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PPGIm
Programa de Pós-graduação em Imunologia

ANAIS DA XVI EXPOPPGIM 2016

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RESUMOS

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SUMÁRIO

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APRESENTAÇÃO

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O Programa de Pós-graduação em Imunologia (PPGIm) há mais de vinte e cinco anos, vem formando recursos humanos de excelência, capacitados para as atividades de ensino e pesquisa em Imunologia e áreas correlatas. O PPGIm tem realizado reuniões científicas anuais visando difusão do conhecimento científico e integração acadêmica com a graduação e a pós-graduação da UFBA e de outras IES.

A ExpoPPGIm, Reunião Anual do Programa, já se tornou um evento tradicional que acontece anualmente desde 2000. Essa reunião tornou-se um fórum de integração de profissionais, pesquisadores e jovens cientistas, alunos de graduação e pós-graduação da UFBA e de outras IES do Estado da Bahia e do Brasil com interesse no amplo domínio da Imunologia. Neste sentido, o objetivo da ExpoPPGIm é divulgar conhecimento científico em Imunologia e áreas correlatas tendo como público alvo estudantes de graduação, pós-graduação e pesquisadores e profissionais da área. Nesta XVI ExpoPPGIm, além de conferências com pesquisadores do Programa e pesquisadores convidados, contamos também com os *workshops* em metodologias envolvendo recentes tecnologias em Imunologia aos participantes interessados, além da sessão de pôsteres e comunicações orais dos melhores trabalhos, incluindo premiações, sendo o melhor trabalho agraciado com a primeira edição do Prêmio Lain Carlos Pontes de Carvalho.

Neste livro de resumos sumarizamos a produção científica gerada durante a XVI ExpoPPGIm com o objetivo de disseminar a Imunologia e promover o nosso Programa, nossos principais objetivos, perspectivas, nossa história e evolução.

Saudações acadêmicas,

Camila A Figueiredo
Coordenadora do PPGIm/ICS/UFBA

#01

SEROPOSITIVITY OF *TOXOCARA* SPP. IN PREGNANT WOMEN IN A POPULATION OF A COHORT DERIVED FROM THE CITY OF QUININDE IN ECUADOR.

Aida Yisela Oviedo Vera[123]; Marcia Barbosa da Silva[1,2]; Luis Fabian Salazar Garces[123]; Mariese Conceição dos Santos[4]; Alana Alcântara Galvao[1,2]; Philip John Cooper[3]; Neuza María Alcântara-Neves[1,2].

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Support: CAPES, CNPq

Introduction: Toxocariasis is a neglected zoonosis caused by larvae of Toxocara spp. (T. canis and T. cati) in parathenic hosts. Human infection with Toxocara spp. occurs in different clinical pictures, such as viscerall migrans (VLM), ocular larva migrans (OLM), meningoencephalitis and asymptomatic forms. This disease has been reported worldwide, but the prevalence is higher in tropical regions and, even more among low-income populations. There are not published data about vertical transmission in humans by Toxocara spp. except for a record of Toxocara congenital infection in a premature infant. Helminth infections during pregnancy may be associated with reproductive disorders; studies investigating the occurrence of toxocariasis in pregnancy are scarce. Objectives: The aim of this study was to determine the seroprevalence of anti- Toxocara spp. IgG antibodies in pregnant women in a cohort study in the city of Quininde, State of Esmeraldas, Ecuador. Methodology: The study was performed on 290 pregnant women. Serum samples were examined by the enzyme-linked assay. Adult female worms Toxocara canis were got from newborn dogs to extract eggs and to get the excreted - secreted antigens from the larvae of the parasite cultured in vitro. The sera were absorbed with Ascaris lumbricoides somatic antigen previously to the ELISA. Results: The prevalence of Toxocara spp. IgG in pregnant women was 80, 7%. Conclusions: Our data showed that the seroprevalence of Toxocara spp in pregnant women is high in this population.

Acknowledgement: The study was conducted in the Laboratory of Allergy and Acarology in collaboration with investigation group of Professor Philip Cooper in Ecuador, and the multidisciplinary group of our laboratory. It was developed with resources from CNPq, Superior Council for Scientific Research and FAPESB, Support Foundation of the State of Bahia Research.
Keywords: Toxocara spp,Maternal seropositivity, Peruvian population.

#02

GENETIC VARIANT IN VITAMIN D RECEPTOR (VDR) IS ASSOCIATED WITH ASTHMA SYMPTOMS

Alana Alcântara Galvão, Emília Belitardo Andrade, Flávia Araújo Sena, Ryan Santos Costa, Maurício Lima Barreto, Camila Alexandrina Figueiredo, Neuza Maria Alcântara Neves

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Support: UNIFESP, ISC, LAA, IMUNOBIO

Introduction: Growing evidences have been showing beneficial effects of vitamin D in several diseases. Currently it is known that several cells express vitamin D receptor (VDR) and this hormone exerts a crucial role in genetic expression by regulating more 3% of all human genome. It also is known that the immune system cells are regulated by vitamin D, giving an protective immunity due to its effects on innate immunity response by triggering the release of antimicrobial peptides. Moreover, this hormone has the capacity to control inflammatory process through a regulatory response. Therefore it has been suggested that vitamin D could be used in the treatment of autoimmune and allergic diseases. Several studies reported beneficial effects of vitamin D in asthma treatment, however there is considerable controversy regarding its benefits. Genetic determinants on vitamin D pathway have been associated with both low- and high-risk of asthma. Objectives: To understand how genetic parameters of vitamin D is associated with atopy and asthma, we analysed some single nucleotide polymorphisms (SNPs) in the vitamin D pathway genes associated with asthma, asthma phenotypes and specific IgE production against different allergenic sources. Methodology: This study involved 639 individuals, including 64 asthma cases. Peripheral blood samples were obtained and whole-blood culture was done. The sera were collected and their supernatant was harvested and stored at -20ºC and -80ºC respectively, until the analysis for allergen specific IgE ; DNA samples were extracted and were genotyped using 2.5 HumanOmni Biochip from Illumina. Statistical analyses were performed in PLINK 1.9. Results and Discussion: Eleven variants (SNPs) in VDR were associated with asthma (rs2254210, rs2853564, rs12721376, rs11168287, rs11168264, rs2853561, rs77193628, rs9729, rs7136534, rs731236, rs2238135) $p < 0,05$. The VDR variant rs7136534 was associated positively with asthma symptoms and negatively with serum production of 25-Hidroxivitamin D, and the variant rs2238135 was associated positively with asthma symptoms and IL-5 production. Conclusions: Vitamin D showed an important role in asthma, the VDR variant rs7136534 reduced vitamin D levels and was associated with higher asthma susceptibility. The SNP rs2238135 had not effect in vitamin D serum levels, however was associated with increase IL-5 supernatant levels, a cytokine involved in asthma physiopathology.

Keywords: Vitamin D, Atopy, Asthma, Genetic Variants

#03

RUTIN MODULATES INFLAMMATORY PROFILE OF MICROGLIA AND NEUROTROFINS UNDER THE INFLUENCE OF GLIOMA CELLS

Silva, A.b., Coelho, P. L.C., Amparo, J.A.O, J.R.P., Soares, Silva, K.C., Silva, V. D. A ., Souza, C. S., Costa, S. L.

Laboratório de Neuroquimica, Instituto de Ciencias Da Saude UFBA

Support: FAPESB

Microglia present an import role in the immune system, actively participating in the defense against neoplastic cells. Human glioblastoma multiform is a highly malignant tumor of the CNS and presents resistance to radio and chemotherapy. Our previous studies have shown that rutin, a flavonoids extracted from seeds of the Brazilian plant Dimorphandra mollis, acts as inhibitor of growth of human glioblastoma cell lines. The objective of this study was to evaluate the chemotatic effect of the flavonoid rutin in C6 glioma cells, their ability to inhibit tumor cell migration and secretion of cytokines during direct and indirect interaction of gliomas cells with CNS microglia. These results show that the flavonoid rutin induces microglia chemotaxis. Rutin also demonstrated to modulate the microglial response reflecting on inhibition of C6 glioma cells migration. Furthermore, the profile cytokines is dose dependently regulated after flavonoid exposure, with increase on secretion of proinflammatory cytokine tumor necrosis factor (TNF-α) and decrease on secretion of regulatory cytokine IL-6. Rutin was able to modulate inflammatory response of microglia during indirect contact with tumor cells to a more responsive profile with increase in secretion of TNF, which in turn may be favorable for killing glioma cells. In C6/microglia co-cultures rutin modulated the expression of mRNA for IL-6, TNF, Arginase, CCL2, CLL-5 and neurotrophins BDNF and NGF. To conclude, these results indicates that rutin induces chemotaxis and changes the immunologic profile during microglia/glioma interactions.

#04

DEVELOPMENT OF A QUANTITATIVE DETECTION TEST OF HIV AND HTLV INFECTED CELLS BY IMMUNOPHENOTYPING

Aline Clara da Silva[1], Alex José Leite Torres[2,3]

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Introduction: HIV infected individuals can be coinfect by other virus, like HTLV 1/2. Both virus presents same transmissions routes, as needles sharing and unprotected sexual contact (KLASE e JEANG, 2013; PILOTI et al., 2013). Clinical complications has been informed in this coinfection and HTLV-1 is associated with AIDS progression (BRITES et al., 2009; BEILKE et al., 2007; CASSEB et al., 2008; REGIS et al., 2009; SILVA et al., 2012). Objectives. To develop a detection test of HIV and HTLV virus in patient coinfected cells by flow cytometry; to determine the intracytoplasmic frequence of HIV and HTLV infected cells; to identify and characterize HIV and HTLV coinfected cells; to compare proviral load with intracytoplasmic virus presence in coinfected cells; to evaluate cytokines expression in coinfected individuals. Material and Methods. Will be collected 10 mL of peripheral blood of pacients with HIV and HTLV in monoinfection and coinfection. Ficoll gradient will isolate mononuclear cells from peripheral blood to flow cytometry. Cytoplasmic virus will be detect by specific probes anti-HIV and anti-HTLV. To determine proviral load, will be used monoclonal antibodies anti-CD4 (APC), anti-CD3(PerCp) and HIV (FITC) probe (to HIV infection) and anti-CD4 (APC), anti-CD3(PerCp) and HTLV (PE) probe (to HTLV infection). The cytokine profile to compare mono and coinfected patients will include IL-12, IFN-α, IFN-γ, IL-5, IL-10, IL-6, IL-2 e IL-4 and it will be done by Cytometric Bead Array. This project was approved by Research Etic Commission (CAAE 56213716.3.0000.5543).

#05

BENZNIDAZOLE TREATMENT INFLUENCE IMMUNE RESPONSE ON INFECTED MICE WITH TRYPANOSOMA CRUZI CLONES FROM DIFFERENT BIODEMES, SUSCEPTIBLE AND RESISTANT TO CHEMOTHERAPY

Amanda C.O Silva; Sonia G. Andrade

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Introduction: Chagas Disease (DC) is an inflammatory, infectious disease is caused by infection with the protozoan parasite Trypanosoma cruzi. The immune response to the disease mobilizes different effector mechanisms of the immune system, with the various cells and mechanisms related to activation of innate immunity and acquired immunity. Several authors have shown that Benznidazole (BZ), drug used in the treatment of DC, also stimulates the immunological system**.** Objectives: In the present study we intend to investigate the influence of treatment with BENZ on the immunological response in mice infected either with the clones Y strain (susceptible) or clones the Colombian strain (resistant). Material and Methods: The experimental groups: clone C5 of Y strain infected and treated with BZ or not-treated ; clone C7 of Y strain infected and treated with

BZ or not-treated ; clone C2 Colombian treated and not-treated ; clone C5 Colombian treated and not-treated treated not infected; Control not treated . Inoculum **:** $1x10^4$ trypomastigotes, (blood forms) injected intraperitoneally. Treatment was initiated in the peak of parasitemia for each strain:7th day for the Y strain and in the 18th day in the infection with the Colombian strain. Chemotherapy was performed in 60 doses (100mg x kg x day) of BZ. Celular responses were evaluated by celular proliferation in the spleen of CD4+, CD8+ and CD4+CD25+Foxp3+ +. Results and Discussion: With 5 doses of treatment was observed higher frequency of CD4 + CD25 + Foxp3 + in the YC5 treated group, CD4 + in the Col C2 treated group and CD8 + in COLC5 treated group. 20 of treatment doses was observed higher frequency of CD4 + CD25 + Foxp3 + in the YC7 treated group, CD4 + in the Y C7 untreated group and CD8 + in the YC5 treated group. 30 doses was observed higher frequency of CD4 + CD25 + Foxp3 + in the Col C2 treated , CD4 + in the Col C2 untreated group and CD8 + in the YC5 treated group. 60 doses was observed higher frequency of CD4 + CD25 + Foxp3 + in the YC7 treated, CD4 + in the Col C5 untreated and CD8 + in the Col C2 treated. Results suggest an influence of treatment with BENZ on the immunological response. Conclusion: Results suggest an influence of treatment with BENZ on the immunological response.

Keywords: Trypanosoma cruzi, Chemotherapy-Benznidazole, Resistance, Susceptibility, Immunological response.

#06

EVALUATION OF THE EXPRESSION AND IMMUNOGENICITY OF HSP60 AUTOLOGOUS IN INDIVIDUALS WITH PERIODONTITIS STIMULATED BY *Porphyromonas gingivalis*

Ana Carla M. Pimentel, Paulo C. Carvalho-Filho, Ellen Karla N. S. Lima, Michelle M. L Falcão, Patrícia N. de Miranda, Rebeca P. B. dos Santos, Roberto M. Nascimento, Marcia T. Xavier, Soraya C. Trindade

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Introduction: periodontitis is a multifactorial aetiology, with its associated pathogenesis to immuno-inflammatory aspects and the oral microbiota of the host, and Porphyromonas gingivalis (Pg) as one of the main pathogens involved in its etiology.Objective: this study will evaluate the expression of heat shock proteins 60 KDa autologous (HSP60) in cells stimulated with Porphyromonas gingivalis antigens of individuals with periodontitis, as well as immunogenic potential by mononuclear cells from peripheral blood culture (PBMC) with human HSP60 isolated and associated with HmuY and somatic antigens, both of Porphyromonas gingivalis.Material and Methods: the registered volunteers in the program will be treated at the clinic of the State University of Feira de Santana, examined and classified according to the clinical descriptors used in the diagnosis of patients with chronic periodontitis. Peripheral blood cells will be cultured with the crude extract and the recombinant protein of Porphyromonas gingivalis HmuY for 48h and the HSP60 expression will be evaluated by RT-PCR in real time. For evaluation of the immunogenic potential after cultivation of PBMC HSP60, HSP60 + HmuY and HSP60 + Pg quantification of the cytokines IL-2, IL-4, IL-6, IL-10, TNF, IFN-γ and IL-17A and anti-human Hsp60 antibodies will be performed by flow cytometry.

Keywords: periodontitis, heat shock protein, Porphyromonas gingivalis

#07

MICROLOCALIZATION OF CD163+ TUMOR-ASSOCIATED MACROPHAGES IN ORAL SQUAMOUS CELL CARCINOMA PATIENTS.

Ana Carolina Valente Santos Cruz de Araujo[1], Camila da Silva Souza[1], Rafael Luiz Vieira Mercuri[1], Deyse Souza Carvalho da Silva[1], Lucas Gomes Silva[2], Ludmila de Faro Valverde[3], Clarissa Araújo Gurgel[3], Roberto Meyer[4], Jean Nunes dos Santos[5], Deise Souza Vilas Bôas[1]

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Support: FAPESB, CNPq and PIBIC

Introduction: Oral squamous cell carcinoma (OSCC) is one of the most common cancers worldwide. Tumor-associated macrophages (TAM) play a dual role in the development of malignant tumors and may influence prognosis of patients with OSCC if alternatively activated. Anti-inflammatory M2-characteristics in macrophages can be distinguished from pro-inflammatory macrophages using M2 markers, and the scavenger receptor CD163 has been considered the major of these. However, the correlations between CD163+TAM and prognosis of OSCC shows to be inconsistent and the microlocalization of CD163+ expression was rarely described in the lesion.Objective: The aim of this study was to investigate the microlocalization of CD163+ TAM in OSCC and analyze the association with the clinicopathological parameters.Material and Methods: CD163 expression was examined in 60 paraffin-embedded OSCC samples by using immunohistochemistry. The microlocalization of CD163+ TAM was assessed in four different OSCC tumor compartments: adjacent non-neoplastic tissues (NT), invasive front (IF), tumor nest (TN) and tumor stroma (TS). For each specimen, CD163+ expression was analyzed in five representative fields by an experient morphologist. Results and Discussion: According to our results, the number of CD163+ TAM was increased from adjacent non-neoplastic tissues (NT) to tumor stroma (TS). The mean value of CD163+ TAM was 4.8 in IF, 2.1 in TN and 22.3 in TS. In epithelia of NT compartment the CD163+ expression was undetectable. We are in the analysis phase of the clinico-pathological parameters. Hu et al. (2016) recently demonstrated that CD163 expression in both parenchymal and stromal cells had significant impact on the

prognosis of patients with OSCCC.Conclusion: Our preliminary data confirm the importance of CD163+ TAM as tumor microenvironmental elements in favor of OSCC progression. Besides that, not intraparenchymal but stromal CD163+ TAM are largely distributed in OSCC.

Keywords: oral squamous cell carcinoma, macrophages, CD163

#08

ASSOCIATION OF POLYMORPHISMS IN THE GENE OXA1L WITH ALLERGY MARKERS IN A BRAZILIAN POPULATION

Anaque De Oliveira Pires[1]; Milca de Jesus Silva[1]; Maria Borges Rabelo de Santana[1]; Raimon Rios da Silva[1]; Hugo Bernardino Ferreira da Silva[1]; Norma Vilany Queiroz Carneiro[1]; Gerson de Almeida Queiroz[1]; Héllen Freitas Fonseca[1]; Sandro de Oliveira Dias[2]; Ryan dos Santos Costa[1]; Maurício Lima Barreto[3]; Camila Alexandrina Figueiredo[1]

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Support: FAPESB, CNPq and PIBIC

Introduction: Asthma and atopy are considered complex diseases linked the environmental and genetic factors. The OXA1L is involved in the biogenesis of proteins from mitochondria membrane. Changes in oxidative stress and calcium homeostasis in bronchial smooth muscle cells increase mitochondrial biogenesis, cell proliferation, and remodeling of the airways. Objectives: Thus, we hypothesize that genetic variants in OXA1L are associated with asthma and atopy in an admixture population from Brazil. Material And Methods: The DNA from 1,307 individuals was genotyped using Illumina Human 2.5-8 Omni Bead chip. Logistic regression analyses were performed to verify the association of polymorphisms in OXA1L with asthma and allergy markers using PLINK 1.9 software adjusted for sex, age, helminth infections and ancestry markers in additive model. In silico gene expression analysis was performed in whole blood tissue using GTEx browser. Results And Discussion: The C allele of rs4981436 in OXA1L was positively associated with asthma (OR:1.41; CI:1.08-1.84; p:0.012). Additionally, the G allele of rs8572 was positively associated with skin prick test to Dermatophagoides pteronyssinus (OR:1.33; CI:1.05-1.70; p:0.020), Periplaneta americana (OR:1.32; CI:1.03-1.70; p:0.029) and dog epithelium (OR:2.21; CI:1.02-4.82; p:0.045). The same allele (G for rs8572) was also positively associated with anti-D. pteronyssinus specific IgE (OR:1.27; CI:1.10-1.56; p:0.027). In relation to the in silico gene expression analysis, the G allele of rs8572 led to a higher gene expression in Whole Blood tissue. Conclusions: Polymorphisms in OXA1L were positively associated with asthma and allergy markers in our population. At least in part, this association can be explained by the increased expression of this gene observed herein.

Keywords: Polymorphism, asthma, atopy, OXA1L.

#09

POLYMORPHISMS IN DENND1B GENE ARE ASSOCIATED WITH ASTHMA PHENOTYPES IN BRAZILIAN CHILDREN.

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Support: FAPESB and PIBIC

Introduction: Asthma is a complex and heterogeneous disease associated with a complex genetic basis involving environmental factors and individual variabilities. The DENND1B gene has an important role on T cell receptor (TCR) down-regulation on Th2 cells and studies have shown that mutations or loss of this factor can be associated with increased Th2 responses and asthma. Genome-wideassociation studies (GWAS) have recently associatedsingle nucleotide polymorphisms (SNPs) in DENND1B gene with asthma in young children. Our objective was to evaluate the association of polymorphisms in the gene DENND1B with asthma phenotypes in Brazilian children. Materials and Methods: Genotyping was performed using a commercial panel from Illumina (2.5 Human Omni bead chip) in 1,309 participants of SCAALA program (Social Change, Asthma, Allergy in Latin American). The study included 73 SNPs for DENND1B. Logistic regressions for asthma and atopy were performed using PLINK software 1.9 adjusted for sex, age, helminth infections and ancestry markers. Results and Discussion: Seven SNPs in DENND1B were associated with both protection and risk for asthma development. The rs6691216 was negatively associated with asthma (atopics + non-atopics) (OR: 0.76; CI: 0.60-0.96) and non-atopic asthma (OR: 0.58; CI: 0.41-0.82). The rs6694441 (OR: 2.37; CI: 1.43-3.94), rs73077640 (OR: 2.3; CI: 1.43-3.94), rs57589685 (OR: 2.39; CI: 1.51-3.79), rs73073636 (OR: 2.00; CI: 1.31-3.05) and rs16841893 (OR: 1.72; CI: 1.05-2.83) were positively associated with non-atopic asthma. The rs6685897 (OR: 1.62; CI: 1.04-2.52) was positively associated with atopic asthma. Conclusion: Polymorphisms in the gene DENN1B are associated with development of asthma. Understanding how DENND1B contributes to childhood asthma can provide new knowledgesinto how this evolutionarily conserved family of proteins can regulate other biological systems and contribute to human disease.

Keywords: Asthma; Atopy; Polymorphism; DENND1B.

#10

EXPRESSION OF miR-16 AND miR-146a IN RELATION TO STROMAL MACROPHAGES IN ORAL SQUAMOUS CELL CARCINOMA

Camila Da Silva Souza[1,2]; Ana Carolina Valente Santos Cruz De Araújo[2], Clarice Ribeiro Lira[2], Rafael Da Fonseca Carvalho[2], Rafael Luiz Vieira Mercuri[2]; Leonardo De Souza Kruschewsky[3]; André Leonardo De Castro Costa[3]; Heleniemarie Schaer Barbosa[4]; Cláudia Malheiros Coutinho-Camillo[5], Fernando Augusto Soares[5]; Roberto Meyer[6]; Deise Souza Vilas-Bôas[2]; Jean Nunes Dos Santos[7]

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Introduction: MicroRNAs (miRNAs) are critical regulators of macrophage polarization, but this approach in relation to the oral squamous cell carcinoma (OSCC) is almost completely unexplored. Objectives: This study aimed to investigate in OSCC the expression of miR-16 and miR-146a, understood as regulators of shifting macrophage polarization, in relation to stromal distribution of total and M2 macrophages. Material and Methods: Using qRT-PCR, we analyzed the expression of miR-16 and miR-146a in 40 paraffin embedded OSCC samples. Additionally, immunohistochemical localization of CD68+ and CD204+ macrophages was developed. Results and Discussion: We observed up-regulation of miR-16 and miR-146a in 71.88% and 73.12% of tumor samples, respectively. The miR-16 was down-regulated in 3.125% of the samples and no case of down-regulation was observed for miR-146a. Our study corroborates to the evidence that unregulated expression of the miR-16 (COUTINHO-CAMILLO et al., 2015; MANIKANDAN et al., 2016) and miR-146 (SCAPOLI et al., 2010; HUNG et al., 2012; HUNG et al., 2013; SHI et al., 2015) are present in OSCC. Positive correlation was observed between miRNAs expression (r= 0.585, P=0.000). The participation of these miRNAs in tumorigenesis probably occurs through a common signaling pathway, as the PTGS2, whose gene presents major role in OSCC and regulation by both miRNAs. All analyzed cases exhibited dense presence of CD68+ and CD204+ macrophages, but no correlation was observed between infiltration of both macrophages populations and expression of miRNAs. This aspect may indicates the diversity in repertoire of genes and proteins that are involved during deregulation of these miRNAs in OSCC. Conclusions: Our study corroborates to the evidence that unregulated expression of miR-16 and miR-146 presents an important role in OSCC tumorigenesis. Furthermore, in OSCC, these molecules possibly not regulate the development of the disease through macrophages signaling pathways.

Keywords: oral squamous cell carcinoma, miRNAs, macrophages

#11

PHENOTYPIC AND FUNCTIONAL CHARACTERIZATION OF B CELLS IN CUTANEOUS LEISHMANIASIS

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Introduction: Leishmaniasis is an infectious disease, not contagious, caused by different species of Leishmania protozoa of the genus, which affects the skin and mucous membranes. It is a zoonotic infection, which can affect animals other than humans. The whole emphasis regarding the pathogenesis of leishmaniasis has been given to the participation of CD4 + or CD8 + cells. However, different B cell subpopulations are often found in leishmaniotic ulcer and peripheral blood of patients. Similar to T lymphocytes, B lymphocytes express regulatory molecules involved in apoptosis and are capable of regulating the innate and adaptive immune response. Furthermore, B lymphocyte effector (Be1 and Be2) different profiles secrete cytokines that stimulate other cells of the immune system. Thus, investigations into the involvement of these lymphocytes in cutaneous leishmaniasis may contribute with new knowledge about the immunopathogenesis of this parasitic disease. Objectives: This study aims to characterize phenotypically the B cell subpopulations in peripheral blood of patients with cutaneous leishmaniasis and evaluate the functional activity of effector and regulatory B cells from patients infected with Leishmania braziliensis. Methodology: Will be included 60 patients, residents of Corte de Pedra, Presidente Tancredo Neves, Bahia, with cutaneous leishmaniasis without prior treatment of both genres and varied age. The control group will consist of 60 healthy subjects matched for age and genre, living in the same region. Parasitological and serological tests of both groups will be realized. Subpopulations of B cells in peripheral blood will be identified and listed by cytometric multiparameter flow using the dye rhodamine R123 and fluorescent monoclonal IgM/APC, IgD/FITC, CD10/FITC, CD19/BIOTIN, CD24/FITC, CD27/PE e CD38/APC. Functional identification of these B cell effector and regulatory will be performed by analysis of intracellular cytokines IFN-gama/APC, TNF-alfa/PE, IL-6/FITC e IL-10/APC, both groups after culturing peripheral blood mononuclear cells. P values < 0.05 will be considered statistically significant. Results: Expected to get a better understanding of the involvement of B lymphocytes in the immunopathogenesis of cutaneous leishmaniasis and generate relevant information to carry out an increasingly effective treatment.

Keywords: leishmaniasis; lymphocytes; flow cytometry.

#12

MICROBIOME PROFILE EVALUATION AND ASSOCIATED IMMUNE PROFILE IN ATOPIC AND NON-ATOPIC ASTHMATICS

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Support: ERC and FAPESB

Introduction: Over the past few years the scientific community widely assumed that asthma was an allergic/atopic disease caused by allergen exposure only. This observation does not explain the global asthma patterns and time trends of the disease, considering that less than one half of asthma cases is attributable to atopy and/or eosinophilic airways inflammation. Recent evidences suggest the microbiome alterations play a role on asthma and atopy. In this way, we hypothesized that asthmatics microbiome differs from non-asthmatics ones. Objectives: To understand how the airways and gut microbiome influence on asthma and atopy development. Materials and methods: This study is part of a large global initiative comprising six different countries applying the same standardized methodology. We will collect data and samples from a cohort of 250 asthmatic and non-asthmatic children, aged 12-16 living in the city of Salvador, Northeastern Brazil. Information including: risk factors questionnaires, clinical characterization, and blood, sputum, nasal lavage and stool samples will be collected. Atopy will be defined on the basis of serum allergen specific IgE and skin prick test positivity. The lung function testing will be conducted according to American Thoracic Society criteria. Microbial community composition from sputum, nasal lavage and stool samples will be studied through high-throughput sequencing of V3-V4 region of 16s rRNA gene. After quality filtering, sequencing reads will be analyzed using QIIME pipeline. We will verify if the similarities of asthmatic and non-asthmatic microbiota using multivariate analyses (e.g. PCA and PERMANOVA) and machine learning techniques (e.g. Random Forests, SEM modeling) will be employed to predict asthma phenotypes from microbial community compostion. Expected results: This study will provide knowledge about novel asthma phenotypes and their relationships with the microbial community. Understanding asthma in a holobionts context may contribute to new treatment methods development.

Keywords: Asthma, Microbiome, Phenotypes.

#13

PREPARATION, CHARACTERIZATION AND STABILITY OF POLYMERIC NANOPARTICLES AS CARRIERS OF ANTIGENS OF LEISHMANIA VACCINE.

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Support: CPqGM-FIOCRUZ; CNPq; Fapesb; UFBA; SENAI/CIMATEC; SIGMA; GATTE-FOSSHE; CIENAM.

Introduction: The search for new delivery systems and adjuvants that enhance the immune response to the vaccine antigens is an important step toward the development of a vaccine against leishmaniasis. In this context, the use of nanoparticles as vaccine antigen delivery systems have been the target of intense research in the last decade. Particularly, polyesters such as poly (ε-caprolactone) (PCL) represents potential inputs for the manufacturing biomolecule-carrying nanoparticles, including vaccine antigens. Objectives: Thus, this project investigated the potential of polymeric nanoparticles based on PCL as nucleosomal histones release systems, to develop a nanovaccine for cutaneous leishmaniasis. Material and Methods: The nanoparticles were prepared by nanoprecipitation method and characterized as the particle size, zeta potential, transmission electron microscopy (TEM), turbidity, pH, electrical conductivity and visual appearance. It was also conducted a study of stability of nanosuspensions by monitoring these parameters for 120 days at 25 ± 1°C. Results and Discussion: The suspensions of PCL nanoparticles were presented fluids, homogeneous and milky-white appearance. Macroscopically, no signs of physical instability were detected, such as formation of precipitates or sediment. The freshly prepared suspensions showed negative zeta potential, with indicatives module values of stable colloidal systems (~ 20 mV). These systems exhibited a mean size of 385 ± 67 nm with polydispersity index of 0.28. The size was confirmed by transmission electron microscopy (TEM), which also provided Information about the morphology of the nanoparticles that exhibited the form of nanocapsules dispersed in medium, without the presence of aggregates. It was also possible to visualize the wall of polymeric nanocapsules delimiting its core. The monitoring of the levels of turbidity, pH and electrical conductivity over 120 days indicated the maintenance of the stability of polymeric nanoparticles. Together, the changes in these parameters indicated no major physical instability phenomena that could compromise the stability of polymeric nanoparticles, which was maintained throughout the trial period of the study. Conclusions: Therefore, we concluded that the polymer nanoparticles showed vesicular features, with optimized stability, being potentials carriers for the incorporation of vaccine antigens, as nucleosomal histones of Leishmania.

Keywords: PCL; Nanoparticles; Histonas; Leishmanaise

#14

TOLL-LIKE RECEPTOR 7 (TLR-7) INDUCTION BY Imiquimod AND granzyme PRODUCTION BY DENDRITIC CELLS DURING EXPERIMENTAL MELANOMA DEVELOPED IN THE EAR OF C57BL/6

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Support: PAPES and CNPq

Introduction and objective- Cutaneous melanoma is a malignant tumor and is associated with high mortality rates. In general, tumor cells can be recognized by the immune system by the absence, or alteration in MHC class I expression, then activating NK and NKT cells. An interesting and alternative approach to potentiate anti-tumor response would be by stimulating innate immunity on a quick and lasting manner by inducing receptor toll-like - 7 (TLR-7). To achieve this, Imiquimod (IMIQ) was used as a topical cream, by inducing TLR - 7 which is widely expressed on B lymphocytes and on dendritic cells (DCs). Methods and Results- 5 x 104 tumor cells were inoculated into the the pinna of the ear, and mice were treated with 10 uL of the topical cream of imiquimod (Aldara) at a concentration of 50 mg/g for 10 consecutive days, 2 times a day. At the end of treatment, splenic ells of mice that were treated (or not) with IMIQ were stimulated and the production of intracellular cytokine was evaluated after stimulation with: 1-PBS as a control, 2- Concanavalin A (Con A) or Con A plus tumor antigens (Con A+tm-ags). As a result, IMIQ was effective by restraining early tumor growth, and an increased INF-γ production by CD8 T cells in both spleen and draining lymph node was observed. TNF-α secreted by CD4 and CD8 T cells were increased in draining lymph nodes of IMQ-treated mice. DCs treated from IMIQ-treated mice had a trend to produce higher levels of Granzyme B (GZB) than untreated animals. In addition, spleen cells stimulated with Con A plus Tm-Ags produced lower levels of GZM when compared to Con A-stimulated spleen cells (p = 0.0286; Mann-Whitney test). Conclusions- These results demonstrate that in the presence of tumor antigen, the DCs presented a reduced functional activity even in IMIQ-treated mice. Tumor antigens may cause an inhibition of the activity of these dendritic effector cells in vivo, decreasing GRZ produced by DCs. Finally, our results indicate a clear efficacy of IMIQ on the initial phase of tumor development, due to its ability to enhance GZM production by DCs.

Keywords: Imiquimod, melanoma, NKT.

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#15

IMMUNOMODULATORY ACTIVITY OF RECOMBINANT PROTEINS OF LEISHMANIA INFANTUM ON CELLS FROM PATIENTS WITH CUTANEOUS LEISHMANIASIS

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Support: SIM, Capes, NIH

Introduction: Leishmaniasis is a complex disease caused by trypanosomes of the genus Leishmania, which parasitize approximately two million people per year (Handman, 2001). In cutaneous leishmaniasis, there is a strong Th1 response that controls the multiplication of the parasite, but leads to tissue injury (Carvalho, Passos and Jesus, 2005). Because of this exacerbation of Th1 immune response in cutaneous leishmaniasis is necessary to discovery of new molecules with immunomodulatory activity having a therapeutic potential. Objective: Determine the activity of the recombinant proteins LCi 3-2R-NT-CT LCi2-NT-CT-SR and LCi2-NT-CT on populations of mononuclear cells of patients with cutaneous leishmaniasis (CL). Material and Methods: Recombinant proteins termed LCi-3-NT-2 R-CT, LCi2-NT-CT-SR and LCi2-NT-CT were prepared in collaboration with Dr. Geraldo Gileno de Sá Oliveira. The selection of these proteins was based on reactivity serum mixtures of naturally infected dogs and humans. Thus, with the proteins produced, the experiment was conducted with mononuclear cells obtained from the blood of patients with LC and healthy individuals. These cells were incubated in the absence or presence of recombinant proteins for 72 hours, after the culture supernatants were collected for measurement of IL-10, IL-1β and IFN-γ by ELISA. To determine the role of monocytes in IL-10 production, mononuclear cells obtained from peripheral blood of healthy individuals were incubated in the presence or absence of recombinant proteins for 6 hours and subsequently added anti-CD14, anti-CD16 and anti-IL-10 for analysis by flow cytometry. Results: The recombinant proteins were not capable of inducing IFN-γ production in healthy subjects cell cultures or CL patients, however the proteins LCi-3-NT-2R-CT LCi2-NT-SR-CT were able to induce IL-10 and IL-1β in cultured cells of healthy individuals. In cell cultures from patients with LC the LCi2-NT-SR-CT and LCi2-NT-CT proteins were able to induce IL-10 production and IL-1β. From the flow cytometric analysis was not possible to observe a statistically significant increase in IL-10 production of different subpopulations of monocytes when stimulated with recombinant proteins. Conclusions: The recombinant protein LCi-3-NT-2R-CT LCi2-NT-SR-CT and LCi2-NT-CT have immunomodulatory activity by inducing mononuclear cells of healthy individuals or individuals with cutaneous leishmaniasis to produce IL-10 and IL-1β. Acknowledgment: Capes, PPGIM e SIM.

Keywords: Cutaneous leishmaniasis, recombinant proteins and immunomodulation.

#16

DETERMINATION OF IL-1 β PRODUCTION PATHWAYS IN PATIENTS WITH CUTANEOUS LEISHMANIASIS

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Support: INCT-DT, CAPES and NIH

Introduction: The activation of inflammasomes components leading to high production of IL-1β is associated with the pathogenesis of a variety of inflammatory diseases. Recent work has shown the importance of the inflammasome activation for IL-1β production in murine leishmaniasis and a role for NLRP3 in IL-1β production was reported. CL due to Leishmania braziliensis infection is an inflammatory disease where skin ulcer development is associated with mononuclear cells infiltrate and high levels of inflammatory cytokines. Therefore, our hypothesis is that CL patients have increased expression of NLRP3 and produce high levels of IL-1β. Methods and Results: Bone marrow-derived macrophages were obtained from C57BL/6 wild type and knockout mice for CASPASE-1, NLRP3, PYCARD, AIM2 and IL-1R. Peripheral blood mononuclear cells (PBMC) and lesion biopsies were obtained from CL patients. To evaluate the IL-1β production pathways in mice cells were treated or not with LPS for 6 hours and stimulated with soluble Leishmania antigen (SLA), L. braziliensis, monosodium urate and lipofectamine for 42 hours. IL-1β was detected by ELISA. In addition, PBMCs from patients were differentiated into macrophages, stimulated with SLA for 2 hours and gene expression of IL-1β, CASPASE-1, NLRP3, PYCARD, AIM2 and IL-1R was determined by real-time PCR. Biopsies and PBMCs from patients were maintained in culture in presence of SLA for 72 hours and IL-1β levels determined by ELISA. We also identified the source of IL-1β by flow cytometry by stimulating PBMC with SLA for 8 hours and staining for CD14, CD16 and IL-1β. In our murine experiments we found that IL-1β production in response to L. braziliensis was dependent on NLRP3 and CASPASE-1. Our patients' results showed increased expression of IL-1β and NLRP3 genes in macrophages stimulated with SLA and high production of IL-1β in supernatants of PBMCs and biopsies from CL patients. The main source of this cytokine was intermediate monocytes (CD14+CD16+). Conclusion: Intermediate monocytes of patients with CL produce high levels of IL-1β, probably through NLRP3 activation.

#17

MICROLOCALIZATION OF CD204+ TUMOR-ASSOCIATED MACROPHAGES IN ORAL SQUAMOUS CELL CARCINOMA PATIENTS

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Support: FAPESB, CNPq and Programa Permanecer - MEC/SESu

Introduction: Macrophages are inflammatory components present in oral squamous cell carcinoma (OSCC) and may influence tumor development and metastasis if alternatively activated (M2). The class A macrophage scavenger receptor CD204 have reported as a M2 macrophage marker. Although the microlocalization of CD68+ (pan-macrophage marker) TAM has already been observed in OSCC (NI et al., 2015), the microlocalization of CD204+ TAM and its prognostic significance has not been described. Objective: The aim of this study was to investigate the microlocalization of CD204+ TAM in OSCC in relation to CD68+ TAM infiltration and clinicopathological parameters. Material and Methods: CD68+ and CD204+ TAM were examined in 60 paraffin-embedded OSCC samples by using immunohistochemistry. The microlocalization of CD68+ and CD204+ TAM was assessed in four different OSCC tumor compartments: adjacent non-neoplastic tissues (NT), invasive front (IF), tumor nest (TN) and tumor stroma (TS). Results and Discussion: CD68+ and CD204+ cells were present in both subepithelial and epithelial compartments in NT. The number of CD204+ TAM was strangely increased from adjacent non-neoplastic tissues (NT) to tumor stroma (TS). According to ours preliminary results, the mean value of CD204+ TAM was 3.4 in NT, 8.2 in IF, 14.3 in TN and 30.7 in TS. These results corroborate the recent study of ESSA et al. (2016), that showed a high presence of CD204-positive cells in the stroma of OSCC. Furthermore, similarly, we observed CD204+ TAM infiltrating presenting similar densities within the same areas in relation to CD68+ TAM infiltrating in correspondent sections. We are in the analysis phase of the clinicopathological parameters. Conclusion: These data confirmed the importance of CD204+ TAM as tumor microenvironmental components in favor of OSCC progression. Most of the CD68+ macrophages were considered to be M2 macrophages.

Keywords: oral squamous cell carcinoma, macrophages, CD204

#18

EFFECT SM29 ANTIGEN OF SCHISTOSOMA MANSONI IN VITRO ON DENDRITIC CELLS AND LYMPHOCYTES PROFILE OF PATIENTS WITH CUTANEOUS LEISHMANIASIS

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Support: UFBA, SIM, INCT-DT, NIH

Introduction: The exacerbated inflammatory response is associated with lesions in cutaneous leishmaniasis (CL). It has been shown that Schistosoma mansoni antigens, such as Sm29, are able to modulate the inflammatory response in immune-mediated diseases. Objectives: The aim of this study was to evaluate the effect of Sm29 antigen in modulate the in vitro immune response of monocyte-derived dendritic cells (MoDCs) infected with Leishmania braziliensis and co-cultured with autologous lymphocytes from patients with leishmaniasis. Material and Methods: The expression of surface molecules on MoDCs and in TCD4+ lymphocytes were evaluated by flow cytometry (FACS). The levels of IL-10, IL-12 and TNF in the supernatant of cultures were evaluated by ELISA. Results: The addition of Sm29 did not alter the mean intensity of fluorescence (MIF) of HLA-DR. The addition of this antigen increased the frequency of MoDCs expressing CD80 [41% (min: 23- máx: 67)], CD86 [92% (65-97)] and CD83 [44% (20-74)], compared to unstimulated cultures [CD80: 24% (19-53); CD86: 86% (41-94); CD83: 27% (13-62), p<0.05]. The Sm29 antigen also increased the frequency of MoDCs expressing IL-10R [19% (6-34)], compared to cultures without stimulation [16% (7-25), p <0.05]. Regarding T CD4 lymphocytes markers, we observed that the addition of Sm29 increased the frequency of regulatory T cells CD4+CD25high [0.5% (0.1-4)] and CD4+CTLA-4+ cells [2.7% (1.3-7.0)], compared to the cultures without stimulation [0.3% (0.1-2) and 0.9% (0.1-2.6), respectively; p <0 05]. On the other hand, we observe a lower frequency of CD4+CD28+ T lymphocytes [35% (22-72)] after stimulation with Sm29, compared to unstimulated cultures [53% (17-77); p<0.05]. The cultures stimulated with Sm29 showed an increase in the levels of IL10 (536 ± 527 pg/ml) and a decreased levels of IL12p40 (257 ± 124pg/ml), compared to the cultures without stimulation [(IL10: 171 ± 134 pg/ml), (IL-12p40: 1126 ± 1029 pg/ml); p <0.05]. We did not observe differences in the levels of TNF in the supernatants of cultures. Conclusions: The Sm29 antigen induced a regulatory profile by MoDCs and lymphocytes that is desirable in the control of exacerbated immune response observed in cutaneous leishmaniais.

Keywords: Leishmaniasis, Sm29 antigen, dendritic cells, lymphocytes

#19

PATHOGENESIS FIBROSIS SEPTAL: A STUDY ON THE ROLE OF KUPFFER CELLS AND HEPATIC STELLATE CELLS.

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Support: FAPESB, CNPq, CPqGM

Introduction - In rats helminth infection *Capillaria hepatica* generates the formation of a peculiar type of fibrosis: septal fibrosis. This fibrosis is characterized by the appearance of fine fibrous septa are spread throughout the hepatic parenchyma, and extends from a portal to another portal zone area connecting the portal spaces between them. Recent studies show that besides the hepatocytes, hepatic stellate cells (HSCs), Kupffer cells and other parenchymal liver cells also participate in the establishment of hepatic fibrogenesis. Objectives- Given these aspects aim to clarify the role of cells of Kupffer cells and hepatic stellate in septal fibrosis induced by *C.hepatica.*Material and MethodsFor this, Wistar rats of both sexes were divided into three groups: control, group *C. hepatica* infected and infected group and treated by intravenous method dichloromethylene bisphosphonate ((Cl_2MDP)) to depletion of Kupffer cells. After a month the animals were sacrificed for tissue collection and serum. Liver fragments of animals of the different experimental groups were fixed in formalin embedded in paraffin and stained with hematoxylin / eosin (H&E) and Sirius-red to evaluate hepatic fibrosis. Was performed also immunostaining for the following antibodies: CD68, α-SMA and desmin.Results and Discussion -Histological analysis obtained through the H&E and Sirius-red staining showed the participation of the various cells that make up the liver parenchyma in fibrogenesis, especially of inflammatory cells and portal space. Was also possible to check the reduction in septal fibrosis in the group treated with Cl_2MDP and infected. The immunostaining for α-SMA and desmin showed a reduction in activated stellate cells during fibrogenesis in depleted animals. The immunostaining for CD68 demonstrated the intense involvement of Kupffer cells during fibrogenesis. However in animals where the Kupffer cells were depleted there was a decrease of CD68 positive cells. Furthermore,these animals, septal fibrosis was clearly reduced. Conclusions - These findings emphasize the importance of these cells in septal fibrosis process induced C.hepatic.

Keywords - Kupffer cells, hepatic stellate cells, fibrosis septal, Capillaria hepatica.

#20

SYNTHETIC PEPTIDES FROM PORPHYROMONAS GINGIVALIS LYS-GINGIPAIN TO STUDY IMMUNE RESPONSE IN CHRONIC PERIODONTITIS

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Introduction: Porphyromonas gingivalis (Pg) is a keystone pathogen in Chronic Periodontitis (CP). Works on its virulence factors immunogenicity may contribute to the understanding of host response to infection. Objective: The present work aimed to identify immunogenic peptides from Lys-gingipain (Kgp) virulence factor of Pg ATCC 33277 (NCBI Taxonomy ID: 431947), which was selected based on previously performed immunogenicity experiments using chromatografic fractions of Pg extract. Material and Methods: Kgp sequence (1723aa) was obtained from the NCBI Protein Database (YP_001929844) and it was scanned for amino acid patterns indicative of MHCII binding using the MHC-II Binding Predictions tool from IEDB (http://tools.immuneepitope.org/mhcii/). This analysis considered nine HLA alleles (loci DQ and DR), which were observed in previous studies involving subjects with CP from Salvador (BA), Brazil. T-cell epitope prediction resulted in 16 peptide sequences (15- mer) from Kgp. Nine Kgp peptides were selected within different regions of the protein (UniProt B2RLK2), they were chemically synthesized and they were tested by indirect ELISA method to verify presence of specific IgG in serum of subjects with CP and without Periodontitis (WP). 41 subjects from Feira de Santana (BA), Brazil, were evaluated. They had no systemic disease and they were clinically classified into CP (20 subjects) and WP (21 subjects) according to periodontal parameters. Their serum samples were pooled into CP and WP pools based on clinical periodontal parameters of the subjects in addition to IgG anti-Pg level of each serum sample tested by indirect ELISA method using Pg extract antigen; such as CP pool (six samples: O.D. 0,53 – 1,10) and WP pool (five samples: O.D. 0,22 – 0,38). Optimal coefficient between CP pool and WP pool was determined for each analysed peptide by checkerboard ELISA. Pg extract (5µg/mL) was used as positive control in the screening of these Kgp synthetic peptides (10µg/mL). Results and Discussion: All nine Kgp peptides were recognized by specific IgG in both sera pools. Kgp12 (within the catalytic subunit) presented the best coefficient value between CP and WP pools, so it was selected to later in vitro experiments. Conclusion: Kgp12 from the Kgp virulence factor seems to be an epitope candidate for experiments with human PBMC to analyse Pg immunogenicity. CAAE 32535914.4.0000.0053

Keywords: Immunoinformatics, Lys-gingipain, Chronic Periodontitis.

#21

EVALUATION OF TOLEROGENIC DENDRITIC CELLS IN THE THERAPY AND/OR PREVENTION OF DEVELOPMENT OF CHAGAS DISEASE CARDIOMYOPATHY

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Introduction: Chronic Chagas disease cardiomyopathy (CCC) is present in 20-30 % of Trypanosoma cruzi-infected people who are in the chronic phase of the infection. Evidence suggests that autoimmune phenomena participate in the pathogenesis of CCC. We have demonstrated that mice in which cardiac immune tolerance to myosin-enriched antigen developed a milder myocarditis than control mice, following infection with T. cruzi. Dendritic cells (DC) have an essential role in the initiation and maintenance of T-cell-dependent immune responses. Immunological tolerance can be induced by immature DC in a non-inflammatory microenvironment. Objective: The aim of the study was to evaluate whether cardiac myosin-sensitized tolerogenic DC are able to interfere with the development of chronic cardiomyopathy in an experimental model of Chagas disease. Materials and methods: DC were produced by culturing bone marrow cells obtained from the femurs of C57Bl/6 mice with RPMI medium supplemented with 30% supernatant of GM-CSF-secreting x-63 cells. Tolerogenic DC were obtained by the addition of dexamethasone (10 µM) after the 3rd day of culture. After 7 days of culture, DC were activated with 1 µg/ml of bacterial lipopolysaccharide for 24 hours, and pulsed overnight with porcine cardiac myosin. Groups of 10 male C57Bl/6 mice received injections of tolerogenic DC intraperitoneally before and after infection with Colombian strain T. cruzi. Two doses with intervals of seven days before infection in two groups were administered, one treated with myosin-sensitized DC and the control only with DC. Two other groups received four injections at intervals of 30 days three months after infection (chronic phase of disease). Naive and T. cruzi-infected control groups received injections of vehicle (saline). Six months after infection, mice were euthanized for histopathological analysis of the hearts. Results and Discussion: Hematoxylin-stained sections showed diffuse inflammatory infiltrates composed mainly by mononuclear cells in the hearts of T. cruzi-infected mice. Morphometric analysis of the hearts did not show a significant difference between the infected groups. Conclusions: Our preliminary results indicate that the therapeutic scheme tested was not efficient in preventing or reducing the progression of chronic Chagas disease cardiomyopathy. Ongoing experiments are being conducted using a protocol with a higher dose of DC.

Keywords: Trypanosoma cruzi, Cardiomyopathy, Dendritic cells, Immune tolerance.

#22

EVALUATION OF THE AIRWAY INFLAMMATION BY CELLULARITY AND CYTOKINES INDUCED SPUTUM IN ASTHMA PHENOTYPES IN SALVADOR - BAHIA.

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Support: Laboratório de Alergia e Acarologia - LAA; PROAR - Núcleo de Excelência em Asma

Introduction. Asthma is a serious public health problem and affects approximately 300 million people worldwide. It is a chronic, heterogeneous and multifactorial disease. According to GINA data (2014) around 5% of asthmatics have asthma refractory to treatment. Objective. To assess airway inflammation through by counting of sputum cells and measurement of the cytokine and chemokine profiles in induced sputum and associate them with the phenotypes of severe asthma in the city of Salvador - BA. Materials and Methods. The study was performed in a total 66 subjects, divided into three asthma groups: severe asthma resistant to treatment (16) severe asthma partially controlled with treatment (22), mild asthma (19) and healthy control group (9). The total cellularity of the samples was counted in a hemocytometer and differential cytology seen in cytospin. The measurements of cytokines and chemokines in induced sputum were performed by Luminex (Upstate / Millipore system "Flex kit"). The cytokines and chemokines panel included Th1-type cytokines (IFN-γ; IL12p40), Th2 (IL-4, IL-5 and IL-13), Th17 (IL-17A), regulatory (IL-10) cytokine and the IFN-I family (IFN-α2), Inflamatory cytokynes (IL-6, TNF) and the chemokines eotaxin, RANTES, MIP1-α, IL-1β, IL1RA. The statistical analysis were performed using Kruskal-Wallis test, with $p \leq 0.05$ considered significant. Results. The neutrophils and eosinophil count showed differential distribution among the phenotypes studied. The severe asthma resistent to treatment presented increase in percentage of neutrophils compared with mild asthma ($p = 0.046$) and eosinophils compared with healthy control group ($p = 0.021$). The cytokine TNF was increased in the severe asthma resistent to treatment group compared the, healthy control ($p = 0.008$), mild asthma ($p = 0.003$) and severe asthma partially controlled with treatment ($p = 0,047$). Individuals with severe asthma resistent to treatment also showed higher IL-6 levels compared to individuals with severe asthma partially controlled ($p = 0.05$). The data suggest that the increase in the severity of asthma in this population is associated with mixed profile of cells (neutrophils and eosinophils) and the presence of pro-inflammatory cytokines (TNF and IL-6). Conclusion. Asthmatic patients with increased production of pro-inflammatory cytokines and higher percentage of neutrophils and eosinophils have more severe disease. The measurement of these cytokine levels and total cellularity in induced sputum might be an aditional tool for monitoring the response to treatment in patients with severe asthma in research centers

#23

PHYLOGENETIC CHARACTERIZATION AND THERAPEUTIC RESISTANCE PROFILE OF HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 (HIV-1) CIRCULATING IN POPULATIONS OF NORTHEASTERN BRAZIL

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Introduction: By the end of 2011, 34.0 million people were living with HIV worldwide. The latest records show that Brazil has 656,701 cases of AIDS and that despite the drop in the incidence rate in the Southeast, there was an increase in other regions of Brazil. The high genetic variability of HIV is a major obstacle both to infection control by the host immune system and to the development of efficient drugs and vaccines. Objective: This study aims to characterize the profile of mutations associated with resistance to different classes of antiretroviral drugs and the phylogenetic relationship of circulating HIV-1 strains in Northeastern Brazil. Material and Methods: Genomic sequences of HIV previously published in GenBank are being collected and organized at a local database. Bioinformatics tools are being used to interpret the data. Associations with epidemiological and clinical characteristics will be investigated through statistical analysis. Results and Discussion: To date approximately 200 nucleotide sequences of the pol and env genes of HIV-1 circulating in Northeast Brazil (Bahia, Pernambuco, Piauí, Alagoas, Maranhão) were obtained. The Los Alamos - Sequence Locator Tool were used to identify the genomic position and the REGA HIV Subtyping Tool were used to identify the sequence subtype. Moreover, a database with socio-epidemiological patient information were created and categorized. Conclusions: This study may contribute to the generation of relevant information for better understanding of the evolutionary properties of the virus to AIDS local epidemic surveillance and the appropriate choice of control measures.

Keywords: HIV-1, resistance, mutations.

#24

ANALYSIS OF NEUROPROTECTION INDUCED BY AUTOPHAGY AND Α-SYNUCLEIN DEGRADATION AS A MECHANISM OF FLAVONOID REGULATORS OF MICROGLIAL TNF-Α

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Support: FAPESB, CAPES

Introduction Parkinson's disease is the second most common neurodegenerative disease in developed countries. Its incidence has increased exponentially since the 60's and according to predictions, it will continue to do so, due to the increase of life expectancy, as Parkinson's is strongly associated with aging. The mechanisms responsible for the loss of dopaminergic neurons that contain neuromelanin in substantia nigra pars compacta remain unknown, however mitochondrial and protein degradation dysfunctions, α-synuclein aggregation for formation of neurotoxic oligomers, oxidative stress, stress in the endoplasmic reticulum and neuroinflammation events are considered to be involved in neurodegeneration. The use and development of neuroprotective agents for the treatment of Parkinson's disease probably represents oneof the major therapeutic strategies today. Based on the disease etiology and its multifactorial characteristic one can see the importance of finding drugs that may relate to the various steps of the development of this pathology. Objective To evaluate the effect of flavonoids on microglial regulation of TNFα and its implications in autophagic flow, degradation of α-synuclein and neuroprotection, and as specific objectives: 1) to investigate the effect of the flavonoidsrutin andapigenin in the regulation of TNFα from LPS-induced expression in primary cultures of rat microglia; 2) to evaluate the role of TNFα released by microglia primary cultures stimulated by LPS in inhibiting the autophagic flow and α-synuclein accumulation in primary culture of midbrain and PC-12 cell lines. 3) to analyze consequential effects of TNFα regulation by flavonoids in the maintenance of autophagicflow, α-synuclein degradation and neuroprotection in primary cultures of midbrain and in PC-12 cell lines. Materials and Methods Therefore, we will conduct primary cultures of mice midbrain, primary rat microglia cultures and PC12 cell lines cultures. They will be treated with LPS and/or flavonoids, and we will assess the cultures viability by MTT test,the gene expression of pro-inflammatory and anti-inflammatory cytokines by RTQ-PCR and TNF dosage will be performed by ELISA. Given these projections, it is expected that at the end of this project it will be possible to clarify neuroprotective mechanisms of flavonoids in experimental models of Parkinson's disease.

Key-words: Parkinson's disease, autophagy , TNFα , neuroprotection, flavonoid

#25

ENHANCING THE YIELD OF SOLUBLE RECOMBINANT PROTEINS IN ESCHERICHIA COLI USING A GENETIC CIRCUIT FOR CONTROLLED INTRACELLULAR PROCESSING (CIP)

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Support: FAPESB/CNPq

Introduction: The production of soluble recombinant proteins in bacterial systems, with high yields, is a cornerstone of modern biotechnology. Nevertheless, the great majority of expression systems used to date were constructed from a limited group of genetic elements, which have not been comprehensively characterized and optimized for production. Objectives:The present work seeks to construct a genetic circuit able to remove in vivo the fusion tags used for enhancement of protein solubility. Methods: In order to create this genetic circuit, two expression-operating units (EOU) were initially tested. The first one composed of the coding sequence (CDS) for superfolder GFP (sf-GFP) controlled by a strong ribosome-biding site (RBS), and an improved T7 promoter, consisting of fragments that led to a higher transcription level. This EOU was then cloned in pUC57 to compose the plasmid pOPT1.0. Another EOU used contains the sf-GFP CDS controlled by a strong RBS and the wild-type T7 promoter (positive control). Results and Discussion: After 20 hours of induction by IPTG, the fluorescence intensity (FI) generated in E.coli transformed with the optimized EOU (70,62 a.u.) was higher than in the positive control (59,63 a.u.). A third EOU, which was composed by an enhanced GFP (eGFP) instead of sf-GFP, was evaluated. The FI using eGFP (108,83) was higher than using sf-GFP (70,63), at the same expression conditions. On the other hand, protein quantification showed that protein concentration (either eGFP or sf-GFP) was higher when using optimized EOU (188 mg/L of bacterial culture) than when using the eGFP EOU (108 mg/L of bacterial culture). Based on these results, the best performing genetic elements were used to assemble the circuit module accountable for producing the target protein fused with a solubility enhancing tag (KDPG-aldolase). Due to its higher FI, and due to the lower yield in soluble form, eGFP was selected as the reporter protein to evaluate the new solubility circuit. Additionally, genetic modules were synthetized to produce the TetR repressor controlled by the Lambda cI repressor; in turn, the the protease responsible for releasing the solubility enhancer, Tabaco Etch Virus (TEV) protease, is produced under the control of a TetR repressible promoter. All these three modules were synthetized and cloned in a pUC57 plasmid and the integrity of the novel plasmids were confirmed using restriction endonuclease digestion. Conclusion: We anticipate that this newly developed genetic circuit will allow for more predictable and efficient production of recombinant proteins in the soluble fraction.

#26

THE ROLE OF IL-10 INDUCED BY *BLOMIA TROPICALIS* EXTRACT IN ALLERGIC AND NON-ALLERGIC INDIVIDUALS FROM LATIN AMERICA

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Support: FAPESB, CNPq, CAPES

Introduction: An increased prevalence of asthma and other allergic diseases are reported all around the world. House dust mites are common sensitizer agents and Blomia tropicalis is one of the most important source of allergens in tropical and sub-tropical areas. Some studies shown that house dust mite extracts are able to induce IL-10 by blood cells even from allergic patients' donors. IL-10 is a pleiotropic cytokine with a well-known immunomodulatory and anti-inflammatory functions. This cytokine is also important in the regulatory function of T-cells. On the other hand, some studies had failed to demonstrate the IL-10 effects avoiding inflammation. There is also a study where IL-10 polymorphisms were associated with persistent cow's milk allergy. Furthermore, we have found in the SCAALA study group that 90% of the B. tropicalis stimulated blood cells had high production of IL-10 suggesting that the IL-10 produced by B. tropicalis antigen may have acquired a Th2-type phenotype increasing allergy. Since members of the IL-10 family are inducers of inflammatory reaction (IL-19, IL-20, IL-22 and IL-24), a possible explanation would be the presence of cross-reaction among members of the IL-10 family leading to an erroneous detection of IL-10 instead of the other family members. Objective: To evaluate the role and cross-reactivity between IL-10 and other members of this family in allergic and healthy volunteers from Salvador, Bahia. Materials and Methods: This study will involve twenty adults, which ten of them will be atopic and the others non-atopics. Whole-blood and serum sampling will be done to perform a PBMC culture and detection of allergen specifc IgE, respectively. PBMC will be stimulated with Blomia tropicalis and Dermatophagoide pteronyssinus and Ascaris lumbricoides extracts, and Toxocara canis excretory-secreted factors. Pokeweed-stimulated and uninstimulated cells will be used as positive and negative controls respectively. Expression of IL-10 and its homolog cytokines (IL-19, IL-20, IL-22 and IL-24) will be detected by real time PCR (ThermoFisher Scientific, Massachusetts, USA). Specific IgE against different allergic sources will be quantified by ImunoCAP (Phadia/ThermoFischer, Massachusetts, USA). IL-10, Th2 and Th1 cytokine will be measurement from PBMC supernatant by ELISA. A parasitological test from all individuals will be done to control the IL-10 produced by geohelminthic infections. Specific IgG4 and IgE against A. lumbricoides and T. canis will be measure by home-made ImmunoCAP. The phenotype of the main cell source of IL-10 will be assessed using the flow cytometry technique. Statistical analyses will be performed using SPSS 22.1 and Graph Pad Prism software. Expected Results: It is expected a better understanding about the function of the IL-10 induced by B. tropicalis. We hope to define which cells are responsible for the IL-10 production. We also intend to identify if Blomia tropicalis could induce IL-10 homologs cytokine that could be mistakenly identified as IL-10 by ELISA.

#27

ANTIMICROBIAL POTENTIAL OF SEVERAL ENDOPHYTIC FUNGI ISOLATATED FROM MANIKARA SALZMANNII

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Introduction: Endophytic fungi are currently being associated with the discovery of novel antimicrobial active substances. Such filamentous fungi are known for colonizing plant tissue and, thus, protect them from other microbial invaders. Objective: the aim of this work was to isolate and test different fugal strains obtained from the commensal interaction with Manikara salzmannii (endophytic interaction). Material and Methods: Nine strains were isolated using solid media technique and culturing for the production of antimicrobial substance were carried out using sabouraud broth (26 ° C , 120 rpm for 8 days). Antimicrobial solutions for the testing were obtained using acetate extraction of cell-free broth. Antimicrobial tests were carried out using 6mm paper disk technique (30µL of broth extracts) and the tests were performed using Gram negative bacteria (E. coli, Salmonella spp.), Gram-positive bacteria (Staphylococcus spp., Enterococccus spp.), (Corynebacterium sp. VD57 and 1002 both isolated from goat) and yeast (Candida albicans). Results and Discussion: The results showed that strains E and H inhibited the growth of all testing microorganisms by forming an inhibition halo which varied from 11-26 mm. However, the strongest antimicrobial effect (25 mm of halo) against some testing bacteria was observed using the extracts from isolate B and C. The isolate D showed specific antimicrobial activity to the yeast Candida albicans (13 mm of halo). This indicates a novel substance for specifically controlling yeast growth, which is currently in demand. The other strains have also showed some antimicrobial activity, but this result was not uniform towards all tested microorganisms. The fact that several strains showed significant effect against Corynebacterium sp increases the expectation for the discovery of a novel substance for the control of lymphadenitis infection (sheep and goats), which is a disease with significant economic impact for the Bahia state.

Keywords: : Endophytic fungi, antimicrobial, microorganisms.

#28

IL1RL1 VARIANT IS ASSOCIATED WITH ASTHMA, PULMONARY FUNCTION AND SOLUBLE ST2 IN AN ADULT BRAZILIAN CASE-CONTROL STUDY

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Support: CNPq, CAPES e FAPESB

Introduction: Asthma and other allergies are caused by type 1 hypersensitivity reaction, initiated by IgE antibody-mediated mechanisms and inflammatory cells. The prevalence of allergies has increased in recent decades, affecting about Million people worldwide. Recent studies have shown that genetic and environmental factors may interact for allergy development. Interleukin-33 (IL-33) IL-1 family, appears to be a potent inducer of Th2 response, since it promotes the release of IL-4, IL-5 and IL-13. This occurs when IL-33 binds to its receptor and activates the IL1RL1 (ST2) cell that releases these cytokines intensifying asthmatic inflammation. The polymorphisms in IL1RL1 are the most replicated genes in Genome-Wide Association Studies (GWAS) for allergy worldwide. Therefore, it becomes of great importance to study the influence of IL1RL1 polymorphism. Objective: The aim of this study was to evaluated the association between the variant rs1420101 with asthma and lung function, never explored before in a Brazilian population. Material and Methods: DNA was extracted from peripheral blood from 1.445 subjects and the samples were genotyped using DNA kit protocol Flexigene@ (Qiagen, Hilden, Alemanha). The study included IL1RL1 variant rs1420101. Logistics and linear regressions were performed to analyze the association between IL1RL1 with asthma, pulmonary function and sST2 production using PLINK software 1.9 adjusted for sex, age and skin color, in all genetics models. Results and Discussion: The A allele of rs1420101 was positively associated with non-atopic asthma (OR=2.07; IC 1.13–3.78 p=0.018) and atopic asthma (OR=1.29; IC 1.05–1.66 p=0.046). This same allele was also associated negatively with pulmonary function (BETA= -2.37; IC -4.67; -0.07, p=0.043). In addition, ST2 soluble (sST2) levels in serum from control subjects were lower in AA genotype for rs1420101 in IL1RL1 compared with individuals with GG (P<0.001) and between AG and GG genotypes (p<0.05). Conclusion: Variant rs1420101 was associated with atopic and non-atopic asthma and decreased pulmonary function; this may be associated with decreased levels of sST2. However, more studies should be conducted to investigate the functional role of this gene that could explain the development of complex conditions such as asthma.

Keywords: Asthma; polymorphism; IL1RL1.

Acknowledgment: CNPq, CAPES, FAPESB.

#29

PRODUCTION AND PURIFICATION OF MOLECULES DERIVED FROM *CORYNEBACTERIUM PSEUDOTUBERCULOSIS* ISOLATED

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DEPARTAMENTO DE BIOINTERAÇÃO, LABIMUNO, UFBA, BRASIL

Support: FAPEX

Introdution: The production of meat, milk, wool, fur and other products derived from sheep and goats is in expansion in brazilian market, but there are still problems that minimizes this production and causes great damage to agribusiness. Among the difficulties in the industry, the caseous lymphadenitis shows being one of the biggest obstacles, once it is an infectious disease caused by Corynebacterium pseudotuberculosis. Objective: Thus, it is necessary to investigate and quantify proteins and other molecules to standardize serological tests and promote the production of vaccines. Material and Methods: The experiment was conducted in LABIMUNO/ICS- UFBA. Four bacterial strains were selected for protein extraction: two were isolated from goats (76 and 21) – strong and negative for biofilm formation – and two isolated from sheep (16 and 60) – moderate and negative for biofilm formation, respectively. For bacterial growth, strains were plated on BHI with 3% Tween 80 and without Tween 80 for analyzing the protein quantification. After the bacterial growth, these were sonicated, centrifuged and extracted by chloroform/methanol/water and the resulting pellet was extracted with butyl at 9%. The resulting protein concentration was determined by lowry method. Results and Discussion: The total protein concentration was determined and the following results were obtained: 0,239 mg/mL, 0,158 mg/mL, 0,542 mg/mL, 0,872 mg/mL, 0,444 mg/mL, 0,908 mg/mL, 0,596 mg/mL, 0,850 mg/mL, respectively for the samples 16, 21, 60 and 76 without tween 80 and 16, 21, 60 and 76 with tween 80, 3%. The results shows that Tween 80 helps to break up the bacterial membrane and release the intracelular material and being a mild surfactant, does not affect protein activity, but their solubilization. Conclusions: These fractions are possibly constituted of glycoproteins, in which the probable antigenic targets will be studied by means of Western blotting. The most reactive fractions through these procedures will be evaluated in mass spectrometer.

Keywords: Corynebacterium pseudotuberculosis, biofilm, molecules.

#30

GENETIC ASSOCIATION STUDY ON GENE ADCY9 POLYMORPHISMS WITH ASTHMA PHENOTYPES AND BRONCHODILATOR RESPONSE IN PATIENTS FROM PROAR, SALVADOR, BAHIA, BRAZIL

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Support: Fapesb, CNPq

Introduction: Asthma is a chronic inflammation of the airways which affects Million of people around the world characterized by bronchial hyperreactivity, mucus production and airway obstruction. Asthma is a multifactorial disease that the genetic factor closely linked to environmental aspects. Adverse effects caused by drugs and lack of response to standard medications have led the search for individualized treatments based on the genetic profile of the patient, it looked for a candidate gene related to asthma, ADCY9 (adenylate cyclase type 9). The ADCY9 is located on chromosome 16 and expressed an integral membrane protein that produces cAMP, a second messenger responsible for activation of several other proteins within the cell. The cAMP is produced in large quantities in regulatory T cells and is described in the literature as a suppressor effector T cells, thereby reducing the inflammatory response. Objective: The aim of this study is to investigate how polymorphisms in the Adenylate cyclase gene 9 are associated with asthma and allergy markers, and the therapeutic response to adrenergic bronchodilators. Material and Methods: Polymorphisms (rs2601796, rs2532019, rs2239313, and rs2230739) will be genotyped using the TaqMan probe-based technology 5'-nuclease assays (Applied Biosystems, Foster City, CA, USA) .The work will be developed from the Program for the Control of Asthma in Bahia (PROAR) where 1,419 patients will be genotyped, these being separated into 3 groups (severe asthma, mild asthma and control). We will evaluate the association of polymorphisms with lung function, atopy markers and response to bronchodilator treatment, among other parameters such as gene expression of ADCY9 in this population. Expected Results: This study will enable the advancement of understanding of asthma with genetic basis and control of asthma symptoms and atopic disease, as well as contribute for personalized medicine based on genetic profile of each individual.

Keywords: Genetic Polymorphism, ADCY9, asthma phenotypes, bronchodilator.

#31

IL10 GENETIC VARIANTS ARE ASSOCIATED WITH ATOPY BUT NOT ON ASTHMA IN A SEVERE ASTHMA CASE-CONTROL STUDY IN SALVADOR CITY

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Support: Universidade Federal da Bahia.

Introduction: Asthma affects about 334 million people worldwide. IL10 is a key immune regulatory cytokine to control immune reactions and has been linked to asthma and atopy. In this way, polymorphisms (SNPs) in the IL10 may affect the production of this cytokine and thus asthma/allergy occurrence. Objective: This work evaluates the associations between IL10 SNPs, different asthma phenotypes, markers of allergy and IL10 levels in a case-control study for severe asthma in adults living in Salvador, Brazil. Material and Methods: DNA was extracted from peripheral blood from 1,406 subjects (448 mild asthma, 436 severe asthma with reversibility, 67 severe asthma without reversibility and 455 healthy individuals) recruited from ProAR (Program for Asthma and Allergic Rhinitis Control in Bahia) and the SNPs were typed by using TaqMan probe. The study included 4 SNPs in the IL10 (rs3024496, rs3024491, rs1800896, rs1878672). Logistics regressions for asthma and skin prick test were performed using PLINK software 1.9 adjusted for sex, age, skin color. IL10 levels in plasma were determined by ELISA. Results and Discussion: The IL10 levels in plasma were significantly higher ($p<0.0001$) in atopic asthmatics patients when compared to healthy individuals. In additive model the SNP rs3024496 was negatively associated with SPT to Dermatophagoides farinae (OR= 0.77; CI 0.65-0.91) and SPT to D. pteronyssinus (OR= 0.83; CI 0.70-0.99).The SNP rs3024491 was negatively associated to D. farinae (OR= 0.81; CI 0.67–0.97) skin reactivity. None of the asthma phenotypes studied (including severe asthma) were associated with IL10 SNPs ($p>0.05$). Conclusion: IL10 SNPs were associated negatively with skin test to aeroalergens, an indicator of allergic sensitization in a Brazilian population confirming the possible role of this gene in atopy but not directly in asthma.

Keywords: IL10, polymorphism, atopy and Asthma.

#32

EVALUATION OF IN VIVO ANTI-INFLAMMATORY ACTIVITY OF HIPPOCAMPUS REIDI HYDROALCOHOLIC EXTRACT

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Support: Universidade Federal de Pernambuco

Introduction: In this study, we have used the marine specie Hippocampus reidi (seahorse) which was one of the most cited natural products used for the treatment of asthma and other inflammatory conditions in an ethnobotanic survey conducted by our research group. Objective: The aim of the present study was to describe the in vivo anti-inflammatory potential of H. reidi hydroalcoholic extract. Material and Methods: BALB/c mice were pre-treated with a vehicle (saline, v.o.), H. reidi extract (100 mg/Kg, s.c. or v.o.) or dexamethasone (0.3 mg/Kg, v.o.). After 1 h, the LPS (1 µg/mL) was intraperitoneally administered, six hours after the intraperitoneal injection of LPS the inflammatory exudate was withdrawn after washing the peritoneal cavity with 3 mL of a saline solution containing EDTA. Aliquots of the peritoneal lavage were used to determine the total cell counts using a Neubauer chamber and differential cell counts were obtained using May-Grunwald-Giemsa-stained cytospin preparations. IL-6 was measured by ELISA. Results and Discussion: Leukocyte and neutrophil counts in the peritoneal cavity were increased ($p < 0.001$) at 6 h after peritonitis induced by LPS compared with corresponding counts in the control group (saline). Total and Differential cell counts showed a reduction of neutrophil and total leukocyte migration via both routes tested compared with the control group ***($p<0.001$). These effects were similar to those obtained after pre-treatment with dexamethasone at 0.3 mg/kg ***($p<0.001$). Pre-treatment with H. reidi extract showed a significant reduction of the IL-6 concentration compared with the LPS group ***($p < 0.001$). Conclusions: Our study revealed that H. reidi hydroalcoholic extract possesses anti-inflammatory activity in vivo and may be a candidate for the isolation of molecules for further study and that H. reidi hydroalcoholic extract might also represent a component of the therapeutic arsenal of inflammatory disorders.

Keywords: Hippocampus reidi; Anti-inflammatory; IL-6.

#33

POLYMORPHISMS IN ADAM33 ARE ASSOCIATED WITH ATOPY IN A LATIN AMERICAN POPULATION

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Support: Department of Bioregulation, Laboratory of Immunopharmacology and Molecular Biology, Institute of Health Science, Federal University of Bahia (ICS), Bahia, Brazil.

Introduction: The ADAM33 gene is located on chromosome 20p13 and codes a member of the ADAM (a desintegrin and metalloprotease) family. The transmembrane protein is mainly expressed in mesenchymal cells, smooth muscle cells and fibroblasts. ADAM33 protein is involved in extracellular domain shedding, cell adhesion and signaling, as well as neurogenesis, muscle development among other biological processes. GWAS and candidate gene studies in diverse populations have shown that ADAM33 is linked with susceptibility for asthma development through bronchial hyperresponsiveness and airway remodeling. The proposed mechanism is proliferation of smooth muscle cells and fibroblasts with excessive deposition of matrix proteins, such as elastic and reticular fibers, collagen, glycoproteins and proteoglycans. Then, ADAM33 polymorphisms may lead to altered protein expression, thus being a risk factor for developing severe asthma. Objective: The aim of this study was to analyze the association between single nucleotide polymorphisms (SNP) on ADAM33 gene atopy markers in SCAALA (Social Change, Asthma, and Allergy in Latin America) cohort. Material and Methods: Genotyping was performed in DNA samples from 1,309 participants from SCAALA program utilizing the commercial panel Illumina 2.5 Omni chip. The study included the analysis of 16 SNPs on ADAM33. As statistical strategy, logistic regression was performed for atopy markers (skin test and IgE levels) by using Plink 1.9 software. Analyses were executed in the recessive genetic model and adjusted for sex, age, helminth infections and ancestry markers. Results and Discussion: The SNP rs553863 was positively associated with atopy (OR: 2.303; CI: 1.11-4.74) and IL-13 levels (OR: 1.449; CI: 1.02-2.05). Both associations lead to atopy susceptibility but not asthma. The rs2280092 was positively associated with specific IgE production for Dermatophagoides pteronyssinus (OR: 2.028; CI: 1.06-3.84) and Blatella germanica (OR: 2.389; CI: 1.17-4.86), as well as with specific skin test for Periplaneta americana (OR: 2.208; CI: 1.08-4.49). Conclusions: Our analyses show the association of ADAM33 polymorphisms with atopy. However, further studies are required in order to describe the mechanisms and routes of how the gene acts on atopy development.

Keywords: polymorphisms;ADAM33 and atopy.

#34

MICRORNAS ASSESSMENT IN PLASMA OF HEALTHY AND INFECTED GOATS WITH WILD STRAIN OF *CORYNEBACTERIUM PSEUDOTUBERCULOSIS*

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Support: FAPESB, CNPq

Introduction: MicroRNAs are an abundant class of highly conserved RNA molecules, formed by a few bases to about 22 nucleotide noncoding and can act as post-transcriptional regulators of gene expression by altering mRNA stability. Objective: This study aims to evalute the miRNA profile of healthy and infected goats with C57 strain of *Corynebacterium pseudotuberculosis.* Materials and Methods: In this experimente, 12 young goats will be used, mixed breed, of both sexes. 06 animals will be part of the control group and 06 animals infected with the C57 strain of *Corynebacterium psedotuberculosis.* These animals are infected with a concentration of 10^7 CFU. The blood of the animal is collected after 90 days of infection in a volume of 5 ml by jugular venipuncture in sterile vacutainer tube with EDTA will then centrifuged at 1400xg for 10 minutes at room temperature to obtain the plasma.For extraction of total RNA will be used MIRCURY RNA ISOLATION Biofluids kit according to the manufacturer's protocol. The cDNA is synthesized using the Universal cDNA SYNTHESIS kit 8-64RXNS (EXIQON) according to the manufacturer protocol. From the list of miR that can be expressed in the serum of humans, present in the serum / plasma panel shape selected 65 miRNAs homologues hircus Capra (Chi). Real-time amplification of miRNAs will be made with the SYBR Green PCR kit exilent Array (EXIQON) according to the manufacturer's protocol. Results and discussion: Detect and analyze an increase or decrease in expression of miRNAs in the plasma of infected goats wild strain C57 *Coryebacterium pseudotuberculosis* compared to healthy animals (control group).

#35

ANTITUMOR PROPERTIES OF THE LEAF ESSENTIAL OIL OF Zornia brasiliensis Vogel (Fabaceae)

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Introduction: Zornia brasiliensis Vogel (Fabaceae), popularly known as "urinária", "urinana" and "carrapicho", is a medicinal plant used in Brazilian northeast folk medicine as diuretic and against venereal diseases. [Objectives] The aim of this study was to investigate the chemical composition, safe and antitumor potential of the leaf essential oil of Z. brasiliensis. Material and Methods: The essential oil was obtained by hydrodistillation using a Clevenger-type apparatus and analyzed by GC–MS and GC–FID. Results and Discussion: Its composition was characterized by the presence of trans-nerolidol, germacrene D, (-)-trans-caryophyllene, a-humulene and farnesene, as major constituents. Material and Methods: In vitro cytotoxicity of the essential oil and some of its major constituents was evaluated for tumor cell lines from different histotypes using the Alamar blue assay. The essential oil, but not the constituents tested, presented promising cytotoxicity. Furthermore, mice inoculated with B16-F10 mouse melanoma were used to confirm its in vivo effectiveness. Results and Discussion: In vivo antitumor study showed tumor growth inhibition rates of 3.78–58.47% (50 and 100 mg/kg, respectively). No significant systemic toxicological signal was seen in essential oil-treated mice. Conclusions: In conclusion, the leaf essential oil of Z. brasiliensis presents trans-nerolidol, germacrene D, (-)-trans-caryophyllene, a-humulene and farnesene as major constituents and is able to inhibit cell proliferation in culture as well in tumor growth in mice.

Keywords: Zornia brasiliensis, Essential oil, Fabaceae, Cytotoxicity, B16-F10.

#36

CORYNEBACTERIUM PSEUDOTUBERCULOSIS EXTRACTED MOLECULES WITH IMMUNOGENIC AND IMMUNODIAGNOSTIC POTENCIALS

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Support: FAPEX

Introdution: The Caseous Lymphadenitis (CL), infectious disease caused by the pathogen Corynebacterium pseudotuberculosis (CP), is responsible for a high level of economic losses in sheep and goat creations in the state of Bahia. This disease has a chronical character, being responsible for negative impacts in the weight gain and milk production, as well as devaluation of leather and carcass condemnation in the slaughterhouse of small

ruminants. Its eradication is very difficult, once it's largely broadcasted worldwide (Dorella et al., 2006). Although low epidemiological monitoring has been done recently in Brazil, the data that we have indicates a high dissemination of the disease. Objective: Develop immunodiagnostic test from Corynebacterium pseudotuberculosis molecules with immunogenic potential. Material and Methods: Some of these molecules immunogenic potential have been identified in our work using mass spectrometry. This molecule was used in the development of an immunodiagnostic test. Results and Discussion: Presented specificity results of 100% and 80% of sensibility. When we compare it with the indirect ELISA, that is usually used, that has 100% of specificity and 67% of sensibility, these molecules show their excellent potential. Conclusions: Complementary analyzes will be conducted as a way to verify its potential in immunodiagnostic tests, and as an immunogen, once preliminary tests in animal models have already demonstrated good results in immune responses. Due to the losses provoked by this disease, these molecules can be a way of reducing or ending with this economic prejudice, besides the decrease in the risk of human contamination, once the agent has zoonotic potential.

Keywords: Corynebacterium pseudotuberculosis, protein molecules, immunogen, immunodiagnostic

Note: The methodology and information about the molecules could not be described, as they'll be part of a patent.

#37

PROFILING OF CIRCULATING MICRORNAS AND SOLUBLE PROTEINS DURING MURINE IMMUNE RESPONSE TO CORYNEBACTERIUM PSEUDOTUBERCULOSIS INFECTION: A PRELIMINARY STUDY

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Support: FAPEX, CNPq

Introduction: Caseous lymphadenitis (LC) is caused by the intracellular facultative bacteria Corynebacterium pseudotuberculosis. The immune response to control it is the gamma-interferon producing Th1, cellular profile. Regulatory mechanisms are based on T regulatory cells (Treg) and on anti-inflammatory cytokines, such as interleukin-10 (IL-10) and Transforming Growth Factor-beta (TGF-β). MicroRNAs (miRNAs) are small, non-coding ribonucleic acids (RNAs) with an important regulatory role in gene expression regulation. Several studies suggest that microRNAs emerged as an alternative immune response regulatory mechanism involved in the control of various biological and pathological processes (e.g. infectious diseases). They are also stably secreted into the bloodstream, turning them into attractive potential serum biomarkers for diseases. Objectives: To evaluate a panel of serum microRNAs and soluble proteins in mice infected by C. pseudotuberculosis, in order to analyze their regulatory mechanism acting on LC parasite-host interaction. In addition, pinpoint potential miRNAs that could function as serum biomarkers of this disease. Material and Methods: C. pseudotuberculosis (virulent strain) infected C57BL6 mice will be euthanized after seven and 60 days of infection. Paneling of 88 miRNAs and 108 proteins related to the animal's immune response will be assessed in both, plasma and spleen crude lysate, respectively, by PCR array and WB. Granuloma presenting organs will be submitted to histological/morphometric evaluation. Expected results: our work will contribute to increase the understanding of the regulatory parasite-host interactions and on the identification of molecular biomarkers that will be further studied and used as an LC serum diagnostic and prognostic tool.

Keywords: Corynebacterium pseudotuberculosis. microRNA. Biomarkers. Immune response.

#38

CHARACTERIZATION OF NEUTROPHILS PROFILE IN SUBCLINICAL INFECTION CAUSED BY *LEISHMANIA BRAZILIENSIS*

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Support: NIH, CAPES

Introduction. Despite *Leishmania braziliensis* infection can promote several clinical manifestations associated with an exacerbated response in skin, this protozoan can also promote asymptomatic infection. Individuals with asymptomatic infection or subclinical Individuals (SC) are characterized by a positive delayed type I hypersensitivity test (DTH) and/or high IFN-γ production to soluble leishmanial antigen (SLA). In contrast to patients with CL, SC individuals produce lower levels of TNF and IFN-γ but are able to control the infection and do not present clinical manifestations. The mechanism which SC individuals are protected against *L. braziliensis* infection is still unknown. Since adaptive immune response is related to immunopathogenesis of cutaneous leishmaniasis, the inate immune response has been investigated in *L. braziliensis* infection. It was already demonstrated that macrophages play an important role in controlling *L. braziliensis* infection but there is no study investigating the role of neutrophils (PMN) in the subclinical infection due to this parasite. Objective. To investigate the profile and functions of neutrophils obtained from individuals with subclinical infection due to *L. braziliensis*. Material and Methods. PMN were obtained from peripheral blood from SC individuals and CL patients and the phenotype of these cells was assessed by CD62L and CD66b expression by flow cytometry. The general production of oxidative burst, specific production of ROS and NO, TLR2 and TLR4 expression were also evaluated by flow cytometry. The percentage of infection and parasite burden were measured by optical microscopic evaluation of cytocentrifuged slides. Results and Discussion. Neutrophils from SC individuals presented lower expression of CD62L and higher expression of TLR2 and TLR4 in comparison to CL patients indicating a more activated phenotype. Despite neutrophils from SC individuals did not present differences in oxidative burst, ROS and NO production when compared to

neutrophils from CL patients, they exhibited lower percentual of infection and parasite burden than CL group. These data suggest that neutrophils microbicidal response are more effective in SC individuals and may *L. braziliensis* can evade these responses in neutrophils from CL. Conclusion. Neutrophils from subclinical individuals are more functionally efficient and may participate in the control of *L. braziliensis* characterized in the subclinical form of infection.

Keywords: human cuatenous leishmaniasis, subclinical infection, neutrophils and Leishmania braziliensis.

#39

IMPAIRED IMMUNOREGULATORY NETWORK FROM PATIENTS WITH SEVERE ASTHMA REFRACTORY

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Introduction: It is estimated that approximately 235 million people suffer from asthma worldwide. The immunopathogenesis of the disease is frequently associated to up regulation of T2-type response. However, there is also a contribution of Th1 and Th17 cytokines in the disease severity. There are only a few published studies regarding the immune markers in response to treatment in individuals with severe asthma. Objective: To evaluate the cytokine profile expressed by TCD4+ cells of patients with severe asthma treated with inhaled corticosteroids. Material and Methods: The studied population included 19 patients with severe asthma, refractory to treatment (SAR), 21 patients with severe asthma controlled or partially controlled with treatment (SAC), 23 patients with mild asthma (MA) and ten healthy controls (HC). Lymphocytes were obtained from PBMC of enrolled individuals and the frequency of different molecules in the cell population was performed using flow cytometry technique. Results and Discussion: We observed that the frequency of TCD4+ cells expressing the regulatory molecule CTLA-4 was higher in individuals with SAC and MA (mean±SD: 1.5%±0.9% and 1.3%±0.7%, respectively), compared to individuals with SAR and HC (0.75%±0.4% and 0.6%±0.2%, respectively). We also observed that the frequency of cells expressing TGF-β was higher in individuals with SAC and MA, compared to individuals with SAR and HC ($p<0.05$). A lower frequency of TCD4+ cells expressing IL-10 in SAR group (1.4%±0.9%), as compared to MA group (2.3%±1.5%) was observed, while the frequency of regulatory TCD4+CD25hi population was higher in SAC group compared to SAR group. The expression of T reg cells expressing FoxP3 was lower in SAR group, as compared to MA individuals ($p<0.05$). The T2-type cytokines were also evaluated and the frequency of cells expressing IL-5 was lower in HC individuals compared to the MA group ($p<0.05$). We did not observe differences among groups regarding the cell expression of IL-13. In relation to the pro-inflammatory cytokines IFN-γ and IL-17A, the frequency of TCD4+ cells expressing these cytokines were higher in SAR group and SAC group, compared to HC individuals ($p<0.05$). Conclusion: In this study the severity of asthma was associated to an increase in Th1 and Th17 inflammatory cells and the refractoriness to treatment was associated to a decreased immunoregulatory network.

Keywords: lymphocytes; immune response; severe asthma

#40

IMMUNE ASPECTS ASSOCIATED WITH DYSLIPIDEMIA IN PATIENTS WITH CARDIOVASCULAR DISEASE LIVING IN BAHIA

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Support: Universidade Federal da Bahia

Introduction: In Brazil, more than two million people died in the last decade due to cardiovascular diseases caused by dyslipidemia. Objective: This study investigated the production of IgA and IgG Saccharomyces cerevisiae antibodies (ASCA) and the serum levels of IgE, C3 and C4 in patients with dyslipidemia from the Cardiology Service of the Ana Nery Hospital in Salvador, Bahia. Material and Methods: Were investigated 81 patients of both genders, while 31 healthy individuals without dyslipidemia from the same local population were the controls. All participants were investigated for CVD risk factors, lipid profile and tested for ASCA (IgA and IgG) by ELISA tests. Total IgE was determined by an automated immunoassay, while the levels of C3 and C4 were quantified by nephelometry. Results: ASCA-IgA were found in 3/31 controls and 7/81 patients ($p>0,05$), whereas ASCA-IgG were detected in 9/31 controls and 23/81 patients ($p> 0, 05$). Total IgE was higher in patient group than in control one (median = 175 UI/mL vs. 76 UI/mL, $p = 0.028$). Lower levels of both C3 and C4 were found in the patient group in comparison with healthy controls (C3, 160 mg/dL vs. 183 mg/dL; C4, 32.9 mg/dL vs. 43.6 mg/dL; $p <0.0001$ and $p = 0.002$, respectively). Conclusion: Immunological alterations represented by an increase in total IgE level and a slight decrease in serum levels of complement C3 and C4 can be observed in dyslipidemic patients living in Bahia. The associations of these immunological changes with CVD risk factors and CVD clinical manifestations are under investigation.

Keywords: Dyslipidemia, Cardiovascular Disease, ASCA, IgE, C3, C4.

#41

PERIPHERAL BLOOD AND TUMOUR INFILTRATING CD4+CD25+FOXP3+ REGULATORY T CELLS DIFFERENTLY CORRELATES WITH CLINICOPATHOLOGICAL FEATURES IN PATIENTS WITH ORAL CANCER

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Support: FAPESB, CNPq, PIBIC-AF UFBA.

Introduction: In oral squamous cell carcinoma (OSCC) contradictory results have been obtained in relation to relevance of Treg cells in tumorigenesis and prognostic of patients. Objectives: This study aimed to investigate peripheral blood and tumor infiltrating regulatory T cell (Treg) subsets in OSCC in relation to clinicopathological parameters. Material and Methods: From peripheral blood and tumoral fresh tissue of 21 patients with OSCC, CD4+CD25+, CD4+FoxP3+ and CD25+FoxP3+ T cells were determined by flow cytometry. Additionally, an immunohistochemical study was carried out to quantify the infiltration of Treg/FoxP3+ T cells in parenchyma (TP) and stroma (TS) of 60 OSCC samples and 14 non-neoplastic adjacent mucosa (NNAM). Results and Discussion: No correlation in corresponding subpopulations was revealed when compared peripheral blood and tumoral levels. Peripheral CD4+CD25+ T cell population was in increased proportion in patients with N1 in relation to N2-N3 individuals (P=0.017). Similarly, we observed an increased proportion of peripheral CD25+FoxP3+ Treg cells in initial size tumors (P=0.053) and tumors with negative muscular infiltration (P=0.051). These results corroborate with Correale et al. (2010), that described a favorable prognosis implied and with Lim et al. (2014), that suggests a key role of Treg in control to the initial inflammation and disease progression. In relation to microlocalization, FoxP3+ cells were present in parenchyma and periparenchymal stroma in 77.78% and 92.59% of tumors, respectively. Comparing TP versus TS, positive correlation was observed in relation to number of FoxP3+ cells (r=658, P=0.000). However, the proportion of the number of Treg in tumoral tissue did not correlated with the clinicopathological outcomes in our preliminary analysis. Conclusion: Our results indicates that increased proportion of peripheral CD4+CD25+ and CD25+FoxP3+Treg cells levels are a favorable prognostic factor in OSCC patients.

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#42

IMMUNOMODULATORY EFFECTS OF N-acil-hydrazone HAH2 IN A MURINE MODEL OF ALLERGIC AIRWAY INFLAMMATION

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Introduction: Allergies affect about 20 to 30 % of the world population and their prevalence, as well as the severity of their symptoms, have been increasing in recent decades. Glucocorticoids are used to treat a large number of immune disorders, such as allergies, however their prolonged use can cause severe adverse effects. Among the synthetic immunomodulatory small-molecules that have entered into pre-clinical tests, a significant number are N-acylhydrazone derivatives. The compound HAH2 belongs to a novel family of immunosuppressive Hydrazone N-AcylHydrazones (HAH) which presents, as main chemical feature, a nitrophenylhydrazone fragment. Objectives: In this work, the effect of oral treatment with HAH2 was evaluated in a murine allergic airway inflammation (AAI) model. Materials and methods: Groups of 8 male BALB/c mice were immunized by subcutaneous injection of 10 µg of ovalbumin diluted in 2 mg/ml alum, followed by a booster injection at day 14. A nasal challenge was performed by inhalational exposure to aerosolised 1% ovalbumin for 15 min/day, on five consecutive days. Two hours before each aerosol delivery, mice were treated orally with HAH2 (5, 20 or 80 mg/kg), dexamethasone (20 mg/kg) or vehicle solution. Bronchoalveolar lavage (BAL) was performed twice by intratracheal instillation of 1 mL of PBS. The total leukocyte number in bronchoalveolar lavage fluid was estimated by counting using a hemocytometer. Differential counts were performed after panotic-stained cytospin preparations. Results and Discussion: Mice challenged with ovallbumin presented an intense airway inflammation, as evidenced by a high cellularity and eosinophilia in BAL fluid. Treatment with HAH2 caused a significant reduction of total cell count and eosinophils in BAL, in a dose-dependent manner. Dexamethasone, a standard drug, also caused a significant reduction in the evaluated parameters. Conclusions: The results show that HAH2 reduces the cellularity and eosinophil numbers in BAL in a murine model of allergic airway inflammation, suggesting the therapeutic potential of this substance. We are currently investigating the mechanisms of action of the drug in the AAI model.

#43

USE OF CXCL10 (IP-10), CD163 AND MR / CD206 IN THE DIAGNOSIS OF LIVER FIBROSIS IN CHRONIC HEPATITIS C

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Support: DECIT-Ministério da Saúde, CNPq, FAPESB, Serviço de Gastro-Hepatologia do Complexo Hospitalar Prof. Edgard Santos e FAMEB-UFBA.
Introduction: Viral hepatitis C (HCV) is considered the leading cause of liver disease worldwide, with high prevalence in populations distributed in various geographical regions. Hepatic changes caused by the virus have been laboratory-diagnosed by histological examination of material obtained by liver biopsy using the classification criteria METAVIR (gold standard). The liver biopsy can not be used in monitoring antiviral treatment, may be subject to errors by insufficient sample of the collected material, variability in the microscopic diagnosis realized by different analysts, in addition to its cost and restricted use in pediatrics. Thus, new bioactive molecules have been studied as potential biomarkers of liver abnormalities in chronic HCV infection. Objective: To evaluate the use of CXCL10 (IP-10), CD163 and CD206 as indirect biomarkers of inflammation and liver fibrosis in patients with chronic hepatitis C. Materials and Methods: This study will investigate new indirect biomarkers associated with inflammation in the diagnosis and monitoring of liver fibrosis in chronic hepatitis C in patients with this viral infection residing in Bahia, not submitted to antiviral therapy. Thus, leukocytes will be evaluated in the expression of the chemokine CXCL10 (IP-10) and two important cellular receptors of monocytes and macrophages, CD163 and MR / CD206, whose involvement in inflammatory diseases, autoimmune and infections has been recent research object. Serum levels of CXCL10 and soluble forms of CD163 (sCD163) and CD206 (sCD206) will also be determined by antigen capture immunoassays, whose results will be compared with those obtained in the serum of healthy controls. Subsequently, the expression and the levels of these immune mediators in hepatitis C carriers will be correlated with the results of histological examination of liver biopsy of patients, carried out before their voluntary entrance and obtained in the present study and obtained with the METAVIR protocol. These findings will also be faced with the results of non-invasive laboratory protocols for fibrosis (APRI index and FIB-4 score), to be obtained in patients with hepatitis C during this study. In parallel, these subjects will be investigated extrahepatic manifestations of autoimmunity and cryoglobulinemia, the latter evidenced by the presence of antinuclear antibodies, smooth muscle and anti-LKM-1. At the end of the study will be sought relevant associations between investigated markers and clinical and laboratory findings of patients.

Keywords: Viral hepatitis C. Biomarkers of inflammation. Liver fibrosis.

#44

PHENOTYPIC AND FUNCTIONAL CHARACTERIZATION OF LYMPHOCYTES IN SEVERE FORMS OF SCHISTOSOMIASIS

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Support: CAPES, FAPESB (APP 0051/2016)
Introduction: Schistosomiasis is a chronic and debilitating disease that affects more than 200 million people worldwide and it is estimated that 700 million live in infection risk areas. About 5 to 10% of individuals infected with Schistosoma mansoni progress to severe forms of the disease. The pathogenesis of schistosomiasis is due the host immune response to egg antigens and studies have emphasized the role of lymphocytes in immunopathogenesis of this disease. Objective: To evaluate the phenotypic and functional profile of lymphocytes from patients with different clinical forms of schistosomiasis and its association with disease severity. Methods: The study subjects will be recruited from the endemic area for schistosomiasis named Água Preta, Bahia, Brazil. It will be performed parasitological examination of feces and upper abdominal ultrasonography in all individuals. Lymphocytes will be obtained from peripheral blood mononuclear cells and stimulated with soluble egg antigen (SEA) and the expression of its molecules will be assessed using the flow cytometry technique. Conclusion: The identification of a phenotypic biomarker expressed by T and B cells of patients with different clinical forms of schistosomiasis will help in the understanding of the mechanisms involved in the pathogenesis of this disease, as also may contribute to the development of new strategies for prevention of severe forms, with consequent decrease in morbidity and mortality associated with this disease.

Keywords: Schistosomiasis, Lymphocytes, T Lymphocytes, Severe Forms.

#45

SEROEPIDEMIOLOGY OF HEPATITIS B IN VOLUNTEERS AGED BETWEEN 30 TO 70 YEARS RESIDENTS IN SALVADOR/BAHIA/BRAZIL - FIRST RESULTS

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Support: Laboratório de Imunologia e Biologia Molecular (ICS-UFBA), Núcleo de Ensaios Clínicos da Bahia NEC-BA (UFBA), Serviço de Gastro Hepatologia do Ambulatório Magalhães Neto, (AMN-HUPES), PIBIC-UFBA

Introduction: Hepatitis B (HB) is caused by HBV and can progress unnoticed to cirrhosis or hepatocellular carcinoma. Cultural behavior interfere in the epidemiology of this infection. Objectives: In order to estimate and associate infection and vaccination markers with sociodemographic covariates in a population sample served by the public health service and born between 1945-1985. [Material and Methods] Transversal study with 650 patients randomly selected from a clinical laboratory. A sociodemographic questionnaire containing items about conduct/habits/health data was applied. Serological tests were performed for HBsAg/TotalAnti-HBc/Anti-HBs (chemiluminescence). Participants with positive results for the first two markers were referred for medical care and participants with negative test for anti-HBs were advised to go to a vaccination station. [Results and Discussion] Of the 650 participants, 56.6%(n=368) were older than 50 years old and 68.0%(n=442) were women. Evaluating serological markers, 2.3%(n=15) were positive for HBsAg, 17.1%(n=111) for Total Anti-HBc and 27.4%(n=178) for Anti-HBs. For age versus virus contact marker (Total Anti-HBc) 66.7%(n=74) were older than 50 years. Regarding exposure to risk factors, 4.5%(n=29) have received blood transfusion, 96.0%(n=624) reported already having unprotected sexual intercourse, 6.7%(n=44) have used illicit drugs, 3.7%(n=24) reported sharing syringes/needles and 52.0%(n=338) personal items (toothbrush/manicure tools/razors). About immunoprophylaxis, 29.7%(n=193) reported to be vaccinated for HB but just 45.1%(n=87) showed Anti-HBs levels more than 10mUI/ml. [Conclusions] Preliminary analysis indicate greater reactivity to viral contact marker in the population over 50 years old. The spread of HBV was probably related to behaviour change. Most of the study population is apparently susceptible to infection.

Acknowledgment: Volunteers participants; Strategic and financial Support: Laboratório de Imunologia e Biologia Molecular (ICS-UFBA), Núcleo de Ensaios Clínicos da Bahia NEC-BA (UFBA), Serviço de Gastro Hepatologia do Ambulatório Magalhães Neto, (AMN-HUPES), PIBIC-UFBA.

Keywords: Hepatitis B; Serological Markers; HBsAg; Anti-HBs; Total Anti-HBc

#46

IL-10 PRODUCTION ON WHOLE-BLOOD CULTURE: A COHORT STUDY OF ASTHMA AND ATOPY RISK FACTOR

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Support: Universidade Federal da Bahia, CNPq.

Introduction: The Interleukin 10 (IL-10) is an anti-inflammatory cytokine that plays role in regulatory immune response. Several immune cells produce this cytokine, as activate macrophages, T regulatory, B cells and others. The regulatory role of IL-10 is due to the fact that it inhibits the antigenic presentation by presenting cell; acting in a negative way in cytokine production of Th1, Th2, Th17 profiles. Objective: Quantify IL-10 production in the supernatant of whole-blood cell culture stimulated by different antigens in order to associate this production with atopy and asthma. Methodology: ISAAC phase III questionary adapted to portuguese was used to identify asthma. Atopy status was defined through specific IgE detection by immunocap; results higher or equal 0,70 KU/L were classified as positive cases. Whole-blood cell culture was carried out with 1:4 diluition in RPMI 1640 enriched with L-glutamin and antibiotics. Áscaris lumbricoides, house dust mites (Blomia tropicalis, Dermatphagoides pteronyssinus), Pokeweed and a negative control were used as stimulus. The culture was incubated for 24 hours in 5% CO2 incubator at 37^{o}C. The supernatant collected was stored at -70^{o}C freezer and the cytokine dosage is being performed using the Enzyme-linked Immunosorbent Assay method (ELISA) following the producer instructions (BD pharmigen, USA). The statistic analyse will be carried out using SPSS 22.1 and the socioeconomic variable will be use as confounder. Expected Results: It is intended to understand how the time modify IL-10 production in this population and what environmental factors help on these changes, since it is a cohort that evaluates IL-10 production in cell cultivated in two different moments. Therefore, It is expected to obtain the understanding of how different IL-10 profiles production are associated with the prevalence of asthma and atopy in the past and in the present.

Keywords: IL-10, Atopy, Asthma.

#47

EXPRESSION OF miR-132 IN RELATION TO PERIPHERAL BLOOD AND TUMOR INFILTRATING NK CELLS IN ORAL SQUAMOUS CELLS CARCINOMA PATIENTS

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Support: FAPESB, CNPq, Programa Permanecer and Hospital Aristides Maltez

Introduction: MicroRNAs (miRNAs) are a class of non-coding small RNAs conferring post-transcriptional regulation and its dysregulation is present in cancer. Natural killer (NK) cells exert important cell-mediated cytotoxicity against solid tumors. It was reported that miR-132 up-regulation reduces STAT4 expression in human NK cells (HUANG et al, 2011). Objective: This study aimed to investigate in primary tumors of oral squamous cell carcinoma (OSCC) the expression pattern of miR-132 in relation to infiltration of tumoral NK cells and STAT4 expression. Material and Methods: Initially, 21 patients with OSCC were selected. From peripheral blood and tumoral fresh tissue, CD56+CD3- and CD56+CD57+CD3- NK cells were determined by flow cytometry. In order to describe the microlocalization of CD57+ NK cells, we conducted immunohistochemistry in 40 paraffin embedded OSCC samples. Using qRT-PCR, we analyzed the expression of miR-132 in corresponding samples. Results and Discussion: NK cells percentage was increased in tumor when compared with peripheral blood, but no correlation was revealed. We observed high expression of NK cells in periparenchymal stroma, however intraparenchymal expression was rare and dispersed. The up-regulation of miR-132 was observed in 90% of cases. Dysregulation of miR-132 expression has been reported in a range of human tumors (CHUNG et al., 2013). However, no correlation between the pattern of miR-132 expression and the density of NK cells was observed. To clarify the relation between miR-132 expression and functionality of NK cells in OSCC, we will compare the preliminary results with expression of STAT4. Conclusion: This is the first study to investigate the expression of miR-132 in a large OSCC sample and our results indicates that up-regulation of miR-132 plays a major role in the lesion. Intraparenchymal NK cells not seems to be an important component in OSCC.

#48

STANDARDIZATION OF ANTIGEN EXPRESSION rSm200 (1) / (2) AND rBHA FOR IMMUNODIAGNOSTIC IN SCHISTOSOMIASIS AND TRICHIURIASIS

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Support: Fapesb

Introduction: Schistosomiasis and trichiuriasis are considered a serious public health problem in Brazil and worldwide. Thousands of people still suffer from the clinical consequences generated by these infections. practical methods of diagnosis with high sensitivity and specificity are necessary to detect these parasites in the blood and are considered essential in helping to control these diseases, since that can identify individuals and communities that have the need for intervention. Objective: The aim of this work was to evaluate in vitro the immunoresponse of the recombinant proteins from Trichuris trichuira Fructose bisphosphate aldolase (rFBPA) and two proteins fragments from Schistosoma mansoni (rSm200 1 and 2) for the immunodiagnostic of the infection. Methods: In silico analysis of the proteins were performed using bioinformatics tools and the codifying sequences were cloned into expression vectors and transformed into several E. coli strain. The purification were done by affinity chromatography using Ni+ columns and the ÄKTA TM Pure 25® (GE Healthcare). The immunoresponse were established by dot blot using S. mansoni and T. trichira infected patients sera from Salvador, Bahia, Brazil that were previously pre-absorbed with A. lumbricoides extract to avoid cross reactivity. Results: The expression and purification methods used were success to obtain high yield and quality proteins. The dot blot analysis with rBPHA was able to detect positive sera against T. trichiura and rSm200 (1) and (2) were also able to detect positive sera against S. mansoni. The negative controls works also fine. Conclusion: This work was able to evaluate the IgG immunoreactivity against the recombinant proteins from T.trichuria and S. mansoni. Further studies are necessary to test the sensitivity and specificity with other immunological test like ELISA.

#49

POLIMORPHISMS IN RORA GENE ARE ASSOCIATED WITH NON ATOPIC ASTHMA IN A BRAZILIAN POPULATION

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Introduction: The prevalence of asthma has increased in recent decades, affecting about million people worldwide. Classically, asthma is caused by type 1 hypersensitivity reaction, initiated by IgE antibody-mediated mechanisms and inflammatory cells. However, non-atopic asthma does not present specific IgE for aeroallergens. Recent studies have shown that genetic and environmental factors may interact for the development of different asthma phenotypes. The Retinoic acid receptor-related orphan receptor-alpha (RORA), which is a member of the nuclear receptor family, plays an important role in the immune response Th2, since it promotes the release of IL-4, IL-5 and IL-13. The polymorphisms in the RORA has been associated with asthma in GWAS worldwide. Objectives: The aim of this study was to associate polymorphisms (SNPs) in the RORA gene with asthma and allergy markers in an admixed population of northeastern Brazil. Material and Methods: Genomic DNA samples of 1,309 individuals participating from Social Changes Asthma and Allergy in Latin America Programme (SCAALA) have been genotyped using Illumina Human 2.5 Omni Beadchip. This study included 2016 SNPs RORA gene. Asthma was defined according to the ISAAC studies as the occurrence of wheezing in the last 12 months. Logistics regressions have been performed to analyze the association among RORA variants and asthma. This task has been carried out using Plink software 1.9 adjusted for sex, age, helminth infection and ancestry markers, using the additive model. Results and Discussion: Thirteen SNPs were significantly associated with non atopic asthma ($P < 0.01$), in which three of them, rs4775309, rs12912031 and rs12915127 were strongly and negatively associated ($P < 0.001$). However, these same SNPs were not associated with IgE anti-house dust mites (Blomia tropicalis and Dermatophagoides pteronyssinus) and IL-5 and IL-13 production, after stimulation by these mites. Conclusions: RORA genetic variants are associated with non atopic asthma, but not-atopy and allergy markers. Therefore, further functional analysis may be performed to verify the role of these genes in the non-atopic asthma physiopathology.

#50

CHARACTERIZATION OF THE IMMUNOLOGICAL MECHANISMS INDUCED IN VITRO BY THE Sm29 ANTIGEN IN MACROPHAGES OF ASTHMATIC INDIVIDUALS

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Introduction: Evidences has been accumulated that chronic helminth infections, particularly by Schistosoma sp or their products, is able to modulate the inflammatory-type 2 response in allergic diseases. Studies suggested that M2 macrophages are the main cells in broncoaveolar lavage fluid from asthmatic patients. Additionally, these cell populations may contribute to severity of the allergic inflammation with an important role in the pathophysiology of the disease. Objectives: The aim of this study is to characterize the immunological mechanisms induced by the recombinant antigen Sm29 from Schistosoma mansoni in vitro in macrophages of individuals with asthma. Material and Methods: They will be evaluated subjects with severe asthma, mild / intermittent asthma, both uninfected with S. mansoni and healthy controls. It will be evaluated the effect of Sm29 antigen in the maturation, activation, cytokines and metalloproteinases production (IL-10, TNF, IL-1β, MMP-2 and MMP-9) by macrophages of patients with severe and mild/intermittent asthma. We will also evaluated the frequency of Toll-like receptors (TLR) -2 and TLR-4 after stimulation with Sm29. The mechanisms underlying the induction of regulation by Sm29 antigen in vitro in macrophages will be evaluated through the signaling of the protein kinases ERK / MAPK, beyond the transcription factors NFκB and the adapter protein MyD88. Conclusions: The identification and characterization of cellular and immunological mechanisms induced by Sm29 in the asthma disease, as proposed in this study, may contribute to produce a feasible antigen for use as control of the exacerbated immune response observed in this disease.

Keywords: asthma, Sm 29, macrophages.

#51

INVESTIGATION, ANALYSIS *IN SILICO*, EXPRESSION, AND PRODUCTION OF THE SEQUENCES CODING FOR PROTEINS OF *Toxocara canis*

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Support: FAPESB, CNPq

Introduction: The parasitic diseases, including those zoonotic affect significant part the population in the world. They are most prevalent in the developing countries. They are neglected by the fact of being little known and have similar symptomology to other more common diseases. IAmong the zoonotic helminthic diseases, toxocariasis has a significant relevance because it produces symptomatology analogous to another illness and produces clinical forms acknowledge as larva migrans visceral; ocular larva migrans and neurological toxocariasis. The treatment for this parasitosis is based on drugs administration. Aim:The aim of this study was using an approach of bioinformatics, to identify possible therapeutic targets that would be used as candidates for the development of a vaccine for the control of this helminth infection in its natural hosts. Material and Methods: In this work; we deal with *in silico* analysis techniques to identify the proteins that may constitute important targets for the development of a vaccine for animal toxocariasis. Firstly, it was performed a literature investigation and identification of sequence genes that are available in the different database and it was selected 30 sequences. After this research differents, parameters were evaluated for identification the best candidates to be tested for vaccine development. Results and Discussion: Four proteins: Tc_03886 G-protein-couple receptors (GPCRs), Tc_014421 (Two pore potassium channel protein sup-9), Tcan_06969 (Cadherins), Tc_18226 (Onchocystatin) were selected. For that, it was necessary to standardize conditions for expression and production of these proteins in heterologous organisms. We expressed three proteins Tc_014421 (Two pore potassium channel protein sup-9), Tc_06969 (Cadherins), Tc_18226 (Onchocystatin)) and the last one Tc_03886 G-protein-couple receptors (GPCRs) was express coming soon. These proteins are already being produced on a large scale to be introduced soon in *invitro* cells to stimulated in vivo assay with the animal model. Conclusions:The use of newtechnologies to identify new therapy targets give new tools to developed new forms to the combat of differents infections that are difficult control and eradication; mainly when it comes to neglected diseases and low-income countries.

Keywords: T. canis, vaccine, recombinant proteins, zoonotic disease.

#52

PERIODONTAL MICROBIOME EVALUATION, IMMUNOLOGICAL AND IMMUNOGENETIC MARKERS OF PERIODONTITIS IN SEVERE ASTHMA

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Support: UFBA e UEFS

Introduction: Asthma is an inflammatory origin chronic disease of the airways, currently considered as a public health problem. Periodontitis, which has also an inflammatory nature, is a multibacterial etiology chronic disease of the protective fabrics and the support of the teeth, which triggers several immunoinflammatory events. Recently, some studies have shown the influence of periodontitis in asthma, especially in its severe form, either by aspiration of pathogenic organisms, either by raising the epithelium reactions triggered by the immune system. The bacterial biofilm adhered to the tooth surface harbors a wide range of gram-negative species, including some typical periodontal pathogens such as Porphyromonas gingivalis, Treponema denticola Tannerella forsythia and Aggregatibacter and actinomycetemcomitans. These pathogens have been reported as inducers of the expression of matrix metalloproteinases (MMPs). In addition, they induce the production of cytokines, which in turn lead to increased MMP levels in periodontal lesions. This aspect corroborates greater tissue destruction in both oral and respiratory mucosa. Objectives: Knowing that the biological plausibility of the association between chronic periodontitis and severe asthma appears to be related to microbiological and immunological components common to these diseases, this study, case-control, intend to evaluate the presence of microbiological and immunological markers periodontitis in individuals with severe asthma and subjects without the disease assessed by PROAR-Salvador / BA. Material and Methods: It will be tested the frequency of five periodontopathogenic microorganisms in the biofilm of all participants, the metagenomic of the presence microbiome in the oral mucosa of part of the individuals, the serum levels of anti-Porphyromonas gingivalis antibodies (ELISA) and, finally, polymorphisms in the MMP-1 gene (rs1799750), MMP-8 (rs11225395), MMP-9 (rs3918242) with real time PCR.

#53

THE USE OF TRYPANOSOMA CRUZI OR OF BCG VACCINE AS IMMUNOMODULATORS IN A MODEL OF MELANOMA TUMOR GRAFTED INTO THE PINNA OF THE EAR OF C57BL/6 MICE.

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Support: CAPES e CNPq

Introduction: Trypanosoma cruzi (Tc) and Baccilus Calmette-Guérin (BCG) may induce strong specific and non-specific immune system activation. We evaluated tumor growth and survival of C57BL/6 mice grafted with B16F0 melanoma tumor cells into the pinna of the ear, after in vivo addition of T. cruzi (Tc) or BCG, either locally or systemically. Material and Methods: Each animal received 5x104 of B16F0 in 0.020mL intra ear (ie.). Mice were inoculated with non-infective epimastigotes, derived from the Y strain (Y-Tc) in situ (ie.) or intraperitoneally (ip.). For ip. inoculation, 30x105 forms of Tc in 0.2 mL were used, one week before tumor cell-grafting. For in situ injections, Tc or BCG were inoculated ie., 10 days after B16F0 cell-grafting. Tc-inoculationin situ, 105 forms/0.020mL were used. BCG were injected at 1,2x107 /0.060mL ie. Additional groups received only tumor cells, Tc or BCG. Results- Ip. Administration of Tc induced an initial inhibition of tumor growth in the first 15 days after B16F0 cell grafting. Results: Tc in situ potentiated initial containment of tumor. The differences when compared to control groups did not reach statistical significance, in spite of the biological difference. It should be noticed that control animals (without Tc) begin to die first, but at the end groups were both equivalent and no survival advantage could be observed. After BCG in situ there was an impressive suppression of tumor growth when compared to control group that received only tumor cells. Statistical difference in tumor size between groups was observed on the 18th day after injection of tumor (p=0.0298, Mann Whitney). Conclusions: BCG was able to decrease tumor size and significantly increase the survival mice. Stimulation with Tc promoted a short-time positive biological effect only within the first 15 days after B16F0 cell grafting. Therefore, BCG is a promising immunostimulant and can be further used as an adjuvant to anti-melanoma therapy.
Acknowledgements: To PPGIm-UFBA (Programa de Pós-Graduação em Imunologia) and to Drs José Mengel, Rosa Pinho and Lain Pontes de Carvalho for helpful discussions: the microorganisms studied may be used to induce protection against tumor cells and to study the mechanisms involved in the control of tumor growth.

Keywords are BCG, Trypanosoma cruzi and experimental melanoma.

#54

IMMUNOPROTEOMIC PROFILE OF TOXOCARA CANIS REVEALED MOLECULES WITH IMMUNOMODULATORY PROPERTIES THAT MEDIATES ALLERGIC DISEASES

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Support: CAPES

Introduction: Toxocariasis is an infection transmitted by *Toxocara canis* and *Toxocara cati*, roundworm of dogs and cats, respectively. The immune response against the parasite consists primarily of a standard predominantly Th2. However, to remain in tissues for long years, the parasite can excrete and secrete substance (TES), can modulate the immune response, inducing the production of regulatory T cells. Infections with *T. canis* may have protective effects against allergic diseases. It is believed that some TES molecules can produce IL-10 and TGF-β by Treg (T $CD4^+$ $CD25^+$ $Foxp^3$), with decreased levels of IL-4. Objective. The objective of the present study was to identify through the proteomics, proteins fro*m Toxocara canis* with immunomodulatory properties, which can be used in the therapy of allergic diseases. For this, puppies infected with *T. canis* were treated with piperazine and mineral oil to eliminate worms. The eggs were taken from adult females and then incubated until the phase of the embryonation. The released larvae were cultured in RPMI medium. The excreted-secreted product was collected every two days and concentrated. The larvae were submitted to a thermal shock, crushed, and the supernatant obtained after centrifugation. Protein concentration of both extracts was determined by the Bradford method. The samples TES and larvae were trypsinized and the peptides were subjected to mass spectrometry using the Q-ExactiveTM Hybrid Quadupole Orbitrape thermal. Search and identification of the proteins were performed with UNIPROT software. The Gene Ontology annotation was performed with the tool Blast2Go. Results**.** A total of 36 proteins were identified by mass spectrometry. These molecules represent a potential candidate for immunotherapy of allergic diseases, such as cystatin, cathepsin, heat shock proteins, MIF, fructose, 1,6-biphosphate, lectins, metalloproteinases, mucins, lectins and carboxypeptidases. Conclusion**.** In this study, through the proteomics combined with mass spectrometry, it was possible to identify proteins involved in regulatory mechanisms that contribute to evasion and survival of the parasite for a long period. These *T. canis* protein has a protective effect against allergic and other inflammatory diseases.

#55

ASPECTS OF IMMUNE RESPONSE AGAINST SHEEP CORYNEBACTERIUM PSEUDOTUBERCULOSIS IN EXPERIMENTALLY INFECTION - PRELIMINARY DATA

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Introduction: Corynebacterium pseudotuberculosis (Cp), is the etiological agent of caseous lymphadenitis (CL), a cosmopolitan chronic disease that affects populations of small ruminants (PINTO et al., 2014). This illness is characterized by the formation of granulomas with infection in superficial lymph nodes and subcutaneous tissue (KUMAR et al., 2013). Few studies with CL have characterized the differences in the proportion of lymphocyte subpopulations. Objectives: Evaluate the expression of markers of cell activation in experimental infeccion. Material and Methods: We used fifteen mixed-breed sheep divided into two groups. Group 1, control, received sterile saline and group 2, infected, received inoculum of Cp VD57 wild strain. The immune response was analysed over five months , and weekly or monthly clinical evaluation and blood samples for ELISA and in vitro stimulation with Cp PAT10 and VD57 antigens and subsequent flow cytometry (CD45, CD4, CD8, CD21, CD335, CD14 and CD11b). Six months after inoculation the animals will be euthanized following the CONCEA guidelines. It will be held inspection anatomopathological-mortem and post-collection and preservation of tissues for further studies. Results: In infected animals there was leukocytosis with fever and positive serology from 42 days. Also the MIFs of CD4 and CD8 were higher with both stimuli, and the MIFs of CD14 and CD335 were lower in the infected group.This Project is now in the final phase of execution. Conclusions: There is some differences of cluster of differentiation molecules in lymphocytes and macrophages in infected group of ovine. The research group expects to fill the gap existing in scientific papers regarding caseous lymphadenitis.

Keywords: caseous lymphadenitis; C.pseudotuberculosis; small Ruminant.

#56

POLYMORPHISMS IN ORMDL1 GENE ARE ASSOCIATED WITH ASTHMA AND ALLERGY MARKERS IN A POPULATION OF LATIN AMERICA

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Introduction: Asthma is a multifactorial chronic inflammatory disease of the lower airways, and its development occurs from genetics and environmental factors. Among the genetic components involved in the pathogenesis of the disease, SNPs are considered the most frequent and important ones. Studies of candidate gene demonstrate that polymorphisms in certain genes lead to susceptibility to the onset of asthma. ORMDL1 gene belongs to the family of ORMDL3, which was associated in GWAS with increased susceptibility to asthma. This gene encodes a protein that acts as a regulator of the sphingolipids synthesis. Objectives: The aim of this study was to investigate how polymorphisms in the ORMDL1 gene are associated with asthma and allergy markers. Material and methods: The study comprised 1,309 subjects of the SCAALA (Social Changes Asthma and Allergy in Latin America) program. DNA was extracted from whole blood samples collected from individuals. The extracted DNA was used to build a genotyping panel using Illumina 2.5 Human Omni bead chip. Logistic regression was used to assess the association between asthma and allergy markers (specific IgE and skin prick test) and ORMDL1 variants in PLINK 1.07 software adjusted for sex, age, helminth infection and ancestry markers. Results: The rs2352709 was positively associated with asthma in the dominant model (OR=1.36; 95%CI=1.03-1.81) and gravity of asthma in the additive and dominant models (OR=1.34; 95%CI=1.01-1.80 and OR=1.47; 95%CI=1.03-2.10). Others 7 SNPs were associated with allergy markers. Conclusions: Polymorphisms in ORMDL3 are associated with asthma and allergy markers. The rs2352709 increases the expression of the gene and leads to increased risk of developing asthma.

Keywords: ORMDL1, polymorphism, asthma, atopy, association study, allergy.

#57

EVALUATION BY MASS SPECTROMETRY (ESI-MS) OF MOLECULES EXPRESSED ON MEMBRANE OF CORYNEBACTERIUM PSEUDOTUBERCULOSIS GROWN IN FETAL BOVINE SERUM

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Support: FAPEX
Introdution: Corynebacterium pseudotuberculosis (Cp) causes Caseous Lymphadenitis in goats and sheep, chronic illness associated with economic losses in livestock. The treatment with antibiotics is not viable and there are not effective vaccines. Objectives: Even with a growing global demand for resolutions for CL, there are few studies of molecular mechanisms virulent of Cp, then, is necessary more researches aimed at understanding the pathogenesis and their associated molecules. Material and Methods: Cp, virulent strain, were grown in Brain Heart Infusion Medium (BHI) or in fetal bovine serum (FBS). Monitoring growth in both medium showed a smaller Iag phase and consequent faster exponential phase for FBS. One gram of bacterial mass obtained in log and stationary phase from both growth was subjected to a hydrophobic extraction. The supernatant (extract) was fractioned by SDS-PAGE, assessed for its reactivity to sheep hyperimmune serum by Western Blot and studied using mass spectrometry. Results: Results obtained showed, in both cases, larger amount of different fractions present in extracts of stationary phase, compared with results of log phase. Also demonstrated, on extracts of stationary phase, the predominant acknowledgement of two bands, one with molecular weight 31 kDa – Phospholipase D (PLD) – and the other one with approximately 60 kDa, identified after spectrometric analysis as a catalase. The use of software for location and virulence prediction indicated, respectively for FBS and BHI extracts, 28 and 19 possible molecules of membrane, and among them, 10 e 9 possible virulence factors. Conclusions: Although is not possible to infer that the FBS cultivation approaches the host infection conditions, this farming model contributed to differentiate expression of membrane molecules of C. pseudotuberculosis.

Keywords: Corynebacterium pseudotuberculosis, antigens e hydrophobic.

#58

IDENTIFICATION OF MECHANISMS INVOLVED IN INJURY AND MIGRATION IN THE IMMUNOPATHOGENESIS OF MYELOPATHY ASSOCIATED WHIT HTLV-1 INFECTION

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Introduction: The human T cell lymphotropic virus type 1 (HTLV-1) infection is characterized by an exaggerated immune response with spontaneous production of TNF and IFN. These cytokines are responsible for increasing the permeability of the blood brain barrier (BHE) and also act on cells to produce metalloproteinases (MMPs) and inhibitor of metalloproteinase (TIMP). MMPs are responsible for degrading extracellular matrix proteins and the basal plate, damaging the BHE. The balance between MMPs and TIMPs is important to keep in homeostasis the BHE. The S100B protein is widely studied in neuropathology as an important marker of CNS damage. The CD147, in turn, is involved in the conversion of inactive pro-metalproteinase in active form and is also used in neurological disorders. Objectives: The aim of this study is to evaluate and determine the role of MMP-3 and MMP-9, TIMP-1 and TIMP-1, TIMP-3 and TIMP-4, S100B and CD147 in the pathogenesis of HTLV-1. Material and Methods: Dosages of MMP, TIMP and S100B will be assayed by ELISA in serum of HTLV carriers, HTLV infected individuals with manifestation of overactive bladder and HAM/TSP. Also, cell migration experiments using transwell will be performed to determine chemokines involved in T cell migration. Keywords: HTLV, MMP, TIMP, S100B and CD147.

#59

EVALUATION OF THE IMUNOMODULATORY POTENCIAL OF TRICHURIS TRICHIURA'S RECOMBINANTS PROTEINS MIF AND FBPA IN EXPERIMENTAL MODEL OF RESPIRATORY ALLERGY INDUCED BY BLOMIA TROPICALIS

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Support: CNPq, UFBA

Introduction: Allergic asthma has emerged as an important public health problem of urban populations in developed countries. Helmints like T. trichiura are described having down regulated effects in allergy and auto immune diseases. The recombinant proteins hMIF e FBPA was estudied by our group and was described leaving to significative increasing of IL-10. Objectives: Evaluate the immunomodulatory effects of hMIF and FBPA in a murine model of respiratory allergy induced by the Blomia tropicalis (Bt) mite. Material and Methods: The respiratory allergy was induced in A/J mice by administration of Bt extract and the treatment was done using 25 µg/animal s.c. of each protein. We then evaluated the changes induced by these drugs on immunological parameters related to the allergic process, which are up-regulated in this allergic model. Results: The most of results are not available yet, but the treatment of animals with 25 µg/animal of hMIF and FBPA led to a significant reduction in IL-4, IL-5 and IL-17 in BAL. Conclusions: These results suggest that hMIF and FBPA have therapeutic potential in this murine model of respiratory allergy to a clinically relevant human sensitizer allergen.

#60

RESEARCH OF MECHANISMS ASSOCIATED WITH THE ACTIVITY OF ANTI-INFLAMMATORY COMPOUNDS AND NEUROPROTECTIVE FLAVONOIDS IN MODEL IN VITRO MULTIPLE SCLEROSIS

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Support: Fapesb

The Multiple Sclerosis (MS) it is an autoimmune disease and neurodegenerative commune irreversible lesions. Basically MS is characterized as a demyelinating disease in which the myelin sheath (MB) is the structure achieved during inflammation by inflammatory response mediated by cytokines of TH1 (IL1-β, IL-4, IL-2, IL-17) stimulating the phagocytes action on the MB, the destruction of this structure leads the individual to a dysfunction in the transmission of nerve impulses, causing the carrier of MS decreased and complicated survival, with frequent outbreaks and considerable anatomical disorders such as loss of vision, constriction of the sphincter among others. MS presents two immunopathological profile: autoimmune where a dysfunction of the blood brain barrier (BBB) by means of an inflammatory response to toxins or pathogens causes a lack of blood brain barrier allowing migration of leukocytes mainly Lymphocytes CD8 which, together with resident cells CNS including microglia, act for their cytotoxicity in MB of neurons resulting in demyelination, which leads the bearer of MS to neurodegenerative profile where desmyelinizate neuron dies via apoptotic mechanisms. Unfortunately, there was observed a treatment that brings the MS patient a cure, current mechanisms increase survival of this individual, reducing the number of frames and side effects of the sclera. Laboratory research group where this work is carried out has observed significant responses of natural molecules Flavonoids between these Rutin in treating induced murine MS, including a setback of injury, mobility improves and neuroprotective and immunomodulatory activity of rutin in these animals . It is necessary before these results to observe the mechanism of these substances in models of in vitro by inflammatory inducers (LPS, Anti-MOG and IL-1β) elucidating the flavonoid activity of pathways on this pathology, being a possible alternative for helping the treatment of MS.

#61

MICROBIOLOGICAL AND IMMUNOLOGICAL BIOMARKERS RELATED TO CHRONIC PERIODONTITIS AND LEPROSY REACTION

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Support: UFBA, UEFS

Introduction: Leprosy reaction is an acute or subacute inflammatory event whose etiology is associated with immune system alterations possibly due to infectious conditions concomitant to leprosy. Objectives: To investigate associations between microbiological and immunological markers of chronic periodontitis, both in saliva and peripheral blood, and the occurrence of leprosy reactions in individuals with leprosy seen at the HUPES university hospital complex. Material and Methods: This epidemiological case control study, which was observational and analytical in nature, will estimate the association between periodontitis and leprosy reactions. The sample will consist of 244 individuals 18 years of age or older, both with and without leprosy reactions. Genotyping for the presence of periodontalpathogens in the subgingival biofilm will be performed via qPCR. Cytokine levels of IFN-γ, IL1- β, IL-12, IL-13, IL-5, IL-10, IL-23, IL 17, IL -6, IL-22, IL-9, TGF-β, TNFα will be quantified in the supernatants by ELISA (Enzyme Linked Immunosorbent Assay). Humoral immune response will be evaluated by measuring serum levels of anti-Porphyromonas gingivalis IgG and salivary levels of anti-Porphyromonas gingivalis IgA by indirect ELISA. Descriptive analysis will be conducted for all variables considered in the study in relation to leprosy reactions. The simple frequencies will be obtained and statistical differences between case and control groups evaluated, using Pearson´s chi-square test, with significance level of 5%.
Acknowledgment: The authors would like to acknowledge the support received from Dermatological service of the Professor Edgar Santos University Hospital and the Immunology and Molecular Biology and the Biochemical Laboratory of the Health Sciences Institute of the Federal University of Bahia. We would also like to thank the Multidisciplinary Research Group of the Feira de Santana State University.

Keywords: Leprosy, Periodontitis, Immunoglobulin, Cytokines.

#62

POLYMORPHISM IN *RIG-I* ARE ASSOCIATED WITH ASTHMA AND ATOPY IN A BRAZILIAN POPULATION

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Support: Cnpq and Fapesb

Introduction: The RIG-I (Retinoic Acid–Inducible gene I),encoded by a gene localized in the chromosome 9p12, is ahomonym cytosolic receptor that acts in the innate immune response, recognizing molecular patterns of double stranded RNA viruses. The receptor-pathogen interaction stimulates the signaling pathways of transcriptional factors such NFkB and AP1.These signals stimulate Interferon Regulatory Factor (IRF3) to initiate IFN-² synthesis and consequently lead the cells foran antiviral status. Also, IFN-I signaling modulates human Th2 development by blocking GATA3, this effect is consistent with the role for IFN-I in suppressing allergic inflammatory processes by blocking granulocyte activation and IL-4 syntheses. Thus, genetics modifications capable of functionally modifying the receptor may affect directly the course of atopic lung diseases such as asthma. Objectives: To evaluate the association between genetic polymorphisms in *RIGI* with asthma and atopy markers. Materials and Methods: Genotyping was performed using a commercial panel (Illumina) in 1,309 participants of SCAALA program (Social Change, Asthma, and Allergy in Latin American). The study included 51 SNPs for *RIG-I*. Logistic regressions for asthma and allergy markers (skin tests and IgE levels) in additive model were performed using Plink 1.9 software adjusted for sex, age, helminth infections and ancestry markers. Results: The rs10813826 was positively associated with atopic asthma when compared with non-atopic asthma (OR:1.82; 95%CI: 1.20-2.76), specific IgE production for *Blomia tropicalis* (OR:1.28; 95%CI:1.09-1.51), *Blatela germanica* (OR:1.37; 95%CI: 1.09-1.71), and *Periplaneta americana (*OR:1.57; 95%CI: 1.21-2.03) and negatively associated with IFN-g production (OR:0.6063; 95%CI: 0.44-0.83).The rs61757209 was negatively associated with atopic asthma when compared with non-atopic asthma (OR:0.39; 95%CI: 0.18-0.85), specific skin test for *Dermatophagoides pteronyssinus* (OR:0.54; 95%CI: 0.29-0.99), for *Blomia tropicalis*(OR:0.41; 95%CI:0.24-0.73), and *Periplaneta Americana* (OR:0.42; 95%CI: 0.20-0.87). Also, this SNP was negatively associated with specific IgE production for *Blomia tropicalis* (OR: 0.56; 95%CI: 0.39-0.81) and for *Periplaneta americana*(OR:0.32; 95%CI: 0.14-0.77). Conclusions: The genetic polymorphisms in the *RIG-I* are associated with atopic phenotype of asthma, atopy makers and IFN-g levels. The interaction between gene and environment factors will be analyzed to better describe the impact of such SNPs in the biologics process of viral infection and asthma and atopy.

Keywords: Asthma, Atopy, Polymorphism, RIG-I.

#63

TH17 AND CD4+ AND CD8+ T CELLS FREQUENCY IN PATIENTS WITH HEPATITIS C

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Support: FAPESB, CNPq

Introduction. The immune response against the Hepatitis C virus (HCV) has a crucial role in the pathogenesis of this infection and a new subtype of helper T cells, called Th17 cells, emerged with the participation reports in the regulation of innate and adaptive immunity of these diseases. Objective. To evaluate quantitatively the frequency of Th17 cells in relation to CD4 + and CD8+ T cells and clinical markers in patients with chronic hepatitis C (HCC) compared to a group of healthy population. Materials and Methods. The patients included 20 subjects with HCC and genotype type 1 without antiviral prior treatment and 10 patients with HCC pre-treated and non-responders to this treatment, and 23 healthy individuals. Immunophenotyping of subpopulations of CD4+T lymphocytes, CD8+T and Th17 of peripheral blood was performed by flow cytometry; while blood count tests, aminotransferases and autoimmunity were performed for clinical characterization of these patients. Informations about liver histopathology and viral load data were obtained from medical records.Results. Most patients had low activity histopathological (F0 -F2), viral load and aminotransferases, and autoimmunity markers present in over 50% of patients. Patients pre-treatment HCC and non- responders had lower percentage frequency of Th17 cells relative to healthy individuals: 0.70%, 0.67% and 2.50%, respectively, $p < 0.0001$. However, the CD4+T cell subpopulations and CD8+T cells showed no differences in the frequencies of same in the different groups ($p=0.85$ and $p=0.76$, respectively). Conclusion. Th17 cells are found numerically reduced in peripheral blood of patients with chronic hepatitis C probably induced by downregulation..

#64

RESEARCH OF NEUROPROTECTIVE POTENTIAL OF POLYPHENOLIC COMPOUNDS IN AN IN VITRO MODEL OF NEURODEGENERATION

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Support: CAPES, CNPq and FAPESB.

Introduction: Neurodegenerative diseases, despite having different etiologies have in similar neural damage associated to oxidative stress and increased inflammatory response, especially through the activation of glial cells. Some flavonoids have been demonstrated antioxidant and anti-inflammatory activities and, in this context may be regarded as promising adjutants for the treatment of neurodegenerative disorders. Objectives: This study aimed to evaluate the neuroprotective potential of flavonoids epigallocatechin gallate (EGCG), epicatechin (EP) and agathisflavone (FAB) in an in vitro model of neurodegeneration or neuroinflammation. Material and Methods: Glia/neurons co-cultures were obtained from the cortex of Wistar rats. To evaluate the neuroprotective potential of flavonoids, co-cultures were exposed for a period of 24h, to rotenone neurotoxin (10 μM) or with IL-1β (10 ng/mL) and then treated with the flavonoids (0,1, 1,0 and 10 μM) for 24h. Cell viability was determined using staining with AnexinV/Propidium Iodide and analysis by flow cytometry, kinetic test to determine lactate dehydrogenase or by Fluoro-Jade B. Results: It was observed in cultures in control conditions (0.01% DMSO), 83.34% viable cells. Rotenone neurotoxin induced reduction of cell viability to 62.95%, and early apoptotic cells increased (4.95%), late apoptotic (23.50%) and necrosis (8.60%). Treatment with flavonoids alone showed no cytotoxic effect on co-cultured glia/neurons. EGCG in a concentration of 10 μM enhanced the rotenone-induced toxicity reducing to 36.98% of viable cells. In contrast, epicatechin seem to have any toxic effect on the highest concentration tested (10 μM), presenting an increase of cell viability to 75.06% when compared to the group treated only with rotenone. Using a model of neuroinflammation induced by IL-1β we observed that FAB reduced neurodegeneration induced by IL-1β. Our data revealed by Fluoro-Jade B or LDH that Agathisflavone reduced the IL-1β-induced neuroinflammation. Conclusions: These data suggest that the flavonoid epicatechin at 10 μM concentration has a protective potential against rotenone-induced neurotoxicity and agathisflavone at 0,1 μM present neuroprotector potential against IL-1β-induced neuroinflammation. However, further studies are needed to elucidate the neuroprotective mechanisms of epicatechin and agathisflavone.

Keywords: Flavonoids, Neurodegeneration, Neuroprotection, Immunomodulatory.

#65

STUDY OF THE NEUROPROTECTIVE AND ANTI-INFLAMMATORY EFFECTS OF FLAVONOID APIGENIN ON IN VITRO MODEL OF NEUROINFLAMMATION

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Support: FAPESB, CAPES e CNPq

Introduction: Many studies showed that oxidative stress and inflammation could be the major cause of neuronal death in neurodegenerative diseases (ND). In this context, neuroprotective strategies would be promising because it slows down the progression of neurodegeneration and recovers the subject from the disease condition. In ND is observed an inflammatory response mediated by glial cells such as microglia and astrocytes, which once activated, release pro-inflammatory cytokines such as IL-1β, resulting in the exacerbation of the inflammatory response and subsequent neurodegeneration. Objectives: This study evaluated the neuroprotective and anti-inflammatory potential of the flavonoid apigenin on in vitro model of neuroinflammation induced by IL-1β. Material and Methods: Glial/neurons co-cultures were cultivated from the cortex of Wistar rats. The cells were exposed for 24h to 10ng/mL of IL-1β and apigenin (1-10μM), control cells was treated with DMSO. It was observed using Fluoro Jade B staining that apigenin was not neurotoxic. The conservation of cellular integrity and glial activation was determined by staining with Rosenfeld and immunofluorescence for β-tubulin III, GFAP and Iba-1 proteins, structural markers for neurons, astrocytes and microglia, respectively. Results: It was observed that IL-1β induced neuronal death, astrocytes and microglia activation. In contrast, treatment with 1μM of apigenin protected neurons from death induced by IL-1β and reduces astrocyte and microglial activation. It was observed that microglia in control conditions (0.01% DMSO) have a branched morphology characteristic of a quiescent state, but when exposed to IL-1β this cells acquired an activated amoeboid morphology. The immunomodulatory effect of apigenin was evaluated by qPCR for pro-inflammatory and regulatory markers. It was observed that treatment with apigenin did not induce changes on expression of TNFα, IL-1β and IL-6. In addition, treatment with 1μM apigenin increases the expression of the regulatory enzyme arginase. IL-1β increased the expression levels of TNFα, IL-1β, IL-6, CCL2 and CCL5, but apigenin (1μM) reduced microglial activation induced by IL-1β and decreased the expression of proinflammatory cytokines such as IL-6 and IL-1β, and also increased the expression of IL-10. Conclusions: These data suggest that apigenin presents neuroprotective and immunomodulatory potential, however more studies are necessary in order to elucidate the apigenin's mechanism of action.

Keywords: Neuroinflammation, Neuroprotection, Anti-inflammatory, flavonoids.

#66

NEUROPROTECTIVE EFFECTS OF RUTIN AND QUERCETIN IN MULTIPLE SCLEROSIS: EVALUATION IN ENCEPHALOMYELITIS AUTOIMMUNE EXPERIMENTAL MODEL

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Support: CNPq, CAPES, Cajal Institute and FAPESB.

Introduction. Multiple Sclerosis is a demyelinating chronic disease of central nervous system. The main characterized is secondary neurodegeneration, infiltrations of the CNS outside cells, microglial activation for T_{h1} profile and astroglial proliferation (gliosis), that can affecting both white and gray matter of brain and spinal cord. The cause of MS is unclear, although, it likely involves a combination of genetic and environmental factors. Recent approaches have demonstrated that flavonoids present in various species of plants present anti-inflammatory activity and may be regarded as potential compounds for treating autoimmune/inflammatory diseases as MS. Objectives. In this study we tested the immunomodulatory effect of flavonoids Rutin and Quercetin in vivo murine model of MS. Methods. Experimental autoimmune encephalomyelitis (EAE) was induced by myelin oligodendrocyte glycoprotein ($MOG_{35–55}$). Adult female C57BL/6 mice were immunized with MOG_{35-55} (150μg/animal), Mycobacterium tuberculosis H37R (1 mg/animal) and Complete Freund's adjuvant (CFA). One and 48 h after immunization, animals were exposed the pertussis toxin from Bordetella pertussis (500ng/animal). Rutin 60mg/kg/day) or Quercetin (40mg/kg/day) were administrated (i.p) for 30 days since the beginning of experiments. Neurological impairment was evaluated using disease scores of motor functions. Microglia was assessed by immunohistochemistry for Iba1. Results and discussion. We observed that Rutin induces reduction in neurological disability, with delay in clinical symptoms, reduction in progression, in severity and duration of disease. Moreover animals treated with Rutin showed decrease in cellular infiltration accompanied by weaker microglial activation. However, animals treated with quercetin showed increase MS symptomatology with very slow remission, and maintained levels of brain cellular infiltration. Conclusions. Our preliminary data suggest that flavonoid Rutin induces anti-inflammatory responses in the EAE model of MS and could be regarded as a possible potential adjuvant for improve therapy for CNS demyelinating diseases. Currently we under evaluation, if these compounds may influence the activation states in the different treatments (EAE^+, EAE^+Rutin, EAE^+Quercetin and control) with in morphologic basis, of microglia (Iba1, iNOS) and astrocytes (GFAP), especially in gliosis sites and cytokine profile by RT-PCR.

Keywords: Flavonoid; immunomodulation; microglia.

#67

IMPACT OF POLYMORPHISMS IN FOXP3 GENE WITH SEVERE ASTHMA AND ATOPY IN BRAZILIAN POPULATION

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Introduction: Genetic and environmental factors may influence the development of asthma and atopy. Several genome-wide association studies (GWAs) have been conducted to understand which genetic components influence asthma. However, genes located on the X chromosome, such as FOXP3, are often underrepresented. This gene encodes a transcription factor that is directly related to the activation and differentiation of regulatory T cells. Objectives: To evaluate the impact of FOXP3 polymorphisms in asthma and atopy. Materials and Methods: DNA was extracted from peripheral blood of 1,418 individuals (443 mild asthma, 523 severe asthma and 452 healthy individuals) recruited from ProAR (Program for Asthma and Allergic Rhinitis Control in Bahia) and the SNPs were genotyped using TaqMan probe. The study included 4 SNPs inFOXP3 (rs2280883, rs2294021, rs2294021, rs2232365). Logistics regressions for asthma and skin test were performed using PLINK 1.9 software with a separate analysis for men and for women. Functional impact, tissue gene expression and linkage disequilibrium were also explored in this study.Results: Analyzing only men the rs2280883 (OR: 0.35; 95% CI: 0.17-0.79), rs2294021 (OR: 0.43; 95% CI: 0.23-0.79) and rs3761548 (OR: 0.39; 95% CI: 0.20-0.79) were negatively associated with asthma. None of the SNPs was significant for atopy, though the rs2294021 (OR: 0.:55; 95% CI: 0.31-0.95) was negatively associated with specific skin test for Dermatophagoidespteronyssinus. In women, the SNPs were not significant for asthma. However, the rs2280883 (OR: 0.68; 95% CI: 0.50-0.93) and rs3761548 (OR: 0.71; 95% CI: 0.53-0.95) were negatively associated with atopy but there was no significance in the specific skin test for Dermatophafoidespteronyssinus in any of the SNPs. Conclusions: In the population studied, some polymorphisms in FOXP3 suggest asthma protection in men and atopy protection in women. However, further analysis is required to better describe the impact of polymorphisms in this gene in Brazilian population.

Key word: FOXP3, asthma, atopy.

#68

ANALYSIS OF HUMORAL IMMUNE RESPONSE AGAINST Porphyromonas gingivalis IN SEVERE ASTHMA

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Introduction: Chronic Periodontitis is a multifactorial disease. Its pathogenesis is related to the host immune inflammatory factors and to the oral microbiota. Porphyromonas gingivalis is a keystone pathogen on its etiology. Asthma is a chronic disease of the airways with an inflammatory origin and it is considered a public health problem in Brazil. Works have suggested an influence of periodontitis on asthma, especially in severe asthma form. It may be due to aspiration of pathogenic microrganisms or to epithelial reactions triggered by the immune response. Thus, there is a biological plausibility of the association between chronic periodontitis and severe asthma. It seems to be related to the presence of common immunological factors for both diseases. It is known that individuals with chronic periodontitis produce higher serum levels of IgG and subclasses specific to Porphyromonas gingivalis. Objectives: Thus, this case-control study aims to evaluate the humoral immune response against Porphyromonas gingivalis antigens in individuals with severe asthma and individuals without the disease. Material and Methods: Serum levels of IgG and subclasses anti- Porphyromonas gingivalis in subjects (n = 220) with severe asthma and subjects without asthma from the Program for the control of asthma and allergic rhinitis in Bahia – PROAR will be evaluated by indirect ELISA. Results: It is expected an association between anti-Pg IgG levels and severe asthma; contributing to the understanding of the association between chronic periodontitis and severe asthma.

Keywords: Asthma, Periodontitis Chronic, Immunoglobulin G.

#69

IMMUNOMODULATORY POTENTIAL OF MESENCHYMAL STROMAL CELLS IN SICKLE CELL DISEASE

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Support: HUPES

Introduction: Sickle cell disease (SCD) is associated with a pro-inflammatory state. Inflammatory cytokines, elevated leukocyte adhesion to vascular endothelium with subsequent endothelial injury, and repeated ischemia-reperfusion injury contribute to disease pathogenesis. Mesenchymal stromal cells (MSC) are multipotent progenitors that promote hematopoiesis and have unique immunoregulatory properties, making them attractive for use as cell-based therapy. Objectives: The aim of this project is to evaluate the immunomodulation potential of MSCs from SCD patients. Materials and Methods: MSC will be isolated from buffy coat prepared from bone marrow from SCD patients or MSC will be obtained from cell bank, clonogenic cell culture will be performed, as well as, Cell Immunophenotyping by specific monoclonal antibodies, Cell multilineage to assess differentiation potential. Lymphocytes will be isolated from peripheral blood, in order to perform a co-culture assay with mesenchymal stromal cells and plasma from sickle cell disease patient and to assess the immunomodulatory profile promoted. These co-cultures will undergo proliferation assays and will also. be analyzed through cytometric beads assay (CBA), both from plasma and from culture. Results: In this work we believe that the MSC will play a immunoregulatory role towards the plasma of individuals with SCD and that therapy based on MSC is a good alternative to minimize the disease pathogenesis.

Aknowledgment: We would like to thank the support recieved from FAPESB, UFBa, ICS, LABIMUNO, HUPES and PPGIm.

#70

FLAVONOID APIGENIN INHIBITS GROWTH, INDUCES DIFFERENTIATION, APOPTOSIS AND ALTERS IMMUNOLOGY PROFILE IN CULTURES OF GLIOMA AND MICROGLIA/GLIOMA CELLS

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Support: Fapesb, CNPQ, CAPES

Introduction: Human glioblastoma multiform is a highly malignant tumor of the CNS and presents resistance to radio and chemotherapy. Microglias present an import role in the immune system, actively participating in the defense against neoplastic cells. Our previous studies have shown that apigenin, a flavonoid extracted from seeds of the Brazilian plant *Croton betulaster*, acts as inhibitor of growth, induces differentiation, apoptosis in a human glioblastoma cell lines. Objectives: The objective of this study was to evaluate the inhibitory effects, antimigratory, chemotatic and immunomodulatory effects of the flavonoid apigenin in microglia cells, microglia/C6 cells and U-251 human glioma cells line. Results and Discussion: Our results show that the flavonoid apigenin induces inhibitory effects in microglia cells and co-culture. Also this flavonoid induces differentiation and changes the immunologic profile to M2 in microglia cells. Otherwise the microglia chemotaxis to C6 cultures was observed. Rutin also demonstrated to modulate microglial response reflecting on inhibition of C6 glioma cells migration through indirect contact. Furthermore, the profile cytokines is dose dependently regulated after flavonoid exposure, with increase on secretion of proinflammatory cytokine tumor necrosis factor (TNF-α) and decrease on secretion of regulatory cytokine IL-6 and IL-10. In addition we observed the apoptosis and authopagy effects in U-251 human glioma cells line. These results suggest that the flavonoid apigenin induced cellular differentiation and reduced growth and cell migration, in addition to changing the profile of regulatory cytokines and immunomodulatory proteins in microglia and microglia/ C6 glioma lineage. Conclusion: Thus, this flavonoid has potential antiproliferative effects and can contribute to the adjuvant treatment of these malignant brain tumors

Key worlds: apigenin, glioma,microglia, immunomodulatory.

#71

EXPRESSION AND EVALUATION IN VITRO OF TRICHURIS TRICHIURA PROTEINS WITH IMMUNOMODULATORY POTENTIAL

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Support: CNPQ

Introduction: Despite being known for thousand years, helminth infections, such as trichiuriasis caused by Trichuris trichiura, are still considered a serious public health problem, especially in development countries as Brazil. Through the escape mechanism of the helminths, there is a release of suppressive cytokines, such as IL-10 and TGF-β, which may be associated with the suppression of inflammatory, autoimmune and allergic

diseases. Objectives: Expression and purification of immunoreactive proteins Trichuris_c27 and Trichuris_c2878 derived from Trichuris trichiura and evaluation of the immunomodulatory potential of these molecules in vitro . Material and Methods: The Trichuris_c27 and Trichuris_c2878 proteins will be expressed with 1 mM IPTG and confirmed using SDS-PAGE 10 % and Western Blotting, later a purification will be performed by affinity chromatography. The endotoxin depleted proteins are going to be cultivated in human peripheral blood cells (PBMC's) of allergic individuals and THP-1 cell line, then stimulated with Blomia tropicalis in order to observe the effect of the immunomodulatory proteins from Trichuris trichiura against allergy. Expected Results: Firstly, it is expected the success of the expression of Trichuris_c27 and Trichuris_c2878 proteins. Moreover, by in vitro analysis, it is aimed to observe the immunomodulatory effect of Trichuris trichiura proteins against allergies, allowing in the future alternatives to immunotherapies.

Keywords: helminth infection, Trichuris trichiura, immunomodulatory potential, allergy.

#72

EXPRESSION OF MAST CELL AND ANGIOGENESIS-RELATED MIRNAS IN RELATION TO TUMOR TRYPTASE-POSITIVE MAST CELLS IN ORAL SQUAMOUS CELL CARCINOMA

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Support: FAPESB, CNPq e PIBIC-UFBA

Introduction: Both microRNAs (miRNAs) and angiogenesis play crucial roles in cancer. Mast cells can stimulate angiogenesis by releasing of tryptase, a potent proangiogenic factor. Nevertheless, in oral squamous cell carcinoma (OSCC) no data are available concerning the expression of mast cell- and angiogenesis-related miRNAs in relation to tumor tryptase-positive mast cells (TPMC).Objectives: The aim of this study was to investigate in OSCC the expression of miR-221, involved in regulation of IgE-mediated activation of mast cells degranulation, besides the miR-195 and miR-17-5p, which respectively exhibit anti and proangiogenic involvement. Material and Methods: The expression of miRNAs was measured in 32 OSCC paraffin embedded samples by qRT-PCR. TPMC were assessed by immunohistochemistry in corresponding samples. Results and Discussion:We observed up-regulation of miR-17-5p, miR-195 and miR-221 in 74.3%, in 33.3% and 70.6% of tumor samples, respectively. Down-regulation of miR-195 was observed in 13.3% of the samples. No case of down-regulation was observed formiR-17-5p and miR-221. These results corroborates that miRNA-195 probably does not have a central role in OSCC (WONG et al., 2008). In addition, positive correlation was observed betweenmiR-17-5p and miR-221 expression (r= 0.716, P=0.000). This result suggests its participation in development of OSCC through a common signaling pathway, as the TGFB1, whose gene presents major role in angiogenesis and regulation by both microRNAs. All analyzed cases exhibited periparenchymal infiltration of TPMC but the presence of intraparenchymal TPMC was observed in only 58.6% of cases. Elevated infiltration of periparenchymal TPMC was present in 68.8% of samples. Conclusion: Overexpression of miR-17-5p and miR-221 is probably associated with tumorigenesis of OSCC. The high infiltration of TPMC observed in periparenchymal stroma demonstrates an importantrole for these cells in OSCC.

#73

STUDY OF HUMAN IMMUNOREACTIVITY IN VITRO TO CORYNEBACTERIUM PSEUDOTUBERCULOSIS ANTIGEN - MASTER PROJECT.

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Support: LABIMUNO

Introduction: Corynebacterium pseudotuberculosis (Cp) is a pathogenic bacillus that causes caseous lymphadenitis in small ruminants. PLD, NanH , SodC, PknG, SpaC Cp antigens have been used in the Laboratório de Imunologia (Labimuno) of the Universidade Federal da Bahia (UFBA) to evaluate infection in goat and sheep using in house methods (Meyer et al, 2005). Human infection with Cp, although rare, has increased in recent decades. Lymphadenitis and pneumonia were observed in individuals who have contact with these infected animals and/or their derivate products or with the laboratory personal working with bacteria in several countries (Heggelund et al, 2015). Justification: The current lack of routine diagnostic tools for humans, limits the identification of infection with Cp and the appropriate treatment for the infected individual. Hypothesis: Cp proteins may be antigenic and serve as efficient tools to identify humans infected by the pathogen. Objective: To evaluate the human immunoreactivity in vitro to Cp antigens in the blood of individuals who have contact with small ruminants and/or with this bacillus. Methods: After approval by the

Research Ethics Committee (CEP ICS) participants will be selected in a reference hospital for respiratory diseases, in institutions that manipulate this microorganism and sheep and/or goat farms. Composition of groups: Group 1 - individuals who have contact with small ruminants and/or the bacteria; no history of tuberculosis, and negative for TRM tests and IGRA for Mycobacterium tuberculosis (Mtb); Group 2 control - individuals without any contact with small ruminants or their raw products, with no history of infectious process characteristic of Mtb and Cp, and negative test for Mtb (IGRA). A sample of 11mL peripheral blood will be collected for the WBC counting, ELISA, analysis of leukocyte cell markers and the production of cytokines after in vitro antigenic stimulation by FACS.
Acknowledgment: volunteers Participants, Farmers, PPGIm, UFBA. Strategic and financial Support: Labimuno-UFBA, HEOM, CAPES.

Keywords: Corynebacterium pseudotuberculosis, diagnosis, ELISA, immunoreactivity in vitro stimulation, lymphadenitis, PLD, NanH , SodC, PknG, SpaC.

#74

STUDY OF THE ACTION OF THE OCIMUM BASILICUM PLANT EXTRACT LINALOOL ON IMMUNE CELL ACTIVITY IN PATIENTS WITH AND WITHOUT PERIODONTITIS.

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Support: Labimuno/Nuppim
Introduction: periodontitis is a multifactorial disease whose primary etiological factor is in the presence of biofilm on the tooth surface, and porphyromona gingivalis a central role in dysbiosis. However, the host response is preponderant in its beginning and development. The treatment is based on mechanical removal of the biofilm. Justification: the use of chemical additives to control periodontitis has also been preconized in various forms of administration, though the use of herbal medicines for this purpose is very limited. On the other hand, several plants of bahia semi-arid region have been used in folk medicine, with varying biological effects, bringing out more viable alternatives to periodontal treatment. Objective: in view of this panorama, this project aims to evaluate the effect of linalool major compound of the plant species ocimum basilicum on mononuclear cells from peripheral blood of volunteers with and without periodontitis. Methodology: mononuclear cells from peripheral blood of 60 subjects (30 with periodontitis and chronic periodontitis 30) are cultured in the presence of porphyromonas gingivalis sonicate extract and linalool for 48h. Then it is intended to verify the role of linalool in lymphoproliferation and cell death by flow cytometry and evaluate the inhibition or induction of il-1beta cytokine production, il-8, ifn-gamma, il-10, il-6 il-13 and il-17 by immunoassay (elisa). After analyzing the data distribution with the kolmogorov-smirnov test, the levels of cytokines between healthy subjects and patients will be compared using the student t test or mann-whitney. The linalool present a modulating effect on the immuno-inflammatory process, already expected to immune cells are crucial in periodontal lesions. It is expected that the compound presents a scientific and biotechnological potential.

Keywords: periodontitis; ocimum basilicum; cytokines, linalool.

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#75

POLYMORPHISM IN IL-12A ARE ASSOCIATED WITH ASTHMA IN A BRAZILIAN COHORT

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Introduction: The interleukin 12 (IL-12) family has its unique structural, functional, and immunological characteristics that have made this family as important immunological playmakers. IL-12A encodes a subunit of some heterodimer cytokines, like IL-12 involved in proinflammatory responses, and IL-35 that possess anti-inflammatory activities. Objective: To evaluate the association between genetic polymorphisms in IL12A with asthma, asthma severity and atopy markers. Materials and Methods: Genotyping was performed using a commercial panel (Illumina) in 1,309 participants of SCAALA program (Social Change, Asthma, and Allergy in Latin American). Logistic regressions for asthma and allergy markers (skin tests and IgE levels) in additive model were performed using Plink 1.9 software adjusted for sex, age, helminth infections and ancestry markers. Functional impact, tissue gene expression and linkage disequilibrium were verified. Results and Discussion: The rs2243131 was positively associated with asthma (OR:1.34; 95%CI: 1,06-1.70), asthma severity (OR:1.37; 95%CI: 1.02-1.83), IL-5 production (OR:1.73; 95%CI: 1,13-2.64) and specific skin test for Blatela germanica (OR:1.56; 95%CI: 1.09-2.23). In silico gene expression analyzing in GTEx (Genotype-Tissue Expression) is possible to infer that the presence of this polymorphism in the gene reduces IL-12A expression in the lung, may result in a lower IL-35 expression, important anti-inflammatory cytokine. Conclusion: The genetic polymorphism in the IL-12A (rs2243131) may be considered a factor of susceptibility / risk for the disease if is leading to a decrease of IL-35 in the tissue. The study of polymorphisms of other subunits - IL12B and EBI3 - which composes the IL-12 and IL-35, respectively, will be studied in the same population to better understand the impact of this cytokines on asthma.

#76

PROFILE ANALYSIS OF GENE EXPRESSION MACROPHAGES INFECTED BY A WILD STRAIN OF CORYNEBACTERIUM PSEUDOTUBERCULOSIS

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Support: UFBA- Universidade Federal da Bahia e FAPESB- Fundação de Amparo ao Pesquisador do Estado da Bahia

The study of gene expression of the host infected by a particular pathogen, with mRNAs profile analysis, has been used in association mechanisms of survival and control of the host, triggered by infection. Although of the genes from common answer of host being induced in different cell types during infection, some gene groups are preferably induced in a cell type when compared to others. Therefore, we will analyze the expression of 88 genes in infected peritoneal macrophages with the wild strain C57 of Corynebacterium pseudotuberculosis from C57BL / 6 PCR Array, besides of the identification and correlation of differences in expression of individual genes with phenotypic characteristics or morpho-functional the infected macrophage detected by Griess reaction and through microscopy, correlating all findings with signaling pathways that can help to elucidate the mechanisms of host-parasite relationship culminating in the success of infection pathogen process. To develop this study will be used 10 animals strain male mice C57BL / 6 with ages 4-8 weeks. The animals will initially be inoculated with 1 - 2 mL sodium thioglycolate intraperitoneally, after three days, cells of five mice infected by concentration of the 107 colony forming units (CFU) , generating an MOI of 1, while mice with controls cells will be treated with saline (0.9%) 0.15M. After 2 hours of infection, the animals are euthanized, macrophages will be harvested by peritoneal washing and cultured in RPMI medium containing 10% fetal bovine serum for 6 and 24 hours. The total RNA extraction and cDNA synthesis and amplification of RNAs are made with the extraction kit, cDNA conversion and amplification with PCR of the Exiqon plates array, as recommended by the manufacturer. The results from the PCR Array will be analyzed by Genex of Exiqon software. It is important to know some gene groups that are preferably induced in macrophages, riding gene networks that can influence the physiological events in infection by Corynebacterium pseudotuberculosis.

Keywords: Corynebacterium pseudotuberculosis, macrophage, Gen, mRNA, PCR Array.

#77

DESCRIPTON OF CELLULAR RESPONSE AFTER IN VITRO STIMULATION WITH ANTIGENIC EXTRACT OF CORYNEBACTERIUM PSEUDOTUBERCULOSIS IN INDIVIDUALS INFECTED WITH MYCOBACTERIUM TUBERCULOSIS – MASTER PROJECT.

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Support: Universidade Federal da Bahia; Hospital Especializado Otávio Mangabeira

Introduction: Tuberculosis (TB) is a worldwide disease that affects the lungs and causes the death of about 1 million people / year. It is caused by an alcohol-acid resistant bacilli (AARB), Mycobacterium tuberculosis (Mtb), belonging to Corynebacterium, Mycobacterium, Rhodococcus and Nocardia (CMRN) Group. Corynebacterium pseudotuberculosis (Cp) is the agent of a disease similar to TB in small ruminants. The two bacterial have some proteins homology and can be used for the evaluation of antigenic cross-reactivity. OBJECTIVE: To evaluate the cellular response after in vitro stimulation with antigenic extract of Corynebacterium pseudotuberculosis in individuals infected with Mycobacterium tuberculosis. METHODOLOGY: The project was submitted to the Ethics Committee. The study population will be composed of volunteers attended at Specialized Hospital Otavio Mangabeira (HEOM) and categorized in: Group I - control (without infection), Group II - Latent (without symptoms) and Group III - Tuberculosis (with the disease). The blood sample will be collected in heparinized tubes and used to perform in vitro stimulation with Cp antigens. After incubation (24 hours) there will be performed the immunophenotyping by using cluster of differenciation molecules for lymphocytes (CD45, CD3, CD4 and CD8) and cytokines. The acquisition will be carried out in flow cytometry in FACScalliburBD® to obtain the mean/median intensity of fluorescence (MIF) of the CD molecules. The data will be analyzed by DakoCytomation Summit and Graph Pad Prism v 5.0.

Acknowledgment: Volunteers participants; Strategic and financial Support: Laboratório de Imunologia e Biologia Molecular (ICS-UFBA), Residual INCT-DT, HEOM, PIBIC-UFBA.

Keywords: Corynebacterium pseudotuberculosis, diagnosis, Tuberculosis, MIF, CYTOKINES.

#78

THERAPEUTIC RESPONSE EVALUATION BASED ON IMMUNOLOGICAL PROFILE IN DOGS WITH GENERALIZED DEMODICOSIS

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Introduction. Demodicosis is a very common inflammatory skin disease caused by Demodex canis. Despite the high incidence, little is known about its pathophysiology and treatment protocols based on clinical outcomes coupled with the negative results of parasitological examinations of the skin are used. T-cell-exhausted phenotype is usually characterized by low production of stimulatory cytokines and high levels of suppressive cytokines, almost all of these changes have been documented in dogs with generalized demodicosis, it is very likely that these dogs suffer from T-cell exhaustion. The objective of this study is to assess the need for such prolonged treatment for canine generalized demodicosis, based on analysis of immunological markers and measurement of the parasitic load by real-time PCR and compare the results of three treatment protocols, two with Ivermectin and one with an Isoxazoline. Forty dogs of either sex with will be included in the study. They were divided into 4 groups with ten dogs each: Group I (control), consisting of 10 healthy dogs without any skin lesion; Group II (Ivermectin), ten dogs treated with ivermectin according to the standard protocol, group III (Ivermectin) with ten dogs treated with ivermectin until the immunological parameters normalize and group IV (Isoxazoline), with ten dogs treated with isoxazoline until the immunological parameters normalize. Cytokines (IL-2, 4, 5, 10, 21, TNF-α and TGF-β) are measured before and after treatment systematically.

Keywords: Dog, Demodicosis, Demodex canis.

#79

SENSITIVITY OF *LEISHMANIA (V.) BRAZILIENSIS* TO FLUCONAZOL IN CORTE DE PEDRA-BAHIA

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Support: CNPq

Introdution: Leishmaniasis is caused by protozoa of the genus Leishmania, considered an endemic disease with wide distribution, and is one of the six major infectious diseases worldwide. In Brazil, Leishmania (V.) braziliensis has a high refractory index to pentavalent antimony. This is observed in the endemic area of Corte de Pedra, Bahia, where the localized cutaneous form (CL) is the most common presentation of leishmaniasis. In Corte de Pedra, about 60% of patients require two or more series of intravenous treatment with antimony, increasing the chance of toxicity and resulting in elevated costs. Moreover, parenteral administration has lower effectiveness in the countryside, due to difficult access to health facilities. Thus, the use of drugs administered orally has the potential to increase the adherence and healing rate in CL patients. Objective: To evaluate the therapeutic response to fluconazole in patients with CL caused by L. (V.) braziliensis in Corte de Pedra, Bahia. Methods: The following methodology was performed: (1) randomized phase III clinical trial, which compared the treatment of patients with ATL employing Fluconazole and pentavalent antimony, and the cure was defined as complete reepithelialization of the ulcer evaluated six months after the last dose treatment; (2) resistance in vitro test of L. (V.) braziliensis promastigotes due to pentavalent antimony, Fluconazole and Miltefosine. Results and Discussion: 53 subjects were included. According to the randomization list, 26 of CL patients underwent treatment with pentavalent antimony (Glucantime®), as recommended by the Ministry of Health, and 27 underwent treatment with fluconazole orally, in 28 days, with a daily dose of 6.5-8.0 mg / kg. In the six-month evaluation, the cure rate in the fluconazole group was 22,2% (6/27) and in the group using Glucantime® was 53,8% (14/26). These data were corroborated by in vitro experiments, which showed greater sensitivity of promastigotes to Glucantime® and Miltefosine compared to fluconazole. Conclusion: The Fluconazole is not effective in the treatment of L. (V.) braziliensis in Corte de Pedra, Bahia.

Keywords : Fluconazole, Leishmania (Viannia) braziliensis, Clinical Trial.

#80

CLINICAL LABORATORY PROFILE ANALYSIS OF INDIVIDUALS WITH PULMONARY TUBERCULOSIS DIAGNOSIS FROM A REFERENCE LABORATORY.

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Support: Hospital Especializado Otávio Mangabeira, Labimuno.

Introduction: Pulmonary tuberculosis (TB) is a infectious disease caused mainly by Mycobacterium tuberculosis (Mtb) and curable in most of new cases. Early diagnosis, especially in individuals with active TB, and the rapid introduction of effective therapy are considered essential actions to control TB. Currently studies have shown promising results using Interferon-gamma (IFN-γ) and IP-10 factor as alternative biomarkers with potential for the development of new testing platforms for latent TB and active TB (I.WERGELAND et al, 2015; MORTEN RUHWALD et al, 2012). With the incorporation of real-time PCR technology in routine laboratory and its use as an automated platform for agent research and rifampin resistance mutations (TRM, XPERT®MTB / RIF, Cepheid, USA), arises the need for interpretation of the results in clinical and epidemiological context. The described sensitivity of this test is 92.2%, which represents a significant gain in relation to smear (MOURE R. et al, 2010; HELB D. et al, 2009). General Objective: To analyze the clinical and laboratory profiles of individuals diagnosed with tuberculosis (positive TRM), both sensitive and resistant to rifampicin. Specific Objective: To characterize the profile of IFN-γ production and IP-10 in individuals, treated or not for sensitive or resistant TB Rifampicin. Material and Methods: After approval by the Research Ethics Committee, this descriptive study, using database of volunteer participants with positive results of TRM, from a reference laboratory for TB in Bahia. Venous blood sample from these participants will be collected for WBC and IFN-γ dosage (IGRA) and IP-10, which will be characterized as the number of previous treatments, body mass index (BMI) and resistance or not to Rifampicin, the primary drug of the therapeutic regimen. Expected results: Data analysis can contribute to the knowledge and characterization of biomarkers profile to improve diagnostic and monitoring the response to anti-TB treatment, a translational approach.

Keywords: Tuberculosis, resistant TB, diagnosis, GeneXpert, IFN-Gamma, IP-10, IGRA.

#81

SEROLOGIC MARKERS TO VIRUSES OF VERTICAL TRANSMISSION IN PREGNANT WOMEN OF SALVADOR: SEROPREVALENCE AND ANALYSIS OF RISK FACTORS

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Support: Faculdade de Farmácia-UFBA

Introduction: Infections that can be transmitted vertically are a major public health problem due to the high frequency occurring and clinical consequences caused to mothers and especially to newborns. They can occur during pregnancy (congenital transmission); during delivery or shortly before (perinatal transmission); and even after birth by breastfeeding. They generate great impact on the health and survival of the embryo and are directly related to miscarriages, premature birth, stillbirth, low birth weight and neuropsychomotor sequelae.OBJECTIVES: This study aimed to determine the prevalence of serological markers for CMV, HSV-1 and 2, Rubella, HIV-1/2, HBV and HTLV-I/II in pregnant women in a maternity hospital in Salvador-BA. The association of these infections with possible risk factors was also analyzed.RESULTS AND DISCUSSION: In the population studied was detected a seroprevalence of 93.3% (513/550), 94.8% (638/673), 94.3% (558/592), 0.3% (2/661), 0.6% (3/544) and 0.4% (3/734) for Rubella, CMV, HSV, HIV, HTLV and HBV, respectively. Statistically significant associations were found between age and seroprevalence to rubella (p=0.002) and HSV-1/2 (p= 0.001), respectively. The seroprevalence of herpes and CMV was higher among those with higher number of previous pregnancies (p=0.011 and p=0.012, respectively); and rubella was higher in those women with higher levels of education (p=0.034) and became pregnant the first time later. The findings showed high seroprevalence of CMV and HSV. Regarding rubella, 93.3% of pregnant women are presented immune and seropositivity for HIV and HBV were similar to those found in other regions of the country and HTLV was higher than that found in other Brazilian states.CONCLUSIONS: The results achieved in the present study confirm the importance of continued screening for CMV infection, HSV, Rubella, HTLV, HIV and HBV in pregnant women. These infections occur frequently in our population of pregnant women and prevention and control measures are possible only when we know the characteristics and peculiarities of individuals. Knowing the serologic profile is the first step towards the broader measures and effective control of maternal-fetal transmission of infection can be adopted and that prenatal screening protocol for pregnant women of our population can be established.

Keywords: Infectious diseases, pregnant women, seroprevalence

ACKNOWLEDGMENT: CAPES and FAPESB for the financial support

#82

ACTIVATED ASTROCYTES AND MICROGLIA IN MICE INFECTED BY *N. CANINUM* VIA INTRACRANIAL

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Support: FAPESB

Introduction: *Neospora caninum* is an obligate intracellular protozoan that infects domestic and wild animals through the oocyst ingestion or through congenital infection by tachyzoites. Cells of the central nervous system can be infected by this parasite. The infection process occurs with the activation of astrocytes and microglia to produce suppressive cytokines of the inflammatory response, able to control parasite growth, while preserving the nervous tissue. Objective: Avaluate the morphological change patterns of astrocytes and microglia in response to tissue infection by *N. caninum* tachyzoites. Materials and Methods. C57BL / 6 mice were submitted to inoculation of tachyzoites by the stereotactic method. After 3 and 7 days of infection, the animals were perfused and brain samples were collected for further processing. Samples were used for immunohistochemistry of float for qualitative and quantitative analysis. Glial fibrillary acidic protein markers (GFAP) and ionized calcium-binding adapter molecule 1 (Iba-1) were used to characterize particular events by cell labeling. Relative expression of cytokines TNF, IL 6, IL-10 and Arg-1 were Analyzed by RT - PCR of brain tissue. Results. During infection with intracranial N. caninum (Nc-1 strain) in mice, were observed reactivity of astrocytes and microglia at 3 and 7 days post infection. Phenotypes were observed in agreement with microglial reactivity in infected animals. The relative cytokine expression TNF, IL 6, IL-10 and Arg-1 no significant differences between the control and infected. Conclusion. The presence of immunoreactivity in astrocytes of infected animals suggests a limiting response lesions and restoring the brain homeostasis. The relative cytokine expression suggests a regulation of the inflammatory response, with reduced cytokine levels in the presence of the parasite *N.caninum*.

Keywords: N.caninum; neuroparasitology, astrocyte; microglia; neuroinflamation.

#83

POLYMORPHISMS IN THE CYSLTR2 ARE ASSOCIATED WITH ATOPY AND HELMINTH INFECTION IN LATIN AMERICAN POPULATION

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Introduction: Atopy is a tendency to produce IgE and inflammatory mediators in response to common allergens, vary in severity and frequency from person to person involving both genetic and environmental factors. The resistance to helminth infection has similar immunological mechanisms as allergic diseases. An initial exposure to helminths antigens leads to activation of a subset of Th2 cells that orchestrate the immune response against exogenous antigen by secreting cytokines, including IL-4, IL-5 and IL-13. Cysteinyl leukotrienes (CysLTs), LTC4, LTD4 and LTE4 are involved in both atopy as the helminth infections they are both inflammatory mediators known to possess potent proinflammatory action, after the interaction with its receptors, cysteinyl leukotrienes receptor type 2 (CysLTR2) a G-protein-coupled receptor. This receptor was already described in several numbers of human organ and tissues, including lung macrophages, airway smooth muscles, and peripheral blood leukocytes. Polymorphism in CYSLTR2 receptor has been associated with asthma and atopy, although the mechanism is not clear. Our aim was to evaluate the association between genetic polymorphisms in CysLTR2 with atopy markers and helminth infection. Materials and Methods: Genotyping was performed using a commercial panel (Illumina), and carried out in 1,309 participants of SCAALA program (Social Change, Asthma, Allergy in Latin American). Logistic regressions for infection (Trichuris trichurias) and allergy markers (skin tests and IgE production) were performed using PLINK 1.9 software adjusted for sex, age, helminth infections and ancestry markers. Results and Discussion: In our study the SNP rs912278 located on 3' untranslated region in CYSLTR2 was positively associated with atopy and negatively associated with Trichuris trichura infection. Other SNPs were also statistically associated with allergic markers: rs9591194, and rs9595965 were positively associated with levels of IgE; rs7330144, rs34494076, rs9568079 were negatively associated with specific IgE against Dermatophagoides pteronyssinus and; rs7330144, rs34494076 were associated with specific IgE against Periplaneta americana. Conclusion: Polymorphisms in CysLTR2 are linked to atopy and Trichuris trichuria infection in a Brazilian population.

Keywords: Atopy; polymorphism; CysLTR2; IgE; Helminth infection

#84

GENETIC POLYMORPHISM ON TGFB1 IS ASSOCIATED WITH SEVERITY OF ASTHMA

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Support: UFBA and FAPESB

Introduction: Asthma is a complex respiratory disease characterized by intermittent airway obstruction resulting from a combination of environmental and genetic factors. TGF-β1 play important role in the regulation of airway inflammation and remodeling in asthma. Polymorphisms in the TGF-B1 gene have been implicated in susceptibility to asthma, however a large number of studies reported inconclusive results. Aim: To evaluate the association of TGF-B1 gene polymorphisms with asthma in case-control study in Salvador, Bahia, Brazil. Methods: This study included 1,418 patients with asthma (465 mild and 510 severe) and 443 control subjects recruited from ProAR (Program for Asthma and Allergic Rhinitis Control in Bahia). Four SNPs (rs1800469, rs1800469, rs2241712, rs2241715) in TGFB1 were genotyped using TaqMan assay. Pulmonary function and skin test responses to common aeroallergens were assessed. Genotypic associations between these SNPs and asthma were evaluated using logistic regression analysis adjusted for sex, age, skin color. Results and Discussion: No significant difference was observed in genotype between the asthmatics patients and controls. However, the SNP rs2241715 was positively with severe asthma when compared to mild asthma (OR= 1.4; CI 1.01 – 1.84). The SNP rs2241712 was positively associated to A. alternata (OR= 1.80.81; CI 1.02 –3.14) skin reactivity. None of the SNPs evaluated herein were associated with pulmonary function tests. Conclusions: In the present study, our results indicate that the rs2241712 polymorphism it is a risk factor for atopy and the rs2241715 polymorphism might be involved in the modulation of asthma severity.

Keywords: asthma, polymorphisms, TGF-B1.

#85

IDENTIFICATION OF MONOCYTES BIOMARKERS ASSOCIATED WITH SEVERE FORMS OF SCHISTOSOMIASIS

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Support: FAPESB, CAPES

Introduction: Approximately 5 - 10% of individuals with Schistosoma mansoni infection develop the severe forms of the disease, which is characterized by portal hypertension and liver fibrosis, pulmonary hypertension, esophageal varices and a variety of clinical manifestations caused by the host immune response to egg antigens. Experimental studies have pointed to the participation of monocytes in the pathogenesis of granulomas. Objective: Identify markers on monocytes associated with progression to severe forms of schistosomiasis. Methods: The study subjects will be recruited from the endemic area for schistosomiasis named Água Preta, Bahia, Brazil. It will be performed parasitological examination of feces and upper abdominal ultrasonography in all individuals. A total of 20 patients with periportal fibrosis and 20 patients with pulmonary hypertension secondary to schistosomiasis will be enrolled in this study. Peripheral blood mononuclear cells (PBMC) will be obtained and stimulated with the soluble egg antigen (SEA) of S. mansoni. The expression of CD14, CD16, HLA-DR, CD80, CD86, CD11b, TNF, IL-6, TGF-β, IL-10, IL-10R , IL-4R, arginase-1, MMP-2 and MMP-9 on monocytes will be evaluated by flow cytometry. Conclusion: The identification of a phenotypic biomarker expressed by monocytes from patients with different clinical forms of schistosomiasis will help in the understanding of the mechanisms involved in the pathogenesis of this disease, as also may contribute to the development of new strategies for prevention of severe forms, with consequent decrease in morbidity and mortality associated with this disease.

#86

COMBINED 1-DNJ AND IBUPROFEN TREATMENT SIGNIFICANTLY DECREASED MICROGLIAL ACTIVATION AND PHAGOCYTOSIS IN MPTP-TREATED MICE

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Support: Fundación Seneca, CIBERNED (Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas), University of Murcia, CAPES e PPGIM

Introduction: Inflammation is a predominant aspect in neurodegenerative diseases. Moreover Introduction: Inflammation is a predominant aspect of neurodegenerative diseases and many studies performed in experimental Parkinsonian models suggest that sustained neuroinflammation exacerbates degeneration of the dopaminergic nigrostriatal pathway. Moreover, altered astrocyte and microglial functions could contribute to neuronal death in Parkinson's disease (PD) and knowing the inflammatory mechanisms associated to PD may help the development of therapeutic strategies specifically targeting these aspects. Objective: The aim of this study was to investigate the neuroprotective response and compare the potency of ibuprofen alone and in association with the iminosugar 1-Deoxynojirimycin [1-DNJ], a molecule which can regulatematrix metalloproteinases (MMPs) and microglia migration. Material and Methods: Parkinsonism in black (C57BL6) mice induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) was adopted as experimental model. Mice ((4 m.o.) were treated with MPTP (50 or 80 mg/kg i.p.) as acute treatment plus saline, or 1 dose of ibuprofen syrup (40 mg/kg), or 1 dose of 1-DNJ (1 mg/kg), 1 h or 30 min before MPTP administration, respectively, or both of them. Animals were sacrificed 48 or 72 h after the first MPTP administration. Postmortem studies of dopaminergic neurons (TH^+), microglia ($IBA\text{-}1^+$) and ($IBA\text{-}1^+$+$CD68^-$), and stereological analysis were used to investigate neuronal integrity, the activation of microglia, microglia-neuron interactions and phagocytosis of dopaminergic neurons in the *substantia nigra pars compacta* (SNpc). Results and Discussion: The results show significantly increased the number of dopaminergic cells in the SNPc after combined 1-DNJ and ibuprofen treatment. Interestingly, were observed increased of number cells microglial after treated combined after MPTP+1-DNJ+IBU. In addition, after treated, predominate anti-inflammatory macrophages M2 ($IBA\text{-}1^+$+$CD68^-$) over inflammatory macrophages (M1). Moreover, analysis of total of contact between activated microglial and dopaminergic neurons processes and body administration revealed decreased significantly of contacts compared to groups treated with MPTP. Conclusions: Our data suggest that inhibition of MMPs by 1-DNJ combined with ibuprofen treatment could decrease glia activation and microglial pgagocytosis and could be an strategy to ameliorate harmful inflammatory outcomes in Parkinsonism by blocking phagocytic signaling. Then, modulation of MMP´s activity combined with anti-inflammatory drugs could provide promising research ways for therapeutic intervention in PD.

#87

IDENTIFICATION OF BACTERIA WITH IMMUNOREGULATORY ACTIVITY IN SKIN SAMPLES OF INDIVIDUALS FROM SALVADOR- BAHIA

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Support: FAPESB, CNPq

Introduction. Asthma is a chronic inflammatory disease of the airways associated with airway remodeling leading to bronchial hyperactivity and mucus hypersecretion. Different asthma phenotypes predominate in different configurations and are associated with distinct etiologies and causal mechanisms. Based on the hygiene hypothesis, it is assumed that children, who grow up in an environment with a high number of pathogens, protect themselves from allergic sensitization. Thus, to test the hygiene hypothesis given the increase in the prevalence of asthma among atopic children, understanding the relationship between skin microbiote diversity with asthma and atopy. Objective. Evaluate the presence of the bacteria DNA from the species Bacillus pumilus, Leuconostoc pseudomesenteroides; Pantoea agglomerans and Acinetobacter spp; in cultivable human skin microbiota of 50 individuals from Salvador city, Bahia, belonging to a prospective study of respiratory allergies, SCAALA. Methods. Bacterial genomic DNA was extracted from cultures swab samples collected from the right arm skin of 50 children, using a commercial kit. DNA amplifications were done using primers specifics to each specie through polymerase chain reaction (PCR). The PCR product was analyzed in agarose gel 2%. Expected Results. Identification of PCR product in agarose gel from the species: Leuconostoc pseudomesenteroides with 168 pB, Pantoea agqlomerans with 1200 pB and Acinetobacter spp with 240 pb. Find increased prevalence of these immune regulatory bacteria in samples from healthy individuals and in the individuals asthmatic remissive for asthma symptoms.
Acknowledgment: CNPQ Project Universal 14/2014; FAPESB Universal 05/2015 responsible for supporting the project.

Keywords: Bacteria, immunomodulatory potential, allergy.

#88

EVALUATION RECOMBINANT PROTEIN FOR VACCINE DEVELOPMENT AGAINST CASEOUS LYMPHADENITIS

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Support: UNIT-SE, UFBA

Introduction: The caseous lymphadenitis is a common worldwide disease in flocks of sheep and goats caused by gram-positive bacteria called Corynebacteruim pseudotuberculosis. Various prophylactic and diagnostic methods have been studied to improve the control of this disease. The bacterial secreted and membrane proteins represent the major components of the pathogen interaction with the host. Therefore, the identification of novel secreted proteins in the outer membrane of C. pseudotuberculosis as targets to develop effective vaccine and diagnostics are necessary to control the disease. Object: Developing recombinant proteins possible to formulate an efficacious vaccine for prevention of lymphadenitis caseous. Metodology: Were asked the cultivation of bacteria and E.coli BL21 C.pseudotuberculosis 1002, cloning and expression of recombinant proteins and expression evaluation used the Western blot test and solubility test. In the characterization of Dot Blot antigenicity of the recombinant proteins. Results and discussion: These results show for the first time the expression of CPR 7041 and rCP5582 of C.pseudotuberculosis in E. coli, this system is widely used in expression of heterologous proteins because it allows to obtain a high yield, due to the fast growth rate of E. coli as well as being a simple and inexpensive technique, a key factor in industrial terms (Porowinska et al. 2013). C.pseudotuberculosis recombinant protein were successfully expressed in E. coli, such as PLD exotoxin (Menizies et al. 1994) and CP40 protein (Droppa-Almeida 2013). The CP40 was also expressed in this work to be tested with recombinant antigen for vaccine production. Conclusions: The importance of recombinant proteins for the development of vaccines against caseous lymphadenitis as imunoprofilático method can develop a large role in the pathogenesis and immuno resulting in viable strategies to control the disease

#89

STUDY OF EXPRESSION OF FACTORS PROMOTERS RESUSCITATION AND IMMUNE RESPONSE IN SHEEP INFECTED CORYNEBACTERIUM PSEUDOTUBERCULOSIS

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Support: UFBA

Introduction: Corynebacterium pseudotubersulosis is a gram-positive bacterium that causes caseous lymphadenitis, chronic disease that affects sheep and goats, which causes great economic losses in the semi-arid northeast as well as in the world. Studies have shown that this bacterium can enter dormancy state after the initial stage of the disease and spend a long time in this condition hindering the action of the immune system and in many cases, it can be reactivated from the cell wall of dormancy breaking causing the disease from a certain point. From studies in Mycobacterium tuberculosis was identified group of genes related to the breaking of dormancy called Factors Resuscitation Promoters (Rpf), a group of proteins present in the cell wall of bacteria Actinomycetales group (Corynebacterium, Mycobacterium, Nocardia, Rhodococcus). The Rpf may be an effective alternative to new antigenic targets to activate the immune response against this bacteria efficiently and controlling the disease. Objective: Study and assess the expression of resuscitation promoting factor in different experimental conditions as well as immune response to these molecules in sheep experimentally infected. Methodology: The study of the expression of the Rpf will start with the making of the design of primers for real-time PCR and rpf expression vector for further evaluation of these factors from the bacterial strains C57 and T1 of C. pseudotuberculosis and immunoassays for evaluation immune response of sheep infected with the T1 line 180 days. Results and Discussion: It was made the design of the rpf primers and expression vector. It is expected that similar results to Mycobacterium tuberculosis are encountered regarding gene expression and elements of the immune response. Conclusion: The understanding of the expression of the Rpf could be an important step to creating new tools, such vaccine for the control of caseous lymphadenitis.

#90

NOTCH SIGNING AND INFLAMMATORY RESPONSE IN PATIENTS INFECTED BY HTLV-1

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Support: CAPES, NIH e SIM.

Introduction: The human T-lymphotropic virus type 1 (HTLV-1) is a retrovirus that infects about 10-20 million people worldwide. The most infected individuals remains asymptomatic (AS) and about 3% develop a chronic neuroinflammatory disease called HTLV-I-Associated Myelopathy/Tropical Spastic Paraparesis (HAM/TSP). The Notch pathway can act as a regulator of proliferation and cell survival through the Notch intracellular domain (NICD), which is released after intracellular cleavage carried out by γ-secretase protein. Believed to deregulate activation of Notch signaling can result in excessive lymphoproliferation in HIV patients. Research with blockers Notch pathway (anti-Notch 1 and anti-Notch-3 blocking the Notch receptor; DAPT and JLK6 blocking γ-secretase) may help to understand the participation of the Notch pathway in the immune response to HTLV-1. Objective: Evaluate the role of Notch signaling in the inflammatory response generated by HTLV-1. Material and Methods: For analysis by ELISA of IL-1β of IL-6, TNF, IFN-γ, CXCL-9 and CXCL-10 levels, peripheral blood mononuclear cells (PBMC) from patients with HAM / TSP and AS were cultured for 72h in the presence or absence of DAPT, JLK6, anti-Notch1 and anti-Notch3. It is intended for use in future experiments the culture supernatant to determine the source of production of IL-1β, IL-6, TNF, IFN-γ, CXCL-9 and CXCL-10 by flow cytometry. The proviral load will be analyzed by RT-PCR and the expression of Notch receptors and their ligands will be obtained by microarray. Results and Discussion: By using JLK-6 decreased significantly of TNF, CXCL9 and CXCL10 levels in AS PBMC cultures when compared to the medium. By using the DAPT decreased significantly of IFN-γ levels in AS PBMC cultures when compared to the medium. It was not possible to observe statistically significant differences in other conditions tested using the JLK-6 and DAPT. It was not possible to observe statistically significant differences in other conditions tested using the JLK-6 and DAPT. There was no significant difference when using anti-Notch 1 and anti-Notch 3 compared to middle. Conclusions: The γ-secretase blockers act on reducing TNF, CXCL9 and CXCL10 (JLK6); and IFN-γ (DAPT) PA, suggesting that the Notch pathway might be participating in the inflammatory response in the HTLV-1 carriers.
Acknowledgment: CAPES, PPGIM, SIM and NIH.

Keywords: HTLV-1, HAM / TSP, Notch, γ-secretase, anti-Notch1, anti-Notch3, γ-secretase.

#91

ASSOCIATION BETWEEN THE GENE GSTP1 AND CHILDHOOD ASTHMA IN SALVADOR CITY - BRAZIL.

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Introduction: Asthma is a chronic inflammatory disease of relevance to global public health, which is influenced by environmental and genetic factors of individuals. One of the explanatory pathways of causation of asthma is intrinsically related to oxidative stress. The enzyme GSTP1 is part of a family of proteins with antioxidant functions associated with asthma and asthma severity, as one of key genes in this biological route. In some publications the GSTP1 gene was associated with asthma severity, due to its high levels of malondialdehyde, an important biomarker used in the evaluation of oxidative stress, and low levels of glutathione, a protein that protects cells against free radicals and improves the immune system individual. The interest in exploring these pathway is due to the findings of a key Genome-Wide association study (GWAS) for asthma in a Brazilian population, where genes linked to oxidative stress were more associated with childhood asthma. GSTP1 is an enzyme leading to the severity of asthma, whether atopic or not atopic. OBJECTIVE: Therefore, the main objective of this project is to study the association of GSTP1 gene, belonging to biological route of oxidative stress, asthma and allergies in a population of children in the city of Salvador. METHODS: This is a cross-sectional study, whose population is highly mixed and coming from a SCAALA (Social change, asthma and allergy in Latin America) cohort. Data collection will be based on information from a questionnaire of the International Asthma and Allergies in Childhood Study (ISAAC, Phase II) adapted and validated for the Portuguese language. From this questionnaire will be identified individuals with asthma and atopy, defined by serology of specific IgE. Statistical analyses such as allelic frequency, linkage disequilibrium and association tests, will be held at Plink software. The tests will be made in different models of genetic heritability (additive, dominant and recessive).

Keywords: Childhood asthma; Allergy; Oxidative stress; Gene Candidate; GSTP1.

#92

SERUM AND TUMOR IMMUNOSUPPRESSIVE CYTOKINES LEVELS IN PATIENTS WITH ORAL SQUAMOUS CELL CARCINOMA

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Support: FAPESB, CNPq and Programa Permanecer MEC/Sesu

Introduction: Oral squamous cell carcinoma (OSCC) is the most common head and neck malignant neoplasm, with high aggressiveness, recurrence and metastasis rates. The main chemical mediators that enable communication between immune and tumor cells are the cytokines and its signals are required for tumor growth and spreading. Particularly, the immunosuppressive cytokines IL-10 and TGF-beta seems to influence tumor microenvironment in favor of tumor progression, however these molecules present controversial data in OSCC. Objectives: This study aimed to compare pre-operative serum and tumoral tissue levels of the immunosuppressive cytokines IL-10 and TGF-β1 in patients with OSCC. In addition, the relationship with clinicopathological parameters was investigated. Methods and Results: The levels of cytokines were measured by flow cytometry using serum samples collected from 46 OSCC patients (36 males and 10 females; aged between 38-85 years) and 46 controls. No difference was observed in relation to serum levels of IL-10 and TGF-β1 in OSCC patients compared to controls. Similarly, no association was observed in relation to clinicopathological parameters. Tumoral IL-10 and TGF-β1 expression was examined in paraffin-embedded corresponding OSCC samples by using immunohistochemistry. The sections were evaluated using a semi-quantitative method in combination with staining intensity score. Our preliminary findings showed that the expression of IL-10 and TGF-β1 are present both in neoplastic and stromal cells of OSCC. With respect to different regions of the same tumor, IL-10 and TGF-β1 expression was quite heterogeneous. Adjacent non-neoplastic epithelium exhibited low or absent expression. Conclusion: The serum immunosuppressive cytokines profile of patients with OSCC is not predominantly elevated. The results of this study will clarify how peripheral and tissue immunosuppressive cytokines IL-10 and TGF-beta contribute to OSCC progression besides evidence differences in relation to serum and tumor expression.

Keywords: oral squamous cell carcinoma, IL-10 and TGF-β1

#93

SCREENING OF GENETIC POLYMORPHISMS ASSOCIATED WITH ASTHMA IN PATIENTS FROM THE PROGRAM FOR ASTHMA CONTROL IN BAHIA (PROAR)

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Support: FAPESB and CNPq.

Introduction: Asthma is a disorder of the lower airways, characterized by inflammation with reversible obstruction of airflow, hyperresponsiveness and bronchial remodeling and eosinophilic inflammation, affecting 300 Million people worldwide. It is a disease determined by genetic and environmental factors and considered as complex and heterogeneous aetiology. In this sense, studies that assess the genetic influence on different phenotypes of asthma have increased during the last decade. Genome-wide association study (GWAS) and candidate gene studies have shown that the presence of certain phenotypes can be inherited, and about 200 genes have been associated with asthma or related phenotypes. Some studies have linked the FCεRI, STAT6, and ORMDL3 gene with a possible modulation of plasma levels of IgE. Objectives: In this study, we will conduct a genomic screening enriched for immune routes using a panel of 700 SNPs and verify the association of such variants with severe asthma and others phenotypes, in a case-control study from Salvador, Bahia, Brazil. Material and Methods: The cases (500 patients with severe asthma), will be recruited from of the Program for the Control of Asthma and Allergic Rhinitis in Bahia (ProAR). The controls will be 500 patients with intermittent or mild persistent asthma recruited mainly from ProAR clinics. These patients will have a diagnosis of asthma confirmed according to the classification of the Global Initiative against Asthma (GINA, 2006) by audit performed by two experts. A second control group will be also studied including 500 individuals with no history of asthma. Initially, DNA extraction will be held, then a custom genotyping SNPs panel 700 using the TaqMan probe technology-based 5 'nuclease assays in Open Array block from Applied Biosystem. The SNPs panel will be designed from previous GWAS for asthma in European, African or American populations, including an estimated total of 192 ancestry markers and 508 markers from candidate

genes for asthma. The techniques to be used for statistical analysis range from exploratory tools to the regression models. Genetic associations will be held in the PLINK program and graphics will be produced in STATA 8.2. Expected results: We will be able to replicate previous results in a completely different population of all studied performed to date and identify possible genetic routes related to severe asthma in this population. The understanding of how genetic issues affect immune routes and are associated with the occurrence of asthma in our population can contribute to understanding of biological mechanisms and to support the development of interventions for this disease.

Keywords: Asthma. Genetics. Immunogenetics. Polymorphisms.

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