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PROGRAMA DE PÓS-GRADUAÇÃO EM IMUNOLOGIA

PPGIm
Programa de Pós-graduação em Imunologia

ANAIS DA XIX EXPOPPGIM 2019
30 anos do PPGIm

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RESUMOS

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Apresentação

O Programa de Pós-graduação em Imunologia (PPGIm) há trinta 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 o conhecimento científico em Imunologia e áreas correlatas, gerado localmente na Bahia. Nesta XIX Edição da ExpoPPGIm, o evento deste ano celebrou um marco importante na história do PPGIm: os 30 anos da criação do programa. O evento, além de ser comemorativo, permitiu a continuidade da interação entre pesquisadores locais de renome nacional e internacional, e suas respectivas colaborações e parcerias, favorecendo não só o intercâmbio científico e tecnológico, mas também a divulgação, dentro de nosso instituto, de tais frutíferas pesquisas científicas. Tivemos duas sessões de pôsteres, comunicações orais dos melhores resumos, incluindo a 4ª. versão do Prêmio Lain Carlos Pontes de Carvalho.

Neste documento, sumarizamos a produção científica gerada para XIX ExpoPPGIm que teve como tema em 2019 "*Imunoterapia e Imunomodulação*". Assim, pretendemos disseminar a Imunologia, divulgar as ideias e projetos em curso desenvolvidos por docentes e discentes de nosso Programa, bem como, ampliar novas perspectivas, resgatando nossa história e instigando nossa evolução.

Saudações acadêmicas,

Camila A Figueiredo
Coordenadora do PPGIm/ICS/UFBA

LINHA 1: Imunologia Aplicada

EVALUATION OF SPECIFIC IMMUNE RESPONSES USING ADJUVANT α-TOCOPHEROL IN ACUTE AND CHRONIC PHASES OF Trypanosoma cruzi INFECTION

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Introduction: Chagas disease is an inflammatory disorder arising from the etiological agent responsible, *Trypanosoma cruzi*. Immune response involvement has been demonstrated in the development and maintenance of clinical forms of *T.cruzi* infection. The development of a more effective response is observed in the acute phase of infection, while a more regulatory response is found in the chronic phase. To mobilize cellular populations, a reagent with potential as an adjuvant treatment was investigated. Alpha-tocopherol (vitamin E) has been described as a potent antioxidant that may be involved in the enhancement of effector cell response seen in other experimental models. Here, we aimed to investigate the pro-inflammatory or specific immune responses resulting from treatment with α-tocopherol in *T.cruzi*-infected mice, which may aid in the elimination of parasites in the acute phase and/or the decrease inflammatory responses seen in chronic stages. **Methods and Results:** C57BL/6 females were divided into the following groups: I-controls, II-treated with Alpha-tocopherol (Alpha), III-treated with α-tocopherol + *T.cruzi* antigens (Alpha +*Tc*Ags), III-treated with Alumen + *Tc*Ags (Al+*Tc* Ags), IV-treated only with *Tc*Ags. Prior to infection with *T.cruzi*, mice were immunized during a 4-week period (once a week). One week after the final immunization, half of the animals in each immunized group were infected with 1000 trypomastigote forms of the Tulahuen strain of *T. cruzi*. Spleen cells from the experimental groups were evaluated by flow cytometry (FACS) 60 days later to identify IL-10 or IFN-γ production by CD4, CD8, CD25, FoxP3, Lap, Gr1 and CD11b cells. Parasitemia and mortality were evaluated throughout the study. Decreased parasitemia was observed in the Alpha and Alpha + *Tc*Ags groups compared to infected non-immunized animals. In addition, these two groups exhibited increased survival in the kinetics analysis, indicating that immunization may lead to increased resistance. We previously demonstrated that infected and immunized groups presented higher numbers of NK, NKT, TCD4 and TCD8 splenic cells than infected non-immunized mice. Higher numbers of INF-γ-producing NK, NKT and TCD8 cells were also found in comparison to infected non-immunized animals. We also show herein that CD8 T cells modulate the production of IL 10 in animals immunized with α+*Tc*Ags compared to the Alpha-only group. **Conclusions:** Effector immunological response was maintained, as evidenced by higher INF-γ production, in the acute phase of infection. The increased production of this cytokine may be involved in the observed reduction of parasitaemia load, as well as linked to greater survival in C57Bl/6 mice immunized with Alpha+*Tc*Ags or with Alpha alone. Finally, proinflammatory response was found to correlate with a modulated regulatory response in mice immunized with **(**Alpha**)** or Alpha**+***Tc*ags during infection.

Keywords: *Trypanosoma cruzi,* alpha-tocopherol, acute, chronic

Support: PAPES-CNPq, FAPESB

ACTIVATION OF TRYPTOPHAN PATHWAY IN GLIAL CELL CULTURES BY GLIOSIS-ASSOCIATED *Neospora caninum* INFECTION

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Introduction: *Neospora caninum*, an Apicomplexa parasite infects many animals species having a tropism for central nervous system where glial cells are responsible for homeostasis. Infection of neurons/glial cells co-cultures by N. caninum tachyzoites activates indoleamine 2,3-dioxygenase (IDO), the first enzyme of tryptophan pathway (TP) with production of kynurenines and preservation of neurons integrity. Catabolites of TP can induce the expression of cytokines, chemokines and their receptors in astrocytes. The present study verified the role of TP in glial cells infected by tachyzoites of *N. caninum* and its relation with astrocyte reactivity and inflammatory response **Methods and Results:** Primary cultures of rat cerebral cortex (P0-2) was infected or not with *N. caninum* (1:1 cell: parasite) during 24h and after, the culture medium was removed, filtered (Millipore 0.22-µm) and used to evaluate tryptophan catabolites by HPLC. Astrocyte and microglia morphology and reactivity was investigated by immunocytochemistry (ICQ) for glial fibrillary acidic protein (GFAP) and for the ionized calcium binding adaptor molecule 1 (Iba-1), respectively. The expression of cytokines, chemokines and of TP enzymes were investigated by qPCR. Infected cultures showed gliosis, with changes on cell morphology and expression of GFAP. Moreover, in infected cultures the expression of mRNA for IL-10, TNF, CCL5, CCL2 and kinurenin monoxidase (KMO) were increased, compared with control uninfected cultures. The conditioned medium by infection presented high concentration of quinolinic acid (QUIN). Although the production of QUIN is associated to activation of KMO in microglia, this catabolite, an agonist of NMDA receptors is also responsible by an increased expression of chemokine receptors in astrocytes and by modulation of the inflammatory response. **Conclusion:** The infection of glial cells by *N. caninum* induces the activation of KMO to produce QUIN and activates astrocytes and microglia which could contribute to parasite survival and consequent neuropreservation.

Keywords: Glia, *Neospora caninum*, quinolinic acid, neuroprotection, tryptophan pathway

Support: CNPq/INNT, FAPESB

EVALUATION OF THE IMMUNOMODULATORY ACTIVITY OF HOUSE DUST MITE HYPOALLERGENIC HYBRID PROTEINS UPON MICE' SPLENOCYTES STIMULATED *IN VITRO*

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Introduction: *Blomia tropicalis* and *Dermatophagoides pteronyssinus* are important house dust mites (HDM), whose allergens are well known as triggers of atopic sensitization and important risk factors for allergic illnesses. An alternative therapeutic approach to current drug usage for these allergic diseases is allergen-specific immunotherapy (AIT). Among AIT technologies, hypoallergenic recombinant proteins have arisen as promising vaccines for displaying reduced IgE-binding activity and similar T-cell priming features, when compared to their parental allergens. Our group have previously showed that two hybrid proteins (BTH2 and QBD1) displayed hypoallergenic features, when tested using sera from HDM-sensitized patients. Thus, we aimed at continuing the characterization of these two potential hypoallergens by *in vivo* immunization of mice and stimulation of their splenocytes. **Methods and Results:** Eight-week-old BALB/c mice were immunized with 5 µg of protein adsorbed to Alum (4mg/mL) in 2 injections of 50 µL administered subcutaneously into the backs of the animals and boosted on days 7, 14, 21, 35, and 42. At day 49, animals were killed, spleens were homogenized, and after erythrocyte lysis, cells were counted. Splenocytes (2 x 105 cells/well) were restimulated with either 40 µg/mL hypoallergenic hybrid proteins (BTH2 and QBD1) or parental

allergen (rBlo t 5 and rBlo t 21 for BTH2; rBlo t 1 and rDer p 1 for QBD1), respectively. Cultures were performed for 24h or 48h and splenocyte supernatants were analysed by capture ELISA for IL-4, IL-5, IFN-γ and IL-10 detection. Splenocytes of immunized mice were stimulated by the addition of either hypoallergens or pokeweed. Restimulation with BTH2 and QBD1 could induce significant production of IL-10, in similar levels to the parental allergens. However, IL-5 was significantly reduced by both hypoallergens. While no significant differences were found in IL-4 levels upon BTH2 and parental allergens stimuli when compared to unstimulated cells, this hypoallergen reduced significantly the production of this cytokine in comparison with Blot 5-restimulated cells. Differently, QBD1 only induced high levels of this cytokine, resembling the levels of Blo t 1-stimulated splenocytes. There were no statistic differences on IFN-γ production after restimulation of splenocytes with proteins, but only with mitogen used as assay control. **Conclusion:** The cytokine profiles induced by BTH2 and QBD1 indicate that the epitope recombination in both hybrids partially preserved the T-cell epitope repertoire of the parental allergens. Therefore, they should allow the induction of specific T-cell tolerance in future therapeutic setups. Nevertheless, further pre-clinical *in vivo* evaluations are still needed to confirm these two hypoallergenic hybrids as allergy vaccines.

Keywords: allergen immunotherapy, *Blomia tropicalis, Dermatophagoides pteronyssinus,* hypoallergen, cytokines

Support: CAPES, Grant nº. 88887.357066/2019-00

EXTRACTION AND CHARACTERIZATION OF POLYMERIC EXTRACELULAR MATRIX OF *CORYNEBACTERIUM PSEUDOTUBERCULOSIS*.

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Introduction: The *Corynebacterium pseudotuberculosis (Cp)* is the etiological agent of caseous lymphadenitis, a chronic disease that affects particularly ovine and caprine animals. The bacteria has many mechanisms of virulence, among them the biofilm formation that protects the bacteria from mechanisms of immunological evasion, increasing the persistence of the infection. During the infection there is the formation of granulomatous lesions in superficial and internal lymph nodes, also called visceral. We aimed to investigate the reactivity of proteins in the polymeric extracellular matrix of *Corynebacterium pseudotuberculosis*, specific strain 76 cap J4 of biofilm. **Methods and Results:** A cell culture of the etiological agent of *C. pseudotuberculosis* in agar BHI (*Brain Heart Infusion*) + glucose 1% (BHIG) were used in this experiment and incubated at 37 oC for 48 hours. After the growth of an isolated colony, isolated and inoculated in 5 ml de Broth BHI for 48 hours with Stirring. The dilution of 1:1000 in 10 mL of BHIG in 50ml falcon tube was tested and incubated until it reached the D.O 0,2 (the samples were tested and duplicated and without agitatiton). After it reached the D.O 0,2 it was incubated at 37 oC for 48 hours without agitation. After that, it was centrifuged at 6.000 RPM for 15 minutes at 25 oC, the supernatant was discarded. In order to extract the MEP, the *pellet* was resuspended with 1 mL of NaCl 1,0 M and then centrifuged at 5000 RPM for 10 minutes at 25 oC . Finally, the supernatant was collected for the quantification of proteins through the method of lowry. Different concentrations were used to sensitize polystyrene plates and determine the best concentration of antigen: 50, 100 e 500 ng by well. To evaluate the reactivity of these proteins, it was used serum from infected and uninfected goats Cp. A protein concentration of 1,29 mg/mL in the MEP extracted from 1,0 M of NaCl. The antigen concentration that showed the best discrimination test result in the indirect ELISA, between samples from negative animals experimentally infected with *Corynebacterium pseudotuberculosis* cepa 76 cap J4 biofilm producer was 500 ng per well. **Conclusion:** The results demonstrated the viability of the extraction of MEP from Cp using protocols previously described for other gram-positive bacteria. In addition, the antigenicity of this fraction against serum from experimentally infected animals were characterized. This study is in the execution phase and new tests will be performed to better characterize the Cp MEP as well as its antigenicity.

Keywords: Biofilme, Polymeric Extracelular Matrices (ECMs), *Corynebacterium pseudotuberculosis*

Support: Fapex, Labimuno

NEUROIMMUNOMODULATION: INFLUENCE OF NEURAL INVOLVEMENT ON DIFFERENT ASTHMA PHENOTYPES

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Introduction: Asthma is a disease characterized by chronic lower airway inflammation and hyperresponsiveness. This process is regulated by complex and diverse mechanisms, with variable disease expression, leading to the emergence of distinct phenotypes. Neural involvement seems to play an important role on asthma. For instance, the autonomic nervous system is linked to the inflammation and hypersensitivity reactions in the nose, skin and lung. The release of neuropeptides that occurs in most tissues, leads to vasodilation, leukocyte adhesion and plasma extravasation. This directly affects the function of various cell types that are associated with the immune system. In this study, we propose to evaluate the presence of some of these neural markers, as well as their association with different immunological phenotypes in asthmatic and non-asthmatic individuals. **Methods and Results:** This is a cross-sectional study of asthma conducted in the city of Salvador, Bahia. The study population consisted of children, adolescents and young adults (aged 12-23 years). During this first part of the study, recruitment and sample collection were successfully concluded. In this way, samples from asthmatic and non-asthmatic patients were collected, totaling 195 subjects in the case group and 50 subjects in the control group. To assess pulmonary function, patients underwent to spirometry. Skin test (SPT) was performed to identify specific allergens. Blood samples were collected and stored for IgE levels. Sputum and nasal wash samples were used to prepare slides (cytospin) for differential identification of leukocytes. The supernatants from such samples were stored for measurement of neural and immunological markers. The following analytes will be evaluated in the present study: IL-4, IL-5, IL-8, IL-10, IL-13, IL-17A and markers of neural involvement like brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF). Data analysis will be performed descriptively, involving comparisons between the asthmatic and non-asthmatic groups. These analyzes will include information on demographic factors, lung function, disease severity, age at onset, persistence, inflammatory markers, neural mechanisms, and atopy. All information obtained (clinical, demographic, pathological) will be used to define new asthma phenotypes with the application of latent class analysis (ACL). Cytokine production and other inflammatory markers will be compared using standard method such as comparing geometric means (t-test). Subsequently, the results of sputum and nasal lavage will be compared through correlation calculations in relation to the results obtained by each method. **Conclusion:** We hope to find relationships that may clarify the existence of some phenotypes through the interaction of the nervous system with the immune system in asthma.

Keywords: asthma, phenotypes, neural involvement

Support: European Research Council — SEP-210203554

ANTI-TUMOR EFFECTS OF COMBINED ADJUVANT THERAPY INVOLVING BCG AND IMIQUIMOD IN EXPERIMENTAL MELANOMA

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Introduction: The study of adjuvant therapies has become increasingly relevant in efforts to treat cutaneous tumors like melanoma effectively. Imiquimod (IMIQ) induces an efficient antitumor immune response by activating the Toll-like receptor seven pathway (TLR7). Another immunotherapy may use *Bacillus Calmette-Guérin* (BCG), which prove useful in mounting an effective antitumor response. The present study aims to use new tools to evaluate the combined adjuvant effect of BCG and IMIQ on antitumor activity by evaluating survival and tumor growth. **Methods and Results**: The present experimental melanoma model involved C57Bl6 mice in which B16F0 tumor cells were inoculated in the pinna of the mouse ear at a concentration of $5x10^4$ cells/20µL. Mice were then treated with 10µL of IMIQ (50mg/mL) twice daily after tumor inoculation. BCG was injected *in situ* ten days after tumor inoculation at a concentration 1.2x107/60µL. Tumor growth and mortality were monitored daily. The following experimental groups were used: mice injected with tumor cells (B16F0), and challenged with BCG vaccine (B16F0+BCG), treated topically with IMIQ (B16F0+IMIQ), or challenged with BCG and treated with IMIQ (B16F0+IMIQ+BCG). Treatment with BCG was observed to promote tumor restrainment in the B16F0+BCG group, on the 18th day ($p= 0.0298$; Mann-Whitney test) and this group also survive longer ($p<0.0001$; Log-Rank test) than the B16F0 group. The treatment with IMIQ also increased survival in the B16F0+IMIQ group ($p= 0.0406$; Log-rank test), and about tumor restrainment, B16F0+IMIQ presented significant differences in tumor growth on days 17($p= 0.0456$; Mann-Whitney test) and 20($p= 0.0076$; Mann-Whitney test) compared to B16F0 mice. The combined treatment protocol B16F0+IMIQ+BCG resulted in significantly increased survival compared to B16F0+IMIQ($p= 0.0415$; Log-rank test) and B16F0 mice($p=0.0001$; Log-rank test).In addition, significantly reduced tumor growth was seen in B16F0+IMIQ+BCG compared to B16F0 on days 10($p=0.0115$; Mann-Whitney test), 12($p=0.0008$; Mann-Whitney test), 15($p=0.0434$ Mann-Whitney test), 18($p=0.0219$; Mann-Whitney test) and 20($p=0.0406$; Mann-Whitney test). **Conclusion**: The combined adjuvant treatment (IMIQ+BCG) was found to promote survival and tumor restrainment in an experimental murine model of B16F0 melanoma. Experiments to investigate the mechanism underlying increased survival by evaluating effector cell functions against melanoma are currently underway.

Keywords: Melanoma, adjuvant therapies, BCG, Imiquimod

Support: PAPES-CNPq, CAPES

SICKLE CELL DISEASE PATIENTS WITH OSTEONECROSIS DISPLAY DIFFERENT PBMC T CD4+ ACTIVATION PROFILE *IN VITRO*

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Introduction: Osteonecrosis(ON) is a chronic severe complication in sickle cell disease(SCD) resultant of microcirculatory obstruction by sickled erythrocytes leading to bone infarction. Mesenchymal Stromal Cells (MSC) therapy has been the best alternative in treating initial stage ON due to their immunomodulatory properties, through secreted cytokines and growth factors. Cytokine and T helper cell (TCD4+) profile from SCD/ON peripheral blood (PB) remains a challenge. Here we assessed PB mononuclear cell (PBMC) TCD4+ phenotype from SCD, SCD/ON and non SCD (nSCD) patients and whether BM derived MSCs from SCD/ON and nSCD/ON patients suppresses TCD4+ proliferation and their supernatant cytokine profile in co-culture. **Methods and Results:** TCD4+ were characterized using BDTritest CD3/CD4/CD8 and

Multitest CD3/CD4/CD45RA/CD45RO. Intracellular cytokine profile (BDTh1/Th2/Th17 intracellular phenotyping kit) showed no significant difference in IFN-γ, IL-4 and IL-17 expression in not-activated nSCD and SCD/ON TCD4+. However when activated, comparison between nSCD and SCD/ON TCD4+ exhibited high expression of all three cytokines with significance values of p<0,002; p<0,02 and p<0,006, respectively. Activated SCD compared to SCD/ON displayed statistical significance in IFN-γ(p<0,05) and IL-4/IL-17 ratio(p<0,03). **Conclusion:** Results show an acute expression of pro-inflammatory cytokine and a strong polarized behavior of TCD4+ in PB of SCD patients with osteonecrosis, supression of both nSCD and SCD/ON TCD4+ in co-culture with MSC and differencial expression of pro-inflammatory cytokine in co-culture supernatant. Registration number 11738, SIPAR/MS: 25000.039812/2005–99

Keywords: T helper, mesenchymal stem cell, osteonecrosis

Support: FAPESB, CAPES, CNPQ

ZIKA VIRUS SINGULAR PEPTIDE PRODUCTION FOR THE DEVELOPMENT OF SEROLOGICAL IMMUNOASSAYS AND EVALUATION OF IMMUNE RESPONSE

RAFAEL RIBEIRO MOTA SOUZA[1], ISABELA BRANDÃO PEIXOTO[1], REJANE HUGHES CARVALHO[1,2], GUBIO SOARES CAMPOS[1], ROBERTO JOSÉ MEYER NASCIMENTO[3], SILVIA INES SARDI[1]

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Introduction: The rapid spread of Zika virus (ZIKV) in endemic regions where other Flaviviruses have already been established, such as Dengue Virus (DENV), has led to difficulties in accurately diagnosing the infectious agent. Both Flaviviruses present very similar acute symptomatic conditions and high similarity among their viral proteins, what leads to serology cross-reactions between Zika and Dengue, producing false positive results. In addition, studies have demonstrated that antibody-dependent enhancement also increase viremia due to the cross-reaction between the tests. Based on the demand for more specific antigens, the present project aims to produce non cross-reactive ZIKV antigens for immunodiagnostic and immunomodulation in vivo. **Methods and Results**: First, by bioinformatics analysis we identified 3 unique and immunogenic epitopes derived from ZIKV proteins; one from the E protein and two from the NS1. The coding region for the E epitope was used to construct an expression vector containing four in tandem uninterruptedly repetitions of the sequence. The other expression vector was constructed using the two sequences from the NS1 protein. Those two regions were placed in a 3 and 2 intercalated repetition manner. The vectors were cloned in bacterial model for expression of the two antigens presenting the ZIKV-specific epitopes. **Conclusion**: Future experiments include validation of expressed proteins, the evaluation of sensitivity and specificity of the antigens using Dengue and Zika IgG+ sera by ELISA and Western Blot, and the evaluation of humoral immune response in BALB / c mice.

Keywords: Zika virus, Singular, Antigens, Immunodiagnostic, Immunomodulation

Support: CAPES, FINEP

PRETREATMENT LEVEL OF HEMOGLOBIN, NEUTROPHIL-TO-LYMPHOCYTE RATIO AND PLATELET-TO-LYMPHOCYTE RATIO AS PROGNOSTIC INDICATORS FOR SURVIVAL IN A LARGE COHORT STUDY OF ORAL SQUAMOUS CELL CARCINOMA

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Introduction: Oral Squamous Cell Carcinoma (OSCC) represents the major tumor of the oral cavity and presents high aggressiveness and metastasis. Particulary in Brazil, OSCC is the fifth most common cancer in males. The use of conventional pretherapeutic assessment of cells and serum markers as adjunvants to improve the clinical managemment of solid tumors has received increasing attention due to its potential to easily provide prognostic information, acting as low-cost biomarkers. Therefore, neutrophil-to-lymphocyte (NLR), lymphocyte-to-monocyte (LMR) and platelet-to-lymphocyte (PLR) ratios as well as serum hemoglobin concentration have been suggested as a new tool in laboratorial investigations in the context of cancer. Thus, this study aimed to investigate the prognostic value of preoperative laboratorial conventional assessment in a large cohort study of OSCC in a Brazilian population. **Methods and Results:** A total of 433 OSCC patients who were treated between 2008 and 2017 at Department of Head and Neck Surgery of Aristides Maltez Hospital (HAM, Salvador, Bahia) were enrolled and evaluated retrospectively. The data were collected from medical and laboratorial records. Overall survival (OS) and progression-free Survival (PFS) were analyzed using the Kaplan-Meier method. Prognostic factors were evaluated using univariate and multivariate Cox regression models. Males represented 68.8% of the sample. In relation to other relevant demographic and clinical informations, 94.5% of patients were black or brown; 68.8% were under 65 years of age and 76.2% were smokers and alcoholics. The most common tumor site was tongue (40.4%), followed by the palate (24%). Most patients were at IVA (35.9%) and II (19.6) TNM stage at diagnosis, respectively. High NLR (≥2.1), LMR (> 4.2) and PLR (≥ 125) were significantly associated with poor OS and CSS ($p < 0.05$). Low hemoblobin concentration (<13.2) was significantly associated with OS and CSS. In the multivariate analysis after adjusting for confounding factors, the group of patients with a high NLR (≥2.1) in comparison with those with a low NLR presented a hazard ratio (HR) of 1.6 (95% CI, 1.19 — 2.15; $p = 0.00$) for OS and 1.3 (95% CI, 0.85-2.0; $p = 0.23$) for PFS. The group of patients with a high LMR (≥ 4.2) in comparison with those with a low LMR showed a HR of 1.4 (95% CI, 1.06 — 1.90; $p = 0.02$) for OS and 1.0 (95% CI, 0.68 — 1.58; $p = 0.84$) for PFS. Additionally, the group of patients with a high RPL (≥ 125) in comparison with those with a low RPL showed a HR of 1.4 (95% CI, 0.94 — 1.95; $p = 0.11$) for OS and 1.4 (95% CI, 0.77 — 2.35; $p = 0.29$) for PFS. The reducted hemoglobin group (<13.2) compared to the normal group presented HR of 1.56 (95% CI, 1.16 — 2.12; $p = 0.00$) for OS and 1.45 (95% CI, 0.89 — 2.37; $p = 0.13$) for PFS. **Conclusion:** Higher pretreatment NLR and LMR as well as lower hemoglobin were demonstrated to be associated with poor clinical outcome and might be prognostic markers for patient stratification in OSCC management.

Keywords: oral squamous cell carcinoma, neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio, lymphocyte-to-monocyte ratio, hemoglobin, South America

Support: FAPESB, CNPq and CAPES

EFFECTS OF SUPPLEMENTATION COPPER IN BROILER CHICKENS DIETS ON MITOCHONDRIAL METABOLISM

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Introduction: Trace elements play a key role as cofactors or structural components of various enzymes. Copper is essential to metabolism as it participates in various biological processes. When used in the supplementation of broiler chickens diet it acts as a growth promoter improving performance. This mineral participates in the enzymatic system of Cytochrome C oxidase, an important enzyme in the oxidative phosphorylation process. In addition, it is a cofactor for several essential metalloenzymes, including copper-zinc superoxide dismutase (CuZn-SOD), and acts on the antioxidant system by combating excess reactive oxygen species that cause oxidative stress. Mitochondria, due to their role in oxidation metabolism, are particularly susceptible to this damage. A change in mitochondrial functioning can cause bioenergetics failure and impair animal performance. Therefore, the aim of this study was to evaluate the effects of copper supplementation on broiler diets and its effects on oxygen consumption in liver mitochondria. **Methods and Results:** 270 day-old Cobb-500 chicks were used. A randomized design with three treatments (T1: CuSO4.5H2O — 10mg / kg ration); (T2: Cu (HMTBa) 2 50mg / kg) and (T3: CuSO4 100 mg / kg) and six replicates was adopted. Oxygen consumption was measured polarographically using a Clark-type oxygen electrode at 37°C. Were determined respiratory control rate (RCR) and ADP/O ratios. A higher RCR rate was observed in the copper supplementation treatments at 21 days of age. Among these treatments, the organic source of copper outperformed the treatment with the inorganic source presenting higher RCR value. The ADP/O ratio, there was no difference between the type of copper source used. **Conclusion:** The use of copper in broiler diets has been shown to be beneficial to mitochondrial metabolism. This mineral provided better flow of electrons in the respiratory chain of mitochondria isolated from animals receiving 100mg/kg copper in the diet supplementation when compared with the control group. This reinforces the importance of copper in the functioning of mitochondria and consequently in respiratory metabolism.

Keywords: immune system, chickens, trace elements, mitochondrial metabolism

Support: UFRB, UFBA

QUANTITATIVE ASSESMENT OF CD271+CD45LOW MUTIPOTENTIAL STROMAL CELLS FROM BONE MARROW IN PATIENTS WITH OSTEONECROSIS

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Introduction: Aspiration of iliac crest bone marrow (BM) remains the most frequent technique used in harvesting multipotential stromal cells (MSCs) for to treat early stages of osteonecrosis (ON). The efficacy of this therapy depends on the quantity of implanted BM mesenchymal stromal cells (MSCs) to induce bone regeneration. CD271, also known as LNGFR (low affinity factor receptor of nerve growth or p75NTR) belongs to the superfamily of necrosis factor tumor. This cell surface marker potentially defines a subpopulation of proliferative MSC precursors with osteogenic potential. Colony-forming unit assay (CFU), the conventional method for the quantification of MSC, is time-consuming, labor-intensive and not suitable for automation. For this reason, this study aims to quantify CD271+CD45low cells and compare it with conventional CFU assay in BM samples from SCD patients. **Methods and Results:** Approval for these studies was obtained from the UFBA ethics committee [registration number 11738, SIPAR/MS: 25000.039812/2005–99], iliac crest bone marrow aspirate was obtained from 11 patients with osteonecrosis. A 50 µl volume of whole bone marrow and 5x105 mononuclear cells isolated from the BM aspirate on a Ficoll density gradient was stained for couting CD271+

CD45low cells using flow cytometry. The proportion of CD271+CD45low cells in BMMC was similar compared with BM aspirates (0,089 ± 0,067% vs 0,070 ± 0,068 %; p = 0,284) respectively. At the same time, the number of CFU-F in BMMC was significantly higher in comparison to BM aspirates (20,33 ± 2,08 vs 1,66 ± 0,57 per 106 nucleated cells; p=0,0044). The percentage of CD34+/CD45low cells (Hematopoietic stem and progenitor cells) in bone marrow aspirate was (0,647 ± 0,289%). Further experiments and multiple correlation analysis are still under investigation. **Conclusion:** We observed CD271+CD45low cells in BM samples of patients with ON, at equivalent levels to those described in the literature for age-related patients without ON. The percentages of CD271+CD45low in BM samples were not comparable to the number of colonies in CFU-F assays. Since BM processing methods can influence MSC concentration and prevalence, we suggest that counting CD271+CD45low in BM aspirate, instead of counting CD271+CD45low in processed and concentrated samples, should be a fast and useful tool to assess BM stromal progenitors population in patients with osteonecrosis.

Keywords: bone marrow aspirate, multipotential stromal cells, osteonecrosis

Support: CAPES, CNPq

LINHA 2: Imunofarmacologia

WOUND HEALING MODEL FOR ASSESSMENT OF IMMUNE RESPONSE AND TISSUE REPAIR IN TRANSGENIC MOUSE

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Introduction: Chronic leg ulcers (CLU) are very common with an estimated prevalence of 0.6 to 5% among patients over 65 years of age. CLUs are recalcitrant, cause a low quality of life and often lead to lower extremity amputation, representing both a significant health risk and a large economic burden. Adipose derived-mesenchymal stromal cells (Ad-MSC) are a potential alternative for treatment of CLUs. These cells secrete cytokines, growth factors, and bioactive molecules responsible for the effects of skin repair and regeneration. Standardized in vivo models are necessary to better understand the wound healing process and the role of MSC in tissue regeneration. **Methods and Results**: MSC were isolated from lipoaspirate samples, characterized (flow cytometry) and assayed for multilineage and immunomodulation potential (*in vitro*). The wound healing model was standardized using metal splints around the wound. Full skin thickness excisional wounds were created on the dorsum of the transgenic C57/Bl6 mice. Wound closure was monitored after surgery and healing rate was calculated based on wound area relative to the original size. The presence of perivascular cells and blood vessels were evaluated by immunofluorescence assay. The standardization of the wound healing model with metallic splints showed a greater delay in the closure of wounds when compared to the control model. Perivascular cells were observed around developing blood vessels after 7 days of injury. MSC conditioned medium demonstrated pro-angiogenic capacity, with higher performance for hypoxia conditioned medium. **Conclusion:** These data demonstrate that the wound healing model adopted is viable for the evaluation of tissue regeneration, without influence of the muscle contraction present in mice, approaching the healing model in humans. The conditioned media of MSC showed good regenerative capacity and pro angiogenic effects. Ongoing experiments will contribute to a better understanding of the potential of MSCs and their role in immune response and regenerative therapy.

Keywords: Mesenchymal stem cells, chronic ulcers, immune response

Support: FAPESB, CNPq, PIBIC-UFBA

CHARACTERIZATION OF THE MICROGLIAL RESPONSE MODULATION BY THE FLAVONOID AGATHISFLAVONE IN AN *IN VITRO* MODEL OF TRAUMATIC BRAIN INJURY (TBI)

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Introduction: Traumatic brain injury (TBI) is a major cause of death and disability in humans and animals. The pathology of TBI is complex and multifactorial. Primary TBI results from mechanical injury and disturbance of brain tissue, often resulting in axonal sectioning, and can lead to bruising and bleeding. A cascade of molecular-level events and biochemical changes begins within minutes after initial impact, as cellular hyperexcitability, astrogliosis and formation of glial scars, edema, hypoxia/ischemia, oxidative stress and inflammation, involving immuno effector

glial cells, that are defined as secondary lesions, and may, in turn, contribute to induce the expansion of the primary lesion. Hence, the development of drugs that can reduce the damage caused by TBI and assist in the recovery of affected patients is extremely important. The pharmacological potential of flavonoids, polyphenolic compounds found in a wide variety of plants, has been investigated by the scientific community, with promising applications for diseases that affect the brain, considering between others, its anti-inflammatory, antioxidant and immunomodulatory activities. Previous studies, conducted in an in vitro model of TBI, demonstrated that the flavonoid agathisflavone modulates the astrocyte response and glial scar formation, favoring the migration of cells (glia and neurons) to the lesion site, and stimulating neural network reestablishment. However, the role of microglial response in this condition has not yet been investigated. The present project aims to characterize neuroprotection mechanisms induced by the flavonoid agathisflavone associated with the modulation of astrocyte and microglial response in an in vitro model of TBI. **Methods:** A mixed culture of glia and neurons will be obtained from the cortex of Wistar rats embryos (16-18 E) and cells maintained in supplemented DMEM/F12 medium. After 10 days culture in vitro, cells will be pretreated (1 hour before injury) with the flavonoid dilution vehicle (0.01% DMSO, control) or agathisflavone (0.1 or 1 μM), and then the cultures will be subjected or not to a mechanical lesion with the aid of a sterile polystyrene pipette tip of 200 μL. After 48 hours the cultures will be processed for experimental analysis. Neuronal integrity will be evaluated by the Fluoro-Jade B reagent and immunocytochemistry (ICQ) for the cytosqueletal protein β-tubulin III. Microglial morphology and reactivity will be analyzed by ICQ for Iba-1, CD68 and CD206 proteins. Moreover, the cells' RNA will be extracted and analyzed by RT-qPCR for expression of M1/M2 inflammatory markers TNF, IL1 β, IL-6, IL10, TGFβ and arginase. **Expected Results:** The clarification of how this compound acts in the integration of neuroimmune signals in conditions of diseases characterized by glial reactivity and neurodegeneration, as in TBI, may contribute to the development of new or combined therapies for this pathology and other related disorders.

Keywords: neuroinflammation, microglia, flavonoid, Agathisflavone, neuroprotection

Support: FAPESB, CAPES, CNPq/INCT—EN, INCT-INNT

LINHA 3: Imunologia das Doenças Infecciosas e Parasitárias

STUDY OF THE MODULATORY EFFECTS OF MAGNETIC STIMULATION IN ASTROCYTES INFECTED in vitro WITH NEOSPORA CANINUM

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Introduction: Magnetic stimulation (MS) is a technic based on electromagnetic theory and has been studied to treat psychiatric and neurologic disorders, and to assess the cognitive function of nervous system. Many research have been produced for achieve this knowledge, but few works were made to know the cellular effect in inflammation and infection of central nervous system. The infection of astrocytes with *Neospora caninum* was validated as an experimental model of nervous system infection. This is an obligated intracellular protozoa which causes abortion and neonatal mortality in cattle. The aim of this study was to investigate the role of magnetic stimulation in *N. caninum* infection in astrocytes. **Methods and results:** Astrocytes were obtained from the cortex of newborn rats (<48 h) and the culture was maintained in DMEM (Dulbecco's modified Eagle's medium) supplemented with 10% (v/v) fetal bovine serum, 24 mM glucose, 2 mM glutamine and 100 UI/ml penicillin/streptomycin. After 10 days, the microglial cells were removed and the astrocytes were plated in 24 wells plates. The cells were infected with *N. caninum* Nc-Bahia strain in ratios 1:1, 1:2 and 1:4 (astrocytes/parasite) and astrocytes not infected were used as control of infection. After 6 hours of infection, MS treatment was performed in the cultures at room temperature with intensity of 0,62T, at 10 Hz for 30s with repetitive pulse. This procedure was repeated once a day for 3 days. Plates without MS treatment were used as control. Cytotoxicity was determined by using the MTT and nitric oxid tests. The results for MTT shown that infected groups had an increase of mitochondrial metabolism of 10,009% (1:1); 13,037% (1:2); 9,978% (1:4). In MS groups, the viability was higher: 8,901% (astrocytes only), 18,257% (1:1), 20,095% (1:2), 24,658% (1:4). The difference was statistically significant between the groups: astrocyte, 1:1, 1:4, with their respectively MS group. The production of nitric oxide (µg of NO/µg of protein) was $2,3x10^{-4}$ in the control plate (astrocytes only), $20,59x10^{-4}$ (1:1), $25,83x10^{-4}$ (1:2), $19,64.10^{-4}$ (1:4), and the plate treated by MS: 3,49x10-4 (astrocytes only), 10,52x10-4 (1:1), 15,61x10-4 (1:2) and 20,51x10-4(1:4). The difference was statistically significant between the groups: astrocytes, 1:1 and 1:2 with their respectively MS group. **Conclusion:** MS improves the cellular viability and reduces the nitric oxid production in astrocytes cultures infected with *N. caninum*.

Keywords: MTT, nitric oxid, repetitive magnetic stimulation

Support: CAPES

EVALUATION OF THE EFFECTS OF PRAZIQUANTEL *in vitro* ON UMBILICAL HUMAN VEIN ENDOTHELIAL CELLS (HUVEC) CHALLENGED WITH *Schistosoma mansoni*

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Introduction: Schistosomiasis is a parasitic disease considered a prevalent health problem in developing tropical countries, and praziquantel (PZQ) is the drug of choice for treatment. However, considering the intravascular habitat of the adult worm and the cytotoxic effect that PZQ has on endothelial cells, it is possible that IL-33 production and activation of other inflammatory pathways by endothelial cells may occur, and may be exacerbated during schistosomiasis treatment. Thus, using the model of human umbilical vein endothelial cells (HUVECs), the aim of this project is to characterize HUVECs cells, evaluating the expression of oxidative stress-associated molecules (SOD1, GPX, GSR, CAT, NRF2) and IL-33 and TSLP secretion after challenge with adult *Schistosoma mansoni* worm and Praziquantel treatment. **Methods:** For this, HUVECs will be cultured under five different conditions. Firstly, a culture with HUVEC will be performed as a negative control. The second condition will be the addition of a couple of adult worms will be placed directly above the HUVEC monolayers. The third condition will be the addition of Praziquantel directly over HUVEC monolayers. The fourth condition will be HUVEC culture with the adult worm couple in the presence of Praziquantel. The fifth condition will be a culture of HUVEC with LPS as a positive control. All these conditions will be performed in 12-well plate wells in triplicate and the experiment repeated three times. Cytokines IL-33 and TSLP will be assayed in culture supernatant by the sandwich ELISA method. MRNA expression for oxidative stress-associated molecules will be evaluated by real-time PCR (qPCR). **Expected Results**: Since the consequences of treatment on endothelial cells during the immunopathogenesis of schistosomiasis are not fully understood, the results of this work will make it possible to describe the characteristics of these cells challenged with the *S. mansoni* adult worm during treatment with PZQ.

Keywords: HUVEC, Praziquantel, *Schistosoma mansoni*, oxidative stress

Support:

SEROPREVALENCE GEOREFERENCING OF CASEOUS LYMPHADENITIS, EVALUATION OF GOAT IMMUNE RESPONSE AND CHARACTERIZATION OF CORYNEBACTERIUM

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Introduction: *Corynebacterium pseudotuberculosis* is a pleomorphic bacillus, facultative intracellular pathogen of macrophage, that commonly affects ruminants, being responsible for Caseous Lymphadenitis in goats and sheep. This endemic disease is widespread in herds throughout Brazil and causes economic losses for producers. Animal diagnosis is relatively simple, but treatment and control of LC have complications. Bacteriological culture from caseous content followed by biochemical tests is currently the gold standard test. We aim to determine the geographic distribution of caseous lymphadenitis and to evaluate the immune response to *Corynebacterium pseudotuberculosis* in goat herds in the state of Bahia. **Methods:** With the help of the Agência de Defesa Agropecuária da Bahia-ADAB, samples from goats will be collected from herds in different regions of the state of Bahia. The municipalities of Bahia were stratified according to the mesoregion to which they belong and the number of animals in the herd. The animals drawn for collection in the breeding establishments visited will be clinically evaluated and blood samples as well as purulent

material will be collected. From the samples collected, serological tests against *C. pseudotuberculosis* antigens will be performed to evaluate seroreactivity and seroprevalence in different regions. From the caseous material, isolation and characterization of strains from different regions will be performed through biochemical and molecular tests. After isolation and confirmation of identity, the strains will be characterized for biofilm production capacity, diphtheria toxin production capacity, cell viability evaluation in culture and electrophoretic profile of secreted proteins. The data obtained in the clinical evaluation and in the different laboratory tests will be used for the formulation of georeferencing maps, which will allow the crossing of these data to identify possible correlations. **Expected Outcome:** Describe the distribution and geographic density of C*. pseudotuberculosis* in the state of Bahia, obtaining a better understanding of the distribution of LC in goats herds in the state of Bahia. To map the incidence of LC in goats and to identify variations of responses and the correlation between strains of *C. pseudotuberculosis* isolated from different regions of the state.

Keywords: *Corynebacterium pseudotuberculosis,* seroprevalence, georeferencing, animal health

Support: LabImuno / Instituto de Ciências da Saúde — ICS / UFBA, Agência de Defesa Agropecuária da Bahia – ADAB, CAPES, FAPEX / UFBA, Programa de Pós-Graduação em Imunologia – PPGIm / ICS / UFBA, Ministério da Agricultura, Pecuária e Abastecimento – MAPA

PRODUCTION AND FUNCTIONAL STUDY OF RECOMBINANT *SCHISTOSOME* ELASTASE

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Introduction: Schistosomiasis is a millenary disease caused by infection with parasites of the genus *Schistosoma*. In Brazil, schistosomiasis occurs in a large endemic area and the number of individuals infected with *Schistosoma mansoni* can reach seven million people. *S. mansoni* parasite has a complex life cycle and the infection of vertebrate hosts occurs by the active penetration of cercariae through the skin. This process involves the vibratory action of cercaria tail and also the emptying of acetabular glands which promotes the secretion of lytic proteases and helps the penetration of host's intact skin. Previous studies have characterized several proteins present in cercaria secretions. Among them, the serine protease elastase (SmCE) has been identified as the most abundant and also as the most important protease in the penetration process. Besides that, cercaria antigens are considered important in immediate immune response developed by hosts that had no previous contact with *S. mansoni* antigens. Based on these data, the present work aims to produce the recombinant(r) SmCE in order to realize its functional study as a serine protease and also its immunological characterization. **Methods and Results:** The heterologous production of rSmCE was conduct in different *Escherichia coli* strains: pLys, BL21 and Rosetta. The plasmid containing SmCE coding sequence was transformed into each strain and the protein production was induced using IPTG. Bacterial lysates were analyzed by SDS-PAGE. All bacterial strains tested were able to produce the protein after a 5-hour induction; however, Rosetta presented a better yield. Following the protein production protocol, bacterial lysates were submitted to a protein solubility test. The samples were evaluated by Western blot and we found a higher concentration of rSmCE in the soluble supernatant. Next, we used the soluble supernatant to purify the rSmCE through affinity chromatography. **Conclusion:** The experiments concluded so far show that the best yield of soluble rSmCE was obtained using the Rosetta strain. The protein produced and purified from this bacterial strain will be used later for this project purposes.
Keywords: *Schistosoma mansoni*, elastase, inhibition, cercaria, infection
Support: CNPq, FAPESB

CD8$^+$ T CELLS AND NK CELLS PROFILES IN THE PATHOGENESIS OF DISSEMINATED LEISHMANIASIS

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Introduction: *Leishmania (Viannia) braziliensis* is the most important causal agent of American Tegumentary Leishmaniasis (ATL). The ATL have different clinical forms, and cutaneous leishmaniasis (CL) is present in more than 90% of the cases. Another clinical form is Disseminated leishmaniasis (DL), represented 4% of cases of ATL. CL is characterized by classical lesion ulcer, and DL is defined by the presence of 10 up to more than 1000 papular, acneiform and ulcerated lesions, located on two more different parts of the body. The lesions in the great most of cases isn't cause by parasites, but an exaggerated inflammatory response. Beyond the monocytes, CD8$^+$T cells is very important cell participate in that response, and the production of proinflammatory cytokines by this cells can be a relation of disease progression. **Methods and Results:** Peripheral blood mononuclear cells (PBMC) and lesions biopsies of patients with CL and DL was used on the study. The PBMC was isolated by Ficoll-Paque cultivated in complete RPMI ($1x10^6$ cells/well), 37º C at 5% of $C0_2$ for cell adhesion. This cells was divided in 4 subgroups, 1-not infected, 2-infected, 3-not-infected and co-cultivated with non-adherent cells, 4 — infected and co-cultivated with non-adherent cells. The infection was with promastigotes forms of *L. brasiliensis*, in 5:1 proportion (promastigotes-cells). After infection and co-cultures the cells were maintained in culture for 48 hours. The biopsies were macerated and cultured for 72 hours. PBMC and biopsies cells were marked for flow cytometry analyses, and the supernatant was collected for cytokine measurement by ELISA. Mann-Whitney, Kruskal Wallis and post tested by Dunn's multiple comparison. The frequency of CD8$^+$T cells on PBMC in patients with DL and CL didn't show differences, but *Natural killer* cells (NK) has more frequency on DL patients, just like more frequency of degranulation marker (CD107) than cells of patients with CL. Although CD8$^+$T cells, on CL biopsies, shown a higher frequency compare with DL biopsies. A less frequency of cells with exhaustion marker (CD57) were observed compare with DL, and even less frequency of cells with markers of exhaustion and degranulation profile. NK cells of biopsies shown more frequency exhaustion and degranulation profile on DL patients compare with CL. On the co-cultures, CD8$^+$T cells producing granzyme B (GzB) presented on the group 4 more frequency in DL and CL compared with group 3, but only on cells of DL patients the frequency of those cells with degranulation profile was higher on group 4 than group 3. NK cells producing GzB showed more frequency in group 4 than the group 3 in DL and CL patients. Culture supernatants were analyzed after 48 hours of culture. The level of GzB is higher in the group 4 when compared with group 3, but only on patients with DL. The biopsies maintained in culture for 72 hours, the frequency of CD8$^+$T cells and NK cells, didn't show differences as well the GzB production. **Conclusion:** CD8$^+$ and CD8$^+$CD45RO$^+$ T cells through cytotoxic action could cause *L. brasiliensis* infected cells lysis with consequent release of parasites contributing to the spread of the parasite in LD.

Keywords: Leishmaniasis, Cutaneous, Disseminated, CD8, NK, *Leishmania braziliensis*

Support: INCT-DT, CAPES, CNPq

HISTOPATHOLOGICAL ALTERATIONS IN INTESTINAL MUCOSA OF MICE INFECTED WITH ASSEMBLAGES AII AND BIV OF *Giardia duodenalis*

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Introduction: *Giardia duodenalis* is a protozoan found in the small intestine of animals and humans. This infection is highly prevalent worldwide, affecting about 280 million people in developed and developing countries. The prevalence rates of *G. duodenalis* verified in Brazil vary according to the region and the population under study. Giardiasis is the name given to *G. duodenalis* infection that colonizes the small intestine of the host. Thus, the aim of this study was to compare the effects of pathogenicity of *G. duodenalis* assemblages AII and BIV on the histological parameters in the duodenum and jejunum of Swiss mice. **Methods and Results:** The study adhered to the guidelines of the Sociedade Brasileira de Ciência em Animais de Laboratório and was approved by the Ethics Committee on Animal Experimentation of the Centro Universitário Integrado, Brazil (statement no. 1070). Forty-two male Swiss, specific-pathogen-free mice, 21 days old, were randomly assigned into three groups: uninfected control group (CG) and mice infected with 1000 cysts of *G. duodenalis* assemblages AII and BIV by gavage (GIA and GIB groups, respectively). After 15 days of infection, the duodenum and proximal jejunum was collected for histological processing and analysis. Histopathological analysis (semi-quantitative and quantitative) was performed on hematoxylin/eosin (HE) stained slides. The presence of suggestive forms of trophozoites, as well as changes in histoarchitecture in the intestinal wall were evaluated directly under the microscope using objective lenses of 10 or 20 or 40 or 100x (20 microscopic fields per animal). The inflammatory infiltrates were quantified (20 microscopic fields at 400x magnification per animal) based on the following classification: i) intensity; according to the number of inflammatory cells observed: absent, discreet, moderate and intense; ii) distribution of the inflammatory infiltrate, distribution per field: focal — a single infiltrate in the visual field; multifocal — more than one infiltrate in the visual field; diffuse — inflammatory cells diffusely distributed in the visual field. All data will be statistically analyzed with level of significance set at 5%. No suggestive forms of *G. duodenalis* trophozoites were observed in the intestinal mucosa of the evaluated mice. Semiquantitative histopathological analysis revealed the presence of diffuse inflammatory infiltrate with mononuclear predominance in the duodenum and jejunum of GIA and GIB mice compared to CG ($p < 0.05$). No changes ($p > 0.05$) were observed in the distribution or intensity of inflammatory infiltrates in the duodenum or jejunum mucosa when comparing assemblages AII and BIV. However, the loss of duodenal mucosa histoarchitecture was more pronounced in GIA. **Conclusion:** This study showed that parasite infection causes structural and histopathological alterations in the duodenum and proximal jejunum. Taken together, histopathological changes suggest that *G. duodenalis* assemblage A is more aggressive to the duodenum of mice.

Keywords: Giardiasis, inflammatory infiltrates, histopathological

Support: Fapesb

KINETICS OF INTRAEPITHELIAL LYMPHOCYTE AND GOBLET CELLS IN COLONIC EPITHELIUM OF RATS INFECTED WITH *Toxoplasma gondii*

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Introduction: *Toxoplasma gondii* is the etiological agent of toxoplasmosis, a parasitic disease with worldwide reach and distribution, with about 10 to 50% of the infected human population. Ingestion of food contaminated with oocysts is the main route of infection by *T. gondii*. After ingestion, in the intestinal lumen, oocysts release sporozoites that quickly cross the epithelial barrier and can be found on the surface of the epithelium and on the ileum and colon lamina propria one hour after inoculation. The intestinal mucosa plays an important role in the mechanical barrier against pathogens, including the *T. gondii*. Among the immune cells which respond to parasite invasion, intestinal intraepithelial lymphocyte (IEL) secretes anti-inflammatory and regulatory cytokines in the intestinal mucosa, which modulate epithelial turnover and repairing of tissue damage. Another protective factor of the mucosa is the production of mucins by goblet cells, which forms a barrier that lubricates and protects the intestinal epithelium. Thus, IELs and goblet cells plays a key role for the intestinal homeostasis. This study aimed to follow the dynamics in number of intraepithelial lymphocyte (IEL) and goblet cells in the distal colon of rats during the first 10 days of toxoplasmic infection. **Methods and Results:** Forty male Wistar rats (Rattus norvegicus), 60 days of age, were randomly assigned into two experimental groups: control group (CG, n=5) and group infected with 5,000 oocysts of *T. gondii* (M49 strain, genotype II, n=35). Five infected rats were killed long the course of the infection at 6 (IG6h, n=5), 12 (IG12h, n=5), 24 (IG24h, n=5), 48 (IG48h, n=5), and 72 hours (IG72h, n=5), and 7 (IG7d, n=5) and 10 days (IG10d, n=5). The distal colon was removed and fragments were submitted to standard histological process in order to obtain sections stained with Hematoxylin and Eosin (HE); Periodic Acid Shiff (PAS) was used to detect neutral mucins and labile sialomucins produced by goblet cells. The number of IELs and goblet cells was counted among 2,500 consecutive epithelial cells in each rat. Counting was performed directly on the microscope Olympus® CX31 with aid of the 40x objective lens. Proportion of IELs or goblet cells/100 epithelial cells was calculated. Statistical tests were performed using GraphPad Prism 5 software. The data were analyzed using one-way analysis of variance (ANOVA) and Dunnett's multiple-comparison post hoc test. The level of significance was 5%. The number of IELs increased progressively until 24 hours post infection and then reduced until reaches values found on the control at 72 hours post infection. Interestingly, another peak of IELs was observed at 10 days post infection. Besides, the infection caused an increase in the proliferation of goblet cells, but the infection groups of 24 and 48 hours it was not observed. **Conclusion:** We conclude that number of IELs in the colonic mucosa changes during the acute infection with *T. gondii*, increasing during the first hours of contact with the parasite and reducing during the following hours. Something similar happens with goblet cells, which has proliferated increasingly mainly after 72h.

Keywords: Toxoplasmosis, Epithelial cells, Mucosal immunity, Lymphocytes, Goblet cells

Support: FAPESB

REVERSE VACCINOLOGY IN MURINE MODEL AND CANINE FOR THE CONTROL OF *Toxocora canis* INFECTION

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Introduction: Toxocariasis is a zoonosis of global importance, toxocariasis has recently been named one of the five neglected parasitic infections in the USA by the Centers for Disease Control and Prevention; *Toxocara* infection in pets and fecal egg shedding are of great public health importance, up to now there is no program to control this infection, and vaccination may provide a good tool to achieve this goal. The objective this research will be applied the methodology of reverse vaccinology in the murine model and canine with the aim of contributing to the control of toxocariasis in canines through immunoprophylaxis. **Methods and Results:** from the genome and proteome of *Toxocara canis*, an *in-silico* analysis and murine model of toxocariasis it was select 2 proteins of immunogenic interest (rTcVcam and rTcCad). These proteins were expressed in bacterial vectors, purified (~ 350µg/mL) and tested in murine model (C57Black mice) of toxocariasis with different adjuvants (25 µg Quil-A® Th1/Th2 profile, 250 µg Alhydrogel® 2% Th2 profile, 10 µg Pam3CSK4 VacciGrade™ Th1 profile). 16 groups of 7 mice will be immunized (SC) with three doses of the proteins (25 µg doses) emulsified in each of the adjuvants with 8 days of interval; following all the groups will be inoculated PO with 500 *T. canis* embryonated eggs. The immunoglobulin profile will be investigated using indirect ELISA, commercially available (IgG, IgG1, IgG2A, IgE) on day 0, 24 and 45; the animal tissues (muscle, liver and brain) will be submitted to count larval analysis using digest tissues and microscopy to quantify the presence of the *T. canis* larvae, and splenocyte culture and cytokine dosage IL-4, IL-5, IL-10, IFN-ɣ in the culture supernatant by commercial ELISA Kit. The best adjuvant will be selected for further immunization of neonates dogs *Toxocara*-free with the proteins following the same protocol of mice (3 group of 6 dogs). RNA from peripheral blood mononuclear cells (PBMC) will be purified and the expression of the cytokines IL-4, IL-6, IL-17A, IL-10, TNF and INF-ɣ will be carried out through RT-PCR. Samples of 5 mL of blood and 50 g of feces will be collected on days 0, 14, 28, 42 and 56 days after *T. canis* inoculation PO (250 *T. canis* embryonated eggs). The immunoglobulin profile IgG, IgG1, IgG2, IgA and IgE will be carried out through commercial ELISA and McMaster for fecal egg count. **Conclusion:** : It is expected that this project will have as a product a vaccine for toxocariasis which might be used for pets and stray dogs, therefore decreasing the environmental contamination with this parasite eggs and possibly will have an impact in the control of human toxocariasis.

Keywords: toxocariasis, immunoprophylaxis, vaccine, zoonosis

Support: RENORBIO/CNpq

EVALUATE OF *in vitro* HUMORAL IMMUNE RESPONSE OF *S. mansoni* INFECTED TO Sm29 AND SmKI VACCINE ANTIGENS

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Introduction: Schistosomiasis is one of the most widespread infectious diseases in the world, affecting 200 million people and posing a threat to more than 600 million individuals living in at-risk areas. As a control strategy, many studies have attempted to identify a vaccine antigen capable of promoting even partial reduction of the parasite burden, considerably reducing pathology and limiting the transmissibility of the disease. In this context, proteins located in the parasite tegument, involved in the immune regulatory processes to favour the parasite survival are promising vaccine candidates. This study aims to evaluate the humoral response profile to *Schistosoma mansoni* tegumental rSm29 and rSmKI antigens in order to contribute to the screening of a vaccine candidate for schistosomiasis. **Methods and Results:** Individuals living in the endemic area from schistosomiasis, city of Conde/BA were included in this study. We collect serum samples and feces to parasitological exams before specific antiparasitic treatment (D0), 1 month post treatment (D30), 6 months (D180) and one year post-treatment (D360) with praziquantel. Parasitological examinations were performed by the Kato-Katz technique. Individuals were classified as negative, low load (<99 opg) and high load (> 99 opg) at D0.Levels of IgG4 and IgE specific to Sm29 and SmKI antigens were quantified by ELISA. In individuals with low parasite burden there was a reduction in Sm29-specific IgG4 in D0 to D180 ($p < 0.05$) and also in D0 to D360 ($p < 0.05$). In subjects with high parasite load, we also observed a reduction in IgG4 levels from D0 to D360 ($p < 0.05$) for the same antigen. When we evaluated the group of paired individuals, we observed that in the group with low parasitic load we observed an increase in SmKi specific IgE levels between D0 and D30. We did not observe differences between the evaluated times in relation to IgE for Sm29. There was a reduction in SmKI specific IgG4 levels in the negative groups and low load between D30 and D360. Regarding Sm29-specific IgG4 levels, there was a reduction in the levels of this antibody in all groups one year after treatment, but without statistical difference. The levels of the Sm29-specific IgE appear to be not affected one year post treatment parasite-specific. This class of antibody appear to be protective in *Schistosoma mansoni* infection. **Conclusion:** Individuals living in an endemic area for schistosomiasis with different degrees of infection have a different profile of IgE and IgG4 response to Sm29 and SmKI antigens. The levels of Sm29-specific IgE over the course of a year suggest that Sm29 is a promising vaccine candidate for schistosomiasis control.

Keywords: *S. mansoni*, antigens, IgG4, IgE

Support: FAPESB

REGULATORY T AND B LYMPHOCYTE-MEDIATED IMMUNITY AMONG INDIVIDUALS WITH DIFFERENT DEGREES of *Schistosoma mansoni* INFECTIONS

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Introduction: Schistosomiasis is a parasitic disease, caused by helminths. *Schistosoma mansoni* is the endemic pathogen in Brazil. Meanwhile the adult worms evades the immune response, the eggs secreted by them generate a strong immune reaction. The immune response against parasite eggs is mainly mediated by $CD4^+$ T lymphocytes. Regulatory T cells (Treg) constitute a subset of $TCD4^+$ cells important in regulating the immune response preventing their deleterious effects. During *S. mansoni* infection there is an increased regulatory response through Tregs induction and increased IL-10 production, reducing Th1 and Th2 cellular responses and allowing the parasite to survive, constituting a probable escape mechanism. In this sense, studies have shown that individuals infected with *Schistosoma haematobium* have a high frequency of $CD4^+CD25^{high}FoxP3^+$ Treg cells and their depletion in vitro causes an increase in T lymphocyte proliferation and in the production of Th1, Th2 and Th17 cytokines, important for the control of helminth infection. We aim to investigate the mechanisms involved in the modulation of *S. mansoni* specific Th1, Th2, and Th17 responses by regulatory T and B lymphocytes in PBMC of individuals with high and low parasitic burden of parasitic infection. **Methods:** It will be evaluated individuals infected with *S. mansoni* with different parasite burden determined by the Kato-Katz technique. T and B lymphocytes will be obtained isolated by separation of PBMC using magnetic beads. In order to evaluate the mechanisms that are dependent of contact cell-to-cell or soluble factors, PBMC and regulatory cells will also be co-cultured in transwell plate and stimulated with soluble adult worm antigen (SWAP). the phenotype of regulatory cells markers in both T lymphocytes (CD4, CD25, CD27, FOXP3, CTLA-4) and B lymphocytes (CD19, CD24, CD38) and cytokines (IL-10 and IL-35) will be performed using flow cytometry applied. In addition TH1, Th2 or Th17 cytokines will be evaluated in supernatants by ELISA technique. **Expected Results:** This work may contribute to a better understanding of the protection mechanisms against this helminthiasis and to the development of therapeutic strategies based on the control of the immune response.

Keywords: Regulatory T lymphocytes, *S. mansoni*, Regulatory B lymphocytes, Immunoppathology

Support: FAPESB

DEVELOPMENT OF A LATERAL FLOW IMMUNOASSAY FOR THE IMMUNODIAGNOSIS OF CANINE VISCERAL LEISHMANIASIS USING *LEISHMANIA INFANTUM*-DERIVED LIPOPHOSPHOGLYCANS AS ANTIGENS

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Introduction: Canine visceral leishmaniasis (CVL) is caused by intracellular parasites of the genus Leishmania and transmitted by Phlebotimine vectors. One of the most prevalent molecules present in the parasite's surface are the lipophosphoglycans (LPG), which present immunogenic and immunomodulatory properties. A high prevalence of canine disease is associated with a high risk of human infection and the limited accuracy of the tests used for diagnosis of this disease in dogs may contribute to the lack of impact on control measures. There are still some problems with the test recommended by the Brazilian Ministry of Health, and this situation points to the development of new tests with

higher specificity and sensitivity. Considering this situation, the objective of this study is to develop a rapid test based on a lateral flow for the CVL diagnosis using LPG as antigen. **Methods:** *Leishmania infantum*-derived LPG will be used as antigen for the imnumochromatographic membrane sensitizing. The revelation of the LPG-antibody interaction will be made using *Staphylococcal* A protein bound to colloidal gold. The immunoassay parameters wil be obtained using 68 serum samples from dogs infected with *Leishmania infantum* and 57 negative control samples obtained from dogs from non-endemic areas. The results of the lateral flow assay will be compared to the PCR results, to the LPG-ELISA and to the DPP-CVL commercial test, this last one recommended by the Brazilian Ministry of Health. **Expected Results:** It is expected that a more efficient test could be developed with the objective to improve CVL immunodiagnosis and to reduce the occurrence of false positives results, which can lead to an improper treatment, and the presence of false negatives results, which can facilitate the spread of the disease.

Keywords: immunodiagnosis, lateral flow, leishmaniasis, lipophosphoglycan

Support: FAPEX

CHARACTERIZATION OF THE IMMUNOLOGICAL MECHANISMS INDUCED *IN VITRO* BY THE SM29 ANTIGEN IN MACROPHAGES OF ASTHMATIC INDIVIDUALS

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Introduction: Chronic respiratory diseases are some of the leading causes of illness and death in the world, such as asthma that affects more than 350 million people worldwide. In asthma, the intensive inflammatory process can affect lung function by limiting patients' airflow, which compromises their daily activities. Evidence has been accumulating that chronic helminth infection, particularly *Schistosoma* sp or parasite products, is capable of modulating the inflammatory response. Recombinant *S. mansoni* proteins, such as the adult worm integument protein Sm29, have been shown to induce IL-10 production by uninfected asthmatic cells. In a murine model of ovalbumin-induced asthma, rSm29 injection was able to modulate the pulmonary allergic inflammatory response. We aimed to characterize the immunological mechanisms induced by recombinant *Schistosoma mansoni* Sm29 antigen in vitro in macrophages of individuals with asthma in different cytokine environments. **Methods and Results**: The effect of rSm29 antigen on the maturation, activation and production of cytokines IL-10, TNF and IL-1β in macrophages of individuals with severe asthma and mild / intermittent asthma in different cytokine environments was evaluated. In addition, ERK / MAPK and NFκB / MyD88 signaling pathways after in vitro stimulated macrophages from asthma patients with rSm29 antigen will be evaluated. The addition of *S. mansoni* antigen reduces the frequency of M1 and M2a macrophages. Preliminary results demonstrate that the reduction in frequency in M1 and M2a populations may be related to the increase in M2c population. There is a reduction in the production of proinflammatory cytokines (TNF and IL1β) by M1 macrophages stimulated with rSm29 antigen. In macrophage cultures of asthmatic patients stimulated with rSm29 antigen in the presence of IFN-γ and in the presence of IL-4 and IL-13, an increase in IL-10 production was observed when compared to cultures without stimulation in the same cytokine environments. **Conclusion:** Preliminary results demonstrate the ability of rSm29 antigen to modulate the macrophage inflammatory response of asthmatic individuals. The rSm29 antigen proves to be a good candidate for developing a therapeutic strategy for the treatment of this disease.

Keywords: asthma, Sm29, macrophages

Support: FAPESB, CNPq

EVALUATION OF HOST AND PARASITE FACTORS FOR FAILURE IN CUTANEOUS LEISHMANIASIS THERAPY

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Introduction: Protozoa of the *Leishmania* genus are the causative agents of leishmaniasis, which kill 30,000 people annually worldwide. In Brazil, Cutaneous Leishmaniasis (CL) has 30,000 new cases annually and the annual incidence is 18.5 cases per 100,000 inhabitants. Bahia is an endemic state for LC, and the district of Corte de Pedra is recognized as one of the most important areas of transmission of *Leishmania braziliensis* with an annual incidence of 8.1 per thousand inhabitants and a prevalence of 14,9%. Depending on the *Leishmania* species and geographical area, the effectiveness of antiparasitic drugs may vary substantially and the most important limitation of leishmanicidal drugs is treatment failure and emergence of drug resistant parasites. The main objective of this project is to identify host and parasite factors that are associated with therapeutic failure so that in the future we can identify new forms of treatments. **Methods:** To achieve this goal, we will establish a cohort of patients with cutaneous leishmaniasis in the early phase (before ulcer onset) (n=60) and in the ulcerated phase 9n=60). Patients will be clinically evaluated on day 0, day 15, 45 and 90, after initiation of treatment with pentavalent antimonial (SbV). Specimens will be collected on days 0, 15 and 90 after initiation of therapy. The parasite will be isolated from biopsies and lesion aspirates to characterize specific polymorphisms, drug resistance and protein expression associated with therapeutic failure. **Expected Results:** The main hypothesis of this project is that the number and genetic difference in parasites are associated with persistent inflammation, host impairment in the modulating immune response, leading to therapeutic failure. The experiments proposed here may lead to the discovery of host or parasite molecules that are associated with therapeutic failure in the early stages of the disease, allowing the administration of alternative therapies.

Keywords: Leishmania, leishmaniasis, sbv, antimonial

Support: NIH (EUA) (AI. R01AI136862-01), CNPq, CAPES

ROLE OF LEUKOTRIENES IN THE PULMONAR HYPERTENSION PATHOGENESIS IN *S. MANSONI* INFECTED PATIENTS

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Introduction: Among parasitic diseases, schistosomiasis ranks second in terms of socioeconomic and public health importance in tropical and subtropical areas. It affects about 200 million people living in developing countries, especially in rural and peri-urban areas, and an estimated 700 million people live in areas at risk of infection worldwide. About 5 to 10% of individuals infected with *Schistosoma mansoni* develop into severe forms of the disease, characterized by liver fibrosis, portal hypertension and pulmonary hypertension, which are responsible for the morbidity and mortality associated with this disease. Leukotrienes are inflammatory mediators involved in recruitment of Th2 lymphocytes, eosinophils as well as in tissue repair processes. **We aim** to evaluate leukotriene-induced immunological mechanisms in the pathogenesis of pulmonary hypertension secondary to schistosomiasis. **Methodos:** Patients diagnosed with schistosomiasis-related pulmonary hypertension from schistosomiasis endemic areas and controls with and without schistosomiasis-related periportal fibrosis residing in an endemic area will be recruited. The gene expression of lipoxygenase (5-LO and 12-LO) and 5-LO activator protein (FLAP) will be analyzed using macrophages derived from peripheral blood monocytes incubated with soluble *S. mansoni* egg antigen (SEA). The activity of 5-LOX will be evaluated through the levels of cys-leukotrienes

in the supernatant. We will also study the *in vitro* effect of 5-LO enzyme blockade on cytokine levels associated with worsening fibrosis (TNF, IL-4, IL-5, IL-13), tissue repair (TGF-β) or regulation of immune response (IL-10) in macrophage and lymphocyte cultures after stimulation with *S. mansoni* SEA. **Expected Results:** The identification and characterization of mechanisms involved in the induction of the leukotriene pathway by *S. mansoni* egg antigens and their *in vitro* participation in the pathogenesis of pulmonary hypertension secondary to schistosomiasis will favor studies for the therapeutic intervention of this pathway and thus prevent the development of severe forms responsible for disease-associated morbidity and mortality. The results of this study may result in the introduction of a clinical protocol for the repositioning of a leukotriene inhibitor drug for the control of such a serious disease.

Keywords: Pulmonary hypertension, schistosomiasis, leukotrienes, macrophages

Support: CAPES / CNPq / INCT

EVALUATION OF MICROBIOLOGICAL AND IMMUNOLOGICAL MARKERS IN THE ASSOCIATION BETWEEN CHRONIC PERIODONTITIS AND METABOLIC SYNDROME

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Introduction: Metabolic Syndrome (MS) is a chronic and progressive disease related to pathophysiological changes caused by central fat deposition and insulin resistance and currently contributes to increased cardiovascular morbidity and mortality and type 2 diabetes mellitus. Regarding the factors associated with MS, some investigations have indicated that its development depends on a complex interaction between factors related to genetic predisposition, lifestyle, and socio-demographic factors, as well as health-disease conditions such as insulin resistance, centralized obesity, hypertension, low HDL-cholesterol levels and periodontitis. Some studies suggest that the periodontal condition may increase the systemic inflammatory load and thus may interfere with the occurrence of MS. As for periodontal disease (PD), it comprises a chronic inflammatory process in response to bacterial antigens from the dental biofilm, resulting in loss of tooth support tissues, resorption of the alveolar bone and formation of periodontal pockets, which may lead to loss of the unit dental. Explanatory theories for this association between periodontal disease and MS have also been presented. Migration of buccal bacteria and their byproducts into the circulatory current, with systemic dissemination of inflammatory mediators of local origin, such as interleukins (IL-6, IL-1β,) and TNF-α, may establish a chronic systemic inflammatory picture. Therefore, increased production of circulating metabolites would be related to the causal process of MS. However, the specific cellular and molecular mechanisms for this association between periodontitis and MS are unclear and, therefore, further studies are needed to better understand this research question. In this sense, the objective of this study is to investigate the association between microbiological and immunological markers in patients with the presence of metabolic syndrome and chronic periodontitis. **Methods:** This is a case-control study that aims to evaluate the immunological and microbiological response in the association of PD and MS. For microbiological analysis will be performed bacterial DNA extraction and genotyping to verify the presence and association of periodontopathogens and MS. In addition, flow cytometry immunoassay will be performed to verify the presence of th1, th2 and th17 cytokines in *Perphyromonas gingivalis* antigen-stimulated peripheral blood mononuclear cell culture supernatant and the presence of metabolic syndrome. To evaluate the humoral immune response, the enzyme immunoassay (ELISA) will be used to evaluate salivary IgA levels and serum IgG levels against antigens present in the sonicated extract of *Porphyromonas gingivalis* in individuals with and without metabolic syndrome. **Expected Results:** It is expected to observe an association between the immunological and microbiological markers of PD and MS, contributing to the understanding of the association between periodontitis and metabolic syndrome.

Keywords: metabolic syndrome, Periodontitis, Immunoglobulin G, Immunogloulin A

Support: CAPES, FAPESB, LABIMUNO (ICS-UFBA), PPGIm (ICS-UFBA)

STUDY OF HUMAN REACTIVITY TO *CORYNEBACTERIUM PSEUDOTUBERCULOSIS* ANTIGENS BY *IN VITRO* IMMUNODIAGNOSTIC METHODS

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Introduction: *Corynebacterium pseudotuberculosis (C. pseudotuberculosis)*, is the etiological agent that causes caseous lymphadenitis, a disease widely known in veterinary medicine because it affects several ungulates, most commonly, sheep and goats. The disease is mainly characterized by the formation of lymph node granulomas, although it also manifests in other organs. Although case reports are rare, human *C. pseudotuberculosis* infection may cause lymphadenitis and pneumonia. Probably the human infection comes from contact with secretions, formites, in manipulating the bacteria; or by ingesting raw food derivatives from infected or diseased animals. Currently, invasive methods of excision or aspiration of the injured site (usually axillary or inguinal lymph node) are used for the diagnosis of human infection, followed by culture and isolation of the pathogen and further discrimination by Api®Coryne (bioMérieux) biochemical test. This project aims to evaluate the antigenicity of *Corynebacterium pseudotuberculosis* molecules and extracts in blood samples from individuals with a history of contact with it bacillus or the small ruminants management potentially infected. **Methods:** the study population will be divided into participants with contact with the bacteria or with their potentially infected derivatives, and participant without this contact, all tested negative for *Mycobacterium tuberculosis* infection. To test protein antigenicity the seroreactivity of the participants will be evaluated by the methods of Indirect Enzyme-Linked Immunosorbent Assay (ELISA) and Western blot, and the cellular reactivity by stimulation *in vitro*, with the proteins extract of *C. pseudotuberculosis*. **Preliminary results:** Western blotting immunoreactivity to *C. pseudotuberculosis* extract with human sera that deal with goats and sheep or have contact with the bacillus has been identified with distinct patterns when compared to individuals without this contact.

Keywords: *Corynebacterium pseudotuberculosis*, diagnosis, immunoreactivity, antigen

Support: Labimuno

ARE ASTHMA ENDOPHENOTYPES AND ATOPY STATUS INFLUENCING ILC2 FREQUENCIES?

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Introduction: Atopy and asthma are increasing their incidences worldwide over the years. The involved factors in allergic diseases are climate change, microorganisms and helminth infections, allergen contacts and genetic factors. The major immune response to allergens is the classical Th2 response trigger IL-4, IL-5 and IL-13 cytokines releases producing a type I hypersensitivity reaction and eosinophilic inflammation involved in the immunopathogenesis of asthma and atopic diseases. The group 2 innate lymphoid cells (ILC2s) also respond to allergens producing type 2 cytokines, which recruit neutrophils, eosinophils, increase IgE production, and thus cause mast cell degranulation likewise Th2 cells. This study aimed to quantify ILC2s frequencies between asthma endophenotypes and associate the number of ILC2 found with aeroallergen skin prink test results, besides to evaluate the ST2 soluble receptor to IL-33 and TSLP receptor serum concentration in the Program of Asthma and Rhinitis Control (ProAR) population. **Methods and Results**: The ILC2s were quantified from peripheral mononuclear cells of 30 individuals (10 bearing severe asthma, 10 bearing mild asthma and 10 non-asthmatic) by flow cytometer. The SPT results for *Dermatophagoides farinae,*

Dermatophagoides pteronyssinus, Blomia tropicalis, Blatella germanica and Periplaneta americana were obtained from ProAR databases. The ST2 and TSLP concentration were quantified through ELISA technique. The ILC2s frequencies did not showed significant results between endophenotypes but the ILC2s frequencies were higher in atopic individuals bearing severe asthma than in non-atopic individuals bearing severe asthma. Severe asthmatic bearing atopy showed higher number of ILC2s and atopic individuals for *B. tropicalis* presented higher ILC2s frequencies. **Conclusion**: none significant association was found between ILC2 frequencies and asthma endophenotype, we suggest that this result may be influenced by the severe asthmatic corticosteroid usage, as well as the TSLP cytokine concentration. ILC2s frequencies showed to be increased by the contact with *B. tropicalis*. Thus, new studies are required to better understanding of ILC2s participation in immune mechanisms in asthma and atopy.

Keywords: Asthma, Atopy, Innate Lymphoid Cells

Support: CAPES, Laboratório de Alergia e Acarologia (LAA)

EVALUATION OF SIGNALING PATHWAYS MAPKP38, JNK AND ERK 1/2 IN CHRONIC PERIODONTITIS

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Introduction: Chronic periodontitis is a disease, immunoinflammatory, of bacterial etiology disease that affects periodontal tissues, in which *Porphyromonas gingivalis* is considered a key pathogen because it has virulence factors that make it able to directly influence the host response and microbiota composition. Among these factors, HmuY and K gingipaines act in nutrient uptake, besides possessing immunogenic capacity, may induce the inflammatory process by activating signaling pathways, especially MAPK. This study aims to evaluate in macrophages the signaling pathways MAPKP38, JNK and ERK1/2 in chronic periodontitis. **Methods:** This research is based on an experimental study with 60 volunteers (30 with chronic periodontitis and 30 without periodontitis), which will be selected at the dental clinic of the State University of Feira de Santana. PBMCs will be cultured, with and without inhibition for MAP38 p38, JNK and ERK 1/2 pathways and stimulated by sonicated extract, rHmuY and *P. gingivalis* K gingipain peptide (Kgp12). Dosage of cytokines in the culture supernatant will be by flow cytometry. The obtained data will be evaluated with the use of ANOVA tests and post-hoc Games-Howell. **Expected results:** Thus, this study is expected to contribute to a better understanding of the action of these pathways in the induction of periodontal inflammation by Porphyromonas gingivalis.

Keywords: *Porphyromonas gingivalis*, Signaling pathways, Macrophages, Gingipaine, HmuY

Support: CAPES

DEVELOPMENT OF A LATERAL FLOW AND SKIN TESTS FOR CASEOUS LYMPHADENITIS IMMUNODIAGNOSIS

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Introduction: Caseous lymphadenitis (CLA) is a small ruminant disease characterized by the development of granulomatous lesions in superficial and internal lymph nodes, as well as in some organs, and causes significant economic losses worldwide at the goats and sheep breeding. The etiological agent of CLA is the bacterium *Corynebacterium pseudotuberculosis.* The commercially available diagnostic tools present problems with regard to specificity, which can lead to false-negative results. This study aims to develop a rapid test based on lateral flow and a skin test using recombinant PLD and CP40 proteins of *C. pseudotuberculosis*. **Methods:** 60 sheep and 60 goats will be use as control animals for the tests standardization, being 30 positive and 30 negative controls from each species. The lateral flow test is going to be developed using the two recombinant proteins in a 1:1 ratio combination, where they will be print in a nitrocellulose membrane; for the specific antibody recognition, the Streptococcal G protein conjugated to colloidal gold will be used. Serum samples will be taken from the animals and use for the validation of the lateral flow test. The skin test is also going to be develop using the two-protein combination, from which 5µg of protein content will be applied intradermally in those animals at a dose of 0.1 mL in the left cervical region. The measurements of the test will be performed with a cutimeter before inoculation and every 24 hours in 3 days; these results will be correlated with the specific production of IFN-gamma after stimulation with the recombinant proteins in the whole blood culture of the animals. **Expected Results:** The lateral flow and the skin tests can be accurate techniques to be used in the field for a faster and efficient diagnosis of CLA in goats and sheep. The development of two biotechnological products and two patent deposits are expected.

Keywords: goats, sheep, immunocromatograph, recombinant protein, intradermal test

Support: CAPES

LINHA 4: Imunogenética, Genômica e Proteômica

POLYMORPHISMS IN THE *PPARα* GENE ARE ASSOCIATED WITH OVERWEIGHT IN A BRAZILIAN POPULATION

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Introduction: Peroxisome proliferator-activated receptors (PPARs) are transcription factors that belong to the superfamily of nuclear hormone receptors and regulate the expression of several genes involved in metabolic processes that are potentially linked to the development of some diseases such as hyperlipidemia, diabetes, inflammation and obesity. *PPARα* is a member of the PPARs family, is expressed in active metabolic tissues including liver, heart, brown fat and skeletal muscle which regulates genes that act on fatty acid catabolism (AG) and associated transcription factors. It regulates the metabolism of lipids, carbohydrates, and amino acids and is activated by ligands such as polyunsaturated fatty acids. We aimed to evaluate the association between *PPARα* gene polymorphisms and obesity in Brazilian children. **Methods and Results:** The study comprised 1,010 children between 4-11 years. Genotyping was done using the Illumina 2.5 Human Omni bead chip. Logistic regression was used to assess the association between IMC and the *PPARα* gene variations in PLINK 1.07 software. The multivariate analysis was carried out adjusting for sex, age and ancestry markers. From 1,010 children included in this study, 15.5% (156) had overweight. In addition, 58% of the overweighted children were male and had an average age of 5 years old. There were overall statistically significant differences for age, but not for sex, between cases and controls. Eight SNVs were associated with overweight, of this total, seven SNVs such as (OR:1.33, 95% CI: 1.03-1.73, OR: 1.45 CL:1.02-2.07), *rs73177040*(OR:3.18,95%CI:1.13-8.95,OR3.18,95%CI:1.13-8.95), *rs1055659* (OR:1.33 95% CI:1.03-1.73, OR:1.45,95% CI: 1.02-2.07), *rs4253624*(OR:2.31, 95% CI: 1.08-5.25, OR:2.64, 95% CI: 1.11-6.26), *rs76078191* (OR:1.95, 95% CI:1.04-3.64) in both additive and dominant model (respectively) and *rs4253617*(OR:5.2, 95% CI:1.27-21.15, *rs4253763*(OR:2.82, 95% CI:1.04-7.69), *rs77561714* (95% CI:2.29, 95% CI:1.03-5.10) in the recessive model, conferring risk for overweight. In the recessive model, rs7286168 (OR: 0.67 95% CI: 0.46-0.96) was associated with protection to overweight. **Conclusion:** *PPARα* gene polymorphisms are associated with overweight in the studied population. Further studies are needed to better understand and elucidate the mechanisms whereby such SNVs are involved with overweight in humans.

Keywords: polymorphisms, ppars, children

Support: FAPESB

IN SILICO ANALYSIS AND IDENTIFICATION OF POTENTIAL ANTIGENIC TARGETS OF *CORYNEBACTERIUM PSEUDOTUBERCULOSIS*

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ABSTRACT: *Corynebacterium pseudotuberculosis* is a gram-positive bacterium that causes caseous lymphadenitis. Caseous lymphadenitis mainly affects small ruminants such as goats and sheep. Controlling the disease is very difficult, since the disease is already established in the herd. We aimed to evaluate in silico antibody binding epitopes on *Corynebacterium pseudotuberculosis* proteins **Methods and Results:** The protease of *Corynebacterium pseudotuberculosis* was subjected to *in silico* evaluation of subcellular localization and protein topology. The *in silico* prediction of linear B cell epitopes was performed and the conservation analysis of these epitopes was performed

on other *C. pseudotuberculo*sis proteomes deposited in the UNIPROT database. 111 proteins were selected from the subcellular evaluation and protein topology, with the subsequent submission of these proteins to a prediction of linear B cell epitopes and their conservation analysis in other proteases of *C. pseudotuberculosis*, resulting in 9 proteins with one or more regions of epitopes conserved in the same protein. **Conclusion:** Conserved regions favor a greater recognition of antigens when evaluated. In addition, a list of epitopes that can be synthesized and tested *in vitro* is provided in this paper.

Keywords: *Corynebacterium pseudotuberculosis*, Epitopes, Immunoinformatics

Support: FAPESB, UFBA

CHARACTERIZATION OF MOLECULAR MARKERS ASSOCIATED WITH BREAST CANCER IN EXOSSOMES OF PATIENTS IN BAHIA

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Introduction: Breast cancer is a malignant tumor that often affects women. In Brazil, in 2018, 59,700 new cases were registered. The host environment in which the tumor develops can be regulated by factors secreted by the tumor cells. Exosomes are vesicles secreted by various cell types that play an important signaling role in the body. They do not contain cell organelles and consist of cytosol surrounded by a lipid bilayer membrane. All of its composition comes from the cell that originated it. Exosome secretion can be strongly induced in cells undergoing inflammatory process or undergoing genetic modification. Exosomes, therefore, are present in most of the biological fluids of patients who are prone to tumor development. Among the exosome components are miRNAs, which are non-coding molecules of approximately 20 ribonucleotides in size. The miRNAsare involved in gene regulation and have been studied as biomarkers for different types of cancer. This project aims to detect and quantify circulating miRNAs from blood serum exosomes of patients in Bahia. The presence and expression of 8 breast cancer-associated miRNAs, as previously reported for European and Asian populations, will be evaluated. **Methods:** Isolation of exosomes will be conducted from blood serum samples.Validation of exosomes will be performedwithwestern blot using secondary anti-CD63 antibody, which recognizes the CD63 protein and is abundant in the exosome membranes. The integrity of exosomes will be evaluated using electron microscopy. RNA will be extracted using a commercial kit and quantified using Qubit® fluorometer (ThermoFisher Scientific). Following reverse transcription of the extracted RNA, the expression levels of the precursor molecule (pre-miRNA) of each miRNA will be evaluated using quantitative PCR (qPCR) andExiLENT SYBR Green master mix (Exiqon). The mir-103 miRNA will be used as an endogenous control in the qPCR experiments. Statistical analyzes will be performed using GraphPad Prism Software (Inc. La Jolla, California, USA). Student's t-test will be used to determine statistically significant differences between control and experimental groups. **Expected Results:** In this project, we expect to profile the expression of breast cancer-associated miRNAs in patients in Bahia, contributing to the development of a diagnostic method possibly including miRNAs not yet studied in this population.

Keywords: Breast tumor, miRNA, biomarkers, exosomes

Support: CAPES

GENETIC VARIANTS IN *DENND1B* ARE ASSOCIATED WITH ASTHMA AND ATOPY IN BRAZILIAN ADULTS

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Introduction: Asthma is a complex and heterogeneous disease characterized by bronchial hyperresponsiveness, obstruction, and chronic airway inflammation. Around 339 million people worldwide suffer from asthma and an increase of over 100 million cases is estimated by 2025. Its heterogeneity is associated with environmental factors and individual variability and, therefore, interest in studying the genetics of asthma has grown over the years. Variants of *DENND1B* have been reported to increase susceptibility to asthma and are related to negative modulation of T cell receptor (TCR) by controlling its internalization in T cells; mutations or decreased expression of this factor are associated with increased Th2 response and asthma. The aim of this study was to investigate the association of *DENND1B* gene polymorphisms, as well as their respective functional impacts, with atopy and different asthma phenotypes, in adult followed up by the Program for Asthma Control in Bahia (ProAR). **Methods and Results:** Genotyping was performed using an Illumina Commercial Panel (MEGA) on 1,177 participants of the ProAR program. Logistic regressions for asthma and atopy markers were performed using PLINK 1.9 software, adjusted for gender, age and ancestry markers. Cytokine level and cell count analyses were performed on GraphPad Prism 7. Fifteen (15) polymorphisms in *DENND1B* were associated with different phenotypes such as asthma, severe asthma, reversibility, and atopy markers such as skin testing for different allergens. The rs2488389 was a protective factor for atopy in asthmatics, in addition to increasing the number of sputum neutrophils and decreasing IL-33 levels. *In silico* data showed that this variant can increase the expression of *DENND1B*. **Conclusion:** A greater *DENND1B* expression is linked to the reduction in time for TCR internalization which, consequently, leads to a controlled production of cytokines by effector lymphocytes. Thus, *DENND1B* polymorphisms are associated with the development of asthma and atopy which can be explained by, at least in part, to a low or lack of expression of this gene.

Keywords: SNP, genetic variants, DENND1B, asthma, atopy

Support: Fapesb, CNPq

POLYMORPHISMS IN *AHR* GENE ARE ASSOCIATED WITH IL-10 AND IL-5 PRODUCTION BY PERIPHERAL BLOOD LEUKOCYTES STIMULATED WITH *A. LUMBRICOIDES* EXTRACT

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Introduction: The aryl hydrocarbon receptor (AHR) is a nuclear receptor that modulates the response to environmental stimuli including microbial, viral and parasitic pathogens. Murine model studies have demonstrated that $AHR^{-/-}$ mice failure in infection control and the modulation of septic shock. Genome-wide association studies and candidate gene studies have identified SNPs in *AHR* associated with atherosclerosis, psoriasis and systemic lupus erythematous. Our objective was to evaluate the association of polymorphisms in the gene *AHR* with peripheral blood cells response in Brazilian children. **Methods and Results:** Genotyping was performed using a commercial panel from Illumina (2.5 Human Omni bead chip) in 1,246 participants of SCAALA program (Social Change, Asthma, Allergy in Latin American). Peripheral blood cells were cultured within 6 hours of collection in the presence or not of *A lumbricoides* antigen and were maintained in a humidified environment of 5% CO2 at 37°C for 24 hours before the supernatants were collected for cytokines measurement by ELISA. The study included 24 SNPs for *AHR.* Logistic regressions for asthma and atopy were performed using PLINK software 1.9 adjusted for sex, age and ancestry

markers. In additive model 3 SNPs in *AHR* were associated with IL-10 and IL-5 production by peripheral blood leukocytes stimulated with *A lumbricoides* extract. The rs2158041 was negatively associated with IL-10 (OR: 0.315; CI: 0.1151- 0.8625) and positively associated with IL-5 (OR: 1.468; CI: 1.015-2.121). The C allele of rs17137616 (OR: 2.278; CI: 1.012-5.125) was positively associated with IL-10 production. The rs6960165 is positively associated with IL-5 production (OR 1.516; CI: 1.044-2.202). **Conclusion:** As demonstrated by functional analysis in peripheral blood cells, the response to environmental stimuli could be modified by polymorphisms in the gene *AHR.* Understanding how *AHR* contributes to the prevalence and severity of many immune and inflammatory diseases has the potential to serve as a target for novel pharmacotherapeutic agents.

Keywords: Asthma, Helminth, Polymorphism, *AHR*,

Support: Fapesb, CAPES, CNPq

TH2-INDEPENDENT PRODUCTION OF PROINFLAMMATORY CYTOKINES IL-6 AND TNF AND DOWN-REGULATION OF THE HIPPO PATHWAY CHARACTERIZE SEVERE ASTHMA PHENOTYPE IN A BRAZILIAN POPULATION

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Introduction: Asthma is a chronic inflammatory, complex and multifactorial disease with several phenotypes. Among the subtypes of severe asthma, there is the 'treatment-resistant asthma', in which control of asthma is not achieved even using maximum maintenance treatment. The Hippo pathway, highly conserved in *Drosophila melanogaster,* regulates organ size by promoting apoptosis and inhibiting cell proliferation in the embryonic developmental stages. Recent studies have shown that Hippo / YAP1 signaling regulates epithelial cell proliferation and differentiation, and performs an important role in embryonic lung maturation and postnatal airway homeostasis. The aim of this study was to search for immunological and molecular biomarkers of treatment resistance in the induced sputum of asthmatic patients. The results may help understanding the biological mechanisms involved in the resistance to treatment of asthma. **Methods and Results**: Sixty-seven subjects were evaluated, divided into three asthma subgroups [17 with severe asthma resistant to treatment (SAR), 22 with severe asthma controlled with treatment (SAC), 19 with mild to moderate asthma (MMA)] and a no asthma (NA) control group including 9 subjects. For immunological analyses, cytokines and chemokines were measured through a multiplex kit by Luminex technology (Merck KGaA, Darmstadt, Germany) and the differential cell count in sputum was carried out using cytospin preparations. For transcriptomic analysis, total RNA was extracted from induced sputa of two severe asthma samples and two mild asthma samples; RNA sequencing was performed in the Ion Torrent S5 platform, using an Ion 540 chip (Life Technologies Corporation, Thermo Fisher Scientific, CA, USA). The genome, annotation, and gene ontology sets for Homo sapiens were obtained from the ensembl database (https://www.ensembl.org), version GRCh38. Differentially expressed genes and miRNAs were determined by fitting the fragment count data to a generalized linear model, GLM, considering a significance level of 5% for the Wald test basically as implemented in the DESeq2 tool. The SAR sputa had higher proportion of neutrophils and of eosinophils when compared with MMA and NA, respectively (p = 0.05; p=0.02). SAR group presented higher production of TNF when compared to NA, MMA and SAC (p=0.001). IL-6 was also higher in SAR in comparison to SAC (p=0.037). The TNF-producing individuals had worse pre and post-bronchodilator FEV1%; FEF25-75% (p = 0.00 for both) and FEV1 / FVC% (p = 0.03 and p = 0.02 respectively). The Hippo cascade genes presented down-regulation in the phenotype of refractory severe asthma when compared to mild to moderate asthma; Furthermore, miRNAs differently expressed between the two groups of asthmatics are mainly associated with the regulation of cell proliferation and inflammatory pathways, including regulation of Hippo. **Conclusion**: Treatment-resistant severe asthma is associated with the presence of proinflammatory cytokines

(TNF and IL-6) and differential expression of the components of Hippo pathway, regulating the expression of these cytokines in a way independent of Th2.

Keywords: Hippo pathway, severe asthma, IL-6, TNF, RNA-seq

Support: CNPq, CAPES, PPGIm/UFBA, Laboratory of Allergy and Acarology

POLYMORPHISMS IN *NLRP3* GENE ARE ASSOCIATED WITH MARKERS OF ALLERGY INDUCED BY *DERMATOPHAGOIDES PTERONYSSINUS* MITE IN A LATIN POPULATION

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Introduction: *Dermatophagoides pteronyssinus* mite is one of the main induced-atopy allergen in tropical and subtropical regions such as Brazil. Allergy is 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. IL-33 appears to be a potent inducer of Th2 immune response. *IL33* and *ST2* are the most replicated genes in Genome-Wide Association Studies (GWAS) for atopy worldwide. In addition, one recent study showed that IL-33 regulates house dust mite extract-induced allergic airway inflammation via NLRP3 inflammasome complex consisting of ASC and caspase-1 downregulating IL-33 production and thus, Th2 response. Therefore, it becomes of great importance to study the influence of *NLRP3* polymorphisms on allergy markers and the aim of this study was to associate variants in the NLRP3 gene with markers of allergy induced by *D. pteronyssinus*, never explored before in a Brazilian population. **Methods and Results:** DNA was extracted from peripheral blood from 1,246 subjects and the samples were genotyped using Illumina 2.5 Human Omni Beadchip. The study included 36 SNPs in *NLRP3*. Logistics regressions were carried out for atopy markers using PLINK software 1.9 adjusted for sex, age, helminth infection and ancestry markers, in the additive model. In addition, haplotype analysis were performed using SNPStats program. The T allele of rs35433972 was positively associated with IL-13 production (OR=1.98; p=0.018), when stimulated with *Dermatophagoides pteronyssinus*. The T, G and A alleles of rs10925023, rs200927356 and rs4925650 also were negatively associated with IL-5 production (OR=0.35; p=0.024), (OR=0.38; p=0.045) and (OR=0.38; p=0.049), respectively, when stimulated with *Dermatophagoides pteronyssinus*. In addition, the haplotype GT (rs200927356 and rs10925023) also was associated with decreasing of IL-15 production (OR=0.38; p=0.042). On the other hand, the C and T alleles of rs3806265 and rs7525979 were positively associated (OR=2.26; p=0.012) and (OR=1.93; p=0.016) with the same phenotype. Regarding the production of specific IgE, the C, A, A and A alleles of rs12137901, rs74154640, rs55914518 and rs4925654 were negatively associated (OR=0.79; p=0.038), (OR=0.72; p=0.030), (OR=0.41; p=0.032) and (OR=0.77; p=0.040), respectively. Regarding the positive SPT for *Dermatophagoides pteronyssinus*, the T and A alleles of rs12728998 and rs4925654 were negatively associated (OR=0.70; p=0.031) and (OR=0.72; p=0.045), respectively. **Conclusion:** Polymorphisms in *NLRP3* gene were associated with markers of allergy induced by *D. pteronyssinus* in our population. However, more studies should be conducted to investigate the functional role of this gene that could explain the development of complex diseases as allergy.

Keywords: NLRP3, Gene, Polymorphism, Atopy, *Dermatophagoides pteronyssinus*

Support: CNPq, CAPES, FAPESB, SCAALA

VARIANTS OF THE *ADCY9* GENE ARE ASSOCIATED WITH THE LACK OF REVERSIBILITY

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Introduction: Asthma is caused by the interaction of genetic variability and environmental factors. The adenylyl cyclase type 9 (*ADCY9*), enzyme, downstream of β2 adrenergic receptor (β2-AR), produce the cAMP. The ADCY9 was associated with treatment response on asthma and cAMP is involved with immunomodulation and bronchial relaxation. The aim of this study was to investigate how variants in the *ADCY9* are associated with lack of reversibility in asthmatics. **Methods and Results:** The study comprised 412 subjects with mild asthma and 401 with severe asthma. The bronchodilator reversibility was considered positive when FEV1 increased at least 200 ml and 12% after use bronchodilator. Regression analyzes was used to assess the association in Plink 1.09 software adjusting for sex, age and principal components. Online platforms such as ensembl.org and Regulome DB were used to characterize variants. The lack of reversibility in severe asthma was associated with risk in the variants rs8045426 (OR: 1,97; IC95% 1.12-3.47) and rs2230738 (OR: 1.65; IC95%: 1.05-2.59) and protection in the variants rs409963 (OR: 0.51; IC95%: 0.31-0.84) and rs72762796 (OR:0.57; IC95%: 0.33-0.94). The lack of reversibility in mild asthma was associated with risk in six variants; of these, two had a two-fold higher risk rs2532013 (OR: 2.10 IC95%: 1.03-4.27) and rs74003488 (OR: 2.05 IC95%: 1.11- 3.76). In addition, protection to lack of reversibility was found for rs147224614 (OR: 0.32; IC95%: 0.11-0.90); rs61042195 (OR: 0.62; IC95%: 0.41-0.92); rs2238448 (OR: 0.63; IC95%: 0.44-0.90); and rs11646996 (OR: 0.65; IC95%: 0.43-0.97). These variants have intronic function that may be related to regulatory characteristics of the expression. In addition, the variant rs74003488 also has lncRNA (non-coding long RNA) function. **Conclusion:** The lack of reversibility may have relation with clinical observations as the loss of prophylactic bronchoprotection and lack of asthma control that has been suggested as important consequence of the β2-AR desensitization. Therefore, these SNVs may help in the asthma pharmacogenetics and the individualized therapy for severe and mild asthma.

Keywords: Asthma, polymorphism, ADCY9, lack of reversibility

Support: CAPES, CNPq

LOCUS 10Q21 IS ASSOCIATED WITH RISK FOR BRONCHIAL OBSTRUCTION IN AFRICAN AMERICANS FROM NORTHEAST BRAZIL

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Introduction: The Diagnosis of asthma and other conditions affecting the respiratory tract is based mainly on the observation of clinical symptoms along with pulmonary function tests. Spirometry is an important tool for the diagnosis, monitoring and to classify the severity of these diseases. Differences in spirometric parameters have been observed between distinct racial and ethnic groups suggesting that biogeographic ancestry may play an important role in pulmonary function. Here, we study the genetic architecture of lung function and risk for bronchial obstruction in the admixed population of Salvador, Brazil. **Methods and Results:** We have used an *Admixture Mapping* strategy, followed-up by *fine-mapping*, to find susceptibility variants associated with European, African or Native American ancestry. The local-ancestry inference was performed using RFmix of ~2.5 million SNPs for child cohort with 950 unrelated individuals from Salvador, Northeast of Brazil from the EPIGEN-Brazil Initiative. We found three

significant admixture mapping peaks associated with bronchial obstruction (FEV/FVC ratio before bronchodilator). African ancestry at 10q22.1 region was associated with decreased lung function and consequently increased risk for bronchial obstruction. Fine mapping of 10q22.1 identified five significant SNPs rs16929777 (G), rs10400019 (C) in *DNAJB12* gene a heat shock protein family (Hsp40) member; and rs11000440 (A), rs75970382 (T) and rs16930172 (G) in *OIT3* gene, a oncoprotein. All polymorphic alleles are more frequent in African populations and also highly frequent in our population. **Conclusion:** Our results point out to a probable contribution of genetic ancestry in lung function, which is a common parameter used to evaluate asthma exacerbation. More studies are needed in order to evaluate the functional impact of these SNPs in bronchial hyper responsiveness.

Keywords: Lung function, bronchial obstruction, biogeographical ancestry, admixture, SNPs

Support: FAPESB, CNPq

ASSOCIATION STUDY OF THE VARIANT RS2275527 IN THE *MTOR* GENE WITH CLINICAL MANIFESTATIONS RELATED TO ZIKA VIRUS INFECTION

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Introduction: Zika virus infections (ZIKV) reported in epidemics have been associated with neurological clinical complications in several Brazilian regions. In 2015, the increase in the number of cases generated national alert, mainly in Feira de Santana, an endemic region in dengue and chikungunia. Due to the cases of twins with different clinical results to microcephaly, a preliminary familiar genetic study by our group revealed a possible genetic marker of predisposition, the rs2275527 in the MTOR gene. The MTOR gene encodes a serine/threonine protein kinase that regulates cell metabolism, growth and survival in response to hormones, growth factors, energy and stress signals and has been linked to neurological clinical manifestations. In this sense, our aim is to evaluate the association of the variant rs2275527 on the MTOR gene with clinical manifestations related to zika virus infection. **Methods and Results:** A cross-sectional incidence survey was carried out in Feira de Santana-BA, collecting blood samples from individuals and recording their clinical history to ZIKV infection. The collected samples were conditioned for DNA extraction following protocol of QIAGEN kit. Subsequently, the DNA was quantified using spectrophotometry and then analyzed for purity by absorbance ratio whose overall quality exceeded 90% of samples. Samples are being standardized for DNA concentration in 96-well plates. Soon after DNA concentration standardization, the samples will be genotyped using QuantStudio 12K equipment and then the results will be evaluated in PLINK software. **Conclusion:** The study of rs2275527 in the MTOR gene is necessary to understand the discrepant clinical manifestations related to ZIKV infectionn as well as their effects.

Keywords: ZIKA virus, genetic variant, MTOR

Support: CNPQ, FAPESB

STUDY OF GENETIC VARIANTS OF miRNAs THAT MODULATE THE B2-ADRENERGIC PATHWAY AND THEIR ASSOCIATION WITH ASTHMA AND THERAPEUTIC CONTROL

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Introduction: Asthma is characterized as an obstructive airway disease that involves chronic airway inflammation with worldwide distribution. Asthma is a multifactorial disease in which the genetic aspect is closely linked to the environmental aspects. The most common bronchodilators used to treat asthma are β2-adrenergic receptor (β2AR) agonists. Studies show that the level of expression of such receptors is directly related to the responsiveness to their agonists and that these levels can be regulated by interfering RNA, such as microRNAs, affecting the control of asthma treatment response. When an agonist binds to the β2AR receptor, it activates adenylate cyclase, which is responsible for regulating cAMP production. From this the protein kinase A (PKA) is activated and plays the role of phosphorylating other target proteins, thus resulting in the balance of phosphorylation and dephosphoritation resulting in bronchodilation, thus contributing to muscle relaxation of the asthmatic patient. It has been shown that miRNA expression can be affected by SNPs (single nucleotide polymorphisms) that are located in the genes encoding miRNA and may alter their biological functions. **Objective:** Investigate the association of polymorphisms in miRNAs that directly regulate ADRB2 expression,with different asthma phenotypes and their therapeutic control. **Methods:** The SNPs to be studied will be those genotyped using the Illead Multi-Ethic AMR / AFR Commercial BeadChip Kit Panel (www.illumina.com), where samples from 1335 patients will be genotyped, which will be separated into 3 groups (severe asthma, mild asthma and control). The frequency of miRNA gene variants that directly regulate the ADRB2 gene, their association with lung function, atopy markers and bronchodilator response, among other parameters, will be evaluated. In addition, a functional study of asthma-associated SNPs will be performed. Formoterol-stimulated cAMP production in PBMC from patients with different main miRNA genotypes will also be evaluated. **Expected Results:** This study will make it possible to advance the understanding of genetically based asthma in the treatment and control of symptoms, as well as the creation of biomarkers for personalized medicine based on each individual's genetic profile.

Keywords*: Genetic polymorphism, ADRB2, miRNAs, Asthmatic phenotypes, Bronchodilator*

Support: Cnpq

STUDY OF GENETIC VARIANTS RELATED TO INNATE LYMPHOID CELLS AND THEIR ASSOCIATION WITH ASTHMA PHENOTYPES

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Introduction: Asthma is a disease that has a major impact on the health and quality of life of millions of people around the world and is associated with high mortality. On average 300 million people worldwide suffer from asthma, consisting of a disease that has chronic inflammation of the lower airways. Asthma has multiple pathogenic mechanisms, caused by genetic and environmental factors, which lead to its clinical presentation in different phenotypes. The pathophysiology of asthma involves innate immune response cells and molecules, with the onset of pulmonary inflammation dependent on the secretion of IL-33, IL-25 and TSLP by epithelial cells present in the alveolar space. These cytokines are responsible for stimulating both T helper 2 (TH2) and lymphoid type 2 (ILC2) cells to produce type 2 cytokines such as IL-4, IL-5 and IL-13, leading to increased bronchial hyperreactivity. Studies point to an important role for ILC2 in asthma and other allergic diseases, and research into genetic variants, especially single nucleotide variants (SNVs), may provide important information regarding asthma heterogeneity and thus help in the discovery of new phenotypes of this syndrome. **Methods and Results:** The study population consists of 1.177 individuals from the Program for the Control of Asthma and Allergic Rhinitis in Bahia (ProAr), divided into three groups: severe asthma, mild asthma and control. DNA extraction was performed from patient

blood samples according to the Flexigene® kit protocol (Qiagen) and genotyping data have already been obtained from the Illumina MEGA® Bead Chip commercial panel. Asthma variables, asthma severity, and atopy were used to perform association analyzes using the Plink program. One variant in the TSLP gene (rs3806933) and ten variants in the IL-33 gene (rs1330383, rs10435816, rs1048274, rs10975519, rs16924243, rs2066362, rs992969, rs77747718, rs928413, rs2026991) were significantly associated with asthma and atopy in this population. Of these, the variant rs2066362 (C > A) has evidence of increased gene expression in homozygous individuals for allele A in skin tissue and is positively associated with atopy in our population. The variant rs3806933 (G> A) in the TSLP gene shows evidence of decreased gene expression in individuals with the allele A and is negatively associated with asthma severity in our population. In the Il-25 gene, no variables significantly associated with asthma and atopy were found. **Conclusion:** The variants present in the TSLP and IL-33 gene are already well described in other populations, but not yet described in populations of African descent, such as ProAr. Further analysis on other study genes will be performed and the genetic score will be created to determine the degree of risk in the presence of more than one allele of SNVs previously associated with asthma severity symptoms.

Keywords: SNVs, asthma, phenotypes, ILC2s

Support: Capes, CNPq, Fapesb

PREDICTIVE GENETIC PANEL ANALYSIS FOR THE DIAGNOSIS OF ASTHMA AND ITS ENDOTYPES THROUGH MACHINE LEARNING

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Introduction: Asthma is a very heterogeneous chronic inflammatory airway disease. In Brazil, the prevalence of asthma is 12.44% and its primary diagnosis is made via clinical findings, whose observable data are relatively generalalist. However, several variants of asthma (phenotypes and endotypes) have been documented, which makes it difficult to manage patients individually, since conventional treatment does not confer remission in all patients. In this sense, great statistical and computational efforts have been made to study complex diseases, for example: Machine Learning (ML). This may be a first step towards elucidating and better understanding their distinct underlying pathophysiological mechanisms, which could facilitate the development of personalized prevention strategies and more effective stratified therapies. Therefore, the main objective of this work is to establish a predictive genetic marker panel for the diagnosis of asthma and its endotypes, using Machine Learning methods. **Methods:** The first two specific objectives will use data from 1179 individuals admitted to Programa de Controle da Asma e Rinite Alérgica da Bahia (ProAR). We will use sociodemographic data, signs and symptoms of asthma based on the patient's history and various biological markers. Statistical and ML analysis will be performed using R and Python programming language. The data will be preprocessed, then the software will be used for machine learning, prediction and ending with the evaluation of ranked models. The performance of each model will be assessed by ROC curve analysis and the area under the curve. A second specific objective will be to evaluate genetic variants to predict asthma and its endotypes, and for this we will use the same previous process, however, with exclusively genetic data. Finally, all described methodology will be validated in another population (Social Changes, Asthma and Allergy in Latin America – SCAALA). The present project will have the infrastructure available at Laboratório de Imunofarmacologia e Biologia Molecular (IMUNOBIO) and the ICS/UFBA IMUNOBIO genomic studies platform.

Keyword: asthma, endotypes, Machine Learning, prediction

Support: CNPq

GENETIC VARIANTS IN AIM2 GENE ARE ASSOCIATED WITH PERIODONTAL DISEASE IN A BRAZILIAN POPULATION

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Introduction: Periodontal disease (PD) is characterized as complex due to multifactorial predisposing factors. The immune response to bacteria contributes to tissue destruction. A cascade system involving cells, inflammatory protein mediators and receptors induces bone resorption, characteristic of periodontitis. Intracellular protein absent in melanoma 2 (AIM2) plays a role in innate immunity, triggering inflammatory responses against the bacteria involved in PD. Studies report that the amount of oral microorganisms is directly proportional to the presence of these proteins. Therefore, the presence of genetic variants in the genes of these proteins may influence PD for both protection and risk. The aim of this study was to verify the association of variants in the *AIM2* gene with PD. **Methods and Results:** 443 individuals of both sexes, aged ≥ 18 years, participating of the Program of Control of Asthma and Allergic Rhinitis of Bahia (ProAR) were classified by the presence (269 individuals) or absence (174 individuals) of periodontitis, as well as by severe, moderate or slight intensity, according to the criteria of Gomes Filho et al. (2007). Genotyping was accomplished using the Infinium Multi-Ethnic Global chip. Statistical analysis used PLINK 1.9 software (logistic regression in three models – dominant (dom), additive (add), and recessive (rec)), with adjustments for (age, educational level, obesity, hypertension, mouth breathing and asthma) the variants with at the least 5% of frequency. The rs2814777 variant was associated in the 3 models as a risk factor for both the presence of PD (dom — OR: 2.33; 95% CI 1.23–4.41; add — OR: 2.31; 95% CI 1.30- 4.91; rec — OR: 7.08; 95% CI 0.86-57.99) and for intensities, severe (dom — OR: 2.53; 95% CI 1.20-5.35; add — OR: 2.45; 95% CI 1.24-4.82) and moderate (dom — OR: 2.46; 95% CI 0.98-6.17) according to the adopted criterion. There were no individuals with slight PD in the sample. The variant rs10489846 was associated in the recessive model as a risk factor for the presence of PD (OR: 4,47; 95% CI 1.13-17.62), as well as for severe PD (OR: 5.15; 95% CI 2.15-21.31). **Conclusion:** The genetic variants of the *AIM2* is associated as a risk factor to the presence and intensity of PD, in different models in a Brazilian population. Understanding the impact of these variants on PD may contribute in the future to the identification of molecular markers for disease diagnosis and prognosis.

Keywords: Polymorphism, gene, AIM2, periodontal disease

Supports: CAPES, UFBA

GENETIC VARIANT ON *FKBP5* IS ASSOCIATED WITH ASTHMA AND LACK OF REVERSIBILITY IN A LATIN AMERICAN POPULATION

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Introduction: Asthma is a disease multifactorial and its development depends of genetic and environmental factors. The protein encoded by *FKBP5* gene, the FK506 binding protein 5, is a member of the immunophilin protein family, which play a role in immunoregulation and basic cellular processes involving protein folding and trafficking. This protein is a co-chaperone probably involved in all steroid receptor complexes, and it attenuates the hormone responsiveness in glucocorticoid and mineralocorticoid receptor complexes, thus may have an impact on asthma and therapeutic control. The aim of this study was to investigate how variants in the *FKBP5* are associated with asthma and lack of reversibility. **Methods and Results:** The study was performed in the ProAR population, where 1179 samples from adult individuals (301 subjects without asthma, 412 subjects with mild asthma and 401 with severe asthma) were genotyped using the Illumina Infinium Multi-Ethnic AMR / AFR-8 Kit. Association analyzes

were performed using Plink software and were adjusted by sex and age. The bronchodilator reversibility was considered positive when FEV1 increased at least 200 ml and 12% from pre-bronchodilator values. The rs3798346 was negatively associated with asthma (OR: 0.71; 95% CI: 0.52-0.98). This same variant was positively associated with lack of reversibility in individuals with asthma (OR: 1.80; 95% CI: 1.09-2.96). **Conclusion:** The rs3798346 may be a protective factor for asthma. However, asthmatic individuals carried this variant have more riskof not being reversible after bronchodilator use. This is the first study to associate this variant with phenotypes of asthma. Further studies are necessary to show if this variant influences in the levels and function of this protein in asthma and reversibility.

Keywords: Asthma, FKBP5, variants, asthma severity

Support: CAPES, CNPq

VARIANTS IN MAP3K14 GENE ARE ASSOCIATED WITH ASTHMA AND ATOPY

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Introduction: The asthma is a chronic airway disease, characterized by mucus production, bronchial hyperresponsiveness, and variable airflow. Affecting around 334 million people worldwide, is a condition which involving genetic and environmental factors, whose prevalence varies between different ethnic and racial groups. In Admixture Mapping, we identify the region 17q21 associated with risk for asthma in individuals with African ancestry, and protection for European Ancestry. After performing logistic regression, the MAP3K14 (mitogen-activated protein kinase kinase kinase 14) gene was found. MAP3K14 or NIK encodes a kinase, which binds to TRAF2 and stimulates NF-kappaB activity. NF-kB acts by inducing pro-inflammatory genes and already demonstrated positive correlation in bronchial biopsy of asthmatics. This study aimed to evaluate the association between SNVs in MAP3K14 gene, asthma and atopy. **Methods and Results:** Data were obtained from the SCAALA (Social Change in Asthma and Allergies in Latin America) cohort, composed of 1,253 children. The DNA was extracted and the genotyping was performed using the Illumina 2.5 Human Omni Bead chip. For statistical analysis, PLINK v.1.9 was used. The allele T of rs11651968 was negatively associated with asthma symptoms (OR 0.70, CI 0.55 – 0.89, $p<0.05$), skin prick test (SPT) for *Blatella germanica* (OR 0.57, CI 0.38 – 0.85, $p<0.05$) and severity symptoms (OR 0.64, CI 0.46 – 0.88, $p<0.05$). On other hand, the allele A of rs11079502 was positively associated with asthma symptoms (OR 1.25, CI 1.03 – 1.52, $p<0.05$) and SPT for *Blatella germanica* (OR 1.36, CI 1.01 – 1.84, $p<0.05$). **Conclusion:** The study of the MAP3K14 gene was important due to the presence of variants in the Nf-kB pathway already known to act in asthma, but further studies should be done to elucidate the role of these variants in asthma immunopathogenesis.

Keywords: Asthma, MAP3K14, Atopy, NF-KB

Support: CNPq, PIBIC-UFBA

rs28763981 IN *ELN* IS ASSOCIATED WITH SEVERE ASTHMA

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Introduction: Asthma is an inflammatory chronic disease characterized by bronchial hyperresponsiveness, remodeling and obstruction of airways. The airway remodeling consists in morphological alteration in structure of airway, which includes loss of epithelium integrity, basement membrane thickening, subepithelial fibrosis, goblet cell and globular cells hyperplasia. Furthermore, in the site of inflammation there is an increased expression of elastin (ELN), a protein that comprise part of the extracellular matrix and confer elasticity to organs and tissues including lungs, ligaments, and blood vessels. Moreover, studies demonstrated association among lung structure changes and asthma severity. Single Nucleotide Polymorphism (SNP) in *ELN* gene can change ELN levels and function. Thus, our aim was to verify the association between polymorphism in *ELN* and asthma. **Methods and Results:** Asthma and asthma control was defined according to the Global Initiative for Asthma – GINA. It was included 364 non asthmatic individuals; 413 subjects with mild to moderated asthma and 401 with severe asthma. Genotyping was performed using the Multi-Ethnic Global Array panel (Illumina). Logistic regressions were performed using PLINK software 1.9. Function and regulatory characteristics of SNPs were analyzed using rSNPbase and HOPE databases. Also, *in silico* gene tissue expression was performed by Gtex. We found that the A allele of rs28763981 was positively associated with severe asthma, when we compare with subjects with mild asthma and severe asthma (OR:1.80; 95% CI: 1.10-3.24 and p-value: 0.04). The A allele of rs28763981 also increased *ELN* gene expression in fibroblasts cells (p-value 0.0015). Furthermore, this polymorphism change Gln in position 279 by Arg leading to a decrease of the protein stability. **Conclusion**: The rs17576 may interfere in asthma control by inducing lung tissue remodeling. Additional studies may clarify the function of such SNP and its correlation with asthma severity.

Keywords: Elastin, asthma, polymorphism

Support: Fapesb, Cnpq

VARIANTS IN THE *ADRB2* GENE ARE ASSOCIATED WITH SEVERE ASTHMA IN A BRAZILIAN POPULATION

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Introduction: Asthma is a heterogeneous disease characterized by a chronic inflammation of the lower airways, which shows its form dependent phenotype genetic and environmental factors. Agonists that act at the β2 adrenergic receptor, encoded by the *ADRB2* gene, promoting the relaxation of smooth muscle of airwaysand consequent bronchodilation being correlated with lung function, as well as the control of asthma. Changes in responsiveness to endogenous adrenergic agonists represent potentialtriggers to impairment of pulmonary function. It is known that polymorphisms of the *ADRB2* gene can promote effects on the development of allergic respiratory diseases and their different presentations, although these observations are still controversial. The objective of this study was to evaluate the association of the genetic variants on *ADRB2* with asthma and severity of the asthma in an urban population of Brazil. **Methods and Results:** The study was conducted by analyzing 1177 individuals, including 401 diagnosed with severe asthma, 412 with mild or moderate asthma and 364 healthy controls recruited by the Program to Control Asthma in Bahia. Six polymorphisms in the *ADRB2* were genotypedusing the Illumina Infinium Multi-Ethnic AMR / AFR-8 kit and the data analyzed by logistic regression. As a result, it was found that the rs1042713 (A allele)

was associated with severity of the asthma in the additive (OR: 1,34; 95%CI:1,06-1,72) and dominant (OR:1,75; 95%CI: 1,21-2,53) genetic model, although not related to the development of the disease itself. The presence of the combination ofrs1042713 (A allele) andrs1042714 (C allele) in heterozygosity was also associated with severity (OR: 2.03; 95%IC: 1.06 — 3.88). **Conclusion:** In conclusion, *ADRB2* variants are not associated with asthma in our population. However, in asthmatic individuals, the presence of variant rs1042713 alone or in combination with variant rs1042714increases by up to 2-fold the risk to a more severe asthma.

Keywords: asthma, immunogenetic, *ADRB2*, severity

Support: CAPES

ASSOCIATION BETWEEN ATOPY AND VARIANTS IN THE LEP AND ADIPOQ GENES IS MODIFIED BY OVERWEIGHT IN CHILDREN

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Introduction: Obesity is considered a condition associated with asthma and its increased severity, being affected by genetic and epigenetic factors, as well as atopy. The LEP and ADIPOQ genes, responsible for the expression and secretion of leptin and adiponectin, respectively, are studied for both diseases independently, and also for the obesity-associated asthma phenotype. Polymorphisms in these genes have been associated with overweight, modification of serum adipokine levels, and chronic diseases such as asthma. Thus, the aim of this study was to investigate variants associated with asthma and atopy, and whether overweight modifies the observed effect. **Methods and Results:** The study involved 1043 participants in the SCAALA (Asthma and Allergy Social Changes in Latin America) program. The BMI / Age Z-Score classification followed the recommendation adopted by the World Health Organization. Genotyping was performed using the Illumina 2.5 Human Omni chip. Logistic regression was performed to identify associations between LEP, ADIPOQ, BMI, Asthma and Atopy. Analyzes were adjusted for age, sex and ancestral markers using PLINK 1.09 software. Allele C of rs3821799 in ADIPOQ was positively associated with atopy in individuals independent of BMI (OR 1.48, 95% CI 1.06-2.06) and in overweight individuals (OR 3.28; 95% CI 1.22-8.77), but not in eutrophic. In LEP, the association was observed in overweight individuals for the G allele of rs6966536 (OR 3.16; 95% CI 1.04-9.60), but not in eutrophic individuals. Associations with asthma were observed for variants in both genes but only independent of BMI. **Conclusion**: These data suggest that SNPs in the LEP and ADIPOQ genes may have an impact on the risk of asthma and atopy, and increased body mass may influence the degree of association. However, further studies should be performed to elucidate the SNP-SNP and Gene- environment interaction in these outcomes, as well as the functional impact of the variants described here.

Keywords: Obesity, Asthma, Leptin, Adiponectin, Atopy

Support: FAPESB

POLYMORPHISM IN *EBI3* ARE ASSOCIATED WITH ATOPY IN A BRAZILIAN COHORT

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Introduction: IL-35 shares the IL-12p35 subunit with IL-12, and EBi3 with IL-27. Mutation in *EBI3* gene can thus affect both IL-27 and IL-35 pathways, leading to distinct imune consequences. We aimed to evaluate the association between genetic polymorphisms in *EBI3* with asthma, asthma severity and atopic markers. **Methods and Results**: 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 atopic 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. The rs428253 was negatively associated with IgE production for at least one common allergen (OR:0.70; 95%CI: 0.51-0.97); rs78749916 was negatively associated with specific skin test to *Periplaneta americana* (OR:0.61; 95%CI: 0.40-0.93); rs77145509 (OR:0.66; 95%CI: 0.46-0.94) and rs76353132 (OR:0.64; 95%CI: 0.43-0.96) were negatively associated with specific skin test for *Periplaneta americana*; and rs4905 was negatively associated with IgE production for *Periplaneta americana* (OR:0.62; 95%CI: 0.40-0.95). **Conclusion**: Studies carried out in Asia have shown that expression of *EBI3* and *IL12A*, subunits of IL-35, are decreased in asthma. The genetic polymorphisms in the *EBI3* may be considered a factor of susceptibility / risk for the disease considering it is leading to a down-modulation of IL-35 in the tissue. Additional studies must be conducted in other to check the gene expression and cytokine quantification according to different genotypes of SNPs within *EBI3* to better understand the impact of IL-35 on asthma.
Keywords: *IL35*, *EBI3*, asthma, atopy, polymorphisms
Support: FAPESB, CNPq

ANALYSIS OF GENETIC AND IMMUNOLOGICAL MARKERS IN PATIENTS WITH ACUTE MYELOID LEUKEMIA

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Introduction: Acute Myeloid Leukemia (AML) is a clonal disease of the hematopoietic tissue. It is characterized by abnormal proliferation of progenitor cells of the myeloid lineage, causing insufficient production of normal mature blood cells. Although its specific cause is not yet fully defined, it is known that genetic and environmental factors play important roles in the molecular pathogenesis of this neoplasm. The imbalance between pro and anti-inflammatory cytokines hinders the proper functioning of the immune system. Variations in cytokine levels are associated with certain allelic variants of cytokine genes. The objective of this work is to describe mutations in *CEBPA*, *FLT3*, *KIT*, and *NPM1* genes, characterize the cellular phenotype and pro/antiinflammatory cytokines in AML. **Methods and Results**: After signing the consent form, peripheral blood or bone marrow aspirates are collected from participants at two times (at diagnosis and at 21 days after treatment initiation). The identification of B and T lymphocytes and NK cell will be performed by immunophenotyping (FACS Calibur). The dosage of cytokines IL-1β, TNF, Lymphotoxin, IL-12, IFN-γ, IL-2, IL-10, IL-8, IL-6, IL-4 and IL-5 will be performed by flow cytometry using the Bito Citokine Beads Array kit (CBA). DNA extraction will be performed with PureLink Kit (Invitrogen). Specific sequences that selectively amplify polymorphic regulatory regions within genes encoding the cytokines TNF (-308G>A), TGF-β1 (10C>T, 25C>G), IL-10 (-1082A>G, -819T>C, -592A>C), IL-6 (-174C>G) and IFN-γ (874T>A) will be characterized using the Cytokine

Genotyping Tray (One Lambda Incorporation). Mutations in *CEBPA*, *FLT3*, *KIT*, and *NPM1* genes will be detected using PCR followed by sequencing (3130xL Appied Biosystems). Electropherograms will be analyzed in the BioEdit v7.2.6.1. Data will be analyzed using the statistical softwares SPSS and Graph Pad Prism, version 5.0. Samples have been collected from 11 research parcipants. The obtained data are under analyses.

Keywords: Acute Myeloid Leukemia, cytokines, FLT3, NPM1, KIT

Support: CAPES

PHARMACOGENOMIC STUDY IN INDIVIDUALS WITH ASTHMA ATTACKS

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Introduction: Asthma is a chronic inflammatory disease of the lower airways that causes episodes of wheezing, dyspnea, chest tightness, and coughing, which vary over time. Asthma affects more than 358 million individuals and is considered a worldwide public health problem. About 6.4 million Brazilians have asthma, which results in a large number of hospitalizations, resulting in high costs for the Brazilian Health System (SUS). In most patients, the disease can be controlled by the combined use of inhaled corticosteroids and bronchodilators β2-adrenergic agonists. However, it is estimated that 5 to 10% of asthmatic individuals have severe asthma, which requires treatment with high doses of inhaled corticosteroids and often supplemented with systemic corticosteroids. However, even today many asthma patients are not monitored and do not use routine medications, using emergency services only during asthma attacks. In addition, there are patients that even using the drugs regularly, they are resistant to treatment, possibly due to poor adherence or misuse of the medicines or to individual genetic variations. Pharmacogenetics seeks to identify which polymorphisms change the response to medications, requiring dose adjustments or even switching to other drugs when there is a risk of serious adverse reactions or ineffectiveness. Therefore, the recurrence of asthma attacks and lack of response to treatment, especially in patients with severe asthma, justifies the search for strategies to prevent such attacks. In this context, in this project we propose to evaluate the impact of the therapeutic accompaniment and the pharmacogenetics associated with the treatment in the prevention of asthma attacks **Methods:** This is a prospective observational study for asthma attacks which will be done in 600 adults attending emergency care in the public health system units in Salvador, Bahia, Brazil. The participants will be halved into a collaborative care group and the remainder in usual care in a larger study. DNA will be extracted from all patients from the peripheral blood, we will perform spirometry, skin test, and patients will also be telemonitored where they will answer questionnaires, such as asthma control questionnaires. Following this, genetic variants will be genotyped into the ADRB2, NR3C1, and ADCY9 genes to identify possible variants associated with asthma phenotypes (such as atopy, lung function) and whether the variants are associated with recurrence of asthma attacks and lack of control. Variants statistically associated will be selected for gene expression assay to investigate whether they affect mRNA levels, It will also analyze the functional impact of these variants by mononuclear peripheral blood cells culture. Statistical analysis will be carried out through statistical packages STATA 8.2, SPSS 20 and PLINK V.1.9, in addition to other platforms. **Expected Results:** To determine genetic factors associated with the recurrence of asthma attacks and lack of response to treatment, investigating since poor adherence to treatment to pharmacogenetics, thus aiming to improve the quality of life of asthmatic individuals.

Keywords: Asthma, Attack, variants, pharmacogenetics

Support: CNPq, ProAr

ANALYSIS OF ALLELIC VARIATIONS IN ENG IN AN ADMIXTURE POPULATION

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Introduction: Endoglin (ENG, CD105) is a coreceptor of the transforming growth factor-beta family and participates in the regulation of cellular processes such as proliferation, differentiation, migration and apoptosis. ENG is most known for their expression in endothelial cells, playing an important role in angiogenesis and vasculogenesis. Furthermore, its expression has already been associated with different pathogenic outcomes, some of them associated with mutations in the ENG gene. **Objectives:** To perform in silico analysis with the allelic variants found in the endoglin gene in the admixture population of Brazil and analyze genetic variants that may be involved in pathogenic processes in other populations. **Methods and Results:** We used the SCAALA and ProAR genomic database for the descriptive study. The individuals DNA was genotyped, and in silico analysis were performed to describe the functional and regulatory characteristics these allelic variants using software programs as RegulomeDB for intronic variants and Polyphen-2, SIFT, MutationTaster2, PMut and HOPE for missense variants. 82 allelic variants were analyzed in silico. The intronic variants rs4837192, rs41492548, rs12551892, rs12553394, rs77946642 and rs373842615 present 2b score and rs34116890, rs41527247 and rs118179851 present 3a score in RegulomeDB, indicate the possible variants that may be involved in regulatory and functional regions. Among the missense variants analyzed, 10 presented damaging score and only 2 variants, rs201393380 and rs200372420, with allele change occurring in the population (frequency of <0.001) were analyzed in HOPE platform indicating this changes occur in orphan domain in the molecule and may disturb interactions with other molecules or other parts of the protein. Allelic variants have been associated in HHT1 phenotype with pulmonary arteriovenous malformations, sporadic cerebral AVM and preeclampsia with the most frequent alleles in the ENG in others populations. **Conclusion:** Further investigation regarding the functional mechanisms of this gene are necessary, since the endoglin participates in a range of important cellular processes and more efforts should be made for genetic studies in the Brazilian population, considering the mixture of populations.

Keywords: Endoglin, CD105 Antigen, Genetic Polymorphism, Single Nucleotide Polymorphism

Support: FAPESB

LINHA 5: Imunodeficiência e Imunopatologia

MONOCARBOXYLATE TRANSPORTER 4 (MCT4) EXPRESSION IN RELATION TO CD204+ M2 MACROPHAGES IN ORAL SQUAMOUS CELL CARCINOMA

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Introduction: The progression of oral squamous cell carcinoma (OSCC) reflects intricate cellular and molecular interactions from tumor cells with immune components of tumor microenvironment. Most cancer cells have an aberrant energetic metabolism with high glucose consumption even in aerobic conditions. Then, high lactate release (so-called "Warburg effect") and consequently extracellular acidification is common in most solid tumors. The MCT4/SLC16A3 is characterized as a low-affinity lactate-preferring transporter that presents a critical role in release and uptake of this catabolite in cancer context. Macrophages require MCT4 up-regulation to maintain a high glycolytic rate during activated inflammatory response and presence of MCT4 positive macrophages within tumor and in the stroma around the tumor has been demonstrated in OSCC. To the best of our knowledge, no study has so far presented the pattern of MCT4 mRNA in a human sample. Furthermore, in OSCC the MCT4 expression in relation to macrophages has only been demonstrated in CD163+ subpopulation. The purpose of this study was to investigate the status of MCT4 mRNA transcripts in human OSCC samples and correlate with MCT4 immunostaining in tumor cells and CD204+ M2 macrophages. **Methods and Results:** This study was approved by Ethics Committees of Aristides Maltez Hospital (HAM) – Salvador, Bahia, Brazil (protocol number 10/2010). Using qRT-PCR, we analyzed the expression of MCT4 mRNA in 30 human OSCC. Additionally, MCT4 and CD204+ M2 macrophages were localized and semi-quantified in corresponding OSCC by immunohistochemistry (IHQ). MCT4 up-regulation was observed in 86.6% (27/30) of OSCC cases. IHQ reveal positive expression of MCT4 in tumor (both cytoplasm and cell membranes) and stromal cells (fibroblasts and inflammatory cells similar to lymphocytes and macrophages) in 61,64% and 76,92% of OSCC samples, respectively. Some cases presented strongly positive expression of MCT4 in tumor cells with rare or absent expression in the associated stromal compartment. In contrast, other cases exhibited a notable absence of MCT4 expression in the tumor compartment, with a marked stromal expression. CD204+ M2 macrophages analysis was not concluded. No correlation was observed between the patterns of gene and protein expression of MCT4 in the paired samples of OSCC evaluated. **Conclusion:** We corroborate with evidence for a metabolic reprogramming of OSCC cells towards Warburg effect. Notably, in OSCC the immnohistochemical expression of MCT4 is compartmentalized. The increased levels of MCT4 mRNA transcripts not accounts for positive MCT4 protein expression, suggesting the existence of an additional post-transcriptional mechanisms involving MCT4 expression in OSCC.

Keywords: oral squamous cell carcinoma, MCT4, SLC16A3, macrophages, qRT-PCR

Support: FAPESB, CNPq

EXPRESSION OF IL-23p19 (INTERLEUKIN-23, ALPHA SUBUNIT p19) mRNA TRANSCRIPTS AND PROTEIN IN ORAL SQUAMOUS CELL CARCINOMA

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Introduction: Interleukin (IL)-23 constitutes a heterodimeric cytokine expressed predominantly by macrophages or activated dendritic cells and comprising IL-12p40 and the IL-23-specific p19 subunit. IL-23 exploration in the context of oral cancer is scarce, but it is known that this cytokine contributes to the inflammatory IL-17 phenotype in premalignant oral lesions. However, the role of Th17 cells in the progression from premalignant lesions to cancer is unclear. Besides that, remains unclear the IL-23 expression pattern in OSCC and whether this cytokine plays a relevant role in oral tumorigenesis. Finally, to the best of our knowledge no study has so far presented the pattern of expression of its mRNA in a human sample. Thus, the aim of this study was investigate IL-23 mRNA transcripts and protein levels in relation to clinicopathological characteristics of OSCC patients. **Methods and Results:** This study was approved by Ethics Committees of Aristides Maltez Hospital (HAM) – Salvador, Bahia, Brazil (protocol number 10/2010). A total of 60 OSCC patients who were treated between 2008 and 2012 at Department of Head and Neck Surgery of HAM were enrolled and evaluated retrospectively. The clinicopathological data were collected from medical records. The tissue expression levels of IL-23 mRNA transcripts (fresh tissue) and protein (paraffin-embedded tissue) were measured in corresponding lesions using TaqMan® qRT-PCR (reference gene 18S) and immunohistochemistry (IHQ) using a mouse anti-human IgG1 (BioLegend clone HLT2736), respectively. In 30 investigated OSCC, 61.11% presented normalike expression of IL23 gene (down-regulation in 33.33% and up-regulation in 5.56% of cases). In non-neoplastic adjacent tissues (margins), 45.45% exhibited normalike expression (down-regulation in 36.36% and up-regulation in 18.18%% of cases). No correlation was observed between IL23 gene expression in OSCC samples in relation to their paired margins. Likewise, there was no association between IL23A gene expression and investigated clinical-pathological characteristics. The IL-23 expression by IHQ was detected in OSCC in both tumor and stromal cells (inflammatory cells similar to macrophages). The IHQ analysis was yet not concluded. **Conclusion**: We consider our partial results unclear regarding the pro or anti-tumor activity of the IL23A gene in oral cancer. A better exploration of our findings is dependent on concluding IHQ analysis. The results of this study will corroborate to clarify the role of IL-23 in oral cancer.

Keywords: oral squamous cell carcinoma, IL-23, IL-23p19, qRT-PCR, IHQ

Financial support: FAPESB, CNPq, PIBIC-UFBA

GPC3 REGULATORY MIRNAS, MRNA AND IMMUNOHISTOCHEMICAL EXPRESSION IN RELATION TO M2 MACROPHAGES IN ORAL SQUAMOUS CELL CARCINOMA

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Introduction: Oral squamous cell carcinoma (OSCC) presents high rates of aggressiveness and metastasis. The oncofetal protein glypican-3 (GPC3) is expressed in diverse malignancies, however little is known about GPC3 in oral cancer. GPC3-positive staining in tumor cell membrane of hepatocellular carcinoma was associated with recruitment of macrophages, immune components with paradoxal roles in tumor microenvironment. Purpose: In this study, we aim to investigate in OSCC the expression pattern of miRNAS considered as GPC3 regulators in relation to tumoral (mRNA levels and immunohistochemical) expression as well as M2 macrophages infiltration. **Methods and Results**: The miR-219a-5p, miR-520c-3p, miR-1271a and miR-4510 expression will be analyzed in 40 paraffin embedded OSCC. In addition, GPC3 expression (cytoplasm and membrane) as well as CD68+ and CD206+ macrophages infiltration will be semi-quantitatively evaluated. Performing qRT-PCR in 20 fresh OSCC samples, 14 Adjacent Non-Tumor Tissues (NAT) and 05 Distant Normal Tissues, our group previously found deregulated expression of GPC3 in 70% of the evaluated OSCC (upregulated in 20% and downregulated in 50% of cases). The GPC3 mRNA expression levels was significantly decreased in OSCC in comparison to NAT (P= 0.009). Immunostaining in 30 OSCC demonstrated heterogeneous distribution of CD206+ macrophages in intratumoral (IT), periparenchymal (PP) and intraparenchymal (IP) compartments. The CD206+ macrophages were mainly distributed in both IT and PP regions, presenting scarce infiltrating in IP compartment. **Conclusion**: Our partial results suggest that GPC3 acts in OSCC preferentially as a tumor suppressor gene in late stages of oral carcinogenesis. In addition, corroborate with previous studies that attribute to CD206+ macrophages protumoral functions in peritumoral region.

Keywords: oral squamous cell carcinoma, Glypican-3, Macrophages, miRNA, IHQ, qRT-PCR

Support: CNPq, FAPESB, PIBIC-UFBA

ACUTE MYELOID LEUKEMIA IN THE STATE OF BAHIA — EXPERIENCE OF 5 YEARS IN A DIAGNOSTIC REFERENCE CENTER

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Introduction: Acute Leukemia (LA) may be from the lymphoid lineage, Acute Lymphoblastic Leukemia (ALL) or myeloid, Acute Myeloid Leukemia (AML). In childhood, ALL predominates (85% of cases) and in adults, AML corresponds to 80% of cases. AML is characterized by both the predominant presence of immature forms with variable and incomplete maturation or the loss of normal hematopoiesis. Acute Myeloid Leukemia is diagnosed when at least 20% immature myeloid cells forms are detected in the blood or bone marrow samples. Immunophenotyping by Flow Cytometry is the mainly technique to determine leukemia diagnosis through monoclonal antibodies profile determination. Pathological or normal cells differentiations are determined by phenotypic patterns that presents aberrant forms and genetic abnormalities. The aim of this study is to characterize Acute Myeloid Leukemia profile of patients from a reference center from Bahia. **Methods and Results:** The study presents a retrospective character for data analysis obtained from a reference diagnosis center of the Federal University of Bahia from 2014 to 2018. Patients register and immunophenotyping reports are being used to evaluate demographic and laboratory data. Variables as gender, age, diagnosis time, clinical data and phenotypic profile are being considered to obtained statistical results. Were reported in the last 5 years a total of 454 new cases of AML in addition to 25.5% of monocytic profile and 17.2% of Promyelocytic Acute Leukemia. Diagnosis average age was lower than World Health Organization data with prevalence to female. Leukocytes subsets range presented a variation to 1.400 and 592.000 cells/mm³. Aberrant cells membrane molecules CD7, CD56, CD2 and CD19 presented higher prevalence. **Conclusion:** The Acute Myeloid Leukemia cases diagnosed in Bahia presents demography and biological characteristics specific and no similar with data published by World Health Organization.

Keywords: Acute Myeloblastic Leukemia, Immunophenotyping, Leukemia

Support: Labimuno

UNBALANCED PRODUCTION OF METALLOPROTEINASE-9 AND ITS INHIBITORS TIMP3 AND TIMP4 IS ASSOCIATED WITH MYELOPATHY DEVELOPMENT IN HTLV-1 INFECTION

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Introduction: Myelopathy / tropical spastic pareparesis (HAM/TSP), one of the diseases associated with HTLV-1, is characterized by demyelination of the Central Nervous System (CNS). Cells infected by the virus, present in the blood, migrate through the blood-brain barrier to the CNS, causing local inflammation. These infected cells are capable of producing metalloproteinases (MMPs) that may affect the integrity of the blood-brain barrier. The TIMPs are inhibitors of MMPs and their presence are important in order to maintain the integrity of the barrier. The aim of this study is to evaluate the production of MMPs and TIMPs and associate their presence with the development of HAM/TSP in HTLV-1-infected patients. **Methods and Results:** The levels of MMP-3, MMP9, TIMP1, TIMP3 and TIMP4 were determined in serum, cerebral spinal fluid (CSF) and peripheral blood mononuclear cells (PBMC) culture supernatants, by ELISA. The levels of MMP9 and the ratio MMP9/TIMP3 were increased in PBMC supernatants from patients with HAM/TSP. Low levels of TIMP4 in serum from probable and HAM/TSP patients was observed, and the ratio MMP9/TIMP4 was increased in HAM/TSP patients. **Conclusion:** Based on the preliminary data, unbalanced MMP9, TIMP3 and TIMP4 production is a characteristic of HAM/TSP patients, suggesting the contribution of MMP9 to the pathogenesis of HAM/TSP and indicating MMP9 as a possible immunotherapy target to prevent the development of HAM/TSP.

Keywords: HTLV-1, MMPs, TIMPs
Support**:** NIH (AI079238; K24078884); FAPESB

EFFECT OF *Schistosoma mansoni* Sm29 ANTIGEN ON EXPRESSION AND ACTIVATION OF LIPID INFLAMMATORY MEDIATOR PRODUCERS IN ASTHMA

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Introduction: Sm29 is a membrane-bound glycoprotein of *Schistosoma mansoni* that exhibits immunoregulatory properties and therapeutic potential in allergic diseases, such as asthma. *In vitro* and *in vivo* studies have shown that this molecule has the ability to regulate the immune response in asthma and that this regulation appears to be dependent on IL-10. As lipid mediators are important molecules involved in the asthma mechanism, we will focus on the possible role of Sm29 antigen in regulate the expression of this mediators by the expression of sm29-specific IL-10. We aimed to evaluate the *in vitro* effect of *S. mansoni* Sm29 antigen on the expression of enzymes involved in the production of lipid inflammatory mediators by macrophages of patients with severe asthma, mild and moderate asthma with aspirin-exacerbated acute respiratory disease (AERD). **Methods:** The effect of recombinant antigen Sm29 on mRNA expression of the enzymes cycloxygenases 1/2 (COX1 / 2), lipoxygenases (5-LO and 12-LO) and 5-LO activator protein (FLAP) by RT-PCR will be evaluated. The effect of Sm29 antigen on the frequency of macrophage subpopulations (M1, M2a and M2c) expressing intracellular enzymes COX 1/2, 5-LO and 12-LO and intracellular expression of cycloxygenases 1/2 (COX 1/2) enzymes will also be investigated *in vitro* and 5-LO and 12-LO by flow cytometry. We also analyze the COXs and LOs activies trough PGE2 and cys-leukotriens level in the supernatant of Sm29-stimulated macrophage and lymphocyte cultures by ELISA. Finally we will verify the effect of 5-LO enzyme inhibition on the levels of asthma-associated cytokines (TNF, IL-4, IL-5, IL-13), tissue repair (TGF-β) or regulation of immune response (IL-10). **Expected Results:** The use of rSm29 antigen, as proposed in this study, as a potential modulator of lipid mediators produced by macrophages in asthma, and may help in the development of new therapeutic strategies for the control and treatment of this disease.

Keywords: asthma, Sm29, macrophages, lipid inflammatory mediators

Support: CAPES, FAPESB, INCT-DT

LINHA 6: Neuroimunoendocrinologia

INVESTIGATION OF THE ROLE OF GLIAL GLUCOCORTICOID RECEPTOR IN THE CONTEXT OF AGATHISFLAVONE SIGNALING IN AN *in vitro* MODEL OF NEUROINFLAMMATION

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Introduction: Oxidative stress and neuroinflammation are characteristic processes of neurodegenerative diseases and result from the release of proinflammatory molecules and the production of reactive oxygen species by glial cells. The co-occurrence of chronic dysregulation of the inflammatory and stress responses in neurodegenerative and psychiatric disorders suggests the participation of the glucocorticoid receptor (GR) in etiological mechanisms of neurodegeneration. Flavonoids are polyphenolic compounds extracted from plants, which have been shown to have neuroprotective, anti-inflammatory, antioxidant, anti-apoptotic and immunomodulatory actions in in vitro and in vivo studies. The biflavonoid agathisflavone is a dimer of the flavone apigenin, with neurogenic, neuroprotective and anti-inflammatory action demonstrated in *in vitro* models associated with estrogen and retinoid receptors. However, the mechanisms by which agathisflavone exerts its effects are still poorly understood. The aim of this study was to investigate whether the anti-inflammatory action of agathisflavone is mediated by GR. **Methods and Results:** An in vitro model of lipopolysaccharide-induced neuroinflammation (LPS; 1 µg / mL) was used in primary culture of cortical astrocytes and microglia obtained from neonatal rats (P0-P2). After 15 days in culture, the cells were treated with or without LPS, with agathisflavone (1 µM), and in the presence or not of mifepristone (1 µM), a GR antagonist. Glial reactivity, an indicator of the inflammatory profile, was evaluated by immunocytochemistry for GFAP and for Iba-1, exclusive proteins of astrocytes and microglia, respectively. Astrocyte proliferation and relative GFAP expression increased in response to LPS treatment, but not in response to co-treatment with agathisflavone, neither in the presence or absence of mifepristone. Microglial proliferation increased in response to LPS treatment and was reduced to control levels when LPS was treated with agathisflavone flavonoid-associated LPS treatment, which was prevented in the presence of RU486. **Conclusion:** These results suggest that agathisflavone modulates microglial proliferation after inflammatory stimulation through GR signaling and contribute to elucidate the mechanisms by which agathisflavone exerts its anti-inflammatory activity.

Keywords: agathisflavone, microglia, glucocorticoid receptor

Support: CAPES (Edital Geral de Cooperação Internacional — PGCI CAPES/2015), FAPESB (Edital de Cooperação Internacional FAPESB/2015, University of Portsmouth), INCT

INVESTIGATION OF THE EFFECT OF AGATHISFLAVONE FLAVONOID ON NRLP3-INFLAMOSOME MODULATION, MICRO-RNAS AND PROINFLAMMATORY CYTOKINES ASSOCIATED WITH GLIAL RESPONSE TO NEUROINFLAMATION

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Introduction: Alzheimer's disease (AD), the most common neurodegenerative disorder in the world, is characterized by the accumulation of β-amyloid protein (Aβ) in the brain parenchyma, formation of neurofibrillary tangles, glial activation and consequent production of inflammatory mediators such as NO and pro-inflammatory cytokines such as interleukin 1β (IL-1β), interleukin 6 (IL-6) and tumor necrosis factor-α (TNF-α), which eventually contribute to neuronal toxicity. Flavonoids, polyphenolic compounds present in a wide variety of plants, have potent anti-inflammatory and antioxidant properties. Here, the anti-inflammatory potential of the flavonoid agathisflavone (FAB), which is derived from the Brazilian plant *Poincianella pyramidalis*, will be evaluated, using the lipopolysaccharide (LPS), IL-1β and Aβ o*ligomers as* in vitro models of neuroinflammation. **Methods and Results:** cultures of microglia and glial cells have been obtained from the cortex of newborn Wistar rats. After 21 days the cell cultures are exposed for 24h to LPS (1µg / mL) or IL-1β (10ng / mL) or for 4h to Aβ oligomers (500 nM) and then treated with agathisflavone (1µM or 10µM) for an additional 24h. After inflammatory stimulation and treatment, immunocytochemical analysis will be performed to evaluate the microglial activation profile and the expression of inflammatory markers. The immunomodulatory effect of agathisflavone on the expression of proinflammatory cytokines (IL1β, TNF-α, INF, IL-2 and IL-6) will be analyzed by ELISA and RT-qPCR. In addition, the immunomodulatory effect on the expression of the NLRP3 inflamosome complex and miR-155 and miR-146a microRNAs associated with regulation of the inflammatory process will be also evaluated by RT-qPCR. At the moment, microglia culture was performed and inflammatory stimulus was made with LPS. Then the cultures were treated with agathisflavone. Cultures were grown on different culture plates for immunocytochemical labeling and RNA extraction. Culture supernatants were frozen for further analysis by ELISA. In preliminary analysis by phase contrast microscopy, we observed that the treatment of microglia cultures with the injurious stimulus (LPS at 1µg/mL), there was an increase in the cell population with amoeboid morphology, indicative of microglial activation. However, in cultures treated with 1µM and 10µM agathisflavone, we observed an increase in activated microglia, but more often in branched-looking phenotype, an indicative of less reactive cells. In culture treated with LPS (1µg/mL) and with 1µM agathisflavone, with the proportion of microglia with amoeboid morphology was similar to cultures exposed to LPS. On the other hand, treatment with 10µM agathisflavone associated to LPS inflammatory stimulus induced increase in the proportion of microglia with branched morphology and inactivated profile. Therefore, 10µM FAB is likely to be more effective in protecting cells against inflammatory stimuli caused by LPS. **Conclusion:** The evidence found shows the potential of the flavonoid to assist in modulating a microglial response, demonstrating that it may exert a neuroprotective effect in vitro, which may be considered as a candidate to contribute as an adjuvant in the treatment of neurodegenerative diseases.

Keywords: Alzheimer Disease, Neuroinflammation, Anti-inflammatory, Flavonoids

Support: CAPES

INVESTIGATION OF THE ROLE OF THE MICROGLIAL RESPONSE ASSOCIATED WITH THE NEUROPROTECTIVE EFFECT OF *Amburana cearensis*-DERIVED COMPOUNDS IN AN *in vitro* MODEL OF NEUROINFLAMMATION

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Introduction: In the central nervous system (CNS) astrocytes and microglia have fundamental roles in the control of homeostasis, fundamental for the functioning of neuronal cells; they are considered protagonists in the inflammatory processes triggered in, and their phenotypic polarization is directly involved in the progression of neuroinflammation associated to CNS diseases. Prospection of drugs of plant origin that act in neuroprotection mechanisms has grown. *Amburana cearensis*, an endemic plant from the Caatinga in Northeastern Brazil, with several applications in the Popular Medicine, including as anti-inflammatory and antioxidant. Previous in vitro studies demonstrated that extracts obtained from *A. cearensis* seeds have neuroprotective effect against glutamate induced excitotoxicity in cerebellar cultures, one of the main features of CNS damages, an effect associated with modulation of astrocyte reactivity, and attributed to coumarins and methyl esters compounds mainly present in the dichloromethane extract (EDAC). However, the effects of isolated compounds, as well as the mechanisms of modulation of the glial inflammatory response have not been clarified yet. In this sense, the present project aims to investigate the mechanisms of microglial response associated with the neuroprotective effect of the *A. cearensis* EDAC and a purified coumarin, against glutamate excitotoxicity. **Methods and Results:** For this, cerebellar cells from Wistar rats (6- 8 days) will be cultured in supplemented DMEM/HAM-F12. After 3 days culture in vitro, cells will be maintained in control conditions (DMSO 0.01%) or submitted to glutamate excitotoxic damage (10 mg/mL) for 4 h, and them treated or not with EDAC (1-10 µg/mL), or with purified coumarin (1 and 10 µM) for 24 hours. After treatments, neuronal integrity will be evaluated by with Fluoro-Jade B reagent and immunocytochemistry (ICQ) for the cytosqueletal protein β-tubulin III, and microglial morphology and reactivity will be analyzed by ICQ for Iba-1, CD68 and CD206 proteins. Moreover, the cells' RNA will be extracted and analyzed by RT-qPCR for expression of M1/M2 inflammatory profile markers TNF, IL1 β, IL-6, IL10, TGFβ and arginase. **Conclusion:** Results obtained could provide elucidation of the neuroprotective mechanisms and association with the modulation of glial inflammatory response of *A. cearensis* compounds, providing important information for the further use of these substances in the prophylaxis and treatment of neurodegenerative diseases, in particular related to the modulation of the inflammatory response in the CNS.

Keywords: neuroinflammation, *Amburana cearensis*, neuroprotection

Support: FAPESB, CAPES, CNPq/INCT—EN

CHARACTERIZATION OF GLIAL RESPONSE TO NICOTINE IN THE AMINOCHROME PARKINSON'S DISEASE STUDY MODEL

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Introduction: Parkinson's disease (PD) is a neurodegenerative disorder that affects brain tissue, especially midbrain dopaminergic neurons. Because of the low levels of dopamine, PD is clinically characterized by motor symptoms such as dyskinesia, muscle stiffness, posture instability and tremors at rest, which appear after years of degenerative processes and are preceded by non-motor symptoms such as olfactory and mood disorders. Studies have been suggested aminochrome as an endogenous neurotoxin responsible for the dopaminergic neurons degeneration in PD. It is a natural molecule in dopaminergic neurons derived from dopamine oxidation that has been used as inductor of PD study model. On the other hand, studies have been demonstrated that nicotine protects neuronal cells against aminochrome-toxicity. The aim of this study is to evaluate the effect of nicotine on glial cell response in models of Parkinson's disease induced by aminochrome. **Methods**: primary microglia cultures will be obtained from neonatal rats (0-2 days) cortex. Primary cultures of mesencephalic neurons/ glial cells will be obtained from wistar rat embryos (15 — 16 days). Primary cultures will be treated with aminochrome and/ or nicotine for a period of 24 h, then the cultures will be analyzed by immunocytochemistry for Tyrosine Hydroxylase, Iba-1, OX-42 and GFAP. Morphological analyzes will be assessed by confocal microscopy followed by qualification of intracellular contacts. Analysis of DT-diaphorase, NRF2, CASP 3 and MAPk will be performed by Wetern Blotting. Anti-inflammatory and proinflammatory cytokines will be analyzed by qPCR. **Expected Results**: At the end of the analysis, we expect characterize the effect of nicotine in glial cells and mechanism involved in its protective effect against aminochrome.

Keywords: Nicotine, aminochrome, midbrain, NRF2

Support: FAPESB, CAPES, CNPQ

NUTRITIONAL STATUS AND CYTOKINE LEVELS IN PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE

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Introduction: Chronic obstructive pulmonary disease (COPD) is characterized by airflow limitation due to changes in airways and / or alveoli, caused by inhalation of harmful particles or gases. COPD patients have chronic inflammation in lungs, with systemic repercussion, that leads to the increase of the levels of inflammatory cytokines. Chronic low-grade inflammation is observed in obese individuals also. The aim of study was to analyse the nutritional status of COPD patients and to investigate possible differences in cytokine profile. **Methods and Results:** The study was performed at the Pulmonology Department of University Hospital, Edgard Santos. Eighty individuals were included

in study: 40 COPD patients (COPD) and 40 control group (CTL), which were matched by sex and age (+ or — 1 year). Nutritional status was calculated according to the following formula: Body mass index (BMI) = weight (kg) / height^2 **(m^2)**. COPD patients and CTLs were classified in: underweight (UW), normal weight (NW) and overweight/obesity (OW/OB), according to the WHO classification. The cytokines IL-6, IL-8, IL-10, IL-12 and TNF were quantified by the flow cytometry technique using commercial kits (BD Biosciences- CBA KIT- HU INFLAMMATION). All statistical tests were performed using GraphPad software version 5.0 (GraphPad Software Inc., San Diego, CA, USA). COPD and CTL groups were compared by Mann–Whitney test and UW, NW and OW/OB by one-way ANOVA. The classification of COPD by nutritional status was: UW 10%, NW 40%, OW/OB 50%. This distribution was not similar to the distribution in CTLs: UW 0%, NW 31,25%, OW/OB 68,75%. Interleukin 12 and TNF were more elevated in OW/OB patients (IL-12: 11.17, 9.65-14.44; TNF: 15.87, 11.11-20.47) than NW (IL-12: 9.65, 8.45-11.96, P= 0,04; TNF: 11.89, 5.15-14.64, P= 0,05). The levels of IL-6, IL-8 and IL-10 were similar in EW, NW and OW/OB groups. **Conclusion:** In COPD, the nutritional status could be related to the differences in the cytokine profile, since OW/OB group exhibit an elevation of inflammatory cytokine levels.

Keywords: COPD, Nutritional status, BMI, cytokine

Support: Fundação de Amparo à Pesquisa do Estado da Bahia (FAPESB)

CHARACTERIZATION OF AMINOCHROME-INDUCED NEUROINFLAMMATION AND DOPAMINERGIC DEGENERATION IN AN *IN VIVO* MODEL OF PARKINSON'S DISEASE AND THE NEUROPROTECTIVE EFFECTS OF QUERCETIN-3-O-RUTINOSIDE.

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Introduction: Parkinson's disease is a multifactorial neurodegenerative disorder that involves the deregulation of various cellular mechanisms that result in loss of dopaminergic neurons in the substantia nigra pars compacta, however the mechanisms responsible for the neurodegeneration remain unknown. Studies have been suggested aminochrome as an endogenous neurotoxin responsible for the dopaminergic neurons degeneration. The aim of this study was to determine glial reactivity induced by aminochrome in an *in vivo* PD model and to explore the protective action of flavonoid rutin in this model. **Methods and Results:** Experimental procedures were sanctioned by the local animal research ethics committee (protocol CEUA –ICS-UFBA- nº. 011/ 2017). Wistar rats (male, 250-270g) were divided. in 6 groups (1: saline control, 2: rutin 10 mg/ kg, 3: 6-hydroxydopamine 21µg/ µL; 4: aminochrome 1,000µM; 5: 6-hydroxydopamine (6-OHDA) + rutin and 6: aminochrome + rutin). The animals were orally dosed with rutina 30 minutes before the stereotaxic injection of aminochrome or 6-OHDA in the striatum and daily orally treated with rutin until the 14th experimental day. After euthanasia, the brains were fixed in 4% PFA, slices were performed in microtome and immunohistochemical analyzes was performed for IBA-1, CD68, GFAP, S100ß and Tyrosine hydroxylase (TH) in the striatum and SNpc. We observed that the aminochrome and 6-OHDA were able to generate increase in the total number of microglia, as well as to increase the quantity of activated microglia evidenced by co-localized IBA-1$^+$/ CD68$^+$ cells. It was also observed that astrocyte activation marked by an increase of co-localized GFAP$^+$/ S100ß$^+$ expression. Dopaminergic neuronal (TH$^+$ cells) loss was also observed in the aminochrome or 6-OHDA-treated groups. On the other hand, rutin presented neuroprotective effect by inhibition effects of aminochrome or 6-OHDA in terms of microglial activation, astrogliosis and dopaminergic neuronal loss. **Conclusion**: rutin inhibits glial activation and protects dopaminergic neurons against 6-OHDA and aminochrome cytotoxicity. This is the first evidence of glial response in an *in vivo* model of PD induced by aminochrome. These results improve a new animal model, suggested as a more

physiological model of PD and contribute to a development of a new therapeutic agent against neurodegeneration.

Keywords: Parkinson's disease, dopamine, neuroprotection, flavonoid.

Support: FAPESB, CAPES, CNPQ

IMPAIRED GASTROINTESTINAL FUNCTION IN EXPERIMENTAL MODEL OF PARKINSON'S DISEASE IN RATS

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Introduction: Parkinson's disease (PD) is the second most important neurodegenerative disorder after Alzheimer's disease and the most prevalent motor disorder. In addition to motor problems, the quality of life of patients with Parkinson's disease is severely impaired by a variety of non-motor symptoms, including gastrointestinal dysfunction. The enteric nervous system (ENS) is responsible for controlling intestinal motility. The involvement of ENS and the gut has been shown to be important in the pathophysiology of PD. We aimed to evaluate the gastrointestinal transit, stool water content and myenteric plexus of the jejunum of rats in a PD model induced by intracranial injection of 6-hydroxydopamine (6-OHDA). **Methods and Results:** Twelve male Wistar rats weighing 250 — 300g were anesthetized and submitted to unilateral nigrostriatal injection of 6-OHDA (21 µg) or Saline (0.9%). Rats were also submitted to behavioral tests (Open-field test, Cylinder test and Rotation test induced by apomorphine [3 mg/kg, i.p.]). Gastrointestinal transit time was evaluated, before euthanasia, by gavage of 0.5 mL of a non-absorbable marker 6% Carmine Red. The fecal pellets were collected, counted and weighed every 3 hours, for 12 hours. Total fecal matter was dried overnight at 60 ° C and total moisture was analyzed. After the trial period, the rats were anaesthetized with ketamine and xylazine intraperitoneally. After, the jejunum was collected, washed with 0.1 M phosphate buffered saline pH 7.2 (PBS), carefully filled with paraformaldehyde fixative solution, and stored in PBS with 0.08% of azide. Performing of the immunohistochemical techniques: The jejunum was microdissected to obtain the tunica muscularis. The enteric nervous system evaluation was performed by analyses of neurons enteric in the myenteric plexus. The immunohistochemical markers used were HuC/D (1:800, Molecular Probes) and β-S100 (1:800, Molecular Probes), respectively. Production of extracellular matrix (collagen) components was determined. Intestinal follow-ups of approximately 1 cm were collected, macerated and suspended in Sirius Red dye solution (Merck, USA) and subsequently quantified by spectrophotometer at 540 nm. The 6-OHDA group showed decreased locomotor activity in the Open-field test (30.0 ± 5.1) compared to the control group (54.2 ± 2.9), also showing reduced performance on the cylinder test. In the rotational test, the 6-OHDA group increased the number of rotations in comparison with the control group ($p<0.05$). The rats in the 6-OHDA group exhibited a significant decrease in fecal water content (22%), reduced fecal yield and delay in gastrointestinal transit time of approximately 2h when compared to the control group. Immunofluorescence demonstrated that there was an increase over 25% in HuC/D and glia protein expression in the jejunum myenteric plexus of the rats of the 6-OHDA lesion group when compared to the control. Collagen dosing results showed that the jejunal concentration increased slightly while the other segments (duodenum, jejunum and ileum) demonstrated collagen reduction thus exhibiting abnormal healing characteristics. **Conclusion:** The results showed that the rats submitted to the 6OHDA lesion, in addition to presenting motor deficit, presented altered intestinal transit, with reduction of gastrointestinal motility, reduction of pellets and fecal moisture. These data suggest that this model is adequate to evaluate the participation of ENS in the pathophysiology of PD.

Key words: Parkinson's Disease, Enteric Nervous System, Enteric Glia

Support: CAPES, CNPq, FAPESB

CHARACTERIZATION OF NEUROINFLAMMATORY RESPONSE IN DIFFERENT *in vitro* MODELS OF CNS CELL CULTURES

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Introduction: Central Nervous System (CNS) is composed of neurons and glial cells, such as astrocytes, microglias, ependydial cells, NG2 cells and oligodendrocytes. Interactions among these cell groups are essential for their development and homeostasis maintenance**.** Astrocytes and microglia are the main immune cells of the CNS, and astrocytes are responsible for homeostasis, detoxification, nutrition and the performance of small synapses. Astrocytes may acquire inflammatory response profile A1 (cytotoxic) or A2 (neuroprotective), whereas microglia, the main defensive cell of the CNS, acts as macrophages, secretting pro and anti-inflammatory cytokines, assuming a profile M1 (proinflammatory) or M2 (regulatory). Neuroinflammation is an inflammatory disorder in the CNS, it can lead to neuronal degeneration and death, is present in Parkinson's disease (PD), Alzheimer's disease (AD), Multiple Sclerosis (MS), among other neurodegenerative diseases. Study models that more reliably characterize neuroinflammation are essential for a better understanding of the processes involved in this pathogenesis. **Methods and Results**: In this study, different protocols will be adopted to characterize neuroinflammation in *in vitro* models: 1. two protocols of co-culture of cortical neurons and glial cells (protocol AI de Silva et al., 2013 and protocol A II by Mecha et al., 2011) and 2. two protocols of primary culture of cerebellar cells (protocol BI of Pereira et al., 2017 and protocol B II adapted from Pereira et al., 2017). The cells will be cultured in supplemented DMEM-HAMF12 medium, kept in a greenhouse with 5% CO2 at 37° C. The neuroinflammatory induction will be assessed by *Escherichia coli* lipopolysaccharide (LPS) treatment. Subsequently, neuronal viability analyzes will be assessed by Fluoro-Jade B; and morphological analyses and glial cell response will be assessed by immunocytochemistry for β-TUBIII, GFAP and Iba1 and qPCR for mRNA for inflammatory factors. **Conclusion**: As future perspectives**,** we expect to choose the best *in vitro* model to characterize cerebral and cerebellar neuroinflammation.

Support: FAPESB, CAPES, CNPq

Keyword: neuroinflammation, experimental models, glial cells

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