidenced by qualitative and quantitative alterations in the CDR3 peaks and in the frequency of the 24 Vβ families analyzed. Furthermore, it was identified four basic patterns of repertoire

reconstitution after transplantation and the pattern that consisted of reconstitution of diversity from a normally diverse repertoire was the most dominant in the analyzed T1D patients. For some Vβ families were observed a pattern that consisted of recovery of diversity from a restricted repertoire, suggesting increased repertoire diversity after HDI/AHSCT. **Conclusions:** Our results suggest that the HDI/AHSCT therapy induced changes in the composition of the TCR repertoire that might explain the induction of remission (insulin-independency) in the T1D patients. Nevertheless, more studies should be conducted to evaluate the T cell repertoire diversity of the T1D patients at later periods after HDI/AHSCT therapy.

Financial support: FAPESP, CNPq

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REGULATION OF FASL GENE EXPRESSION BY PGE_2 IN $CD4^+$ T LYMPHOCYTES

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Introduction: $CD4^+$ T lymphocytes are responsible for orchestrating adaptive immune responses. A typical adaptive immune response is characterized by a multistep process: antigen presenting to T lymphocytes by APCs, T lymphocyte activation and clonal expansion, differentiation of activated T cells in effector and memory cells, and, finally, cell death to maintain homeostasis of T cell populations. This homeostasis occurs by apoptosis via AICD (activated-induced cell death) and/or ACAD (activated cell autonomous death). AICD occurs by FAS/FASL whereas ACAD depends mostly on BIM activation. Our group showed that PGE_2 is released by APCs in response to TOLL like receptor (TLR) stimulation and prevents TcR/CD3-mediated FASL upregulation and consequent AICD in $CD4^+$ T lymphocytes (WEINLICH *et al*., 2008). In the present work, we aimed to define the molecular mechanism responsible for PGE_2 inhibition of FASL expression. Here, we show preliminary results suggesting that PGE_2 regulates FASL by inducing the expression of Inducible cAMP early repressor (ICER). **Methods and Results:** FASL promoter was mapped through UCSC Genome Bioinformatics (http://genome.ucsc.edu/) and NCBI (http://www.ncbi.nlm.nih.gov). After this, we used *MacVector 12.5.1* software to search for transcription factor sites and found out a consensus sequence for ICER, an important transcription repressor (BODOR *et al.,* 1996). To confirm ICER modulation by PGE_2, we performed a dose-response and time-course analysis of DO11.10 hybridoma cells. RT-PCR analysis followed by agarose gel electrophoresis revealed that PGE_2 is able to induce ICER expression with optimal concentration of 10^{-8}M. In addition, the expression of ICER was already significant in 30 minutes and reaches maximum at 1-2 hours after treatment. **Conclusion and Perspectives:** We provide preliminary evidences to support our hypothesis that PGE_2 controls FASL expression by inducing the transcription repressor ICER. Future experiments based on gene reporter assays, shRNA technology and ChIP analysis are current under way in our lab, in order to prove our hypothesis.

Financial support: FAPESP, CNPq.

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IDENTIFICATION OF MOLECULAR TARGETS IN HPV ASSOCIATED CERVICAL TUMORS

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Introduction: High oncogenic risk Human Papillomavirus (HPV) infection is the main cause for cervical cancer and other anogenital and oropharingeal tumors. Cervical cancer is the second most frequent cancer among women in developing countries. Specific molecular targets in tumors may be a tool for diagnostic, prognostic, and even therapy. To investigate the presence of such targets in cervical tumors we used the peptide phage display method. The peptide phage display based biopanning consists in the enrichment of peptide sequences with affinity for a given target (proteins, cells, tissues, tumors), through several cycles of incubation with target and re-expansion of the selected sequences. We used this method with cervical cancer derived cell lines, both *in vitro* and *in vivo*, and cervical high grade lesion biopsies to identify peptide sequences that recognize specific molecular targets in HPV associated tumors.

Methods and Results: Three approaches were used in our study, biopanning against cultured cell lines, against tumors grown in immunodeficient mice, and against cervical tumor biopsies. We used the M13 bacteriophage PhD7 CXC library from New England Biolabs (MA, US).

We have isolated 4 peptide sequences from the cervical tumors and 7 peptide sequences from cultured cell lines. We are conducting the *in vivo* biopannings and doing specificity tests with the already sequenced bacteriophages.

Conclusion: We have identified peptide sequences with homing propertied towards cells or tumors previously infected with Human Papillomavirus.

Financial support: CMA has a PIBIC/CAPES fellowship and this project is supported by INCT-HPV 2012 – 573799/2008-3.

IMMUNO 2012
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ACTIVATED AND QUIESCENT HOST CELLS INFECTED BY HIV-1 DISPLAY OPPOSITE EPIGENETIC PROFILES

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INTRODUCTION: The term “epigenetic modifications” refers to a number of changes in gene expression encompassing several molecular changes such as DNA-methylation and histones post-translational modifications that define the structure of chromatin and its transcriptional activity. Besides environmental factors such as lifestyle, nutrition, sunlight, chemicals and/or physical, another common source of cellular stress is contact with pathogens. The differential methylation of the host genome has been documented under several viral infections. The latest highlights on HIV-1 and epigenetics research tried to elucidate how the viral genome and their protein products may be affected by differential methylation or histone modification, moreover, how it affects the ability of the virus to infect the host cells, replicate and produce effective progenies or even, in an antagonistic manner, to remain latent and integrated in host cell genome in order to produce and disseminate a future infection. **OBJECTIVE:** The main goal of this work was to evaluate putative epigenetic modifications in PBMCs after infection by the HIV-1. **METHODS:** Epigenetic modifications were analyzed by three approaches:(i) measurement of global methylation on CpG islands in genomic DNA using specific restriction enzymes;(ii) qPCR using array to evaluate the expression of 84 genes related to epigenetic modifications;(iii) western blot to detect protein levels of classical epigenetic markers (H3K9, H3K27 and H3K4 tri-methylated). **RESULTS:** HIV-1 seems to negatively modulate levels of methylation in genomic DNA in previously activated infected cells, but not in quiescent cells. The same effect is observed for the epigenetic markers H3K9 and H3K27, showing a tendency of transcription repression in activated cells and of transcription activation in quiescent cells infected by HIV-1. Moreover, in activated HIV-1 infected cells, it was possible to detect by qPCR array strong up-regulation in expression of SEBD2 and RSK2 transcripts, which are related with silencing of gene expression. However, the same transcripts were down-regulated in quiescent HIV-1 infected cells. **CONCLUSIONS:** These results suggest that HIV-1 infection exerts an opposite modulation of epigenetic

mechanisms in activated and quiescent cells. Thus, we speculate that cellular activation status influence in the HIV-1 and host-cell interaction and may influence in infection prognostic.

FINANCIAL SUPPORT: CNPq, FAPESP, CAPES.

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GENERAL BIOCHEMICAL AND IMMUNOLOGICAL CHARACTERIZATION OF *BOTHRIOPSIS OLIGOLEPIS* SNAKE VENOM

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Introduction: This communication describes the general biochemical and immunological properties of venom from the Peruvian arboreal snake *Bothriopsis oligolepis*.
Methods and Results: The LD_{50} determined, in mice for the soluble venom was 2.30 mg/Kg. This value is highly lethal when compared to the other species of Bothrops and Bothriopsis venoms. The venom displayed proteolytic, phospholipasic, hemorrhagic and oedema activities. A specific anti-*B. oligolepis* sera was produced in rabbits and used in Western blot experiments. Polyvalent anti-bothropic therapeutic antivenoms from Peru and Brazil were also tested. Western blotting analysis showed that proteins ranged between 15-130 kDa were immunogenic. ELISA was also used to verify the cross-reactivity between anti-*B. oligolepis* sera and others snake venoms. These experiments showed cross-reactivity with other Peruvian venoms such as *Bothrops atrox*, *B. pictus*, *B. bilineata*.This venoms are used to produce the Peruvian commercial antivenom.; Reactivity with Brazilian venoms such as *B. jararaca*, *B. jararacussu*, *B. moojeni*, *B. alternatus*, *B. neuwied*i, venoms used to produce Brazilian commercial serum,and Crotalus were also observed.
Conclusion: The Peruvian snake *B. oligolepis* venom is toxic to mice and display proteolytic, phospholipasic, edema and hemorrhagic activities. *B. oligolepis* anti-venom displayed consistent cross-reactivity with venom antigens from the others species Bothrops, Bothriopsis and Crotalus, showing that same Peruvian and Brazilian venoms have common epitopes and can be possible the production of common antivenoms.

Financial support: CNPq, CAPES and FAPEMIG.

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

MUTATED BIP IMPAIRS ACTIVATION OF ER STRESS SENSORS AND LEADS TO HYPOGAMMAGLOBULINEMIA IN HUMANS

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Introduction: Common variable immunodeficiency (CVID) is a disease with heterogeneous causes that are not well established yet. The main symptom is the significant reduction or even absence of at least two isotypes of serum immunoglobulins (Ig). During terminal differentiation of B lymphocytes into plasma cells, the latter cells produce increased levels of Ig. To accommodate the Ig overload, an expansion of the endoplasmic reticulum (ER) occurs. This activates an intracellular pathway named Unfolded Protein Response (UPR) that rescues ER homeostasis. In the absence of misfolded proteins within the ER lumen, three transmembrane proteins (IRE-1α, PERK and ATF6) are linked to BiP protein, the main ER chaperone. When there are misfolded proteins in the ER, BiP binds to them, releasing the transmembrane sensors to start the UPR pathway. Earlier studies from our group identified one CVID patient (Patient P) whose activation of IRE1α-XBP1 arm of the UPR pathway occurs at a slower rate when compared to a matched healthy donor, resulting in Ig accumulation into the ER. In vitro treatment of immortalized B cells from this patient with chemical chaperones (DMSO) partially rescued secretion of IgM and IgG. We hypothesized that BiP from Patient P might have mutations that interfere with its functioning, explaining patient P's inability to fold and secrete Ig and her hypogammaglobulinemia (low levels of Ig in the serum).
Methods and Results: We sequenced the transcripts of IRE-1α, XBP-1 and BiP genes. Our analyses found many nucleotides substitutions or gaps in the BiP from Patient P. But these mutations are not caused by a switch of the reading frame, most of them are not present in the exons junctions and they are not Single Nucleotide Polymorphism (SNP). Besides, due to an early stop codon, the translated protein has only an incomplete nucleotide-binding domain. We also found a second translation initiation site that, if functional, generates a partial C-terminal substrate-binding domain.
Conclusion: Our results suggest that the mutations found in patient P's BiP impair ER sensors releasing from BiP (at least for IRE-1α), justifying the slower

activation of the Patient P's UPR. The mutations might disturb chaperone's immunoglobulin-folding activities, contributing to the accumulation of Ig inside the ER and hypogammaglobulinemia observed in patient P.

Financial Support: FAPESP (2011/04826-5 for FMCS, 2009/51326-8 for MMDC) and CNPq (474056/2011-2 for MMDC).

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EVIDENCE FOR IMPAIRED REGULATORY T CELL RESPONSE IN PEMPHIGUS FOLIACEUS

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Introduction: Recent studies have detected Foxp3$^+$ regulatory T (Treg) cells in skin lesions of pemphigus, an autoimmune blistering skin disease, but have overlooked other important molecules involved in T cell response and suppression. The aims of this study were to characterize lesional skin compared to uninvolved skin of pemphigus foliaceus patients based on the expression of marker molecules and to evaluate the proportion of T-helper 2 (Th2) and regulatory T (Treg) cells in peripheral blood of patients at different stages of disease activity, and of healthy controls.
Methods and Results: mRNA expression of a set of molecules was measured by real-time PCR for lesional and uninvolved skin, while magnetic cell sorting was performed to quantify Th2 and Treg cells in peripheral blood. We focused on markers of Treg cells and of the Th1/Th2 responses. Genes found overexpressed in lesional skin were *FOXP3* (fold=1.6, P=0.06), *CTLA4* (fold=5.1, P=0.06), *IL10* (fold=2.5, P=0.03), *LILRB2* (fold=4.7, P=0.001) and *CXCR4* (fold=5.6, P=0.03), while downregulated genes in lesional skin were *GATA3* (fold=0.1, P=0.03) and *TNFRSF18* (fold=0.3, P=0.03). Most genes upregulated in lesional skin of patients are characteristic of Treg cells. The frequency of these cells is decreased in patients with active disease in relation to healthy controls (P=0.02) and patients undergoing a healing process (P=0.03). Moreover, we found no detectable mRNA of *IL4* and *IFNG* in lesional and uninvolved skin, indicating that Th responses are concentrated elsewhere.
Conclusion: Our data suggest that Treg cells are present in the lesional skin, but possibly by impaired functionality are failing to control the initial inflammatory insult and subsequent disease progression. Taken together with the positive results after transfer of Treg cells in a murine model of pemphigus vulgaris (Int Immunol. 23:365-73, 2011), our findings magnify the perspective regarding new Treg-based therapeutic strategies against pemphigus. In addition, the chemokine receptor CXCR4 and the leukocyte immunoglobulin-like receptor, subfamily b, member 2 (LILRB2) genes were for the first time proposed to participate of the pathogenesis of pemphigus.

Financial support: CNPq, CAPES and Fundação Araucária.

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GENE EXPRESSION PROFILING OF BONE MARROW MESENCHYMAL STROMAL CELLS FROM TYPE 1 DIABETES PATIENTS AFTER AUTOLOGOUS HEMATOPOIETIC STEM CELLS TRANSPLANTATION

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Introduction: Bone marrow multipotent mesenchymal stromal cells (BM-MSCs) are currently being considered as potential therapeutic agents in various inflammatory autoimmune diseases for their tissue-repair and immunomodulatory properties. They also provide a substantial contribution to the creation of the hematopoietic stem cell (HSCs) niche and play a crucial role in the hematopoiesis, suggesting their role in the HSCs engrafment after autologous hematopoietic stem cells transplantation (AHSCT) in type 1 diabetes (T1D). **Methods and Results:** To evaluate the global gene expression profile of BM-MSCs after AHSCT in T1D patients BM-MSCs cultures were derived from BM-aspirates of T1D patients before (N=4) and after (N=4) AHSCT. Total RNA was isolated and the gene expression profiling was analyzed by using human whole genome oligonucleotide microarrays from Agilent Technologies. Analyses of differential gene expression were performed using the R package of the Linear Models for Microarray Data (LIMMA). Differentially expressed genes ($p < 0.05$ and fold change > 2.0) were imported to the Ingenuity Pathway Analysis (IPA) software for identification of significantly regulated gene networks and canonical pathways. The microarray gene expression analysis revealed significant differential expression of 391 probes. Of these, 214 probes were up-regulated and 177 were down-regulated in BM-MSCs from T1D patients after AHSCT. In summary, the top networks in IPA analysis were associated with cellular movement, hematological system development and function, immune cell trafficking. Regarding the biological functions, cell spreading related genes (VCAM1, TPM1, VEGFA, ITGAV, PXN) were up-regulated. Accordingly, paxillin signaling canonical pathway was also up-regulated. **Conclusion:** Initial analyses of global gene expression showed that BM-MSCs from T1D patients after AHSCT may have greatest spreading potential. Further functional analysis may prove the gene expression data in order to understand the relevance and biological/clinical significance of this finding.

Financial support: FAPESP, CNPq, CAPES

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PREPARATION AND CHARACTERIZATION OF AN INTERLEUKIN-21/IgG3 FUSION PROTEIN FOR ENHANCING IN VIVO BIODISPONIBILITY

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Introduction: Interleukin-21 is secreted primarily by activated $CD4^+$ T cells during infection or other forms of activation; this cytokine increases the function and proliferation of $CD4^+$ T cells, $CD8^+$ T cells and NK cells. Interleukin-21 modulates the immunity against tumors and viruses.Thus, IL-21 is a potential candidate for use in immunotherapy against tumors, however it is produced only at the time of adaptive immune induction and is almost exclusively released by activated CD4+ T cells. Like other cytokines the half- life of IL-21 is too short. Immunoglobulins (Igs) are proteins with long half-life and the combination of Ig parts with cytokines to increase the biodisponibility was previous reported for IL-10, IL-2 and IL-23. The aim of this was to construct a fusion protein using cDNA from murine IL-21 and the cDNA that codes for the constant portion of the IgG3. The half-live of the hybrid protein was tested *in vitro* and *in vivo* as well as the capacity to stimulate CD4+T cells, CD8+T cells and NK cells.
Methods and Results: A cloning strategy was designed to construct a hybrid cDNA coding to murine IL-21 and constant portion of murine IgG3 into pCR3.1 plasmid. The genetic construction pIL-21/IgG3 or pIL-p21 was used to transfect VERO cells. The IL-21 produced was quantified by ELISA in the supernatant from 24, 48, 72 and 96h after transfection. For *in vivo* analysis the IL-21/IgG3 produced was concentrated and injected intravenously in female C57BL/6 mice. The cytokine was quantified in the serum 2h, 6h, 12h, 24h and 48h after treatment. The percentage of $CD4^+$T cells, $CD8^+$T cells and NK was analyzed in the spleen from mice treated with the cytokine. The fusion protein was efficiently constructed and the identity with original sequence from IL-21 and IgG3 was more than 98%. The in *in vitro* analysis showed that the quantity of IL-21 in the supernatant from cells transfected with pIL21 was higher than cells transfected with pIL-21/IgG3 in 24, 48 and 72h (pIL-21: 1385pg/mL ; 1000pg/m; 720pg/mL. and pIL-21/IgG3: 144pg/mL; 413pg/mL; 756pg/mL). However, after 72h the level of IL-21 decreases in cells transfected with pIL-21, but increased on pIL-21/IgG3 (pIL-21: 918pg/mL; 2098pg/mL). *In vivo* the also IL-21/IgG was detected in the serum till 48h by the other hand IL-21 was not detected after 12h. There was no significantly difference in NK cells when IL-21 or IL-21/IgG3, however the percentage of CD4+ T cells and CD8+ T cells were higher when IL-21/IgG3 was used.

Conclusion: The IgG3 fraction improved the stability and biodisponibility of IL-21 in vitro and in vivo. This optimization also influenced the action of IL-21 stimulation of CD4+ and CD8+ T cells.