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EXPRESSION OF VEGFR-1, ENDOGLIN AND TNF-ALPHA IS INCREASED IN PLACENTA OF PREGNANT WOMEN WITH PREECLAMPSIA

INGRID CRISTINA WEEL (PG)[1]; JOSÉ CARLOS PERAÇOLI[1]; VANESSA ROCHA RIBEIRO (IC)[2]; CAMILA FERREIRA BANNWART-CASTRO[1]; MARIANA ROMÃO (PG)[1]; VERA THEREZINHA MEDEIROS BORGES[1]; MARIA TEREZINHA SERRÃO PERAÇOLI[2]

[1] Departamento de Ginecologia e Obstetrícia – Faculdade de Medicina- UNESP-Univ Estadual Paulista, Botucatu, São Paulo, Brazil, CEP 18618-970

[2] Departamento de Microbiologia e Imunologia – Instituto de Biociências, UNESP-Univ Estadual Paulista, Botucatu, São Paulo, Brazil , CEP 18618-970

Introduction and Objectives*:* Preeclampsia (PE) is a human pregnancy-specific syndrome characterized by the onset of hypertension and proteinuria after the 20th week of gestation. Although PE is a disease of etiology not well established, it is accepted that this disease originates in the placenta, probably due to factors involved in its formation and development. The imbalance between angiogenic/anti-angiogenic factors and cytokine profile expression affects the process of placentation and immune response. The aim of this study was to analyze the expression of cytokines, such as tumor necrosis factor alpha (TNF-a), interleukin-10 (IL-10) and transforming growth factor beta (TGF-b_1), as well as angiogenic factors such as endoglin (Eng), vascular endothelial growth factor (VEGF) and vascular endothelial growth factor receptor-1 (VEGFR-1) in placental tissues from preeclamptic, and normotensive pregnant women.

Methods and Results: The subjects were 30 pregnant women of whom 10 were normotensive (NT) and 20 were preeclamptic. Women with PE were classified according to the onset of clinical manifestations in early-onset (< 34 weeks of gestation, n=10) and late-onset PE (≥ 34 weeks of gestation, n=10). A tissue fragment of the placenta was obtained immediately after delivery weighing 1-2 g and was cut between the umbilical cord insertion and placenta border. The samples were prepared for immunohistochemistry analysis, and segments of 4 μm thick were placed on histological slides. The results were analyzed employing Image J software and differences between groups were evaluated by parametric tests with significance set at $p < 0.05$. TNF-a, VEGFR-1 and Eng expressed in

placental villous of pregnant women with PE were significantly increased when compared with NT. On the other hand, IL-10 and VEGF expression was higher in NT than in preeclamptic patients. Comparison between early and late-onset PE showed significant differences in relation to TNF-a, TGF-b_1 and Eng.

Conclusions: The results demonstrated that the high expression of TNF-a and low expression of IL-10 generates an inflammatory profile complicating the success of pregnancy. High VEGFR-1 and Eng as well as low VEGF expression may lead to the low trophoblastic invasion characteristic of this inappropriate placentation in pregnant women with PE.

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IL-6 AND IL-21-SECRETING T CELLS ARE DIRECTLY ASSOCIATED WITH NEUROLOGICAL DISABILITY IN NEUROMYELITIS OPTICA PATIENTS

PRISCILA O. BARROS[1]; TAISSA M. KASAHARA[2]; ULISSES C. LINHARES[3]; PATRICIA B. SCHIAVONI[3]; BRUNA TEIXEIRA[2]; THAIS B. FERREIRA[2]; REGINA ALVARENGA[3]; JOANA HYGINO[1]; VERA CAROLINA B. BITTENCOURT[2]; REGIS M. ANDRADE[2]; ARNALDO F. B. ANDRADE[1]; CLEONICE A. M. BENTO[2]

From ; [1]Department of Microbiology, State University of Rio de Janeiro, Rio de Janeiro, Brazil [2]Department of Microbiology and Parasitology/Federal University of Rio de Janeiro State and [3]Department of Neurology/Federal University of Rio de Janeiro State.

Introduction: Once considered as a subtype of Multiple Sclerosis, Neuromyelitis optica (NMO), also known as Devic's disease, is an autoimmune, inflammatory disorder of the central nervous system in which the immune system attacks myelin of the neurons located at the optic nerves and spinal cord, thus producing a simultaneous or sequential optic neuritis and myelitis. Although the etiopathogenesis of the disease is unknown, humoral immunity appears to play an important role in the pathogenesis of NMO. In addition to involvement of humoral immunity, T cells may also play a pivotal role in the initiation and perpetuation of NMO lesions. To date, studies about the cytokine profile produced by activated T cells of NMO patients during clinical remission have been lacking.
Objective: To evaluate the T-cell function in NMO patients during clinical remission.
Methods and results: The PHA-activated peripheral blood mononuclear cells (PBMC) from healthy (control group, n=20) or NMO patients (n= 22) were maintained from 3 days and T cell proliferation, measured by [3H] thymidine incorporation, and cytokines profile, quantified by ELISA, were analyzed. The impact of glucocorticoid on the cytokine production was evaluated following addition of pharmacological doses of dexamethasone (DEX) at the beginning of cell cultures.In our study, the *in vitro* T cell proliferation and the production of Th1 cytokines were significantly lower in cell cultures from NMO patients, as compared with healthy individuals. In contrast, a dominant Th17-like phenotype, associate with higher IL-23 and IL-6 production by LPS-activated monocytes, was observed among NMO patients. The release of IL-21 and IL-6 by polyclonally activated $CD4^+$ T cells was directly correlated to neurological disability. In addition, the *in vitro* release of IL-21, IL-6 and IL-17 was significantly more resistant to glucocorticoid inhibition in NMO patients.
Conclusion: The results indicate dominating Th17-related response in NMO patients that was directly proportional to neurological disability. Furthermore, our

results can help to explain why NMO patients trend to be more refractory to corticoid treatment.

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THE IMPACT OF PREGNANCY ON THE HIV-1-SPECIFIC T CELL FUNCTION IN HIV-1-INFECTED PREGNANT WOMEN

JOANA HYGINO(1); MORGANA M. VIEIRA(1); TAISSA M. KASAHARA(2); LUCIANA F. XAVIER(2); BERNARDO BLANCO(2); LANDI V. C. GUILLERMO(2); RENATO G. FILHO(2); CARMEN S. SARAMAGO(2); AGOSTINHO A. LIMA-SILVA(2); ARIANE L. OLIVEIRA(3); VANDER GUIMARÃES(4); ARNALDO F. B. ANDRADE(1) AND CLEONICE A. M. BENTO(2)

(1). Department of Microbiology, Immunology and Parasitology, State University of Rio de Janeiro, Rio de Janeiro; (2). Department of Microbiology and Parasitology/Federal University of Rio de Janeiro State; (3). Leprosy Laboratory, Oswaldo Cruz Institute, Rio de Janeiro; and (4). Fernandes Figueiras Institute, Oswaldo Cruz Institute, Rio de Janeiro, Brazil.

Introduction: As intracellular pathogen, HIV-specific $CD4^+$ Th1-mediated immune response plays a pivotal role in protecting HIV-infected patients from disease progression. However, during pregnancy, the hormonal changes operate to dampen the cellular immune response against the semi-allogeneic fetus by inducing maternal regulatory T cells (Treg) at the foetal-maternal interface. Nevertheless, the risk of disease progression to acquired immunodeficiency syndrome (AIDS) in HIV-infected women does not increase during pregnancy.
Objective: To evaluate, *in vitro*, the dual impact of HIV-1-infection on maternal immune profile and the effect of pregnancy on HIV-1-specific T cell responses.
Methods and Results: Peripheral blood mononuclear cells (PBMC), B cells-depleted or $CD4^+$ cells-depleted PBMC from HIV-1-infected pregnant (n=25) and non-pregnant (n=25) women which controlled the plasma viral load (PVL) were activated *in vitro* with HIV-1 antigens, anti-CD3/anti-CD28 or with PMA/ionomycin, and the lymphoproliferation and cytokine network were measured by [^{3}H] thymidin uptake and ELISA, respectively. The phenotypes of T cells were analyzed by cytometry through both surface and intracellular staining by using different monoclonal antibodies (mAb). Finally, to evaluate the impact of endogenous IL-10 on *in vitro* HIV-1 replication, the anti-IL10 mAb was added to the cultures and the HIV-1 RNA quantification was performed by RT-PCR. As compared with non-pregnant women, B-cells-depleted PBMC cultures from HIV-1-infected pregnant women demonstrated a lower T cell proliferation and a higher IL-10 production following addition of both mitogens and HIV-1 antigens. The $CD4^+Foxp3^-$ T cells are the main T lymphocyte subset involved in producing IL-10. The depletion of $CD4^+$ cells from whole PBMC culture elevated TNF-α and IFN-γ release and diminished IL-10 production. Interestingly, the *in vitro* HIV-1 replication was lower in PBMC cultures from pregnant patients, and it was inversely related to IL-10

production. In these cell cultures, the neutralization of endogenous IL-10 by anti-IL10 mAb elevated TNF-α release and the HIV-1 replication.

Conclusion: Our results reveal that pregnancy-related events should favor the expansion of HIV-1-specific-IL-10-secreting $CD4^+$ T cells in HIV-1-infected women; which should, in the scenario of pregnancy, help to reduce the risk of vertical HIV-1 transmission.

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STATISTICAL EVALUATION OF THE EFFICIENCY OF IMMUNOTHERAPY ON RESPIRATORY ALLERGY

SAMARA ALVES SÁ TELES[1]; JOSÉ ROSA FIGUEIRÊDO FILHO[2]

(1,2) Universidade Estadual de Feira de Santana

Introduction: Allergic reactions are the result of pre-sensitization to repeated contacts of the individual, usually genetically predisposed to such response, with the antigens that cause hypersensitivity reactions (allergens). The most common allergens are mites found in dust, foods, pollen, molds, drugs, food dyes and other food additives.[1] Allergic rhinitis, a major respiratory allergy, is a disease of great impact in the current population, because its prevalence ranges from 24 to 25% in the general population, and it can reach greater variations according to the lifestyle and age of the group studied. Moreover, it must be taken in account that patients diagnosed with allergic rhinitis may experience many limitations for performing their daily activities, either by discomfort caused by specific nasal symptoms or by associated symptoms such as malaise, headaches and sleep disturbs.[2] Thus, since immunotherapy is one strategy employed to reverse this situation, it is necessary to evaluate the effect of this treatment. **Methods and results:** We collected data regarding the RAST test results for total IgE of 50 patients from the Department of Allergy and Clinical Immunology of Cardiopulmonary Clinic in Feira de Santana - Bahia, aged 06-30 years, who were diagnosed with respiratory allergies and started using immunotherapy. The RAST tests analyzed were those conduced before starting the treatment and those made 01 year later. The data collected was analyzed using the t test, using the software package *Statistica*, in order to infer if whether or not there is a difference between before and after the treatment. Divided into three groups, children, teenagers and adults, it was observed that none of the groups was significant ($p > 0.05$), indicating that 01 year of treatment was not sufficient to reverse the allergic symptoms. However, a visible trend was observed in a Box plot, from the median and the limits of the post treatment data, the decrease of IgE. When all data are analyzed together by MANOVA, they begin to show a significant difference with treatment ($p < 0.05$). **Conclusion:** Overall, the result confirms that immunotherapy is an effective treatment for the improvement of respiratory allergy.

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EFFECT OF AGE ON PHYTOHEMAGGLUTININ-INDUCED IFN-g PRODUCTION AMONG CHILD AND ADOLESCENT HOUSEHOLD CONTACTS OF PULMONARY TUBERCULOSIS PATIENTS

IUKARY TAKENAMI[1]; CAROLINA COELHO CUNHA[1,2]; CAROLINA CAVALCANTE DE OLIVEIRA[1]; ALMÉRIO MACHADO JR[3]; KRISZTINA EMODI[4]; LEE RILEY[4]; SÉRGIO ARRUDA[1,2]

[1]Laboratório Avançado de Saúde Pública, Centro de Pesquisas Gonçalo Moniz, Salvador, Bahia; [2]Escola Bahiana de Medicina e Saúde Pública, Salvador, Bahia; [3]Hospital Especializado Octávio Mangabeira, Salvador, Bahia, Brazil; [4]School of Public Health, University of California, Berkeley, California, USA

Introduction: Interferon-gamma release assay (IGRA) has been used worldwide for the detection of latent tuberculosis infection (LTBI). However, data on the performance of this assay in children is relatively limited. In younger children the performance of the test could be impaired because the child's immune system does not completely mature until about 6 years of age, thus resulting in indeterminate results (low positive mitogen control). The aim of this study is to evaluate the effect of age upon the quantitative performance of the IGRA among children and adolescents evaluated for LTBI. **Methods:** Seventy-three children and adolescents up to 18 years of age with household contacts of pulmonary tuberculosis were enrolled in the study. We collected peripheral blood specimens at enrollment and performed a commercial IGRA according to the manufacturer's recommendations. Participants were categorized into 2 age groups: children < 11years and adolescents >12yrs. **Results:** IGRA were positive in 17 of 41 (41.5%) children and 11 of 32 (34.4%) adolescents. IFN-g production in response to mitogen (positive control) was positively correlated with younger age (r=0.357, p=0.0019), but no significant difference was found between IFN-g production induced by antigen-specific T cells and age of the children (r=0.095, p=0,426). Among LTBI patients, adolescents had significantly higher IFN-g response to mitogen than children (33.1±27.6 IU/mL vs. 6.1±5.6 IU/mL; p=0.0064).

Conclusion: The quantitative result of the IFN-g production in response to mitogen is affected by patient age. Adolescents with LTBI tended to have a higher mitogen-induced IFN-g production than children. These findings support the hypothesis that the immune system's ability to effectively respond to antigens, particularly T cell-mediated, is impaired in younger children. Moreover, IGRA responses to tuberculosis-specific antigens were not compromised by young age.

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HIGH FREQUENCY OF HIV-1-SPECIFIC TR-1 CELLS IN HAART-TREATED AGED PATIENTS WAS DIRECTLY RELATED TO LOWER *IN VITRO* HIV-1 REPLICATION

TAISSA M. KASAHARA[2]; REGIS M. ANDRADE[1,2]; JOANA HYGINO[2]; MORGANA M. VIEIRA[2]; LUCIANA F. XAVIER[2]; BERNARDO BLANCO[2]; PAULO V. DAMASCO[1]; RODRIGO M. SILVA[3]; DIRCE B. LIMA[4]; ARIANE L. OLIVEIRA[5]; ALBERTO S. LEMOS[6]; ARNALDO F.B. ANDRADE[3] AND CLEONICE A.M. BENTO[2]

From [1]Discipline of Infectious and Parasitic Diseases, School of Medicine, and [2]Department of Microbiology and Parasitology, Federal University of the State of Rio de Janeiro, Brazil; [3]Department of Microbiology, Immunology and Parasitology and [4]Discipline of Infectious and Parasitic Diseases, Medical School, State University of Rio de Janeiro, Brazil; [5]Leprosy Laboratory, Oswaldo Cruz Institute, Rio de Janeiro and [6]Department of Infectious and Parasitic Diseases, University Hospital, Federal University of Rio de Janeiro, Brazil.

Introduction: Aging is now a well recognized characteristic of the HIV-infected population. It is not only a consequence of successful treatment and care of people living with HIV/AIDS, but also an effect of increasing sexual transmission of HIV among older individuals. By 2015, it is predicted that greater than half of all HIV-1-infected individuals will be older than 50 years of age. Therefore, studies are needed to determine the impact of immunosenescence on T cell functional reconstitution in older AIDS patients submitted to highly active anti-retroviral therapy (HAART).

Objective: To elucidate the effects of age and HIV infection on the frequency of different T-cell subsets and the cytokine profile in response to HIV-specific and non-specific stimuli.

Methods and results: PMBC from young and older HIV-1-infected individuals who were successfully treated with HAART were freshly stained with different monoclonal antibodies to quantify the frequency of different T cell subsets by cytometry. The cytokine profile, determined by ELISA and cytometry, was evaluated after activating PBMC cultures with polyclonal activators (PMA/Ionomycin) or ENV peptides. Finally, the level of HIV-1 replication *in vitro* was determined by RT-PCR. In this context, as compared with the younger AIDS group, the frequency of naïve ($CD45RA^+CD62L^+$) and central memory ($CD45RO^+CD127^+$) in both $CD4^+$ and $CD8^+$ T cell compartments was significantly lower in aged HIV-1 infected patients. In contrast, the frequency of effector T cells ($CD45RO^+CD62L^-CD127^-$) was significantly higher in the aged infected group. A dramatic loss of classical Treg cells ($CD4^+FoxP3^+CD25^+$) was observed in aged AIDS patients. With regard to cytokine profile, reduced IFN-γ production associated with high IL-10 release was observed in aged-derived PBMC cultures following polyclonal activation. In our system, the production of IL-10 in the aged AIDS patients was mainly derived from ENV-specific $CD4^+FoxP3^-CD152^+$ T cells. Finally, while blockade of IL-10 activity by monoclonal antibody clearly enhanced de release of IL-6 and IL-1β by ENV-stimulated PBMC cultures from aged AIDS patients, this monoclonal antibody enhanced *in vitro* HIV-1-replication.

Conclusions: In conclusion, HIV infection and aging undoubtedly contribute synergistically to a complex immune dysfunction in HAART-treated older HIV-infected individuals.

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SERUM LEVELS OF CYTOKINES AND C-REACTIVE PROTEIN IN POSTMENOPAUSAL WOMEN WITH METABOLIC SYNDROME

CLAUDIO LERA ORSATTI[1]; FÁBIO LERA ORSATTI[2]; JORGE NAHAS NETO[1]; STEVEN S. WITKIN[3]; ELIANA AGUIAR PERI NAHAS[1]
[1]University Estadual Paulista (UNESP),[2] Triângulo Mineiro Federal University (UFTM), [3]Weill Cornell Medical College, New York, USA

Introduction and objectives

Prevalence of the metabolic syndrome (METS) and its components significantly increase after the menopause. Related increased cardiovascular risk may partially be explained by a pro-inflammatory state. Several inflammation markers such as the prototypical inflammatory cytokines TNF-α and IL-6 have been suggested as mediators in atherogenesis. Furthermore, a large number of studies have shown that C-reactive protein (CRP) is a reliable marker of CVD and has thus been incorporated into CVD risk prediction protocols. The objective was to assess serum levels of cytokines and C-reactive protein in postmenopausal women with and without the METS

Methods and Results

Serum of 311 postmenopausal women (last menstrual period >12 months ago and who were > 45 years old) was analysed for tumour necrosis factor-alpha (TNF-α), interleukin 6 (IL-6), interleukin 1 (IL-1), interleukin 10 (IL-1), HOMA, Glucose, PCR and profile lipids. Cytokine and cardiometabolic makers levels were compared among those with and without the syndrome. The concentration of cytokines in plasma was determined by commercial ELISA (Colocar a metodologia do PCR, glucose, lipídios, circunferência da cintura, etc.)**.** Statistical analyses were done using X^2 e *t* test. Women with the METS (n = 134) significantly (p<0,001) had higher body mass index prevelence (65.7% vs 23.7%), and higher rates of abdominal obesity (96.3% vs 45.0%), hyperglycemia (44.0% vs 2.8%), hypertriglyceridemia (84.4% vs 26.7%), hypercholesterolemia (62.7 vs 36.2%), hypertension (64.9% vs 9.0%), lower HDL-C levels (77.1% vs 13.0%) and high PCR level (21.6% vs 6.8%). Women with the METS significantly (p<0,01) had higher HOMA values (IC95% 2.7-3.5 vs IC95% 1.4-1.6) and higher age values (IC95% 52.3-54.1 vs IC95% 54.0-56.3). Cytokine levels did not differ among women with or without the METS.

Conclusion

Circulating CRP levels are associated with METS more that the proinflammatory cytokines.

Female mice show increased leukocyte recruitment in adipose tissue than male mice in a food allergy model

PEREIRA R. V. S.[1]; BATISTA, N. V.[2]; FONSECA, R. C.[3]; PEREZ, D. A.[4]; ALVARENGA, D. M.[5]; CARA, D. C.[6].

1. Rafaela Vaz Sousa Pereira. Departamento de Morfologia do Instituto de Ciências Biológicas da Universidade Federal de Minas Gerais. Av. Antônio Carlos, 6627- Pampulha - Belo Horizonte - MG, Brasil. CEP: 31270 901. Fone: +55 (31) 3409 2808. rafaelavazsp@gmail.com
2. Nathália Vieira Batista. Departamento de Morfologia do Instituto de Ciências Biológicas da Universidade Federal de Minas Gerais. nathaliavb@gmail.com
3. Roberta Cristelli Fonseca. Departamento de Morfologia do Instituto de Ciências Biológicas da Universidade Federal de Minas Gerais. beta_cristelli@hotmail.com
4. Denise Alves Perez. Departamento de Morfologia do Instituto de Ciências Biológicas da Universidade Federal de Minas Gerais. deniseperez86@yahoo.com.br
5. Débora Moreira Alvarenga. Departamento de Morfologia do Instituto de Ciências Biológicas da Universidade Federal de Minas Gerais. deboraalvarenga@yahoo.com
6. Denise Carmona Cara. Departamento de Morfologia do Instituto de Ciências Biológicas da Universidade Federal de Minas Gerais. Av. Antônio Carlos, 6627- Pampulha - Belo Horizonte - MG, Brasil. CEP: 31270 901. Fone: +55 (31) 3409 2808. deniseccm@gmail.com

The recruitment of leukocytes is an essential event in the inflammatory process which is also present in the adipose tissue (AT) in an experimental model of food allergy (FA). Studies have shown that the development and prevalence of some immunomediated diseases are higher in women. The aim of this study was to evaluate the difference between gender on the leukocyte recruitment in the AT

microvasculature in this FA model. For this, BALB/c mice were sensitized with OVA (allergic group) and then were challenged with an OVA diet. The control group wasn't sensitized. In allergic groups, the level of anti-OVA IgE was significantly higher but there was no significant difference between genders. Both genders ate less in the first week of allergenic challenge due to the immunological aversion, but in allergic males (AM) this consumption was 31,8% lower and in allergic females (AF) it was 25,7% lower. On the other hand, the significant loss of AT was more evident in AF, nevertheless the adipocyte area wasn't different. The decreased food consumption is not the only cause of weight loss seen in allergic animals. This is also due to the increased metabolism induced by the inflammatory process which was more marked in AF, confirmed by further increase of leukocyte adhesion in the microcirculation of AT in these animals. Our data show that the inflammatory response in female, induced by FA, is more intense.

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SIGNALING PHENOTYPES IN MONOCYTES IN SYSTEMIC JUVENILE IDIOPATHIC ARTHRITIS (SJIA)

CLAUDIA MACAUBAS[1]; ELIZABETH WONG[2]; KHOA D. NGUYEN[1]; ARIANA PECK[2]; JANE L. PARK [1]; TZIELAN LEE [1]; AND ELIZABETH D. MELLINS[1]

[1]Department of Pediatrics, Program in Immunology, Stanford University; [2]Department of Biology, Stanford University

Introduction: Systemic juvenile idiopathic arthritis (sJIA) is a chronic autoinflammatory condition of childhood, characterized by remitting fever, transient rash, and relapsing arthritis. The disease course is variable (monocyclic, polycyclic or persistent), and macrophage activation syndrome occurs in approximately 10% of patients. The absence of typical characteristics of autoimmunity (such as autoantibodies, auto-reactive T cells and association with MHC alleles), together with good clinical results with anti-IL-1 and anti-IL-6 therapies suggest that dysfunctions of the innate immune system may play an important role in sJIA. Specifically, cells of the monocytic lineage have been implicated in sJIA pathogenesis. We have previously reported that monocytes are expanded during sJIA flare, and that, although the distribution of $CD14^{hi}CD16^{-}$ and $CD14^{lo}CD16^{+}$ monocyte subsets is normal in sJIA, both subsets show a mixed M1/M2 activation phenotype during sJIA flare, and evidence of a M2-like polarization persists at quiescence. **Methods and Results:** We investigated the functionality of the monocytes in sJIA, regarding signaling response to in vitro stimulation, using the flow cytometry based 'phospho-flow'. Thawed and rested $CD14^{hi}CD16^{-}$ and $CD14^{lo}CD16^{+}$ sJIA monocytes exhibit normal levels of pSTAT1,-3,-5, pERK and pp38. Following stimulation with IFNg and IFNa, STAT1 phosphorylation was decreased in sJIA monocytes, in both flare and quiescence groups, in comparison to controls. Phosphorylation of STAT5 in response to GMCSF was decreased in sJIA quiescence samples, but not in flare samples as a group. Other cytokine signaling pathways were not altered: responses to IL-10 as seen by levels of pSTAT3; pSTAT1, pSTAT3 and pERK in response to IL-6; and pSTAT6 in

response to IL-4 stimulation. Phosphorylation of p38 in response to LPS was also similar to controls. We also observed that HLA-DR expression following IFNg stimulation was lower in sJIA monocytes, indicating a functional consequence of the defective IFNg/pSTAT1 response. **Conclusions:** The decreased IFN/pSTAT1 response may contribute to sJIA pathology, as it has been shown that IFNg can inhibit IL-1. The low IFN/pSTAT1 response may also be associated with M2 polarization of sJIA monocytes, as a protective mechanism in response to a highly inflammatory environment.

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DIETARY AND AIRBORNE ANTIGENS IN HUMAN MILK

PATRICIA MACCHIAVERNI[1]; AKILA REKIMA[2]; MERI TULIC[2]; VALÉRIE VERHASSELT[2] AND ANTONIO CONDINO-NETO [1]

[1] Department of Immunology, Institute of Biomedical Sciences, University of São Paulo-SP, Brazil

[2] EA 6302 Tolérance Immunitaire, Université de Nice Sophia-Antipolis - Hôpital de l'Archet, Nice-France.

Introduction: In adult, allergy prevention is classically based on allergen avoidance. This approach has been extended to foetus, neonates and infants by promoting the avoidance of allergen exposure during pregnancy, lactation and the first years of life. However, such politics has not yielded the expected results as prospective studies assessing allergen avoidance have failed to show a significant long term reduction in food allergies. In a mouse model, Verhasselt *et al* demonstrated that breastfeeding can protect against allergic diseases by oral tolerance induction in the progeny if two conditions are met in breast milk: (1) presence of the allergen (2) presence of TGF-beta and/or maternal IgG allergen-specific. In human, the influence of breastfeeding on allergic diseases remains controversial.

Methods and results: In the present work, we quantified by ELISA the concentration of environmental and dietary allergens (*Der p* 1 and OVA), TGF-beta and allergen-specific IgG and IgA in breast milk in a coorte of 300 colostrum samples; which we have the clinical follow up of the infant until five years old. We detected OVA in 52% of the samples in a mean concentration of 133pg/mL (range from 0,0pg/mL to 2233pg/mL). OVA concentration in breast milk was not correlated with OVA-specific IgG nor IgA levels. Surprisingly, we also detected the presence of *Der p* 1 in 78% of the colostrums samples assayed in a median concentration of 62pg/mL (range from 0,0pg/mL to 771pg/mL). *Der p*-specific IgG and IgA was present in almost all samples assessed (97,2% and 99,6%, respectively) in a wide range of concentrations. TGF-beta was also much variable from one mother to the other. The mean concentration was 594pg/mL and was not correlated with specific-IgG nor specific-IgA levels in colostrum.

Conclusion: Our study describes for the first time the efficient transfer of an airborne allergen and it's specific-IgG through colostrum. This is a important step for the translation of rodent data to the human model. We are now investigating if these factors can also exert a protective role in their infant and how the levels of these compounds in breast milk are controlled.

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PATTERNS OBSERVED IN THE GRANULOMAS DEVELOPED IN PARACOCCIDIOIDOMICOSIS IN ORAL LESIONS OF PATIENTS AS COMPARED TO THOSE DEVELOPED BY EXPERIMENTALLLY INFECTED MICE.

[1]Eva Burger, [2]Marta Miyazawa, [3]Denise Tostes Oliveira, [1]Alessando Costa Pereira, [2]João Adolfo Hanemann

[1]Federal University of Alfenas, Biomedical Sciences Institute, Alfenas, MG
[2]Federal University of Alfenas, Odontology School, Alfenas, MG
[3]Sao Paulo University, Odontology School, Bauru, SP

Introduction: Severe clinical paracoccidioidomycosis (PCM) forms are characterized by the presence of numerous disseminated granulomas (Gr), in contrast with mild forms that have few compact Gr. Formation of G can be interpreted as a host defense mechanism to destroy or contain *P. brasiliensis* (Pb) by immune mechanisms. Our objectives were to characterize the oral lesions observed in PCM patients and compare these data with those previously described in the experimental murine model of PCM.
Material and methods: For the studies using patients, the demographical data of patients were retrieved from anatomopathological files. Microscopical analysis was done in 38 cases of HE and Grocott stained slides. The architecture of the Gr, the presence of morphologically preserved or destroyed fungi, the composition of the cellular infiltrate and the pattern of deposition collagen fibers was compared to those previously observed in lesions of susceptible and resistant mice infected with Pb, after similar preparation of the material.
Results: The best indicators of control of experimental PCM were the presence of compact Gr, containing few Pb with altered morphology, delimited by continuous deposit of collagen arranged in concentric orientation, and of multinucleated giant cells (MGC) containing Pb. In patients the most frequent sites of lesions were gengiva, hard and soft palate, and oral mucosa. Microscopy revealed predominance of intense, diffuse inflammatory infiltrate, mainly constituted by lymphocytes and plasmocytes. In most cases, no compact Gr were found, while MGC of the Langhans type were observed in 68% of cases. Pb were frequently found dispersed in connective tissue and inside MGC. A patient showed clinical symptoms suggestive of PCM, which was confirmed by microscopy following biopsy. An intense diffuse mononuclear infiltrate was observed at the lamina propria, with formation of compact Gr and presence of MGC containing Pb. The overall aspect of his lesions was similar to that observed in resistant mice, in contrast to the disseminated Gr of susceptible mice. He was treated for 6 months

with 200mg per day Itraconazol, resulting in complete cicatrization of the lesions and is nowadays completely asymptomatic.

Conclusion: We can conclude that few Gr may indicate depression of cellular immunity in some patients and presence of compact Gr suggest control of the infection by local immune mechanisms in both, experimental animals and patients.

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IMPROVED CANINE AND HUMAN VISCERAL LEISHMANIASIS IMMUNODIAGNOSIS USING COMBINATIONS OF SYNTHETIC PEPTIDES IN ENZYME-LINKED IMMUNOSORBENT ASSAY

MÍRIAM MARIA SILVA COSTA (PhD) (1), MARCOS PENIDO (PhD) (1), MARIANA SILVA DOS SANTOS (PG) (2), DANIEL DORO (IC) (2), ELOÍSA DE FREITAS (PhD) (3), MARILENE SUSAN MARQUES MICHALICK (PhD) (3), GABRIEL GRIMALDI (PhD) (4), RICARDO TOSTES GAZZINELLI (PhD) (1), ANA PAULA FERNANDES (PhD) (2)

(1).Departamento de Bioquímica e Imunologia, Instituto de Ciências Biológicas Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brasil; (2).Departamento de Análises Clínicas e Toxicológicas, Faculdade de Farmácia, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brasil; (3).Departamento de Parasitologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brasil; (4).Departamento de Imunologia, Fundação Oswaldo Cruz, Instituto Oswaldo Cruz, Rio de Janeiro, Brasil.

Introduction: Zoonotic visceral leishmaniasis (VL) is a severe infectious disease caused by protozoan parasites of the genus Leishmania and the domestic dogs are the main urban parasite reservoir hosts. In Brazil, indirect fluorescence antibody tests (IFAT) and indirect enzyme linked immunosorbent assay (ELISA) using promastigote extracts are widely used in epidemiological surveys. However, their sensitivity and specificity have often been compromised by the use of complex mixtures of antigens, which reduces their accuracy allowing the maintenance of infected animals that favors transmission to humans.
Methods and Results: In this context, the use of combinations of defined peptides appears favorable. Therefore, they were tested by combinations of five peptides derived from the previously described Leishmania diagnostic antigens A2, NH, LACK and K39. Combinations of peptides derived A2, NH, LACK and K39 antigens were used in ELISA with sera from 44 human patients and 106 dogs. Improved sensitivities and specificities, close to 100%, were obtained for both sera of patients and dogs. Moreover, high sensitivity and specificity were observed even for canine sera presenting low IFAT anti-Leishmania antibody titers or from asymptomatic animals.
Conclusion: The use of combinations of B cell predicted synthetic peptides derived from antigens A2, NH, LACK and K39 may provide an alternative for improved sensitivities and specificities for immunodiagnostic assays of VL.

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THE ROLE OF IN VITRO HOST SOLUBLE FACTORS INDUCED BY MOREAU BCG FOR THE INITIATION OF MONOCYTE APOPTOSIS IN HEALTHY VOLUNTARIES

LEANDRO PERES[1]; SUELEN MARINHO[1]; JESSICA LIMA[1]; MARIANA SIQUEIRA[1]; THAÍZE PEDRO[1]; DOLORES SILVA[1]; CARLOS PONTE[1]; PAULA DE LUCA[2]; LUÍZ CASTELLO-BRANCO[1] & PAULO ANTAS[1].

[1]Laboratório de Imunologia Clínica, and [2]Laboratório de Imunoparasitologia, Oswaldo Cruz Institute, FIOCRUZ, Rio de Janeiro.

Introduction: Tuberculosis (TB) remains the world's leading cause of mortality. For its control, studies of TB vaccines are needed. Since BCG is the only vaccine against TB currently in use, studies addressing the protective role of BCG is urgent required. **Methods and Results:** Cohorts of HIV-negative voluntaries have been enrolled: Adult Control Donors (CD; n=18) and neonates Umbilical Vein (UV; n=10). BCG Moreau was primarily used for in vitro monocyte infection at both 24h and 48h. After that, harvested conditioned medium (CM) was added to autologous resting cells for an additional 24h or 120h, and Annexin V-FITC and PI were used for apoptosis detection. Also, in those cultures the remaining CD4+ lymphocytes were stained for PD-1 and CD25/FoxP3, and concurrent caspases were detected in monocytes. Supernates were assayed for Nitric Oxide (NO2). P levels were setup at $\leq$ 0.05. Here, CM induced higher apoptosis levels in the CD group only. NO2 were released equally during BCG infection in both groups, but higher levels were found in CD, when compared to UV group ($p<0.05$). For PD-1, higher levels were also observed in CD-derived CD4 lymphocytes only. On the other hand, mean levels of Caspases in monocytes and Treg cells (CD4+/CD25high+/FoxP3+) were not induced in either group of individuals, but expression of CD25dim+ as an activation marker was dependent on BCG infection only in the UV group. **Conclusion:** The vast majority of the world's current population has been vaccinated with BCG, with the possible requirement for a booster immunization in adulthood for TB protection. Sustained data have shown an enhancement of in

vitro apoptosis levels in adult monocytes during long-term incubation with CM from BCG Moreau RDJ cultures, but neonate monocytes remained viable when cultured with autologous CM. That was followed by higher NO2 released and PD-1 expression on CD4+ T cells from the primed group, but not in naïve individuals, although only neonates showed in vitro activated lymphocytes. A booster immunization with BCG may protect the immunized individuals and further studies are needed to better evaluate these findings. *Financial support: FAP; Fiocruz; Faperj; CNPq*

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IL-6, IL-8, G-CSF, GM-CSF, MCP-1 AND MIP-1b AS CANDIDATES FOR DIAGNOSING PLEURAL TB

MARIANA SIQUEIRA[1]; JESSICA LIMA[1]; CARLOS PONTE[1]; LEANDRO PERES[1]; SUELEN MARINHO[1]; MAYARA BASTOS[2]; PATRICIA SOARES[2]; MARCIA BELO[2]; ANETE TRAJMAN[2] & PAULO ANTAS[1]

[1]Laboratório de Imunologia Clínica, Oswaldo Cruz Institute, FIOCRUZ, and [2]Santa Casa de Misericórdia, Rio de Janeiro.

Introduction: The production of IFNg and TNFa are associated with effective cellular immune response against *Mycobacterium tuberculosis* infection, through cytokine-mediated macrophage activation. In extrapulmonary tuberculosis (TB), such as pleural TB, a wide array approach for cytokine responses has not been well studied in such patients. **Methods and Results:** *Ex vivo* non-stimulated cytokine responses in the pleural fluid from 43 patients with confirmed pleural TB, of whom 5 were HIV-infected, and 18 with other diseases were compared. A multiplex assay was performed using a commercial kit covering 17 cytokines and chemokines, and ELISAs were made to detect C-Reactive Protein (CRP in house) and Matrix Metalloproteinase (MMP)-9. Median values in three groups (TB/HIV+, TB/HIV-, other diseases) were compared using the Mann Whitney test. Median IL-6, IL-8, G-CSF, GM-CSF, MCP1 and MIP-1b were significantly higher ($p<0.05$) in pleural fluid of TB than other disease patients as well as the already expected higher levels of IFNg and TNFa. HIV- infected patients had a different cytokine profile: three-fold higher levels of IL-6, and the lowest production of CRP (< 360-fold). **Conclusion:** The multiplex results points to possible strategies to identify *ex vivo* profiles of soluble factors that may help diagnosis, and together, possibly provide insight into TB. The sample will be increased in order to build ROC curves to establish cut-off values for accurately distinguishing pleural TB from other diseases. The profile in HIV patients suggests abnormalities in adaptive and innate immune functions. *Financial support: Fiocruz; Faperj; CNPq*

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MODERATE PHYSICAL EXERCISE IMPROVE THE HUMORAL IMMUNE RESPONSE OF ELDERLY PEOPLE AGAINST THE INFLUENZA VIRUS VACCINE

[1]**ANDRE LUIS LACERDA BACHI**, [1]ANGELICA VICTORINO, [2]VINICIUS SUGURI MAGALHÃES, [2]SUEMY CIOFFI IZU, [2]MAURO VAISBERG, [1]JOSE DANIEL LOPES

[1]Departamento de Micro/Imuno/Parasitologia, [2]Departamento de Otorrinolaringologia e Cirurgia de Cabeça e Pescoço - Universidade Federal de São Paulo (UNIFESP), Brasil.

Introduction and Objective: Aging is a multifactorial phenomenon characterized by important changes in many systems and one of the most affected being the immune system, called immunosenescence. These changes can result in greater susceptibility to infection, reduced response to vaccination, development of neoplasias, among other problems. Although immunosenescence is an irreversible process, regular moderate exercise can attenuate some aspects of the decline in the immune system. The aim of this study was to investigate humoral immune response in physically active elderly individuals against influenza virus vaccine.

Methods and Results: Seventy-eight (78) individuals aged between 60 and 85 years (mean age 69,7±6,97 years) living in the city of São Paulo were recruited and divided in two groups: sedentary [SE, n=34 (mean age 70,4±6,9 years)] and physically active [PA, n=44 (means age 69,2±6,35 years)] that performed a routine of physical activity (4-days a week - 1 hour each exercise session, with aerobic and resistance exercises), at least for 12 months. All volunteers received the same sample of influenza virus vaccine and none of the participants had severe diseases. Serum and saliva samples were collected at two different times: before and 30 days after the vaccination to influenza virus to determine immunoglobulin M and G levels produced in response to vaccine and concentration of secretory immunoglobulin A, all by ELISA. Before the vaccination the IgM and IgG levels no differ between the SE [IgM= 0.016(0.0-0.08), IgG=0.14(0.04-0.37)] and PA [IgM= 0.035(0.0-0.79), IgG= 0.22(0.11-0.33)] groups. After the vaccination there was a significant increase of antibodies levels in both groups [SE, IgM= 0.05(0.01-0.17) and IgG= 0.3(0.15-0.42); PA, IgM= 0.16(0.05-0.26) and IgG= (0.46(0.29-0.57)] in relation to previous levels. Furthermore, the increased levels of IgM and IgG observed post-vaccination in the PA group was statistically higher than in the SE group levels. The levels of secretory IgA in saliva of individuals in both groups were similar before or after the vaccination.

Conclusion: We were able to demonstrate that moderate physical exercise improved immune response of elderly as can be viewed by the higher levels of IgM and IgG antibodies against the virus of the influenza vaccine. Thus, this effect could protect the elderly people - at least partially - from the functional changes of aging.

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Expanding the clinical and genetic spectrum of human CD40L deficiency: The occurrence of paracoccidioidomycosis and other unusual infections in Brazilian patients

Otavio Cabral Marques, MSca,b, Paulo Vítor Soeiro Pereira, MSca, Mary J. Hackett, BScb, Paolo Ruggero Errante, PhDa, Lena-Friederike Schimke, MDa,b, Cristina Worm Weber, MDc, Janaíra Fernandes Ferreira, MDd, Nelson Augusto Rosário Filho, MD, PhDe, Hans D. Ochs, MDb, Troy R. Torgerson, MD, PhDb, Beatriz Tavares Costa Carvalho, MDf, Antonio Condino-Neto, MD, PhDa

[a]Department of Immunology, Institute of Biomedical Sciences, University of São Paulo, SP
[b]Department of Pediatrics, University of Washington School of Medicine, and Seattle Children´s Hospital, Seattle, WA
[c]Pediatric Allergy & Immunology Clinic, Caxias do Sul, RS
[d]Albert Sabin Hospital. Fortaleza, CE
[e]Department of Pediatrics, Federal University of Paraná, Curitiba, PR
[f]Division of Allergy and Clinical Immunology and Rheumatology, Department of Pediatrics, Federal University of São Paulo , São Paulo, SP

Introduction: CD40 ligand (CD40L) deficiency or X-linked hyper-IgM syndrome (X-HIGM) is a well-described primary immunodeficiency in which Pneumocystis jiroveci pneumonia is a common clinical feature. We have identified an unusual high incidence of fungal infections and other not yet described infections in a cohort of 11 X-HIGM patients from nine unrelated Brazilian families. Among these, we describe the first case of paracoccidioidomycosis (PCM) in X-HIGM.

Objective: To analyze the clinical and genetic spectrum of Brazilian patients with CD40L deficiency

Methods. Relevant clinical data were obtained from the clinical records. Genetic and functional analysis of CD40L was performed by gene sequencing and evaluation of CD40L protein expression on activated T cells by flow cytometry.

Results: Nine of these 11 patients (82%) had fungal infections. These included fungal species common to CD40L deficiency (P. jiroveci and Candida albicans) as well as Paracoccidioides brasiliensis. One patient presented with PCM at age 11 years and is now doing well at 18 years of age. Additionally, one patient presented with a simultaneous infection with Klebsiella and Acinetobacter, and one with condyloma caused by human papilloma virus. Molecular analysis revealed four previously described CD40L mutations, two novel missense mutations (c.433 T>G

and c.476 G>C) resulting in the absence of CD40L protein expression by activated CD4+ cells and one novel insertion (c.484_485insAA) within the TNFH domain leading to a frame shift and premature stop codon.

Conclusions: These observations demonstrated that the susceptibility to fungal infections in X-HIGM extends beyond those typically associated with XHIGM (*P. jiroveci* and *C. albicans*) and that these patients need to be monitored for those pathogens.

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DOPAMINE ENHANCES IL-17 PRODUCTION IN MULTIPLE SCLEROSIS PATIENTS

BRUNA TEIXEIRA[1]; VERA CAROLINA B. BITTENCOURT[1]; THAIS B. FERREIRA[1]; TAISSA M. KASARAHA[1]; PRISCILA O. BARROS[1], JOANA HYGINO[1], ULISSES C. LINHARES[2]; REGINA ALVARENGA[2]; ARNALDO F. B. ANDRADE[3] AND CLEONICE A. M. BENTO[1]

From [1]Department of Microbiology and Parasitology/Federal University of Rio de Janeiro State and [2]Department of Neurology/Federal University of Rio de Janeiro State; [3]Department of Microbiology, State University of Rio de Janeiro, Rio de Janeiro, Brazil.

It is now known that the immune system is regulated by central nervous system. This is mainly achieved by cathecolamines, such as dopamine (DA), which interact with different effector immune cells and thereby ultimately regulate the homeostatic response of an individual to different stimuli. As DA is primarily a neurotransmitter in the central nervous system (CNS), this cathecolamine should influence the clinical course of inflammatory pathologies in the CNS, such as multiple sclerosis (MS). Multiple sclerosis (MS) is a clinically and pathologically heterogeneous disease most often characterized by inflammatory and demyelinating lesions throughout the CNS. MS is characterized by macrophage and lymphocyte infiltration which correlates with demyelination, axonal injury and loss of neurological function, clinically determined through the Kurtzke Expanded Disability Status Scale (EDSS). Although recent studies have implicated the Th17 cytokines in the MS pathogenesis, until now, none study was performed to evaluate the impact of DA on the T cell behavior from MS patients.

Objective: To investigate the impact of DA on the T cell proliferation and cytokine profile following addition of T-cell polyclonal activator.

Methods and Results: The peripheral blood mononuclear cells (PBMC) from healthy (control group, n=20) or patients suffering from MS (n=16) were activated *in vitro* with PHA (1 µg/mL) in the presence or absence of stress-related dose of DA (5 x 10^{-6v}M). After 3 days, the lymphoproliferation and cytokine profile were determined through [^{3}H] thymidin up-take and ELISA technique, respectively. In this context, while DA diminished the T cell proliferation in the control group, this neurotransmitter elevated the thymidin up-take in MS-derived PBMC cultures. With regard cytokine profile, DA enhanced IFN-γ, TNF-α and IL-17 release only in the MS patients-derived cell cultures. Furthermore, DA addition diminished the release of IL-10 by polyclonally-activated T cell from MS patients. Finally, as production of IL-17 was directly related to EDSS score among MS patients, the ability of DA in enhancing this cytokine suggest a detrimental role for this central neurotransmitter on the MS course disease.

Conclusion: Our results demonstrated that DA, by up-regulating the *in vitro* Th17-related cytokines, should have a deleterious impact on the clinical course of MS.

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THE ROLE OF OBESITY AND CYTOKINES IL12/ IFNγ IN THE MODULATION OF ULCERATIVE COLITIS INFLAMMATORY RESPONSE AND TREATMENT WITH SHORT CHAIN FATTY ACIDS.

DENIS GONÇALVES SILVA(IC)[1]; ANGELA CASTOLDI[2]; FREDERICK WASINKI[3]; VINÍCIUS DE ANDRADE OLIVEIRA [2]; PEDRO M. M. M. VIEIRA [2]; NIELS OLSEN SARAIVA CÂMARA[2].

(1) Departamento de Medicina, Universidade Federal de São Paulo; (2) Departamento de Imunologia, Instituto de Ciências Biomédicas, Universidade de São Paulo; (3) Departamento de Biofísica, Universidade Federal de São Paulo.

Introduction: The precise etiology of ulcerative colitis (UC) remains unclear, although it is believed that intestinal inflammation results from dysregulated mucosal immune responses and a hypertrophy of mesenteric adipose, besides a deficiency of short chain fatty acids (SCFA) may play an important role. Thus, this work aimed to study the role of obesity and cytokines IL-12/ IFN-γ in ulcerative colitis severity and possible mechanisms of the disease, as well as a possible treatment with SCFA. **Methods:** Male mice, IL-12KO, IFN-γKO and WT, all C57BL/6J were used and obesity induced with a high-fat diet (HF) (45% kcal from fat) for 8 weeks. After this time, UC were induced by 25g/L dextran sulfate sodium in water and the survival analyzed or sacrificed on the ninth day. On the final day was collected serum (for circulating cytokine by Bioplex), colon tissue (histological analysis to gauge disease severity), and mesenteric lymph nodes (FACS analysis of mucosal lymphoid tissue responses). For the study of SCFA, WT were divided into groups with acetate or propionate or butyrate or without SCFA (CTL); all groups had 5 days of pretreatment with its respective SCFA, diluted in water at a concentration of 150 mM or CTL, and also the same concentration in the 40 days of induction of colitis. **Results**: The groups WT HF and IFN-γ HF displayed higher weight compared with their controls ($p<0.001$) and IL12KO ($p <0.001$), on the other hand the knockout for IL-12 does not gain weight compared to controls or the other two groups. The UC results showed that the group WT HF has lower survival than the control animals ($p<0.001$) and knockouts for IL-12 ($p<0.001$) and IFN-γ ($p<0.001$). Also showed in the same group a decrease in mesenteric lymph nodes of macrophages CD11b+F4/80+ ($p<0.05$) and CD4+ T cells ($p<0.05$). And finally, the results revealed less weight loss ($p<0.001$) and a significant reducing of death in all groups of SFCA, highlighting the butyrate group, which all survived the 40 days (CTL had a survival mean of 14 days). **Conclusion:** In WT obese mice, induced by HF diet, the low state of chronic systemic inflammation increases the severity of colitis, and IL-12 and IFN-γKO showed a lower severity of UC,

demonstrating the importance of these pro-inflammatory cytokines in the disease. Moreover, the anti-inflammatory effects attributed to SFCA are actually doing effect, protecting mice against the death and proved to be a promising treatment of UC.

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PHENOTYPIC EVALUATION OF SURFACE RECEPTORS AND TRANSCRIPTION FACTORS AND CYTOKINES PRODUCTION IN RECURRENT WHEEZING CHILDREN

Angela Falcai[1], Paulo Vítor Soeiro-Pereira[1], Christina Arslanian Kubo[1], Isabel Rugue Genov[2], Vera Rullo[3], Dirceu Solé.[2], Antonio Condino-Neto[1]

[1]Department of Immunology, Institute of Biomedical Sciences, University of São Paulo, São Paulo-SP, Brazil; [2] Division of Allergy, Clinical Immunology and Rheumatology, Department of Pediatrics, Federal University of São Paulo Medical School, São Paulo-SP, Brazil; [3] Santos Medical School, Santos - SP, Brazil

Introduction: Wheezing in infancy is frequent and its etiology is often difficult to establish. In general, recurrent wheezing (RW) has been associated with asthma diagnosis. It is assumed that at least 20% of children up to 2 years old show transient wheezing. A study in São Paulo, Brazil, showed that 52% of the children presented wheezing episodes until 30 months of age, and 43% had three or more acute episodes. **Aim:** Evaluation of phenotypic surface receptors, transcription factors and cytokines production in RW children. **Materials and Methods:** We collected blood from 25 RW and 9 non-RW children with age around 30 months. We analyzed surface markers and transcriptional factors on fresh blood cells by flow cytometry. We evaluated CD14, TLR4, CD119, HLA-DR, CD16, CD18, CD32, CD11b, Stat 1 for monocytes. CD14, TLR4, HLA-DR, CD18, CD15, CD36, CD32, CD11b for neutrophil. And CD4, CD8, CD212, TLR4, stat-3, GATA-3, T-BET for lymphocytes. We also evaluated cytokine production profile of peripheral blood mononuclear cells (PBMC) in response to *in vitro* stimulation with TLR2, 3, 4, 5, 7 and NOD agonists, f-MLP, *Der p*, *Blo t*, *Bla g*, IFN-gamma and IL-12. We performed Mann Whitney statistical test. **Results:** We observed that monocytes from RW children showed a tendency for lower expression of CD18, CD11b and HLA-DR and higher expression of CD36, Stat 1 when compared with non-recurrent wheezing (non-RW). In neutrophils, we observed a tendency for lower expression of HLA-DR, CD18, CD11b, CD15 and CD32 and higher expression of CD36 in RW children compared with non-RW. In lymphocytes, we observed a tendency for lower expression of CD4/CD212 and higher expression of Stat 3 and T-bet in RW children compared with non-RW. Expression of GATA3, CD14, TLR4, CD119, CD4 and CD8 had no differences between groups. We observed that PBMC from RW released lower amounts of TNF-alpha in response to TLR3 (*p=0,01), TLR5 (*p=0,01) agonists, and *Der p* (*p=0,02), and also released lower amounts of IL-12 (*p=0,02) after stimulation with IFN-gamma compared with non-RW. **Conclusion:** We concluded that RW children have a deficiency on the expression of surface receptors, transcription factors and cytokines production; suggesting a failure in the

immune system of RW children, confirming our previously results that RW children have impairment in Th1 response. However, the number of non-RW children samples have to be increased for a better statistical analysis.

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CYTOKINE PROFILE IN SERUM OF INDIVIDUALS WITH ACTIVE TUBERCULOSIS AND LATENT

ROSALINA GUEDES DONATO SANTOS[1,,2 ,7] VALDIRENE LEÃO CARNEIRO [1, 7,10.];; MARILDA CASELA BATISTA [3,7]; FILIPE SAMPAIO REIS DA SILVA [4,7]; CATARINA ALFAYA MOREIRA[4,7]; ROBERTO MEYER [5,6]; CLEUBER FRANCO FONTES [5,8,9] ; JACY ANDRADE[5,6]; EDUARDO MARTINS NETTO[6,7] LUCAS PEDREIRA CARVALHO[6,7]; JULIANA SACRAMENTO MOTA SOUZA[3]; SONGELI MENEZES FREIRE [5,6,7 ,10]

1.PhD student PPGIm/UFBA , 2. Professor FTC. 3..MSc student PIOS/UFBA 4.Undergrad. student/FAPEX 5.Undergrad. student/CNPq. 6. Professor UFBA. 7.INCT-DT/MCT/CNPq member 8.CNPq Researcher 9.IBIT-FJS 10. Professor UNEB.10-Professor FBMSP

Introduction: Tuberculosis (TB) remains a significant threat to public health worldwide. A major challenge in tuberculosis control is the early diagnosis and correct treatment during latent infection. Previously there were only the tuberculin skin test (TST), largely considered simple and inexpensive to the diagnosis of the Latent Tuberculosis Infection (LTBI), in spite of the several limitations including subjective results and false positive results mostly due to technical problems, cross-reactivity with BCG vaccination or responses to environmental mycobacteria. In the last decade some Interferon-Gamma Release Assays (IGRA) emerged as attractive alternatives. *Another alternative would be to seek an association between cytokines in order to discriminate between latent and active tuberculosis.* The aim of the proposed study was to evaluate the cytokine TH1/TH2 profile in serum of individuals with active tuberculosis and latent. **Methods and Results**: The study groups included 65 patients with active TB (culture and BAAR positive), 87 individuals with LTBI (positive TST) and 76 healthy controls (with negative TST and no clinical symptom or sign of TB), all of them from Reference Hospital and Institute for TB (IBIT and HEOM) in Salvador-Bahia-Brazil. The cytokines IL-12p70 IFN-γ IL-2 IL-10 IL-8 IL-6 IL-4 IL-5, IL-1β TNF-α TNF-β were analyzed by CBATh1/Th2 (BD™) with detection limits from 20 to 20,000 pg / ml. The median age of patients in the TB, LTBI and control groups were 25, 40 and 36 years old, respectively. To evaluate the difference between the groups it was used the Kruskal wallis ($p<0,05$). There were higher expression of IL-6 and TNF-beta among all cytokines quantified. . The median IL-6 was 0, 32.2, 39 in the control, LTBI and TB groups and difference was found between Control and TB and LTBI and TB.

(p<0.05). Median TNF-beta values were 0, 149 and 0 in the control LTBI and TB, respectively, difference was found between Control and LTBI. **Conclusion:** The data show that IL-6 serum levels were elevated in the vast majority of patients with active TB. In addition, the Lymphotoxin (TNF-beta) also was higher in LTBI individuals which may indicate its importance in maintaining the granuloma.

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REDUCTION OF CD4, CD8 AND CD19 CELLS IN PERIPHERAL BLOOD OF PATIENTS WITH PULMONARY TUBERCULOSIS.

SONGELI MENEZES FREIRE [5,6,7] VALDIRENE LEÃO CARNEIRO [1, 9]; ROSALINA GUEDES DONATO SANTOS[1]; MARILDA CASELA BATISTA [2]; FILIPE SAMPAIO REIS DA SILVA [3]; CATARINA ALFAYA MOREIRA[4]; JACY ANDRADE[5,6]; LILIA MOURA-COSTA. [5,6]; EDUARDO MARTINS NETTO[5,8]; JULIANA SACRAMENTO MOTA SOUZA[3]; ROBERTO MEYER [5,6]

1.PhD student PPGIm/UFBA 2.MSc student PIOS/UFBA 3.Undergrad. student/FAPEX 4.Undergrad. Student/CNPq. 5. Professor UFBA. 6.INCT-DT/MCT/CNPq member 7.CNPq PQ[2] researcher 8.IBIT-FJS 9. Professor UNEB; 10. Professor EBMSP

Introduction: For decades Tuberculosis (TB) has been considered as a serious worldwide public health problem. The immunological mechanisms that lead to control of *Mycobacterium tuberculosis* are not fully understood. Cellular immune response is crucial for controlling infection and T lymphocytes are involved more directly in the bacterial growth control. Some authors report reduction of lymphocyte phenotypes, such as helper T lymphocytes, cytolytic T lymphocytes and B-lymphocytes in TB patients. They suggest using these parameters to identify patients with active or latent TB. The lymphocyte subsets quantification has been used to monitor several infectious diseases and could be a useful tool in the TB management. The aim of this study was quantify lymphocyte phenotypes Th, Tc, B and NK cells in TB patients comparing with other groups. **Methods and Results**: we included 125 volunteers subjects, divided into three groups: 34 patients with TB (BAAR and/or positive Culture); 50 patients with latent infection (positive TST) and 41 healthy individuals (negative TST, with no TB history or symptoms). In each sample the lymphocytes phenotypes were analyzed by cytometry (FACSCalibur, BD Biosciences CellQuest software) using single step kits, Lymphogram® and ™PerfectCount (Cytognos-Spain). The statistical analysis was performed by Kruskal-Wallis statistical test. The number of lymphocytes was higher in the healthy group than in those with positive TST and TB groups ($p = 0.001$). TB patients had less total lymphocytes and $CD3^+CD4^+$, $CD3^+CD8^+$, and $CD19^+$ phenotypes as compared with the control and latent groups ($p = 0.001$, 0.003, 0.002 and 0.002, respectively). The variable (percentage of NK cells that expressed CD56) was higher with significant difference ($P<0,03$) in TB group when the groups were compared. **Conclusion:** In our population the absolute and relative lymphocyte phenotypes must be routinely

counted to help in the differential diagnosis or prognosis of latent and pulmonary tuberculosis.

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EVALUATION OF PERIPHERAL HUMAN NEUTROPHILS FUNCTIONS AND SYSTEMIC COMPLEMENT ACTIVATION IN RHEUMATOID ARTHRITIS

LARISSA FÁVARO MARCHI (PHD)[1]; ADRIANA BALBINA PAOLIELLO PASCHOALATO (PHD)[1]; ANA ELISA CALEIRO SEIXAS AZZOLINI (PQ)[1]; CLENI MARA MARZOCCHI-MACHADO (PQ)[1]; EDUARDO ANTONIO DONADI (PQ)[2]; YARA MARIA LUCISANO VALIM (PQ)[1]

(1) Departamento de Física e Química, Faculdade de Ciências Farmacêuticas de Ribeirão Preto da Universidade de São Paulo.
(2) Departamento de Clínica Médica, Faculdade de Medicina de Ribeirão Preto da Universidade de São Paulo.

Introduction: Rheumatoid arthritis (RA) is a chronic immune inflammatory disease. Although the etiopathology of this condition is not fully understood, it is known that neutrophils are participants in the joint inflammation detected in RA patients. Evaluation of the respiratory burst induced by receptors FcγR and CR was carried out in peripheral neutrophils in RA patients with active and inactive disease. Simultaneously, cooperation between these receptors and their expression, chemotaxis, and complement system systemic activity were also investigated.
Methods and Results: Neutrophils were stimulated with IC-IgG opsonized with normal human serum (NHS) or not, or with IC-IgG opsonized with RA human serum (RAHS). ROS production was increased ($p < 0.05$) in neutrophils of patients with active or inactive RA stimulated of IC-IgG opsonized with NHS compared to the response of the cells mediated by ICIgG. However, there was poor FcγR/CR cooperation in these RA neutrophils, as indicated by decreased ROS production upon stimulation with IC-IgG opsonized with RAHS. In the case of active RA patients, neutrophils presented significantly higher CR1 and CR3 expression, as well as slight elevation in CD32 and CD16 expression ($p < 0.05$ for each comparison). Positive correlations between FcγR and CR, complement alternative pathway activation, and increased RA serum ($p < 0,05$) chemotaxic activity were only detected in active RA patients.
Conclusion: Taken together, these results indicate that several abnormalities of the complement system exist at the systemic level, namely impaired membrane receptor cooperation, alternative pathway activation, and presence of pre-existing chemoattractant factors in the serum, as reflected by the neutrophil function in the particular case of active RA patients. All, these abnormalities may synergistically contribute to RA pathogenesis.

Financial Suppport: CNPq

Bronchopulmonary and osteoarthritis caused by *Aspergillus fumigatus* as the primary clinical manifestation of a girl with autosomal chronic granulomatous disease

Chronic granulomatous disease (CDG) is a primary immunodeficiency characterized by early onset of severe recurrent infections. We report on a 10 year old girl whose primary clinical manifestation was a bronchopulmonary infection caused by *Aspergillus fumigatus* at age 7 years followed by osteoarthritis of the left hip. The control of the infection was accomplished by antifungal therapy and surgical procedures, resulting in sequelae of lungs and hip. The laboratory investigation revealed an abnormal DHR test. 30nM PMA-stimulated patient neutrophils were 372 MFI compared to 2463 MFI from healthy controls. 30nM PMA-stimulated patient monocytes were 78 MFI compared to 234 MFI from healthy controls. The superoxide release was also abnormal. 30nM PMA-stimulated patient neutrophils and monocytes released respectively 0,35 and 1,27 nmol of superoxide/10^6 cells/hr. Healthy controls data were respectively 16,72 and 15,55 nmol/10^6 cells/hr. Molecular genetic sequencing revealed a GT deletion at the beginning of exon 2 of *NCF1* gene, the most frequent genetic alteration of this gene. This case became clinically relevant as bronchopulmonary and osteoarthritis of the left hip caused by *Aspergillus fumigatus* were the primary clinical manifestations leading to the diagnosis of autosomal CGD at age 7 years in a girl with a previous history of no clinical relevant infections.

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RESTRICTED AND SKEWED TCR Vβ REPERTOIRE IN CHROMOSOME 22Q11.2 DELETION

Jerusa Marilda Arantes[1]; Marcília Sierro Grassi[1]; Natália Moreira Santos[2]; Leslie Kulikowski[1]; Luísa Guilherme[2]; Roberta Dutra[1]; Letícia Watanabe[1]; Cristina Jacob[1]; Cláudia Augusta Zago[1]; Magda Carneiro-Sampaio[1]

(1) Instituto da Criança-Hospital das Clínicas-USP, São Paulo, Brazil; (2) Instituto do

Coração-Hospital das Cínicas-USP, São Paulo, Brazil

I**ntroduction**: Chromosome 22q11 deletion is the most common human deletion and is found in the majority of patients with DiGeorge and velo-cardio-facial syndromes. Many patients have a mild to moderate immunodeficiency, and most have cardiac anomaly.

The objective is to evaluate TCR repertoire diversity in infants with 22q11.2 deletion identified at FMUSP ward for congenital heart diseases.

Methods and Results: TCR Vβ variable chain repertoire was analyzed by the TCRBV CDR3 lenght spectratyping technique, and repertoire diversity was quantified utilizing the complexity score (CS), that represents the sum of the number of peaks for each one of the 24 BV families. 22q11.2 deletion was detected utilizing multiplex ligation-dependent probe amplification.

First case report: A 9-month-old boy was identified in a survey among infants with complex congenital heart anomalies. He was born from non-consanguineous parents, weighing 2845g and presenting microcephaly, micrognathia, ocular hypertelorism and low set left ear, renal involvement, left atrial isomerism and pulmonary atresia. He also had hypocalcemia and hypoplastic thymus. He has lymphopenia=3,800 cells/mm3 (CD3=1,454 cells/mm3, CD4=888cells/mm3, CD8=537 cells/mm3), thrombocytopenia=55,000, IgG+=1,285mg/dL, IgM=123mg/dL, IgA=132mg/dL.

The patient presented CS=49, in contrast with 2 healthy age-matched male infants with 127 and 135. Four young healthy adults presented CS between 165 and 178. The patient presented mostly olygoclonal distribution and even absence of TCRBV families, while healthy donors exhibited mainly polyclonal non-Gaussian distributions.

Conclusion: The evaluation of new cases as well as the follow-up the patients will demonstrate if the repertoire diversity correlates with clinical severity.

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MESENCHYMAL STROMAL CELLS ISOLATED FROM MULTIPLE SCLEROSIS PATIENTS HAVE DIFFERENTIAL GENE EXPRESSION PROFILE AND DECREASED SUPPRESSIVE EFFECT

GISLANE LELIS VILELA DE OLIVEIRA[1]; KELEN C. RIBEIRO MALMEGRIM[1]; KALIL W. ALVES DE LIMA[1], DANIEL GUARIZ PINHEIRO[1], AMANDA COLOMBINI[1], PATRICIA B. VIANA[1], RODRIGO PANEPUCCI[2], DIMAS T. COVAS[1,2]; JULIO CESAR VOLTARELLI[1,2], EDUARDO A. DONADI[2]

[1]National Institute of Science and Technology in Stem Cells and Cell Therapy, Regional Blood Center of the School of Medicine of Ribeirão Preto, University of São Paulo, Ribeirão Preto, Brazil. [2]Department of Clinical Medicine, School of Medicine of Ribeirão Preto, University of São Paulo, Ribeirão Preto, Brazil.

Introduction: Mesenchymal stromal cells (MSCs) suppressive effects on immune cells have been the subject of extensive investigations and their use have been suggested in treatment of autoimmune diseases. We evaluated the gene expression profile and the *in vitro* immunomodulatory properties of MSCs isolated from multiple sclerosis patients. **Methods and Results:** Bone-marrow derived MSCs from patients and controls were isolated by plastic adherence. Total RNA was extracted and gene expression profile was performed by agilent microarrays slides. For the immunomodulatory assays, peripheral blood mononuclear cells (PBMCs) were labeled with CFSE and cocultivated (1:2 and 1:5 ratios) with patients' and controls' MSCs in the presence of PHA at 37°C in a 5% CO_2. After 5 days, T-cell proliferation was assessed by CFSE method and the percentage of $CD4^+CD25^{hi}Foxp3^+$ was assessed by flow cytometry. IL-10 and TGF-β was measured in coculture supernatants by CBA flex kit. The results were analyzed by limma t-test and one-way anova. This study was approved by the local ethics committee. We found 1317 genes significant differential expression of 641 genes differentially expressed in patients' MSCs ($p<0.05$). Of these, 578 genes were downregulated, including *tgfb1* and *hgf* and, 739 were upregulated, among them *il10*, *il6* and *ifngr1*. In coculture assays, we found differences ($p=0.002$) in

proliferation percentages when comparing PBMCs plus PHA (71.3 ± 12.9%) with patients' MSCs plus PBMCs at 1:2 (33.3 ± 13.4%) and 1:5 ratios (39.1 ± 21.9%). The inhibitory effect of patients' MSCs (52.15 ± 5.65%) on T-cell proliferation was significantly reduced (p=0.024) compared to controls' (69.37 ± 7.25%). There were no differences in percentages of $CD4^{+}CD25^{hi}Foxp3^{+}$ cells in cocultures with patients' and controls' MSCs. IL-10 and TGF-β secretion were significantly diminished ($P<0.05$) in supernatants from cocultures with patients' MSCs (IL-10: 53.8 ± 49.4; TGF-β: 59.4 ± 24.6 pg/mL) compared to controls (IL-10: 109.9 ± 64.6; TGF-β: 226.6 ± 153.2 pg/mL). **Conclusions:** Patients' MSCs have different gene expression profile and we found altered expression in genes involved in immunomodulatory functions. Additionally, although the MSCs isolated from MS patients were able to suppress T-cell proliferation, they showed decreased inhibitory effect and lower secretion of IL-10 and TGF-β cytokines. These data suggest that more studies should be conducted before their use in autologous clinical applications.

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HIGH LEVELS OF SOLUBLE CD40 LIGAND AND MMP-9 IN SERUM ARE ASSOCIATED WITH FAVORABLE CLINICAL EVOLUTION IN HUMAN VISCERAL LEISHMANIASIS

FABRÍCIA ALVISI DE OLIVEIRA [(1)]; PRISCILA LIMA DOS SANTOS [(1)]; ALINE SILVA BARRETO [(1)]; CARLA VANESSA OLIVEIRA SILVA [(1)]; NAYRA PRATA DAMASCENA [(1)]; RODRIGO OLIVEIRA PASSOS [(1)]; MALCOLM DUTHIE [(2)]; JEFFERY GUDERIAN [(2)]; AJAY BATHIA [(2)]; TATIANA RODRIGUES DE MOURA [(1)]; STEVEN REED [(2)]; ROQUE PACHECO DE ALMEIDA [(1, 3)]; AMÉLIA RIBEIRO DE JESUS [(1, 3)]

(1) Universidade Federal de Sergipe – Aracaju, Brazil; (2) IDRI- Infectious Disease Research Institute; (3) Instituto de Investigação em Imunologia (iii)

Introduction: Soluble CD40 ligand (sCD40L) and matrix metalloproteinase 9 (MMP-9) are inflammation markers and have been poorly described in infections disease. In this prospective study we describe the sera kinetics of these two molecules in the course of treatment follow up in human visceral leishmaniasis (VL).

Methods and results: Sera from VL patients were collected before and during follow up of regular Antimony treatment. sCD40L and MMP-9 were measured by Luminex assay.

While sCD40L and MMP-9 were not observed in sera from healthy control subjects at low risk of *Leishmania chagasi* infection, elevated levels were observed in sera from VL patients, and an increase in sCD40L and MMP-9 levels were detectable during the follow-up of VL patients undergoing antimony treatment. sCD40L levels were also high in individuals living in endemic settings at high risk of infection. Additionally, negative correlations were found between spleen sizes and MMP-9 before treatment and sCD40L at day 15 of treatment.

Conclusion: Our findings suggest that elevated sCD40L and MMP-9 levels are associated with resistance to infection and resolution of VL.

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Evaluation of leishmanicidal activity of macrophages isolated from treated and asymptomatic visceral leishmaniasis subjects

LUANA CELINA SERAPHIM CUNHA(1); CÁRCIA SANTANA PASSOS(1); PRISCILA LIMA DOS SANTOS(1); PAULO DE TARSO GONÇALVES LEOPOLDO (1); AMÉLIA RIBEIRO DE JESUS(1,2); TATIANA RODRIGUES DE MOURA(1); ROQUE PACHECO ALMEIDA(1,2)

(1) Universidade Federal de Sergipe – Aracaju, Brazil; (2) Instituto de Investigação em Imunologia, São Paulo, Brazil

Introduction: Visceral leishmaniasis (VL) is a systemic disease that is the most severe form of leishmaniasis with high morbidity, leading to death if not adequately treated. Depending on the interaction between the parasite and the host immune response, different clinical manifestations may develop: asymptomatic, oligosymptomatic and symptomatic. Macrophages play a central role in immune response. However, their microbicidal activity may be impaired, leading to the survival and proliferation of the parasites inside them. We report, for the first time, the leishmanicidal activity of macrophages from treated and asymptomatic subjects after infection by *Leishmania chagasi*.

Methods and results: Mononuclear cells were isolated from peripheral blood of 07 subjects (05 treated VL - the blood was taken 6 months after treatment and the patients were considered cured of the disease - and 02 asymptomatics, DTH positive) to obtain monocytes and further differentiation into macrophages. The macrophages were infected with *L. chagasi* for 2, 72 and 96 hours in conditions with and without LPS and IFN-□ stimulation□□ Macrophages of treated subjects, after stimulation with LPS and IFN-γ, were more infected with *L. chagasi* (40.78 ± 21.36) than those of asymptomatic (21.75 ± 13.81). Upon 72 hours, we observed that the infection was reduced in macrophages of the treated (27.48 ± 21.61), although the reduction was more evident in the macrophages of asymptomatic (8.75 ± 3.5). At 96 hours, macrophages of the treated did not control the infection (25.71 ± 22.46), independent of stimulus, unlike asymptomatic, that controlled the infection (6.87 ± 3.96) ($p = 0.008$, Mann-Whitney test).

Conclusion: We demonstrate that macrophages of treated subjects were more infected *Leishmania chagasi*, and they did not control infection, even in the

presence of stimulus, unlike asymptomatic's macrophages, which controlled infection.

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ANTITUMOR IMMUNITY INDUCED BY TRANSGENIC *Trypanosoma cruzi* IS ASSOCIATED WITH THE GENERATION OF EFFECTOR ANTIGEN-SPECIFIC CD8$^+$ T CELLS

LUARA ISABELA DOS SANTOS [1,2]; BRUNO GALVÃO FILHO[1,2]; PAULA CRISTINA BATISTA DE FARIA[1,2]; CAROLINE FURTADO JUNQUEIRA[2]; MAURÍCIO MARTINS RODRIGUES[3], DOUGLAS T. FEARON[4], LIS RIBEIRO DO VALLE ANTONELLI[2], RICARDO TOSTES GAZZINELLI[1,2].

[1] Department of Biochemistry and Immunology, UFMG, Belo Horizonte, MG, Brazil; [2] Rene Rachou Research Center, FIOCRUZ, Belo Horizonte, MG, Brazil; [3] Department of Microbiology, Immunology and Parasitology, UNIFESP, São Paulo, SP, Brazil; [4] Wellcome Trust Immunology Unit, University of Cambridge School of Clinical Medicine, MRC Centre, UK

Introduction: The identification of the Cancer Testis Antigen (CTA) allowed the development of new tools for immunotherapeutic approaches, such as cancer vaccines. Although multiple clinical trials have resulted in the development of measurable immune responses only a minority of patients has experienced clinical benefit, such as tumor regression. The use of a transgenic attenuated *Trypanosoma cruzi* strain (CL-14) as a vaccine vector expressing the CTA NY-ESO-1, proposed by our group, was able to prevent tumor growth in a vaccine model. In the current study, we propose elucidate the mechanisms involved in tumor inhibition afforded by vaccination with transgenic parasite and explore their therapeutic potential. **Methods and Results:** Mice immunized with transgenic parasite were challenged with melanoma cell line B16F10 expressing NY-ESO-1 and the tumor growth was scored by measuring perpendicular diameters. The results showed that the animals that received the boost dose were able to control tumor growth and and had their survival rate increased. However, over time the effectiveness of the vaccine was decreased. We observed that transgenic parasite were able to trigger both effector and memory effector CD8$^+$ T cells and that this first phenotype correlated to the capacity of tumor control while the second with decay. Furthermore, we found a higher number of antigen-specific effector CD8$^+$ T

cells in the time periods of better prognosis as well as higher production of IFN-gamma and IL-2 upon recombinant NY-ESO-1 stimulation. To increase T cell response induced by transgenic parasite we propose, in therapeutic protocol, the blockade of inhibitory signals mediated by cytotoxic T lymphocyte-associated antigen 4 (CTLA-4). The results showed promise since was observed increase of the percentage of effector cells, migration of $CD8^+$ T cells to tumor-infiltrating, production of IFN-gamma and IL-2 in animals treated with the proposed combination. Finally, we demonstrated that the treatment was able to efficiently control the growth of a very aggressive tumor.

Conclusion: This study showed that the stimulation of the immune system employing a transgenic parasite in combination with the neutralization of CTLA-4 increases the response effector providing control of melanoma growth and improving the survival of mice.

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Title: Proteomic analysis as a tool for identification of alterations in rheumatic and myxomatous mitral valve lesions

Authors: CARLO DE O. MARTINS[1]; KEITY SOUZA SANTOS[1]; PABLO M. A. POMERANTZEFF[2]; CARLOS M. A. BRANDÃO[2]; RONEY SAMPAIO [2], GUILHERME SPINA[2], JORGE KALIL[1]; EDECIO CUNHA-NETO[1]; LUIZA GUILHERME[1]

[1]Laboratory of Immunology of Heart Institute and LIM-19, [2]Surgical and Clinical Departments of Heart Institute, School of Medicine, University of São Paulo, São Paulo, SP, Brazil)

Introduction: Rheumatic Heart Disease (RHD) is a serious autoimmune disease following *S. pyogenes* sore throat in susceptible and untreated individuals affecting irreversibly the mitral and aortic valves. Myxomatous valve degeneration (MVD) with unknown etiology also affects preferentially mitral and aortic valves, causing prolapse, chordae rupture and myxomatous material deposition in the extracellular space. We present, for the first time to our knowledge, a comparison between protein expression in both RHD and MVD diseases.
Methods and Results: Human biopsies were collected according to the Ethics Committee of Heart Institute, Clinical Hospital. We extracted proteins from heart mitral valves in a chaotropic buffer, which were labeled with DIGE® (GE Healthcare) and submitted to 2-DE. A total of 28 spots, corresponding to 36 proteins were differentially expressed, when compared to normal valves. These spots were picked out and identified by LC-ESI-MS/MS. Of note, the extracellular matrix proteins collagen (COL6A1) and prolargin (PRELP) were decreased in RHD, whereas lumican (LUM) and vimentin (VIME) were increased. The spot corresponding to VIME was observed at 34 kDa, possibly due to cleavage in RHD. Vimentin is also target of antibodies from RHD sera, as observed by electroblotting. For MVD, LUM and biglycan (BGN) and superoxide dismutase (SOD2) are decreased.
Conclusion: This work supports evidence for different extracellular matrix disorganization mechanisms between RHD and MVD. Involvement of proteases in matrix degradation of MVD valves is a possible mechanism. The *in silico* analysis using Ingenuity Pathway Analysis (IPA) method suggested the participation of TGF-β in fibrosis and neovascularization in RHD lesions, as well as a decrease in NF-κB signaling. In addition a possible cleavage of VIME in RHD can account for cytoskeleton disassembly and autoantigenicity.

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ATOPIC DERMATITIS IN ADULTS: ELEVATION OF IGG4 AND IGE ANTIBODIES SERUM LEVELS DIRECTED AGAINST ENTEROTOXIN B FROM *STAPHYLOCOCCUS AUREUS*.

RAQUEL LEÃO ORFALI[1], TIAGO DE OLIVEIRA TITZ[1], VANESSA GONÇALVES DOS SANTOS[1], ALBERTO JOSÉ DA SILVA DUARTE[1], MARIA NOTOMI SATO[1], VALÉRIA AOKI[1]

[1]Laboratory of Dermatology and Immunodeficiency (LIM-56), Dermatology Department, University of Sao Paulo School of Medicine - Sao Paulo-SP – Brazil.

Introduction and Objectives: Atopic dermatitis (AD) is a chronic recurrent inflammatory disease, with prevalence around 10 to 20% in children and 1-3% in adults, and its diagnosis is based on clinical features. *Staphylococcus aureus* is present in 80-100% of atopic skin and is related with worsening of the disease by the action of enterotoxins (A, B, C, D, E, F and TSST-1). The aim of this study was to evaluate the profile of anti-*Staphylococcus aureus* enterotoxin B (SEB) antibodies in adults with AD.
Methods and Results: We selected 38 patients (19 male and 19 female) with AD, diagnosed by Hanifin & Rajka's criteria, aged between 18 and 65, and 26 non-atopic controls (6 male and 20 female), aged between 20 and 47 years. The severity of the disease was established according to EASI (Eczema Area and Severity Index) and patients graded as mild (28%), moderate (58%) and severe (14%). Blood samples from both groups were collected to evaluate IgG subclasses, IgA, IgM and IgE in the respective sera by ELISA directed against SEB. When comparing patients with AD and non-atopic controls, we observed an increase in IgG4 levels in patients. Five non-atopic controls and seven adults with AD did not show any IgG1 anti-SEB production. Elevated IgG1 and IgG3 serum levels were observed in patients with mild AD, when compared to severe cases. Adults with AD showed elevated IgE levels and low IgA and IgM levels, in comparison to controls.
Conclusion: In AD, the immunoglobulin-related subtype Th2 lymphocytes, such as IgE and IgG4, appear to be relevant in the humoral response targeted by superantigens. Moreover, there is a deficit of IgA production in the analyzed AD patients. These results emphasize the compromise of the humoral immune response profile in AD.

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IgE, IgG1 AND IgG4 REACTIVITY TO ANTIGENIC ISOFORMS FROM *DERMATOPHAGOIDES FARINAE* IN ATOPIC AND NON-ATOPIC PATIENTS

LEANDRO HIDEKI YNOUE[1]; JULIANA SILVA MIRANDA[1]; KARINE C. DE ALMEIDA[1]; ISABELLA LIMA SIMAN[1]; JAIR PEREIRA DA CUNHA-JÚNIOR[1]; DEISE A. O. SILVA[1]; ERNESTO AKIO TAKETOMI[1]

(1). Institute of Biomedical Sciences, Federal University of Uberlândia, Uberlândia, MG, Brazil.

Introduction: *Dermatophagoides farinae* (Df) is considered one of the major house dust mite and it is an important source of indoor allergens worldwide. Several Df allergens occur as isoallergens that present changes in amino acid sequences or glycosylation, leading to differences in allergen sensitization. The aim of this study was investigate the reactivity of IgE, IgG1 and IgG4 antibodies to Df antigenic isoforms in Brazilian atopic and non-atopic subjects, with potential application for the allergy laboratorial diagnosis and follow-up of specific allergen immunotherapy.
Method: Atopic (n=60) and non-atopic (n=30) subjects were selected on the basis of the respiratory allergy history and skin prick test (SPT) to house dust mite allergens. Sera were analyzed by enzyme-linked immunosorbent assay (ELISA) for measuring levels of IgE, IgG1 and IgG4 antibodies to Df allergen. Crude Df extract was separated by one (1-D)- and two (2-D)-dimensional electrophoresis with subsequent 1-D and 2-D immunoblot for evaluating the profile of IgE-, IgG1-, and IgG4-binding components from the Df allergen extract in sera from atopics and non-atopics. Levels of IgE, IgG1 and IgG4 specific to Df and frequencies of 1-D reactivity profile were used to select sera to 2-D analyses. 2-D IgE, IgG1 and IgG4 reactivity profile shown interestingly patterns of recognition and some spots were characterized.
Result: Single IgE and double IgE/IgG4 binding in atopic migrated only below 37 kDa and single IgG1 binding migrated exclusively above 37 kDa. The immunoproteomics approach in both atopic and non-atopic groups showed a great number of antigenic components and many of them were related to already characterized allergens.
Conclusion: The immunoreactivity of these proteins observed may be potentially useful for serodiagnosis and opens further opportunities for the development of a personalized immunotherapeutical composition for treating patients with allergic disease.

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EVALUATION OF TUMOR NECROSIS FACTOR BETA (TNF-B) *NCO*I GENETIC POLYMORPHISM AND SERUM CYTOKINE LEVEL IN PATIENTS WITH MULTIPLE SCLEROSIS FROM LONDRINA AND REGION, PARANÁ

ANA PAULA KALLAUR[1]; SAYONARA RANGEL OLIVEIRA[1]; ANDREA NAME COLADO SIMÃO[2]; ELAINE REGINA DELICATO DE ALMEIDA[2];HELENA KAMINAMI MORIMOTO[2]; LARISSA MULITERNO PELEGRINO[3]; WILDEA LCJ PEREIRA[3]; DAMACIO RAMON KAIMEN-MACIEL[3,4] EDNA MARIA VISSOCI REICHE[1,2]

[1]Health Sciences Postgraduate Program, Health Sciences Center, State University of Londrina, Londrina, PR, Brazil; [2]Department of Pathology, Clinical Analysis and Toxicology, Health Sciences Center, State University of Londrina, Londrina, PR, Brazil; [3]Outpatient Clinic for Multiple Sclerosis, Health Sciences Center, State University of Londrina, Londrina, PR, Brazil; [4]Department of Clinical Medicine, Health Sciences Center, State University of Londrina, Londrina, PR, Brazil.

Introduction: Variations in genes coding for the expression and regulation of cytokines may play a role in multiple sclerosis (MS). The aims of this study were to determine the frequency of TNF-β *Nco*I polymorphism among MS patients, to investigate the association between TNF-β *Nco*I polymorphism with the susceptibility for MS, clinical progression, and activity of the disease, and to evaluate the serum cytokines levels in relapsing-remitting MS (RR-MS) patients.

Methods and Results: 220 MS patients and 292 blood donors from Londrina, PR were enrolled. The disability of patients was evaluated using the Expanded Disability Status Scale (EDSS) at the time of the enrollment and five-year follow-up. The disease activity was evaluated using magnetic resonance imaging (MRI) with the gadolinium (Gd). Genomic DNA was extracted from PBMCs and a 782 base-pair fragment of the TNF-β gene was amplified using PCR. The PCR products were subjected to *Nco*I restriction digestion and analyzed by RFLP. The cytokines IL-1β, IL-4, IL-6, IL-10, IL-12, IL-17, IFN-γ, and TNF-α level was

assessed in serum from 169 RR-MS patients and 132 controls. The frequency of TNFB2/B2 genotype of MS patients differed from the TNFB1/B2 genotype in MS patients and controls (p=0.0449) and when compared with the TNFB1/B1 genotype in MS patients and controls (p= 0.0225). MS patients carrying the TNFB2/TNFB2 genotype presented 1.49 more chances to develop MS than controls when compared with the heterozygous genotype carriers (95% CI: 1.023-2.170); and 2.058 more chances to develop MS when compared with the TNFB1 homozygous carriers (95% CI: 1.099-2.855). Individuals carrying the TNFB2 allele exhibited 1.427 more chances to develop MS than TNFB1 allele carriers (95% CI: 1.093-1.863). Serum IFN-γ, IL-6, IL-12, and IL-4 levels were higher among the RR-MS patients than controls (p<0.05). Serum IL-4 level was higher in RR-MS patients with mild disability than those with moderate and severe disability (p=0.0375). Serum TNF-α and IL-10 levels were higher between RR-MS patients with negative Gd than those with positive Gd (p=0.0457 and p=0.0533, respectively).

Conclusion: TNF-β *Nco*I polymorphism may contribute for the MS susceptibility and the RR-MS patients exhibited a balance of the cytokines with the modulation of the pro-inflammatory, Th1, Th17, and Th2 cytokines. The elevated TNF-α and IL-10 serum levels may reflect the immune response during the remission of the disease.

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MUSCLE ATROPHY DEVELOPMENT IN MURINE MODEL OF ARTHRITIS IS RELATED TO DISEASE SCORE

LIDIANE ISABEL FILIPPIN(1), VIVIAN DE OLIVEIRA NUNES TEIXEIRA(2), PRISCILA SCHMIDT LORA(3), PAULA RAMOS VIACAVA(4).

(1) - Serviço de Reumatologia, HCPA
(2) - Faculdade de Medicina, UFRGS
(3) - Faculdade de Medicina, UFRGS
(4) - Serviço de Reumatologia, HCPA

Introduction: Rheumatoid arthritis (RA) is an inflammatory autoimmune disease of unknown etiology, affecting mainly the joint but also other tissues. RA patients usually present weakness and muscle atrophy, non-articular manifestations of the disease. Although causing great impact, the understanding of muscle atrophy, its development and the mechanisms involved are still very limited. The objective of this study is to evaluate the development of muscle atrophy in skeletal muscle of a murine model of arthritis. **Methods and Results:** The experimental murine model of collagen induced arthritis (CIA) was used (Nature Protocols 2: 1269-75, 2007). DBA/1J mice were randomly divided into three groups: control (CO, n=25), sham arthritis (SA, n=25) and arthritis (CIA, n=28), analyzed in different time-points: 25, 35 and 45 days after the induction of arthritis. Significance was considered $p<0.05$. The arthritis development was followed by clinical scores and hind paw edema three times a week. These parameters were enhanced increasingly in CIA in all experimental times, demonstrating greater difference than the other groups in 45 days (clinical score – CO: 0±0, SA: 4.1±1.6, CIA: 15±1.6). Spontaneous exploratory locomotion and weight were evaluated weekly. CIA was lighter during all experimentation period with a difference of 6% from CO in 45 days (CO: 22.1±0.4, SA: 22.6±0.4, CIA: 20.9±0.4 g). CIA animals also demonstrated progressive decrease in distance walked, with a reduction of 54% in 35 and 74% in 45 days (CO: 164.7±21.6, SA: 145.9±15.7, CIA: 42.6±10.4 cm). Cytokine analysis was evaluated by CBA in serum collected before the death of the animals in different time-points and identified increased IL-6 in CIA than CO and SA in all experimental times. Cross-sectional area (CSA) of the myofiber of gastrocnemius (GA) skeletal muscle was measured and was decreased 26% in CIA in 45 days

after the induction of disease (CO: 808±84, SA: 813±116, CIA: 549±120 μm^2). There was significant and inverse correlation between the disease clinical score and myofiber CSA in 45 days (GA: r= -0.71). **Conclusion:** These observations are relevant to understand the development of muscle loss, as well as for the design of future studies trying to understand the mechanisms involved in muscle wasting. As far as we concern, this is the first study to evaluate the relation between disease score and muscle atrophy in a model of arthritis. **Financial support:** CAPES, CNPq, FAPERGS, FIPE-HCPA.

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ENHANCED SPONTANEOUS TCD4$^+$ APOPTOSIS AND SPREAD CHANGES IN APOPTOSIS-RELATED GENES EXPRESSION CORRELATE TO LYMPHOPENIA AND CD4/CD8 INVERSION IN CVID PATIENTS

BUZZETO, LILIAN CRISTINA [1;2]; KOKRON, MARIA CRISTINA [3,4]; COLÓ, ANNA LUIZA ESTELA [2]; SARDINHA, LUIZ ROBERTO [2], AMARANTE-MENDES, JOÃO GUSTAVO PESSINI [1], RIZZO, LUIZ VICENTE [2].

[1] Department of Immunology, University of São Paulo, Brazil. [2] Hospital Israelita Albert Einstein, São Paulo, Brazil. [3] Laboratory of Medical Investigation 60, University of São Paulo, Brazil [4]Division of Clinical Immunology and Allergy, University of São Paulo, Brazil.

Introduction and aim: Common Variable Immunodeficiency (CVID) is the most frequent diagnosed primary immunodeficiency in humans and it is characterized by low immunoglobulins levels and higher susceptibility to infections. T cell dysfunctions and lymphopenia affect up to half of CVID patients. To address the status of the T lymphocytes in our cohort, we analyzed cell distribution and spontaneous apoptosis in peripheral blood and apoptotic-related genes expression in CD4$^+$ and CD8$^+$ sorted cells in CVID patients. **Methods:** Peripheral blood mononuclear cells were isolated by Ficoll gradient. Quantitative analysis of CD4$^+$, CD8$^+$, CD45RA$^+$, CD45RO$^+$ and Annexin V$^+$ cells were performed by flow cytometry. Spontaneous apoptosis *in vitro* was evaluated after 2 hours. CD4$^+$ and CD8$^+$, sorted by FACS, were analyzed for apoptotic-related gene expression by quantitative PCR. **Results:** Nineteen CVID patients (mean age 40.7, range: 20 to 77 years) and 19 healthy subjects (mean age 38.7, range: 21 to 75 years) were enrolled. Patients showed decreased frequency of CD4$^+$ (p = 0.005) and CD4$^+$CD45RA$^+$ (p = 0.02), higher frequency of CD8$^+$ (p = 0.02) and lower CD4/CD8 ratio (p = 0.0008) when compared to controls. CVID subjects presented enhanced apoptosis in CD4$^+$ cells (p = 0.04) and total lymphocytes (p = 0.01). CD4$^+$ cells from patients showed lower expression of death receptors TRAIL-R1 (p = 0.03) and TRAIL-R2 (p = 0.01), anti-apoptotic Bcl-2 (p = 0.0009), death receptor inhibitor cFLIP$_{Long}$ (p = 0.03) and death receptor adapter molecule FADD (p = 0.0004); CD8$^+$ cells displayed reduced expression of death ligand TRAIL (p = 0.0003),

death receptor adapter molecule FADD ($p = 0.003$), anti-apoptotic Bcl-2 ($p < 0.0001$) and BCL –xL ($p = 0.03$) and enhancement of death receptor inhibitory molecule $cFLIP_{Short}$ ($p = 0.01$). **Conclusion:** CVID patients have reduced frequency of $CD4^+$, enhanced $CD4^+$ apoptosis and spread changes in apoptosis-related genes expression. Lower levels of anti-apoptotic molecule Bcl-2 in T cells from CVID patients helps to explain the frequency reduction and apoptosis of $CD4^+$. $CD8^+$ also enhancement of extrinsic pathway of apoptosis inhibitory molecule $cFLIP_{Short}$, what could contribute to the higher survival rate of $CD8^+$ cells when compared to $CD4^+$. These results help to partially clarify mechanism responsible for lymphopenia and CD4/CD8 inversion in CVID and reinforces T that cells compartment is also impaired in the disease.

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CHANGES IN THE PRODUCTION OF IL-10 AND TNF-α IN SKELETAL MUSCLE OF MICE WITH HEART FAILURE SECONDARY TO G-CSF AND STEM CELLS ADMINISTRATION

ELISA BROSINA DE LEON (1,2); NADJA PINTO GARCIA (2); LIZIARA FRAPORTI (2); GLEICY ALMEIDA (2); JOÃO PAULO DINIZ PIMENTEL (2,3); ADRIANA MALHEIRO (1,2).

(1). Universidade Federal do Amazonas; (2). Fundação de Hematologia e Hemoterapia do Amazonas; (3). Instituto Leônidas e Maria Deane/Fiocruz-AM.

Introduction: Despite intense research efforts, the symptons, as exercise intolerance, due to heart disease remains at an unacceptable level. A better understanding of the peripheral mechanism involved in heart failure is necessary to develop specific therapeutic strategies, as stem cells (SC) or granulocyte colony-stimulating factor (G-CSF) to slow or prevent muscular atrophy present in this syndrome. Recent studies show that the proinflammatory cytokine tumor necrosis factor-α (TNF-α) is overexpressed and the anti-inflammatory cytokine interleukin-10 (IL-10) is downregulated in heart failure. These changes have been demonstrated both in the plasma and heart muscle and in skeletal muscle. This study aimed to investigate the production of TNF-α and IL-10 in hamstrings muscles of animals with heart failure. **Methods and Results:** We used female Swiss mice (n=5) that underwent ligation of the left coronary artery. One week after this procedure, the animals were submitted into treatment with PBS (Control), G-CSF (G-CSF), G-CSF associated with SC using intracardiac route (G-CSF+$SC_{(IC)}$) or SC using intramuscular route ($SC_{(IM)}$). They remained under observation for a further period of 4 weeks, then, were collected peripheral muscle for analysis. Cytokines levels were measured by Enzime-Linked Immunosorbent Assay and the data of cytokines are shown as mean fluorescence intensity (MFI). Our results showed significant ($p<0.05$) increase of cytokine TNF-α level by 38% after G-CSF administration (Control, MIF=0.76; G-CSF, MIF=1.06). There were no changes in G-CSF+$SC_{(IC)}$ (MIF=0.71), but we found a decreased by 61% in TNF-α levels after SC administration in the hamstrings muscles ($SC_{(IM)}$, MIF=0.47). IL-10 levels also were measured in hamstrings muscles and the results not shown statistics difference (Control, MIF=0.5; G-CSF, MIF=0.36; G-CSF+$SC_{(IC)}$, MIF=0.56; $SC_{(IM)}$, MIF=0.25; $p=0.1$). The decrease (50%, $p<0.05$) in the IL-10/TNF-α ratio was due to both increased tissue levels of TNF-α and decreased tissue levels of IL-10 in G-CSF group. In G-CSF+$SC_{(IC)}$ group there was an increase in IL-10 that alter the rate, increasing 29%. The IL-10/TNF-α ratio decreased 10% due both decrease in TNF-α and IL-10 levels. **Conclusion:** Our results showed significant changes in the IL-10/TNF-α ratio, which were changed according to the treatment. The date

suggests that G-CSF promoted an increase in TNF-α and an imunommodulation after SC administration in peripheral muscle.

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SKELETAL MUSCLE: EDEMA AND LOCAL AND LONG-DISTANCE INFLAMMATION CAUSED BY Crotalus VENOM - BAHIA - BRAZIL

NATALY SOUZA DE OLIVEIRA[1]; FLAVIANE SANTOS DE SOUZA[1]; DULCINÉA F. ANDRADE[1]; ILKA BIONDI[1]

1. UEFS - Feira de Santana State University (Portuguese: Universidade Estadual de Feira de Santana, short UEFS) - Laboratory of Venomous Animals and Herpetology (Portuguese: Laboratório de Animais Peçonhentos e Herpetologia, short LAPH). Email: naty.cbio@yahoo.com.br

Introduction - Accidents caused by *Crotalus durissus* in Brazil are classified as moderate and severe, mainly due to neurotoxic activity induced by crotoxin. On the other hand these accidents are from minor local changes such as pain and edema. In the State of Bahia, the clinical data of *Crotalus* accidents demonstrate the occurrence of edema and the patients reported pain at bite site. The purpose of this study was to evaluate the real local injuries caused by the venom of *Crotalus*. **Methods and Results** – It was used murine as model experiment, by the following tests: (i) the local edema induced activity, (ii) local inflammatory response induction (iii) measurement of cytokines (IL-1β and IL-6) at the site of inoculation, and (iv) histopathological analysis of the footpad and skeletal muscle regions. We observed dissociated dermal collagen fibers and vascular endothelium swelling, with accumulation of mononuclear inflammatory infiltrate with leukocytes diffusely distributed. Histopathologic analysis demonstrates edema, vacuolation, degeneration and destruction of muscle fibers, and acute diaphragm myositis and inflammatory infiltrate with presence of lymphocytes. **Conclusion** - It was concluded that the venom induces at the inoculation site a severe interstitial edema with recruitment of inflammatory cells around venules (perivascular leukocytes) and induces the production of IL-1β and IL-6.

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DEVELOPMENT OF A SIMPLIFIED METHOD TO ANALYZE HUMAN MONOCYTE FUNCTION IN RESPONSE TO *Mycobacterium tuberculosis* INFECTION

MILENA SOBRAL ESPÍNDOLA (PG)[1,2]; PATRÍCIA APARECIDA ASSIS (PG)[1,2]; LUANA SILVA SOARES (PG)[1,2]; LEONARDO JUDSON GALVÃO DE LIMA (PG)[1,2]; MAIRA DA COSTA CACEMIRO (PG)[2]; LÚCIA HELENA FACCIOLI[1,2]; FABIANI GAI FRANTZ[1,2]

(1) Pós-Graduação em Imunologia Básica e Aplicada, Departamento de Bioquímica e Imunologia – Faculdade de Medicina de Ribeirão Preto – USP; (2) Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Departamento de Análises Clínicas, Bromatológicas e Toxicológicas – USP

Introduction: *Mycobacterium tuberculosis* is an intracellular pathogen, capable of survive and replicate in human macrophages. Host cells developed various mycobactericidal and immunoregulatory mechanisms to control bacterial growth. However, cells from immunocompromised hosts, such as HIV+ infected individuals, may not be able to control infection. Thus, the aim of this work was to develop a method that could measure the main functional activity of human monocytes in a simplified manner.

Methods and Results: Peripheral blood mononuclear cells from healthy individuals were obtained using Ficoll-Paque protocol. Monocytes were separated by adherence overnight, and then infected with different MOI of *Mycobacterium tuberculosis*. Phagocytosis, bacterial growth control and cell viability were evaluated by resazurin reduction test and analyzed in fluorimeter. Supernatants of cell culture were collected after 24h and used to measurement of nitric oxide and cytokines, using Greiss reaction and ELISA, respectively. In additional experiments, to evaluate the monocytes function regulation, LPS, GM-CSF and PMA were added in cell cultures 18h before infection. After *in vitro* infection, we only detected phagocyted bacteria using MOI 10, 5 and 2, after 24h of resazurin metabolization. However, infection with MOI 10 caused reduced cell viability. Therefore, MOI 5 was chosen to be used in other experiments. The infection induced high levels of MCP-1, IL-1 beta and IL-6. In contrast, there was no nitric oxide production. Further, we verified high phagocytosis index and bacterial growth control in 4 healthy individuals, which was not enhanced after priming with LPS, PMA or GM-CSF.

Conclusion: In conclusion, resazurin reduction test is an efficient technique to evaluate phagocytosis and mycobacterial growth control in human monocytes, showing that it can be effectively used to compare functional activity from groups of research interest.

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MOLECULES PRODUCED BY FERMENTATION OF THE INTESTINAL MICROBIOTA PROTECT FROM ACUTE KIDNEY INJURY.

VINICIUS ANDRADE-OLIVEIRA[1]; MARIANE T. AMANO[1;] MATHEUS CORREA-COSTA[1]; ANGELA CASTOLDI[1]; PEDRO M. MORAES-VIEIRA[1]; JEAN S. PERON[1]; ENIO J. BASSI[1]; MEIRE H. HIYANE[1]; CAROLINE M. FERREIRA[2]; MARCO A. VINOLO[2]; RUI CURI[2]; NIELS OLSEN CÂMARA[1]

[1]Department of Immunology; [2]Department of Physiology and Biophysics, Institute of Biomedical Sciences, University of Sao Paulo, Sao Paulo, Brazil.

Introduction: Short chain fatty acids (SCFA) such as acetate, propionate and butyrate, are the end-products released from the fermentation of complex carbohydrates by bacteria of the intestinal tract, for which have been ascribed anti-inflammatory roles. **Objective**: to evaluate the role of SCFA in models of acute kidney injury whereas inflammation plays a major role. **Methods:** Mice were submitted to two model of AKI injury (ischemia-reperfusion injury bilateral (IRI) and sepsis). SCFA were given individually (200 mg/kg) or in pool (200 mg/kg of each), 0.5h before ischemia and at the time of reperfusion. After 24h, samples were collected for analyses. A p value was considered significant when ≤ 0.05. **Results:** SCFA induced better renal function, when administered isolated or in pool when compared to untreated group, (n=5 for all groups and $p<0.0001$), being acetate associated with the best protection. This improvement was due to lower protein levels of inflammatory molecules as IL1-β, IL-6 and MCP-1. A reduction in the influx of macrophages, activated dendritic cells and neutrophils was also seen in SCFA-treated animals. Interestingly, an amelioration of the systemic inflammatory response was also achieved, better seen by the reduced serum levels of MCP-1, IL1A and RANTES. All together, these data was corroborated by a decreased activation of NF-κB pathway. The level of apoptosis also reduced in kidney of animals treated with SCFA. In vitro, it was observed that these SCFA modulate of the activation of dendritic cells and inhibition of lymphocyte proliferation. Finally, we observed that treatment with acetate modulated the expression of genes related to chromatin modification (epigenetic regulation) in the kidneys of animals submitted to IRI. **Conclusion**, the SCFA is a promising tool in the protection of the AKI. Support: FAPESP, CNPq (LIA, CNPq/Inserm). Complex Fluids INCT, and CEPID-FAPESP.

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ANTI-INFLAMMATORY MEDIATORS IS ASSOCIATED WITH REGULATORY PROFILE IN PATIENTS SERA WITH ACTIVE DIFFUSE CUTANEOUS LEISHMANIASIS (DCL)

JAQUELINE FRANÇA-COSTA (PG) (1); VIVIANE BOAVENTURA (1,2); ANA SALDANHA (3); HAYNA MALTA (IC)(1); NÍVEA LUZ (PG)(1); DANIELA CAMPOS (IC)(1); MURILO OLIVEIRA (IC)(1); ALDINA BARRAL (1,2); JACKSON COSTA (1); VALÉRIA DE MATOS BORGES (1,2).

(1). GONÇALO MONIZ RESEARCH/OSWALDO CRUZ FOUNDATION/FIOCRUZ, SALVADOR - BA - BRASIL; (2). FEDERAL UNIVERSITY OF BAHIA/INCT/III, SALVADOR - BA – BRASIL; (3). FEDERAL UNIVERSITY OF MARANHÃO, SÃO LUIS - MA – BRASIL; .

Introduction: Diffuse Cutaneous Leishmaniasis (DCL) is a rare clinical manifestation of tegumentary leishmaniasis caused by *Leishmania amazonensis*. It is characterized by an inefficient parasite-specific cellular response and heavily parasited macrophages. It has been demonstrated “in vitro” that murine macrophage infection by *L. amazonensis* increases arginase I, TGF-β and PGE2 contributing to parasite proliferation enhancement. However, the relevance of these mediators for DCL pathogenesis remains unknown. Here, we evaluate systemic release of inflammatory mediators in DCL patients.
Methods and Results: Sera from 12 active DCL patients and 35 endemic health controls (HC) from Maranhão were obtained between 1980 and 1990. All patients had clinical and laboratory diagnosis of DCL, whereas 15 HC had positive skin response to leishmanin (DTH+) and 20 had DTH-. Serum samples were evaluated for arginase I, TGF-β, PGE2, LTB4 and MCP-1 levels by ELISA. The levels of arginase, TGF-β1 and PGE2 , mediators involved with macrophage deactivation, were increased in the active DCL sera (3,4; 603; 379 pg/ml, respectively) compared with DTH+ (0,16; 294; 43 pg/ml) and DTH- (0,12; 262; 36 pg/ml), indicating a involvement of these anti-inflammatory mediators in DCL clinical state. Additionally, MCP-1 amount, a chemokine that stimulates the oxidative burst in macrophages, was decreased in DCL patients (23 pg/ml) compared with DTH+ (524 pg/ml) and DTH- HC (185 pg/ml). In another hand LTB4, an eicosanoid known by opposite PGE2 effects, did not show difference between DCL (283 pg/ml) and DTH+ (378 pg/ml) or DTH- HC (386 pg/ml). However, PGE2/LTB4 ratios were higher in DCL patients compared with DTH+ or DTH- HC, supporting an anti-inflammatory immune profile in DCL patients. Interestingly, arginase and TGF-β were also higher in DCL patients (603; 4,4 pg/ml, respectively) when compared with sera from patients which present the responsive poles of

Leishmaniasis, Localized (126; 0,7 pg/ml) and Mucocutaneous Leishmaniasis (164; 0,4 pg/ml).

Conclusions: Our data suggest that a regulatory profile might be implicated in the inability of DCL patients to mount an efficient immune response against *L. amazonensis.* Investigating the involvement of arginase and eicosanoids in DCL pathogenesis can favor the development of new strategies for parasite proliferation controlling.

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EFFECTS OF FISH OIL SUPPLEMENTATION ON PRODUCTION OF INFLAMMATORY MARKERS AND WEIGHT LOSS IN PATIENTS WITH COLORECTAL CANCER IN CHEMOTHERAPY

MICHEL CARLOS MOCELLIN[1]; JULIANA DE AGUIAR PASTORE E SILVA[2]; PAULA FERNANDA DE OLIVEIRA[1]; EVERSON ARAUJO NUNES[1]; YARA MARIA FRANCO MORENO[1]; MARIA EMÍLIA DE SOUZA FABRE[2]; SCHEILA GEVAERD[2]; CAROLINA DE QUADROS CAMARGO[1]; ERASMO BENICIO SANTOS DE MORAES TRINDADE[1]

(1). Federal University of Santa Catarina; (2) Ambulatory Care Clinic and Oncology Research Center

Introduction: Cells and inflammatory mediators are essential components of the tumor microenvironment. The presence of a chronic inflammatory response associated with cancer can result in worse clinical outcomes. The aim of this study was to investigate whether fish oil supplementation results in changes on production of inflammatory markers and weight loss induced by colorectal cancer and its chemotherapy treatment.

Methods and Results: Eleven patients with colorectal cancer and indication for chemotherapy treated at the Ambulatory Care Clinic and Oncology Research Center of Florianópolis, SC – Brazil, were enrolled in this prospective, randomized, clinical trial. Patients were randomly allocated to consumption 2 g/day of fish oil encapsulated containing 600 mg of EPA plus DHA (Omega 3®, PHITOMARE, Governador Celso Ramos, SC, Brazil) for nine weeks starting on the day of first chemotherapy session (Supplemented Group – SG, n=6) or no placebo formula (Control Group – CG, n=5). Weight, height, plasmatic cytokines, C-Reactive Protein (CRP) and albumin were evaluated the day before the first chemotherapy and nine weeks later. The means of age, weight and BMI were 54.5 years (±9.8 y), 70.4 kg (±11.6 kg) and 27.66 kg/m² (±5.16 kg/m²), respectively. IL-1β, IL-10, IL-17A and TNF-α values were not affected by supplementation or chemotherapy, did not differ between times and study groups ($p>0.05$). Plasma CRP levels decreased in the supplemented group whereas increase at control group (medians of mean difference: -1.24 *vs* 9.93 mg/L, $P = 0.006$). Plasmatic albumin decreased slightly in the CG while remained in the SG ($p>0,05$). Patients in the supplemented group maintained or gained weight during the nine weeks of chemotherapy while the control group tended to lose (medians of mean difference: 1.2 *vs* -0.5 kg, $P=0,72$).

Conclusions: The supplementation of 2 g / day of fish oil in colorectal cancer undergoing chemotherapy has resulted in positive immunomodulatory effects, reducing the levels of PCR, keeping the levels of albumin, and preventing weight loss induced by cancer and its treatment.

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PHENOTYPIC CHARACTERIZATION OF CD4 T CELLS IN ASTHMATIC PATIENTS IN PORTO ALEGRE/RS

PATRÍCIA DIAS DE ARAÚJO[1], ANA PAULA SOUZA[1], BÁRBARA PORTO[1], GIOVANA DOS SANTOS[1], MARCUS JONES[1], PAULO MARCIO CONDESSA PITREZ[1], RENATO STEIN[1], CRISTINA BONORINO[1]

1. Instituto de Pesquisas Biomédicas, Pontifícia Universidade Católica do Rio Grande do Sul (PUCRS).

Email: patriciadiasdearaujo@hotmail.com

Introduction: Asthma is a problem of public world health that mainly affects children and it is characterized by airway hyperresponsiveness (AHR), recruitment of leukocytes to lung and tissue remodeling. Asthma can be divided in atopic and non-atopic status. The aim of this study was to characterize the phenotype of TCD4 cells in asthma measuring their receptive transcription factors: Tbet, GATA-3, RORγT and Foxp3. **Methods and Results:** Forty-three school children aged 8 to 14 years from schools of Porto Alegre were included in the study if they were diagnosed with asthma without cold in the last 12 months or use asthma medications (n=37) and without symptoms as a control group (n=6). This study was approved by ethics commitee of PUCRS and written informed consent was obtained from children's responsible. Peripheral blood mononuclear cells (PBMC) were purified from patient's whole blood using a Histopaque-1077 gradient. PBMC were stimulated with anti-CD3/anti-CD28 or left unstimulated and the expression of transcription factors Tbet, GATA-3, RORγt and Foxp3 were analyzed by flow citometry. When we analyzed the unstimulated cells we found that the asthmatic patients have a higher percentage of Th1 and Th17 phenotype comparing with healthy children. The mean percentage of CD4+GATA+ cells were 3.9 ± 5.22 in asthmatic group and 0.12 ± 0.12 in healthy children (p=0.001), and the mean percentage of CD4+ RORγt + cells were 0.65 ± 0.52 in healthy children and 4.70 ± 5.00 in asthmatic patients were (p=0.41). When we analyzed the stimulated cells with anti-CD3 and anti-CD28 we found that asthmatic patients maintained a higher percentage of Th2 cells, however they have an increase amount of Th1 (mean 15.6 ± 15.4 versus 6.7 ± 4.7) and Treg (mean 4.7 ± 3.4 versus 1.2 ± 1.3) comparing to control group. **Conclusion:** These results suggested that asthmatic patients have two predominant phenotype Th2 and Th17 comparing to healthy children and after we

can modulate phenotype. This data are preliminary we will also analyze this cells after stimulation with Derp1 protein from HDM and compare atopic and non-atopic patients.

Keywords: Asthma, T helper cells, asthmatic children

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SERUM LEVELS OF CYTOKINES ASSOCIATED WITH ATHEROSCLEROSIS IN BRAZILIAN PATIENTS LIVING IN BAHIA

MARIANA M. PEREIRA[1]; TACIANA P. SANT'ANA SANTOS[2]; ISABELA S. OLIVEIRA[1]; ROQUE ARAS[3]; RICARDO DAVID COUTO[4]; AJAX M. ATTA[4]

[1] Programa de Pós-Graduação em Imunologia da UFBA, [2] Programa de Pós-Graduação em Farmácia da UFBA, [3] Serviço de Cardiologia do Hospital Ana Nery, [4]Departamento de Análises Clínicas e Toxicológicas da UFBA

Introduction: Atherosclerosis is an important world health problem whose immunopathogenesis has the involvement of macrophages, lymphocytes and cytokines. However, the immune mediation of this disease has been little studied in Brazil. **Objective:** The aim of this study was to investigate the serum levels of some important cytokines associated with atherosclerosis in Brazilian patients living in Salvador-BA that had clinical diagnosis of cardiovascular diseases and were treated for dyslipidemia with statin. **Methods and Results:** One hundred and sixty-two patients, 80 women (age = 60 ± 10 years) and 82 men (age = 59 ± 8 years), from the Cardiology Service of the Hospital Ana Nery (Bahia, Brazil) participated of this study. One hundred and thirty-nine out of 162 (85.8%) patients had hypertension, 103 out of 162 (62.0%) showed abnormal coronary angiography (CA) and 67 out of 162 (41.4%) had a previous documentation of myocardial infarction (MI). The serum levels of IL-1β, IL-6, IL-8, IL-10, MCP-1, TNF-α and TGF-β were determined by capture ELISA. Female patients with previous documentation of myocardial infarction in their medical files were compared with female patients without MI and had higher median level of both TNF-α (9.5 pg/mL vs. 5.8 pg/mL, $P < 0.05$) and IL-8 (15.8 pg/mL vs. 8.2 pg/mL, $P < 0.05$), but they had lower median level of IL-10 (6.3 pg/ml vs. 5.4 pg/mL, $P < 0.05$). No difference was observed in the serum levels of cytokines and chemokines when male patients were classified using their results of coronary angiography or previous documentation of MI ($P > 0.05$). **Conclusion:** The serum levels of TNF-α, IL-8 and IL-10 must be investigated in female patients with dyslipidemia to evaluate their importance to predict MI.

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REGULATORY T CELLS AND T, B AND NK LYMPHOCYTES IN CHRONIC HEPATITIS C

ISABELA OLIVEIRA 1, BARTIRA MENEZES 2, LUCAS CARVALHO 3, AJAX M. ATTA 4, MARIA LUIZA B SOUSA-ATTA 4

1 Programa de Pós-graduação em Imunologia, Instituto de Ciências da Saúde, Universidade Federal da Bahia, Salvador, Bahia, Brasil

2 Programa Institucional de Bolsas de Iniciação Científica (PIBIC/CNPq/UFBA), Faculdade de Farmácia, Universidade Federal da Bahia, Salvador, Bahia, Brasil

3 Serviço de Imunologia, Hospital Universitário Prof. Edgard Santos, Universidade Federal da Bahia, Salvador, Bahia, Brasil

4 Departamento de Análises Clínicas e Toxicológicas, Faculdade de Farmácia, Universidade Federal da Bahia, Salvador, Bahia, Brasil

Introduction: The hepatitis C virus (HCV) causes a chronic infection in most infected individuals. The mechanisms that promote viral persistence are not completely known. Thus, some mechanisms have been proposed: mutations in HCV epitopes, depletion of effector T cells, defects in antigen presentation and suppression of regulatory T cells (Treg) by viral proteins. **Objective:** The aim of this work is to investigate the frequency of CD4+CD25+ FoxP3+ Treg cells and T, B and NK cells in patients chronically infected with HCV before and after antiviral treatment with α-interferon plus ribavirin. **Methods and Results:** The frequency of the cells was carried out by flow citometry and analyzed by the FlowJo software (Tree Star). Ten patients (5 men and 5 women, mean age 48.2 ± 8.4 years) and 10 healthy individuals (5 men and 5 women, mean age 48.7 ± 7.3 years) were included in the study. Preliminary results show a decrease in the frequency of CD4+T cells in HCV carriers (median = 38.0%) compared to controls (median = 45.2%, P = 0.04) and increased frequency of B lymphocytes in the patients (median = 10.1%) compared to that of the controls (median = 5.7%, P = 0.03). The frequency of NK-cells, CD8+T cells, CD3+T cells and monocytes were similar when patients (13.9%, 20.0%, 69.7% and 2.2%, respectively) and healthy controls were compared (20.4%; 18.3%, 66.4% and 2.8%, respectively) (P >0.05). The frequency of CD4+ CD25+ FoxP3+ Treg cells in HCV patients and controls was similar (1.2% and 1.7%, respectively; P >0.05). **Conclusion**: Our preliminary results suggest that chronic HCV infection causes opposite effects on CD4+T cell

and B cell populations in HCV carriers. However, CD4+CD25+FoxP3+ Treg cell population is not apparently influenced by this viral infection.

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ANTIPHOSPHOLIPID ANTIBODIES IN PATIENTS WITH DYSLIPIDEMIA AND CARDIOVASCULAR DISEASES

TACIANA P. SANT'ANA SANTOS[1]; MARIANA M. PEREIRA[2]; ISABELA S. OLIVEIRA[2]; ROQUE ARAS[3]; RICARDO DAVID COUTO[4]; AJAX M. ATTA[4]

[1] Programa de Pós-Graduação em Farmácia da UFBA, [2] Programa de Pós-Graduação em Imunologia da UFBA, [3] Serviço de Cardiologia do Hospital Ana Nery, [4]Departamento de Análises Clínicas e Toxicológicas da UFBA

Introduction: Cardiovascular diseases (CVD) are the main cause of death worldwide. The involvement of antiphospholipid antibodies has been investigated in these disorders, mainly in acute myocardial infarction (AMI) and stroke (CVA), but the results of these studies are controversial. **Objective:** This study investigated the presence of serum antiphospholipid antibodies in CVD patients living in Salvador-Bahia. **Methods and Results:** 150 patients (79 men and 71 women, 60 ± 9 years) having clinical diagnosis of CVD and treated for dyslipidemia with statin (Cardiology Service of the Hospital Ana Nery) were included in the study. 60 healthy individuals (30 men and 30 women, 34±9 years) were the controls. The presence of serum IgA, IgG and IgM against β2-glycoprotein I (β2GPI)and cardiolipin, IgG and IgM anti-annexin, IgG and IgM anti-phosphatidylserine and IgG and IgM anti-prothrombin were determined by indirect ELISA. IgA anti-β2GPI antibodies were the most prevalent antibodies in patients (24/150,16%) and controls (5/60, 8%). IgG and IgM against β2-glycoprotein I and cardiolipin, IgG and IgM anti-annexin, IgG and IgM anti-phosphatidylserine and IgG and IgM anti-prothrombin were detected in low prevalence in these groups, varying from <1% to 5%. IgG and IgM anti-phosphatidylserine antibodies were not observed in the patients and controls. The lipid profiles (serum levels of cholesterol, LDL, HDL, triglycerides and apolipoproteins A and B) were similar in patients that were seronegative or seropositive for IgA anti- β2GPI ($P > 0.05$). Seven out of 14 patients with previous stroke had IgA anti-β2GPI, whereas 17 out of 112 patients without previous stroke were seropositive for these antibodies ($P=0.047$). **Conclusion**: The presence of IgA aβ2GPI antibodies may be associated with an increased risk of cerebrovascular accident in patients with dyslipidemia.

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Key-words: atherosclerosis; cardiovascular disease; stroke; antiphospholipid antibodies; β2GPI IgA antibodies.

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PLASMA LEVELS OF NEUROTROPHIC FACTORS AND TNF RECEPTORS IN PATIENTS WITH ALZHEIMER'S DISEASE AND MILD COGNITIVE IMPAIRMENT

MAYARA CHAVES FARIA[1]; GISELE SANTOS GONÇALVES[1]; NATÁLIA PESSOA ROCHA[2]; KARINA BRAGA G. BORGES[1]; ANTÔNIO LÚCIO TEIXEIRA[2]; LIRLÂNDIA PIRES DE SOUSA[1]; MARIA DAS GRAÇAS CARVALHO[1]

[1] Departamento de Análises Clínicas e Toxicológicas, Faculdade de Farmácia; [2] Laboratório de Imunofarmacologia, Departamento de Bioquímica e Imunologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais.

Introduction: Alzheimer's disease (AD) is the most common form of dementia in the elderly and it becomes more worthy of study as our life expectancy rises. The progressive and irreversible characteristics of AD have prompted researchers to look for biomarkers that could work as useful tools for either its diagnosis or for understanding the course of the disease. Recent findings have highlighted alterations in the peripheral level of neurotrophic factors in AD patients. Those findings, however, remain controversial.
Methods and results: In this study we measured the plasma levels of brain derived neurotrophic factor (BDNF), glial cell-derived neurotrophic factor (GDNF) and neuronal growth factor (NGF), as well as the levels of the tumor necrosis factor- alpha (TNFα) soluble receptors sTNFR1 and sTNFR2 in the plasma of 59 AD patients, 57 patients with mild cognitive impairment (MCI) and 58 healthy elderly controls. As results we have found that BDNF levels were significantly higher for MCI (3089 ± 329.4 pg/ml) and AD patients (3905 ± 387.8 pg/ml) compared to controls (1965 ± 240.4 pg/ml) and demonstrated an increasing tendency along with the severity of the disease. This increase might be due to a compensatory mechanism to fight early neurodegeneration and could also be linked to an activation of immune cells (known sources of neurotrophins) as the levels of sTNFR1 and sTNFR2 showed to be increased as well. The level of GDNF and NGF did not show significant differences between the studied groups.
Conclusion: Our findings indicate that there is a progressive increase in the peripheral level of BDNF that follows cognitive decline until the early stages of AD, after which a decrease seems to take place.

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NUCLEOSOME ANTIBODIES IN BRAZILIAN PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS

[1] RODRIGO C. OLIVEIRA; [2]JOÃO PAULO MEDEIROS; [1]ISABELA S. OLIVEIRA; [3]MITTERMAYER B. SANTIAGO; [3]PRISCILA SANTIAGO, [3]FELIPE OLIVEIRA, [4]MARIA LUIZA B. SOUSA ATTA; [4]AJAX M. ATTA

[1] Programa de Pós-Graduação em Imunologia da UFBA, [2] Programa de Pós-Graduação em Farmácia da UFBA, [3] Serviço de Reumatologia do Hospital Santa Izabel e EBMSP, [4] Departamento de Análises Clínicas e Toxicológicas da UFBA

Introduction: Systemic lupus erithematosus is mainly characterized by chronic inflammation and exuberant autoantibody production. Nucleosome autoantibodies are immunoglobulins that have been investigated by different groups to know their association with clinical manifestations of this disease. **Objective:** To investigate the prevalence of nucleosome antibodies and their association with biomarkers of SLE in Brazilian patients living in Salvador-Bahia. **Methods and Results**: Eighty-one female SLE patients from a reference center of rheumatic diseases (Hospital Santa Izabel, BA) were included in the study. Complement C3 and C4 were determined by nephelometry, Erythrocyte Sedimentation Rate (ESR) by Westergreen method, whereas Ht was measured by automated cytometry. Nucleosome antibodies were found in 72 out of 81 (88.9%) patients with a median level of 163 U/mL, whereas the presence of dsDNA antibodies was detected in 50 out of 81(61.7%) subjects (median level = 216 IU/mL). However, there was a positive correlation between the levels of these two antibodies ($r = 0.31$, $P = 0.031$). Nucleosome antibody levels were positively correlated with ESR (median = 45 mm; $r = 0.39$, $P = 0.0007$) and an inverse correlation was observed between the levels of these antibodies and Ht (median = 36.4%; $r = - 0.44$, $P < 0.0001$), complement C3 levels (median = 92.1 mg/dL; $r = - 0.31$, $P = 0.007$) and complement C4 levels (median 14.6 mg/dL; $r = - 0.24$, $P = 0.039$). The serum levels of IL-10 (median = 6.5 pg/mL) was positively correlated with nucleosome antibody levels ($r = 0.31$, $P = 0.008$). **Conclusion:** Nucleosome autoantibody levels show significant correlation with biomarkers of inflammation and disease activity present in Brazilian patients with systemic lupus erythematosus.

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WHOLE-BLOOD ASSAY OF LEPROSY PATIENTS AFTER *in vitro* STIMULATION WITH PGL-I or *Mycobacterium leprae*

ANGÉLICA RITA GOBBO (IC) (1); AMANDA CRISTINA GODOT THOMAZ (IC) (1); MOISES BATISTA SILVA (1) and CLAUDIO GUEDES SALGADO (1).

(1) Dermato-Imunology Laboratory UFPA/MC, Belém, Pará, Brazil.

Introduction: Leprosy is a chronic infectious disease caused by the intracellular bacteria *Mycobacterium leprae.* It is characterized by a spectrum of clinical manifestations and the differences are due to variability in host immune response to *M. leprae*. Phenolic glycolipid-1 (PGL-I) is a specific antigen present in cellular wall of *M. leprae*. The aim of this work was to evaluate immune response of leprosy patients cells *in vitro*, when stimulated with *M. leprae* or PGL-I. **Methods and Results:** Plasma samples were obtained from new leprosy cases without treatment and healthy endemic controls (HC) attended at Reference Unit in Dermatology of Pará State "Dr. Marcelo Cândia" (URE Marcelo Candia), Marituba, Pará, Brazil. Whole-blood assay (WBA) were performed collecting 10mL of peripheral blood by venipuncture, followed by dilution 1:2 with RPMI 1640 10% BFS, and distribution of 2mL total blood on each well of 12 wells culture plate. The antigens were tested at the concentration of 40 µg/mL of *Mycobacterium leprae* sonicate extract (MSE), 5 µg/mL of native PGL-I and 5 pg/mL of LPS. After 24h incubation at 37ºC, 5% CO_2 supernatants were collected, centrifuged and tested for IFN-γ and IL-4 by ELISA. Thirteen plasma samples were obtained, 8 from patients and 5 from HC. MSE and PGL-I stimulation showed for IFN-γ 21.172 and 20.742 pg/mL to Tuberculoid (TT), 13.614 and 22.724 pg/mL to Borderline-tuberculoid (BT), 13.446 and 8.712 pg/mL to Borderline-lepromatous (BL) and 18.542 and 19.366 pg/mL to Lepromatous (LL) patients. IL-4 showed, respectively, 91.774 and 92.524 pg/mL to TT, 73.882 and 81.588 pg/mL to BT, 85.884 and 92.712 pg/mL to BL and 18.584 and 20.248 pg/mL to LL patients. **Conclusion:** PGL-I, in comparison to MSE, induced better immune response on cytokines analyzed. TT patients produced more IFN-γ and IL-4, what may explain better immune response and giant cell formation than that found in LL patients. **Financial support:** Universidade Federal do Pará (UFPA); Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq/PIBIC); Secretaria Executiva de Saúde Pública do Estado do Pará (SESPA).

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ASPECTS OF THE IMMUNE RESPONSE IN REACTIVE HBV BLOOD DONORS

LAURA PATRÍCIA VIANA CHAVES[1,2]; ANDRIELE HUPPES PEDROSO MENDONÇA[1]; VALDETE LOPES MARTINS DE CASTRO[1]; SILVIA AMORIM LEITE[1]; EDSON LIRA[1]; ADRIANA MALHEIRO[1,3]

[1]Fundação de Hematologia e Hemoterapia do Amazonas, [2] Universidade do Estado do Amazonas, [3] Universidade Federal do Amazonas,

Introduction and objectives: Hepatitis B infection is a serious public health problem at the western Amazon. Several risk behaviors, adopted before or during the imprisonment, accounts for that. Among them, the use of intravenous illicit drugs, sharing of needles, tattoos and unprotect sexual activity are the most important. The immune response may modulate liver damage in HBV infection. The aim of this study was to evaluate the anti-HBc and HBsAg antibodies prevalence and the profile of the cellular immune response in blood donors of the Manaus - AM. **Methods and Results:** Serological assays (ELISA) and flow cytometry were done; monoclonal antibodies anti-CD3, anti-CD4, anti-CD8 and anti-CD69 were used in cytometry to identify lymphocyte sub-populations. Among the 65 blood donors presented reactive results for anti-HBV assays and 1 for HBsAg, demonstrating a seroprevalence of 2,61%. The analysis of the cellular profile demonstrated a statistically significant difference between the percentile average of total lymphocytes in the control group (18.58±6.18%) and HBV^+ (28.79±13.22%) group. The analysis for cytometry demonstrated significant increase of $LTCD8^+$ in HBV^+ blood donors (25±0.90%) in relation to control group (20,97±0.87%). It was not observed any significant difference in $LTCD4^+$ between the two groups, positive HBV (41.18±0.88%) and control (42.79±0.91%). The percentile averages of monocytes activated ($CD69^+$) were significantly larger in the HBV^+ patients when compared with other cells. **Conclusion:** We conclude that donors who had chronic infection had a response with a predominance of TCD4+ lymphocytes, which favors the chronic infection.

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key-words: Hepatitis B, cellular immune response, blood donors

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ASSOCIATION BETWEEN PLASMA LEVELS OF HSP70 AND TNFR-1 IN PREGNANT WOMEN WITH EARLY-ONSET PREECLAMPSIA

VANESSA ROCHA RIBEIRO (IC)[1] MARIA TEREZINHA SERRÃO PERAÇOLI[1]; CAMILA FERREIRA BANNWART-CASTRO[2]; INGRID CRISTINA WEEL (PG)[2]; MARIANA ROMÃO (PG)[2] ; VERA THEREZINHA MEDEIROS BORGES[2]; JOSÉ CARLOS PERAÇOLI[2]

[1] Departamento de Microbiologia e Imunologia – Instituto de Biociências, UNESP-Univ Estadual Paulista, Botucatu, São Paulo, Brazil , CEP 18618-970

[2] Departamento de Ginecologia e Obstetrícia – Faculdade de Medicina- UNESP-Univ Estadual Paulista, Botucatu, São Paulo, Brazil, CEP 18618-970

Introduction and Objectives*:* Preeclampsia (PE) is a specific disorder of human pregnancy characterized by an excessive maternal inflammatory response to pregnancy. Oxidative stress as well as high levels of tumor necrosis factor-alpha (TNF-a) plays a central role in the pathogenesis of PE. Expression of heat shock proteins (Hsp) is an adaptive response to cellular stress. Stress induces TNF-a production that in turn, induces Hsp70 expression. This study investigated the levels of Hsp70, p55 TNF-a receptor-I (TNFR-I) and Interleukin-10 (IL-10) in plasma of pregnant women with PE.

Methods and Results: The subjects were 80 preeclamptic pregnant women, classified according to the onset of clinical manifestations in early-onset (< 34 weeks of gestation, n=40) and late-onset PE (≥ 34 weeks of gestation, n=40). Plasma was obtained from peripheral blood and Hsp70, TNFR-I and IL-10 were determined by enzyme immunoassay. Results were analyzed by parametric tests, with significance level set at 5%. Plasma levels of Hsp70, TNFR-I and IL-10 obtained from early-onset PE women were significantly higher than in late-onset PE women. A significant positive correlation between Hsp70 and TNFR-I levels ($r = 0.4927$; $p = 0.0069$) was detected in the early-onset PE group. No significant correlations were observed in the late-onset PE group. Higher plasma levels of IL-10 detected in early-onset PE patients might be involved in the modulation of the increased levels of Hsp70 and TNFR-I produced by patients with early-onset PE.

Conclusions: The relationship between increased plasma Hsp70 and TNFR-I levels found in patients with early-onset PE suggests that circulating Hsp70 might

be involved in the development of the maternal systemic inflammatory response in this more severe form of PE.

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IL-10 CYTOKINE AND CD4(+)FOXP3(+) REGULATORY T-CELL PROFILE CORRELATE WITH SYSTEMIC LUPUS ERYTHEMATOSUS ACTIVITY

ANDREA ALICE DA SILVA (1), ANA LUIZA BARBOSA DOS SANTOS (1)(PG), ALINNE CHRISTINA ALVES PIRES (1)(IC), NATALIA FONSECA DO ROSÁRIO, (1)(TCT), CARLA GAGLIARDI (1)(MD).

(1) Faculdade de Medicina, Departamento de Patologia, Universidade Federal Fluminense, Niterói, RJ, Brasil

Introduction: The Systemic Lupus Erythematosus (SLE) is an inflammatory chronic disease characterized by increase of circulating autoantibodies and cytokine imbalance. It is shown that the Treg defective aspects, both quantitative and qualitative, are involved in SLE pathogenesis and flares of activity. Based on this, it is tempting to correlate the frequency of T cell population and other inflammatory molecules with disease activity, and speculate a possible role as a SLE activity marker. **Methods and results:** The inflammatory profile was investigated in the serum sample from 36 SLE diagnosed patients, including 23 with active (SLEDAI>6) and 13 inactive (SLEDAI<6) disease and compared to 15 non-SLE individuals. We quantified and compared the frequency of T-cells and CD4+Foxp3+ Treg subsets**.** Independent of activity, plasma levels of oxide nitric (p=0.015), IL-1 (p=0.0287) and IL-6 (p=0.0081) showed statistic differences compare with non-SLE individuals. However, only the IL-10 levels were correlated with activity of SLE (p=0.0033). Decrease of leucocytes and lymphocytes was observed in active SLE patient and it was positive correlated with SLEDAI index. Interesting, CD4+ and CD8+ T-cell frequency observed in SLE patient was lower than non-SLE patients, but not correlated with activity disease. The flow cytometry analysis showed that SLE patients presented significant higher frequency of CD4(+)CD25(-)FoxP3(+) T cells than those non-SLE individuals (5.73±4.41 and 4.11±1.18; *p*=0.0037). The active SLE patients presented a significant reduction in the percentage of CD4(+)CD25highFoxP3+ T cells (0.63±0.23 e 0.94±0.36; *p*=0.0031) and CD4(+)CD25lowFoxP3+ T cells (1.69±0.55 e 2.47±0.88; *p*=0.0259), as well as a significant augmentation in CD4(+)CD25(-) FoxP3+ T cells frequency (7.46±4.90 and 4.68±2.23; *p*=0.0058), when compared with inactive SLE patients. In addition, there was a negative correlation between the SLEDAI score and CD4(+)CD25highFoxP3(+) T cells (*p*= 0.0018) and CD4(+)CD25lowFoxP3(+) T cells frequencies (*p*=0.0283). According, a significant positive correlation between SLEDAI score and CD4(+)CD25(-)Foxp3(+) Treg cells frequency (*p*=0.0155). **Conclusion:** These results suggest that the IL-10 cytokine and CD25high and CD25low Treg subsets were involved with pathogenesis of SLE patients and it

laboratorial analysis may be tools of SLE activity markers. To confirm this hypothesis a longitudinal study is necessary.

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METABOLIC AND INFLAMMATORY PROFILE OF VISCERAL ADIPOSE TISSUE DURING CANCER CACHEXIA DEVELOPMENT

FELIPE DOS SANTOS HENRIQUES[1]; RODRIGO XAVIER DAS NEVES[1,2]; ROGÉRIO ANTÔNIO LAURATO SERTIÉ[1,4]; ANDRÉ R.G. PROENÇA[4]; FELIPE DE OVIVEIRA FRANCO[1]; CLÁDIO SABORU SHIDA[1]; SIDNEY BARNABÉ PERES[3]; FABIO BESSA LIMA[4]; MARILIA SEELAENDER, M[1]; MIGUEL LUIZ BATISTA JÚNIOR[1,2]

1 - Laboratory Adipose Tissue Biology - NIB - University of Mogi das Cruzes.

2 - Group of Molecular Cell Biology – ICB I - University of São Paulo.

3 - Department of Physiological Sciences – State University of Maringá.

4 - Laboratory of Adipose Tissue Physiology- ICB I - University of São Paulo.

Introduction: Several studies have demonstrated that cancer cachexia induces loss of fat mass that accounts for a large part of the dramatic weight loss. To evaluate the onset of cancer cachexia in visceral adipose tissue (VAT) at a metabolic and inflammatory level we assessed different VAT depots in a cancer cachexia model at three different time-points after tumour implantation.

Methods: Male Wistar rats, 8 weeks old, were subcutaneously inoculated with 1 mL (2×10^7) of tumour cells (Walker 256). Samples of different VAT depots (mesenteric and retroperitoneal) were collected at day 0, 4, 7 and 14 and stored at -80^{o} C (5 animals per each day/group). Metabolic alterations were evaluated by lipolysis (isoproterenol stimulation, 10^{-5}M) and lipogenesis (D-[U-^{14}-C]-glucose incorporation induced by insulin stimulation, 10^{-9}M) assays on day 4 and 14 from isolated adipocytes. The inflammatory profile was determined measuring the gene expression (qPCR) of CCL3, 5 and CXCL2 in the stromal vascular fraction (SVF) and immunohistochemistry for CD11b from VAT during development of the cachexia.

Results: In mesenteric depot, lipogenesis was reduced on day 4 in basal and insulin-stimulated condition (55 and 64%, respectively, $p < 0.05$) compared to day 0. Lipolysis increased (6-fold, $p < 0.005$) on day 14 just at basal stimulation, compared to control situation. Gene expression of CXCL2 and CCL3 increased (10 and 4-fold, respectively, $p < 0.05$) significantly on day 7 with no change on the other evaluated time-points in samples from mesenteric depot compared to day 0 in the same tissue. CD11b was positively marked just on day 7 in mesenteric depot.

Conclusion: In the present study we demonstrated time-dependent changes at metabolic and inflammatory parameters in VAT of tumour-bearing rats. Lipogenesis seems to be reduced at early stages of cachexia followed by increase of *polymorphonuclear* leukocytes and of lipolysis at end stages of cachexia.

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PASSIVE TRANSFER OF ANTIBODY ANTI-RUBELLA THROUGH THE PLACENTA IN NEWBORNS OF DIABETIC MOTHERS

ESTÉFANI GONÇALVES (PG) (1); PAULA CRISTINA DE SOUZA SOUTO (1); PATRÍCIA FERNANDES MASSMANN (PG) (1); ROSA JACINTO VOLPATO (IC) (1); IRACEMA DE MATTOS PARANHOS CALDERON (2); EDUARDO LUZIA FRANÇA (1); ADENILDA CRISTINA HONORIO-FRANÇA (1)

(1) Instituto de Ciências Biológicas e da Saúde, Campus Universitário do Araguaia, Universidade Federal de Mato Grosso.
(2) Departamento de Ginecologia e Obstetrícia, Faculdade de Medicina, Campus de Botucatu, UNESP

Introduction: Pregnant women are capable of producing amounts of antibodies and the transfer of such antibodies to the fetus through the placenta. This transfer occurs during the last 4 to 6 weeks of pregnancy. Most IgG class antibodies are capable of crossing the placenta barrier. Placental transfer of IgG antibodies is an important mechanism that provides protection for the newborn, while the response is inefficient and contributes to the maturation of the immune system. This mechanism results in neonatal serum IgG levels equivalent to maternal, and with the same patterns of antigen recognition. Women with diabetes have an increased risk of acquiring certain diseases during pregnancy due to increased glucose coupled with transient immunosuppression, and when attacked by infections can transmit them vertically. Rubella and hepatitis B are in Brazil, some of the major infectious diseases found in pregnant women that can be transmitted from mother to fetus or the newborn. This transmission can occur during pregnancy, at birth or during breastfeeding. The objective of this study was to evaluate maternal-fetal transfer of total and specific IgG through the placenta in newborns of diabetic mothers.
Methods and Results: We evaluated samples of maternal and umbilical cord blood. The groups were divided according to maternal glycemic status: normoglycemic (N = 12), mild hyperglycemic (N = 12) and diabetes (N = 12). The concentration of total IgG was measured by the turbidimetry methods. The available of antibody anti-antigen of hepatitis B virus (HBsAg) and rubella-specific IgG were determined by ELISA. We demonstrated that IgG levels in cord blood were higher in the hyperglycemic group. Plasma antibody levels were lower in hyper- than in normoglycemic women. None of the samples proved to be positive for the detection of the antigen HBsAg. 83% (N = 10) of serum samples from normoglycemic mothers and their newborns and 100% of mothers and newborns from hyperglycemic groups were positive for IgG antibodies specific for rubella.

Conclusion: These results suggest that maternal hyperglycemia alters levels of total IgG, but the maternal-fetal transfer of IgG anti-rubella is not associated with maternal blood glucose level.

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IMMUNOLOGICAL BIOMARKERS FOR LEPROSY SURVEILLANCE: FINDING NEW CASES BY NOVEL APPROACHES

CLAUDIO GUEDES SALGADO[1,2]; LAYANA DE SOUZA GUIMARÃES[1,3]; DENIS VIEIRA GOMES FERREIRA[1]; MARCO ANDREY CIPRIANI FRADE[4]; PATRICIA SAMMARCO ROSA[5]; MOISÉS BATISTA DA SILVA[1,2]; JOSAFÁ GONÇALVES BARRETO[1,6]

[1]Laboratório de Dermato-Imunologia UEPA/UFPA/Marcello Candia, Marituba, Pará; [2]Instituto de Ciências Biológicas, Universidade Federal do Pará, Belém, Pará; [3]Unidade de Referência Especializada em Dermatologia Sanitária Dr. Marcello Candia, Marituba, Pará; [4]Departamento de Dermatologia, Faculdade de Medicina da Universidade de São Paulo, Ribeirão Preto, São Paulo; [5]Instituto Lauro de Souza Lima, Bauru, São Paulo; [6]Universidade Federal do Pará, Campus Castanhal, Pará, Brasil.

Introduction: Leprosy, a chronic infection caused by *Mycobacterium leprae*, causes cutaneous lesions, peripheral neuropathy and anesthesia with related disability along with a social stigma. Despite the enormous success of worldwide leprosy control programs since the implementation of multidrug therapy in the mid-1980's, the reality is that 228.474 new cases were recorded in 2010 among the 130 countries reporting, with 9% of these in children and 5.8% presenting with advanced grade-2 disability. The occurrence of leprosy among children is correlated with recent disease and active foci of transmission in the community. **Methods and Results:** We performed clinical and serological examinations of 1592 randomly selected school children (SC) during a cross-sectional survey in 8 hyperendemic municipalities of the Brazilian Amazon region. Sixty-three (4%) SC, with a mean age of 13.3 years (SD = 2.6), were diagnosed with leprosy, and 777 (48.8%) were seropositive to anti-PGL-I. Additionally, we evaluated 256 household contacts of those students diagnosed with leprosy: 24 (9.4%) of them were also diagnosed with leprosy and 107 (41.8%) were seropositive. Seroprevalence was significantly higher among girls, students from urban areas and students from

public schools (p<0.0001). Forty-five (71.4%) new cases detected among SC were classified as paucibacillary, and 59 (93.6%) did not demonstrate any degree of physical disability at diagnosis. These results suggest a high rate of undiagnosed leprosy and subclinical infection among children in the Amazon region. A new method for finding new cases was developed by our group using anti-PGL-I results on visited municipalities. At Oriximiná, Northwest Pará, we found 23 new cases among anti-PGL-I+ SC and their contacts, while only 7 were found on the anti-PGL-I negative group. Using the same approach, examining only anti-PGL-I+ SC and their contacts, or anti-PGL-I+ contacts of leprosy cases, our group found more than 70 new cases in one week work at Castanhal, a medium city 65 Km from Belém, capital of Pará State. **Conclusion:** The advantages of school surveys in hyperendemic areas are finding early cases of leprosy with no physical disabilities, preventing the spread of the infection in the community and breaking the chain of transmission. We are now on a joint effort with Colorado State University, to find if new biomarkers, as LID-I, LAM and others may be useful to further increase our capability on finding new cases of leprosy.

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TNFα: A screening tool in occupational

Marcello Mascarenhas, Norberto Luiz Souto Neto, Nelson Wilhelms Junior, Caroline Borges Rosa, Alessandra Peres

Centro Universitário Metodista, do IPA

Occupational diseases are responsible for musculo-skeletal problems from various sources, but mostly by violent movements and are also attached to a painful condition. The objective of evaluating the level of tumor necrosis factor α (TNF α) of workers exposed to drills and pain. The study is constituted by a transversal, with survey data obtained from workers (n = 85) of a refrigerator chickens city of Farrukhabad Rio Grande do Sul, which was approved by the Ethics in Research with protocol number 24/2011. The research subjects were invited to participate after an aliquot was collected venous blood for determination of TNF αby Elisa method. The data were analyzed using the Student T-test, where the p value was considered 95% reliability. The results showed that 79.48% were individuals with pain (79.48%) and 20.51% no pain when combining TNFα noted that a significant difference between the groups group (p = 0.030). Therefore, it is possible to use the biomarker as a way to assess pain and chronic process thereby serving as a form of diagnostic routines Occupational Research.

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Cytokines profile and oxidative stress in a hepatic encephalopathy murine model

BRUNO E FALEIROS(1); ALINE S MIRANDA(1); ALLINE C CAMPOS(1); LUCAS M KANGUSSU(1); MILENE A RACHID(1); ANTÔNIO L TEIXEIRA(1).

[1] *Universidade Federal de Minas Gerais, UFMG, Belo Horizonte, MG;*

Introduction: Hepatic encephalopathy (HE) is a neuropsychiatric syndrome resulting from hepatic failure and comprises a wide spectrum of neurological symptoms. Although hyperammonemia has been traditionally implicated, the pathogenesis of HE remains unknown. Some recent studies have suggested that neuroinflammatory processes may play a role on HE development. In this context, the aim of this experimental study is to investigate the neuroinflammatory process in a thioacetamide (TAA)-induced HE experimental model in C57BL/6 mice.

Methods and Results: Eight to 12 week-old C57BL/6 female (20-25 g) mice were used. The HE was induced by TAA administration via intra-peritoneal (i.p.) route. A single dose of 600 mg/kg dissolved in saline (300 mL) was injected. The control group received the same volume of saline. Liver enzyme ALT was measured for injury quantification and behavior was assessed through Open Field test monitoring all movements of the animals, both at 24 hours post-induction (p.i.). The concentration of cytokines (TNF-a, IL-1b, KC, MCP-1, MIP-1a and RANTES) was evaluated in brain tissue and serum by ELISA at 24, 36 and 48 hours p.i.. Oxidative stress was measured by lipid peroxidation (TBARS) and by the activity of three antioxidant enzymes: SOD, GSH and catalase. Blood-brain barrier (BBB) integrity was evaluated at 24 hours p.i. by Evans Blue method. Liver damage was confirmed by high ALT levels and Open Field showed no difference between controls and HE-induced mice. Higher brain levels of cytokines were observed in HE mice at 24 and 36 hours p.i. ($p < 0.05$) in comparison to control group. Oxidative stress was evident by increased TBARS levels and decreased antioxidant enzymatic activity ($p < 0.05$). Finally, BBB disruption was evidenced by increased permeability to Evans Blue in HE-induced mice.

Conclusion: In the light of an acute liver injury, we observed normal behavior in HE-induced mice at 24 hours. Moreover, there was an up-regulation of cytokines and oxidative stress in the brain of HE-induced animals in parallel with BBB disruption. These preliminary findings suggest that involvement of neuroinflammatory mechanisms in HE pathogenesis may develop early in the disease, possibly before symptoms become present.

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