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IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

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ABSENCE CCR4 CONFERS RESISTANCE TO TOXOPLASMA GONDII INFECTION

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Introduction and Objective: The oral infection with Toxoplasma gondii (T. gondii) induces an intense intestinal inflammation in C57BL/6 mice, which succumb to infection due to the exacerbated Th1 response. Moreover, chemokines produced by intestinal epithelial cells are involved in the migration and activation of inflammatory cells. In this work, we evaluated the role of CCR4 chemokine receptor during experimental toxoplasmosis. **Methods and Results:** For this, resistant (BALB/c), susceptible (C57BL/6-WT), and C57BL/6-CCR4$^{-/-}$ mice were orally infected with 10 cysts T. gondii (strain ME49) and the survival was monitored daily. We verified that CCR4$^{-/-}$ mice are highly resistant to infection similar the BALB/c mice, whereas the majority of C57BL/6 mice died at acute phase. To explore why the absence of CCR4 confers resistance to the host, we evaluated the parasite burden by qPCR. CCR4$^{-/-}$ mice presented significant reduction parasite burden in small intestine and liver in the 10 days post-infection than C57BL/6 mice. To verify the immune response pattern induced by T. gondii infection in the absence of CCR4, the spleen and mesenteric lymph node (MLN) were collected at 10 days post-infection, CCR4$^{-/-}$ mice showed lower activation of CD4^{+} T lymphocytes compared to C57BL/6 mice. Moreover, we observed that CCR4$^{-/-}$ and C57BL/6 mice presented similar frequency of Th1 cells (CD3^{+}CD4^{+}IFN-gamma^{+}), however the CCR4$^{-/-}$ mice showed an increased frequency of Th17 cells (CD3^{+}CD4^{+}IL-17^{+}) compared to C57BL/6 mice. On contrary to C57BL/6 mice that presented decreased regulatory T cells (Tregs - CD4^{+}Foxp3^{+}), the CCR4$^{-/-}$ and BALB/c mice exhibited an accumulation these cells in the MLN after infection by T. gondii. **Conclusion:** Taken together, these results suggest that in the absence of CCR4 confers resistance the to T. gondii infection due balance among Th1, Th17 and Treg cells elicited during inflammatory immune response by T. gondii infection.

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ABSENCE OF MICROBIOTA IMPAIRS MACROPHAGE MICROBICIDE ACTIVITY AND PRODUCTION OF NITRIC OXIDE AND REATIVE SPECIES OF OXYGEN

MATEUS EUSTÁQUIO DE MOURA LOPES; MATHEUS BATISTA HEITOR CARNEIRO; LILIANE MARTINS DOS SANTOS; LEONARDO GOMES VAZ; FLAVIANO DOS SANTOS MARTINS; LEDA QUERCIA VIEIRA.

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Introduction: Animals are colonized by their indigenous microbiota from the early days of life. The estimated number of associated bacterial cells is around 10^{14} per individual. Several studies have investigated the microbiota-host relationship and the use of germ-free animals has been an important tool in these studies. These animals, when infected with a pathogen, have shown to be sometimes more resistant and other times more susceptible than conventional animals, as during infection by Leishmania major. Previous studies showed that in the infection by this parasite, Swiss/NIH germ-free animals developed a typical Th1 immune response, but failed to heal lesions, while conventional mice developed the same response and controlled the infection. Th1 response is clearly related in the literature with healing and parasite clearance in this infection especially due strong production of IFN-γ and consequently expression of iNOS by macrophages. **Objective**: Our aim is to evaluate a possible adjuvant effect of the microbiota in the microbicidal activity (production of $^{\bullet}$NO and ROS) of the macrophages, since these are the cells that kill the parasite. **Methods:** We stimulated bone marrow-derived macrophages from germ-free and conventional Swiss/NIH animals with LPS+IFN-γ (classic activation), IL-4 (alternative activation). Macrophages were also infected in vitro with L .major and L. amazonensis amastigotes. The production of $^{\bullet}$NO, ROS and inflammatory cytokines (TNF-α, IL-12) were assessed. Furthermore, we analyzed the expression of iNOS, arginase I and FIZZ1 to better understand the phenotype of activation of these cells. **Results:** Our results showed that macrophages from germ-free animals produced less $^{\bullet}$NO and ROS and showed higher arginase activity. Furthermore, they showed lesser production of TNF-α and IL-12 and a higher expression of FIZZ1. These data suggest that macrophages from germ-free mice have impairment in microbicidal activity which can be related with the fact that in vivo these mice are not able to control infection with L. major.

Support: CNPq, CAPES, FAPEMIG, INCT REDOXOMA

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AEROBIC AND RESISTANCE EXERCISES INTERFERE IN ANTI-TOXOPLASMA GONDII ANTIBODY PROFILE OF PRE-TRAINING C57BL/6 MICE IN A MODEL OF EXPERIMENTAL TOXOPLASMOSIS.

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LABORATORY OF IMMUNOPARASITOLOGY, INSTITUTE OF BIOMEDICAL SCIENCES, FEDERAL UNIVERSITY OF UBERLÂNDIA, UBERLÂNDIA - MG - BRASIL.

Introduction: Regular physical exercise has been implicated to improve general human health and to decrease susceptibility against many diseases, including those triggered by protozoan parasites. Objectives: The aim of this work was to investigate whether pre-training could improve the susceptibility to Toxoplasma gondii by determining the antibody profile from IgM, IgGtotal, IgG1 and IgG2a isotypes produced by C57Bl/6 mice. Methods and Results: A total of 30 male (23.7±1.09g) were used for this study. The animals were allocated in five groups (6 per group), as follows: i) non infected sedentary (NIS), ii) infected sedentary (IS), iii) non-infected exercised (NIEx), iv) infected exercised (IEx), v) infected exercised that stopped after 30 days infection (IEx+30). The animals were housed in an animal facility with a 12 h light/dark cycle (the lights were switched off at 7:00 a.m.) maintained at controlled temperature (22-24°C) with food and water provided ad libitum. The 6 week-old mice started exercising (NIEx, IEx and IEx+30) and two groups (NIEx and IEx) stopped 6.5 weeks later, then three groups were infected (IS, IEx and IEx+30) with 5 cists of T. gondii and 30 days later all five groups were euthanatized and blood was collected. We found no significant differences among all groups and negative control. We did a similar 5 cists infection in 6 week-old C57Bl/6 mice and we found a significant different using ELISA index among infected to non-infected and negative control. These data suggest that it is possible to increase the amount of cists when infection is carried out in older mice. In addition, we could observe that 50% of mice from the IEx+30 group and 16.7% from IEx presented up to 50% of IgG2a ELISA index when compared to all other groups of animal. Conclusion: As IgG2a is a characteristic antibody isotype induced by Th1 immune response in murine model, the most significant mechanism to control T. gondii infection, it can be concluded that the exercise it is important to improve protective immune response in this model, when compared to sedentary infected animals. Financial support: FAPEMIG, PROPP-UFU, CAPES.

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ALANYL-GLUTAMINE EFFECTS ON INTESTINAL CELL PROLIFERATION AFTER ACUTE INFECTION WITH ENTEROAGGREGATIVE ESCHERICHIA COLI

ANA KAROLINA SILVA DOS SANTOS; MARA DE MOURA GONDIM PRATA; PEDRO HENRIQUE QUINTELA SOARES DE MEDEIROS; ANTONIO VINICIOS ALVES DA SILVA; PALOMA ARAUJO CAVALCANTE; ROSA ELAYNE MARQUES DE FREITAS; TAMARA SILVA RODRIGUES; CASSIA RODRIGUES ROQUE; ILA FERNANDA NUNES LIMA; JOSIANE DA SILVA QUETZ; ALDO ANGELO MOREIRA LIMA; ALEXANDRE HAVT.

FEDERAL UNIVERSITY OF CEARA (UFC), FORTALEZA - CE - BRASIL.

Introduction: Enteroaggregative Escherichia coli is a pathogen of great relevance in diarrheal diseases worldwide and it is also linked to malnutrition in children of Fortaleza-Ceara-Brazil. Mechanisms involved on diarrheal diseases as proliferation and renovation of intestinal epithelial cells may be impaired. Alanyl-glutamine plays a role on protection and immunomodulation of epithelium. This study aimed to evaluate the potential role of Ala-Gln on intestinal damage induced by EAEC, focusing on cell proliferation and innate immune response. **Methods and Results:** Rat intestinal epithelial cells (IEC-6) were cultured and infected with one of the following bacterial strains: EAEC 042 strain and EAEC wild type strain (isolated from a malnourished child). IEC-6 were seeded in 96-well plates at $2,5x10^4$ cells/well and cultured for 24 hours. Cells were then infected for 3 hours. Ala-Gln at 1mM was added to the wells and after 12, 24 and 48 hours, cell proliferation was evaluated by adding WST-1 reagent, following measurement at spectrophotometer (450nm). For mRNA analysis, cells were seeded in 12-well plates and the same infection protocol was performed, followed by mRNA extraction, cDNA synthesis and RT-qPCR. Transcription levels of TLR-5, NF-kB and IL-8 genes were quantified at 6 and 12 hours after treatment. Statistical analysis was performed with ANOVA and Bonferoni test. It was shown that antiproliferative effects induced by EAEC (for both strains) were significantly reduced with Ala-Gln treatment at 1mM ($p<0.05$). Increased transcription levels of NF-kB gene at 12h were not reverted by the treatment of Ala-Gln. TLR-5 and IL-8 transcription levels were increased after 6 hours, followed by moderate reduction after 12 hours, but not influenced in the presence of Ala-Gln. **Conclusion:** Ala-Gln supplementation promoted protection to the epithelium against damage induced by EAEC. However, the antiproliferative effect found after supplementation with Ala-Gln could not be associated with the transcription levels of IL-8 and NF-kB.

Financial support: IBISAB/CNPq.

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ANNEXIN-A1 AND GALECTIN-1 ANTI-INFLAMMATORY PROTEINS ARE CHANGED IN COLORRETAL CARCINOGENESIS?

ANA ELIZABETE SILVA[1]; LUIS OTÁVIO MAIA GONÇALVES[2]; ALAOR CAETANO[3]; WAGNER COLAIACOVO[4]; DALISIO DE SANTI NETO[5]; PATRÍCIA MATOS BISELLI-CHICOTE[6]; ÉRIKA CRISTINA PAVARINO[7]; ENY MARIA GOLONI-BERTOLLO[8]; SONIA MARIA OLIANI[9]; MAYSA SUCCI[10].

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Introduction: Colorectal cancer is one of the models of inflammation-cancer association that progresses from normal epithelium to adenoma (AD) and adenocarcinoma (ADC), and has been associated to Fusobacterium nucleatum that varies in its pathogenic and proinflammatory potential. Alterations in expression of inflammatory modulators, such as Annexin-A1 (AnxA1/ANXA1) and Galectin-1 (Gal-1/LGALS1) have been reported during carcinogenesis. The aim of this study was to evaluate the mRNA and protein expression levels of AnxA1 and Gal-1 in biopsies of AD and ADC and adjacent normal mucosa (NM); to investigate the occurrence of correlation between the expression levels of both mRNA, and association with risk factors (age, gender, smoking and drinking habits), anatomic site of lesion origin and presence of F. nucleatum. **Methods and Results:** The quantitative real-time PCR (qPCR) technique was used to quantify the mRNA levels (RQ) of ANXA1 and LGALS1 in 27 AD and 43 ADC samples, and the immunohistochemistry assay was used to characterize the protein expression in 10 AD and 15ADC. F. nucleatum were assayed by PCR in the same samples of lesions. The mRNA expression of ANXA1 was significantly increased in AD (RQ=1.11) and ADC (RQ=2.33) compared to adjacent NM, while LGALS1 showed overexpression only in ADC (RQ=1.85), with AD showing basal expression (RQ=0.90), but both genes were significantly more expressed in ADC compared to AD (ANXA1: P=0.039; LGALS1: P=0.019) and in both lesion groups it was observed positive correlation between the mRNA expression of these genes (AD: r=0.63, P=0.0004; ADC: r=0.73, P<0.0001). No association was observed between mRNA expression of these genes and age, smoking and drinking habits, anatomic site of lesion origin and presence of F. nucleatum, but in ADC group, the ANXA1 gene showed relative expression 2-fold higher in female (RQ=3.61) compared to males (RQ=1.79). The protein expression confirmed the gene expression data, with intense immunostaining in ADC and moderate in AD for AnxA1, while Gal-1 showed moderate immunostaining in epithelium and stroma in both lesions. The presence of F. nucleatum showed association with late stages of disease (P<0,0001). **Conclusion:** AnxA1 shows overexpressed in AD-ADC sequence, while Gal-1 seems overexpressed only in ADC, suggesting that these proteins are involved in anti-inflammatory pathways of sporadic colorectal carcinogenesis, with participation of F. nucleatum. F. support: FAPESP,CNPq, CAPES

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ANTI-INFLAMMATORY ACTIVITY OF EXTRACT HARPAGOPHYTUM PROCUMBENS DURING EXPERIMENTAL SCHISTOSOMIASIS MANSONI

ELISANDRA DE ALMEIDA MONTIJA; RICARDO DE OLIVEIRA CORREIA; JOICE MARGARETH DE ALMEIDA RODOLPHO; ANA CAROLINA MARAGNO FATTORI; SANDRA REGINA PEREIRA DE OLIVEIRA; PAULO CEZAR VIEIRA; FERNANDA DE FREITAS ANIBAL.

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Introduction: Schistosomiasis is a endemic disease caused by trematode of the genus Schistosoma. The inflammatory response developed is not yet completely understood, and parasite control measures are deficient, it's may lead to environmental damage and population, so the step is more efficient treatment of infected patients. Currently, the drug of choice is praziquantel (PZQ), but some studies have shown the resistance of some strains of the parasite to the routine use of this drug. Therefore, in this study we assess eosinophilia during experimental infection with S. mansoni (Sm) after treatment with extract of Harpagophytum procumbens (Hp), which it's may a potential future candidate for formulation of a compound for the treatment of schistosomiasis. **Methods and Results:** To develop this study, we used Balb/c female mice between 15-18g. Subcutaneously infected with 50 cercariae/mice, divided into 4grupos (n= 6 mices): Control (no infected mice), Infected with Sm (Sm), Infected with Sm plus PZQ 500mg/Kg with treated on 1, 42 and 43 days after infection (Sm+PZQ) and Infected with Sm and treated with of extract Hp (Sm+Hp) daily for 49 days.After the mice were euthanized on the 49th day of infection, where blood was collected by cardiac puncture, bronchoalveolar cavity lavage and peritoneal cavity lavage for perform differential cell count in these compartments. The results showed that there was a reduction in the number of eosinophils in the blood, bronchoalveolar and peritoneal cavity lavage after 49 days of infection in the Sm+Hp group when it is compared with others groups. **Conclusion:** These study suggest that the extract of Hp shows activity in modulating the immune response interferes in the eosinophils recruitment.

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ANTI-TOXOPLASMA GONDII IGG ANTIBODIES IN GOATS FROM THE SEMIARID REGION OF BAHIA STATE, BRAZIL

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Introduction: The infection by Toxoplasma gondii can result in economic losses in goat breeding, being an important cause of abortion and fetal abnormalities (Vet Parasitol. 149:25–28, 2007). Moreover, the meat and milk from contaminated animals are a source of infection to humans (Arq Bras Med Vet Zootec. 60:36-41, 2008). A previous study in Bahia has observed the frequency of 41.97% (n=274) and 7.27% (n=165) seropositive goats by the latex agglutination test, respectively, in regions of humid and dry climate (Vet Parasitol. 82:273–276, 1999). The aim of this study was to determine the occurrence of antibodies against T. gondii in goats of Bahia state, Brazil, by ELISA test.

Methods and Results: Blood was collected from crossbreds adults goats, males and females, in Juazeiro, Casa Nova and Jaguarari (50 animals in each one), cities located in a semi-arid region of Bahia state, Brasil, in 2012. The levels of anti-T. gondii IgG antibodies was verified in the serum of each animal, by indirect ELISA (Comp Immunol Microbiol Infect Dis. 24:197-206, 2001). Microtitre plates were coated with 100µL well of an T.gondii antigen solution (0,1 g/dL of total protein), diluted at 1:100 in carbonate buffer pH 9.6 by incubating at 4 ºC for 24 h. Afterwards, 100 µL of the serum samples (1:100 diluted in PBS with 0.05% Tween 20 and 0.25% defatted powdered milk) were distributed in duplicates and incubated for 1 h at 37 ºC.

After washing, it was added 100µL per well of anti-Goat IgG whole molecule-peroxidase (1:5000 diluted in the same previous solution), the plates were incubated for 45 minutes at 37 ºC and washed again. Finally, it was added 100 µL/well of substrate (40mg of o-phenylendyamine in 100mL of citrate phosphate buffer pH 5.6 and 150µL of H_2O_2) and incubated for 20 min. The reaction was stopped with 50 µl/well of 0.5M H_2SO_4 and the optical density (OD) was measured at a wavelength of 492nm. The procedures were approved by the Ethics Committee for the Use of Animals of UFBA. In Jaguarari, 2 seropositive animals (4%) for T. gondii were found, however, anyone was found either in Juazeiro or Casa Nova. Among 150 samples, it was observed a frequency of 1.3% positives.

Conclusion: There was a low frequency of positive animals for T. gondii in the samples. Nevertheless, it is necessary to investigate the importance of T. gondii and other parasitic zoonoses in domestic animals.

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ARTIFICIAL IMMUNE SYSTEM: PROSPECTS FOR RESEARCH IN PLASMODIUM FALCIPARUM MALARIA

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UNIVERSIDADE FEDERAL DE VIÇOSA, VIÇOSA - MG - BRASIL.

INTRODUCTION: Malaria is a serious public health problem in the contemporary world. Cases of severe malaria are related to infection by protozoa of the Plasmodium falciparum specie, although there is an increase in the number of cases of infection by Plasmodium vivax. The main consequences of severe malaria are: metabolic acidosis, acute renal failure, shock, pulmonary dysfunction, and hypoglycemia. Increasingly, the concern with this neglected disease is growing in a globalized world, making relevant the quest for new forms of research. In this context, the possibility of alliance between biology, medicine, and computer science makes experiments in silico an effective way to research on malaria. Thus, the objective of this abstract is to present a system for computational modeling of the immune system (IS) for performing in silico experiments in Plasmodium falciparum malaria (PFM). **METHODS AND RESULTS**: We performed a literature review – conducted in databases of PubMed (U. S. National Library of Medicine) and SciELO (Scientific Electronic Library Online) in order to identify the previously published immune system (IS) simulations and strategies applied to the study of PMF. The performed investigations point to the use of the following models: ordinary differential equations, cellular automata, and multi-agent systems (MAS). We chose to focus on MAS, opening possibilities for the verification of hypotheses about the way in which cells and cytokines interact in the IS, as well as for the possibility of therapeutic intervention and vaccines in MPF. The literature review provided subsidies for the construction of a computational model based on MAS – denominated AutoSimmune – using the framework Repast Simphony. Models were made of: (1) agents – antigen (P. falciparum), antibody, cells; (2) zones – tissue, lymph node, circulation, bone marrow and thymus; and (3) diffusion of substances (cytokines). Initial tests performed to investigate autoimmune events showed that the model presents behavior coherent with the current biomedical literature. The requirements for the investigation of the immune response in PFM were also raised and are being implemented in AutoSimmune. **CONCLUSION**: Despite decades of research on PFM, numerous points of immunology are still unclear. In silico research will contribute to knowledge about the mechanisms of disease and the vaccine development.

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ASSOCIATION OF POLYMORPHISMS AT MBL2 PROMOTER REGION WITH HUMAN T-CELL LYMPHOTROPIC VIRUS TYPE 1/2 (HTLV-1/2) INFECTION AND CLINICAL OUTCOME

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Introduction: The HTLV infection is endemic in Brazil and can cause various clinical manifestations, including the HTLV-I associated myelopathy/tropical spastic paraparesis (HAM/TSP). However, most carriers remain asymptomatic, which may be related to individual genetic factors. Mannose binding lecting (MBL) is an innate immunity molecule and a complement system activator. It has been reported that single nucleotide polymorphisms (SNPs) at MBL2 gene could be involved with the HTLV infection. Thus, we investigated the association of the MBL2 gene SNPs at promoter and structural regions with the HTLV infection and its clinical status.

Methodology and results: MBL2 genotyping was done by Real Time PCR technique, using Taqman probes for promoter regions (-550 and -221) and SYBER GREEN chemistry for the exon 1 region. We enrolled 232 seronegative donor individuals and 150 HTLV carriers attended at HUOC/UPE, which 25 are symptomatic (HAM/TSP) and 125 are asymptomatic carriers.The analysis of the -221 region showed that genotype YY frequency was higher in all HTLV carriers (72.7%, p=0.002;OR=2.08;IC=1.28–3.38) and in asymptomatic patients only (74.4%, p=0.001;OR=2.27;IC=1.35-3.84) than in control group (56.5%), compared to variant genotypes YX+XX (27.3%, 25.6%, 43.5%, respectively). Besides, referent to -550 region, it was observed a higher frequency of LL genotype in symptomatic patients with HAM/TSP than in asymptomatic ones (68% vs. 44%) compared to variant genotypes HH+HL, whose frequency was 32% and 56%, respectively (p=0.028;OR=2.70;IC=1.01-7.44). No differences were observed for other genotypic comparisons. Also, neither allelic nor haplotype frequency were significantly different among the groups.

Conclusion: It seems that MBL serum levels caused by SNPs at MBL2 promoter regions may influence the establishment of neurological disease related to HTLV virus, and also, the susceptibility to infection. However, a larger number of individuals are necessary to better investigate these associations.

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ASSOCIATION OF THE ESTIMATED BONE MARROW PARASITE BURDEN AND PARASITEMIA WITH THE CLINICAL AND SERUM CYTOKINES OF PATIENTES WITH NEW WORLD VISCERAL LEISHMANIASIS

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Introduction: Visceral leishmaniasis (VL) is lethal but the pathogenesis of severe disease is poorly understood. Little is known about the influence of the number of parasites and the role of serum cytokines on mortality. This study was conceived to evaluate the association parasite burden as estimated by quantitative PCR (qPCR) of the agent Leishmania infantum bone marrow parasite load (BMPL) and parasitemia with disease severity and serum cytokines concentration.

Methods and results: The study included 241 patients with a confirmed diagnosis of VL. A single experienced physician prospectively and carefully examined each patient to classify according to the presence of symptoms and signs of severe disease. Bone marrow and blood DNA isolation was performed by QIAmp DNA Blood Mini Kit, according to manufacturer's instructions. qPCR was based on TaqMan probe. Specific primers based on kDNA and albumin (housekeeping gene) were used. Amplification and detection was performed in StepOne™ Real-Time PCR System (Applied Biosystems). The cytokines IL-8, IL-1β, IL-6, IL-10, TNF-α, IL-12, TGF-β and IL-17 were measured in BDFacsArray™ Immunocytometry System. Statistical analysis was performed by Mann-Whitney and t-Student test to analyzed clinical status. Correlation was evaluated using Pearson tests, been statistically significant at $p < 0.05$. The estimated amount of parasite in blood and BMPL was correlated with the positivity of the extension slide of bone marrow aspirates. Parasitemia was higher in male, age upper 18 years, HIV-1co-infected patients and in those with bleeding, death, diarrhea, epistaxis and weight loss. BMPL was higher in males, with age upper 18 years, longer time of fever, and with epistaxis, edema, altered lung and malnutrition. Parasitemia and spleen size was correlated with BMPL ($r = 0:45$; $p < 0.001$) and ($r = 0.188$; $p = 0.005$). Cytokines IL-8, IL-6 and IL-1β were correlated with clinical factors of severe leishmaniasis. IL-10 was associated with HIV-1 co-infection. TGF-β was associated with fever. IL-17 had low detection (9,8%). There was no correlation between the amount of parasites and serum cytokines studied.

Conclusion: Clinical and demographic data of severe VL are associated with the estimated amount of parasites both in blood and bone marrow. Patients with the severe form of the infection by Leishmania infantum also had higher concentrations of some cytokines, but there was no correlation between amount parasites and cytokines.

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AUTOSIMMUNE: ARTIFICIAL IMMUNE SYSTEM FOR THE STUDY OF IMMUNOLOGY OF INFECTIOUS DISEASES

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Introduction: The immune system (IS) is organized to respond to events that produce imbalances in homeostasis, presenting itself as a network of cells and substances of complementary and synergistic action. Are notorious immune responses to infectious diseases, which in most cases can be observed attempt to eliminate microbial agent. Recently, software tools have been developed to study the IS using different methods. In this communication we report the *AutoSimmune* system developed to investigate *in silico* the IS. **Methods and Results:** The *AutoSimmune* [Rev Bras Ter Intensiva 2012; 24(3): 294-301], a computational model based on the multi-agent systems (MAS), in terms of the bottom-up approach, was built using the Repast Symphony framework. The system was based on the definition of "individual aspects, relating to the agents in such a way as to permit emergence of the collective aspects" [Ann Math Artif Intell 2011; 62(1-2):27-53]. Model was made of: (1) agents – *antigen* (*bacteria, protozoan and viruses*), *antibody* and *cells* (basophils, eosinophils, lymphocyte, mast cell, mononuclear phagocytes, natural killer cells, neutrophil); (2) zones – *tissue*, *lymph node*, *circulation*, *bone marrow* and *thymus*; and (3) diffusion of substances (cytokines). Initial tests performed to investigate autoimmune and inflammatory events in infectious diseases – related to sepsis, poststreptococcal glomerulonephritis and Chagas disease – showed that the model presents behavior coherent with the current biomedical literature, especially in terms of immune specificity. **Conclusion**: The development of *in silico* models of SI – like *AutoSimmune* – may contribute to the investigation of the mechanisms involved in infectious diseases, to the extent that allow the development of hypotheses that can be tested, later, *in vitro* and in *vivo*.

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CCR4-DEFICIENT MICE INFECTED WITH MYCOBACTERIUM TUBERCULOSIS EXHIBITED DIFFERENT PATTERN OF LYMPHOCYTES, CYTOKINE AND CHEMOKINE PRODUCTION.

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Introduction: The control of M. tuberculosis infection requires granuloma formation as consequence of the leukocyte recruitment, partially dependent on chemokine and chemokine receptor. The pattern of chemokine and chemokine receptor expression drives the recruitment of specific leukocyte populations, including lymphocyte subsets. Chemokine receptor 4 (CCR4) is mostly expressed in Th2 $CD4^+$ cells and $CD4^+Foxp3^+$ cells. The aim of this study was to assessment the participation of CCR4 in response to pulmonary infection with M. tuberculosis. **Methods and Results:** CCR4 deficient ($CCR4^{-/-}$) and wild type (WT) mice were infected with $1x10^5$ bacilli by intra-tracheal route. Seventy days post-infection, Colony-Forming Unit (CFU) number in the lung and spleen, cell subsets, cytokine and chemokine production were evaluated in the lungs of $CCR4^{-/-}$ and WT mice. At the chronic phase (70 days), $CCR4^{-/-}$ (n=15) mice were more susceptible to M. tuberculosis infection compared to WT (n=15) mice ($p<0.05$). In addition, 70-day infected $CCR4^{-/-}$ mice showed an increase in the total number of $CD4^+$ and $CD8^+$ cells, an increase in the frequency of $CD8^+$ cells, followed by a decrease in the frequency of NK and $CD4^+Foxp3^+$cells compared with WT mice. The lungs of infected $CCR4^{-/-}$ mice also exhibited a significant reduction of IL-17 and CCL-17, beyond a higher production of IFN-γ compared with those observed in infected WT mice. **Conclusion:** These data show that CCR4 interfere in the chronic phase of the infection which leads to a change in the pattern of leukocytes, cytokines and chemokines production, which is associated the resistance for tuberculosis.

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CD161 AS A NOVEL IL-17-INDEPENDENT BIOMARKER IN HUMAN CUTANEOUS LEISHMANIASIS: A COMBINED FLOW CYTOMETRY AND IN SITU TRANSCRIPTOMICS STUDY

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Th17 cells secrete IL-17 and are regulated by IL-6+TGF-beta/IL-23, RORC (IL17 master regulator) and STAT3, among other factors, being involved in the pathogenesis of many inflammatory conditions with tissue damage, such as autoimmune diseases. Although the C-type lectin CD161 has been discovered as a NKT cell marker, several groups have demonstrated CD161 as a bona fide human Th17/Tc17 marker. However, this molecule is totally unexplored in human leishmaniasis. Therefore, we quantified CD161 levels by multiparametric flow cytometry in NK, NKT, T cells as well as CD4 and CD8 subsets in 30 localized cutaneous leishmaniasis (LCL) patients and 20 healthy controls. We found that T cell CD161 levels at diagnosis were significantly correlated to posterior healing time (r=0.42, p=0.039), following standard Sb^V treatment. Hence, we performed a comprehensive in situ transcriptomic approach of the CD161/Th17/Tc17 network. Using nCounter (NanoString), we quantified >600 host and parasite RNAs at femtomolar detection level by multiplex hybridization with bar-coded fluorescent probes. RNA was extracted from skin and mucosal biopsies, respectively from patients with LCL and mucosal leishmaniasis (ML), covering the whole clinical spectrum of tegumentary leishmaniasis caused by L. braziliensis, as well as healthy skin and mucosal biopsies. LCL biopsies displayed higher CD161 RNA levels (p=0.03) vs healthy skin, but IL-17A/B/F RNA levels were low or undetectable in both ML and LCL biopsies. In LCL, CD161 also correlated with cytokine receptors IL6R and IL23R. T-bet-related transcription factor Tbx21 RNA levels were also increased, confirming LCL Th1 profile. However, in ML, CD161 correlated to IL23, IL23R, CCR6 and RORC, suggesting a differential cytokine profile in ML vs LCL. As CD161 strongly correlated with CD8 (r=0.80, p=0.0096, but only weakly with CD4) as well as GZMA/B RNA levels, we conclude that LCL lesions have a predominant CD8 effector rather than Th17 profile, characterized by $CD161^+/GZMA^+/CD8^+$ cells, while STAT3-regulated $Tc17/RORC^+$ cells were increased in ML. These data indicate that different factors regulate human leishmaniasis progression to ML or LCL, depending on Th1/Th17 fine-tuning or imbalance. Finally, we propose CD161 as a novel clinical biomarker capable to predict healing time in LCL, apparently independent of IL-17 function.

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CD8+ T LYMPHOCYTES IN HEALING PROCESS OF HUMAN CUTANEOUS LEISHMANIASIS EVALUATED DURING AND AFTER ANTIMONIAL THERAPY

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OSWALDO CRUZ FOUNDATION, RIO DE JANEIRO - RJ - BRASIL.

Introduction and Objective: Human cutaneous leishmaniasis (CL) is endemic in Rio de Janeiro, Brazil, where is caused by Leishmania braziliensis. The adaptive immune response is accountable for the cure of CL, however little is known about the immunological profiles of these cells during the antimonial therapy. Though the essential role of $CD8^+$ T cells has been well established in some studies, the actual participation of the effector $CD8^+$ T-cell subpopulation and the modulation of these cells by apoptosis have not been elucidated so far, which was our goal. **Methods**: Ex vivo and in vitro assays were evaluated, by flow cytometry, to define phenotypic profiles and apoptotic rates in total and effector $CD8^+$ T cells of blood samples obtained from patients during treatment (PDT) and patients clinically cured (PCC) after treatment as well as healthy subjects (HS). **Results:** The higher apoptotic rates of total $CD8^+$ T lymphocytes in PDT seems to be related to the lower frequency of $CD8^+$ T lymphocytes as observed in ex vivo and after L. braziliensis-antigen stimulation assays. Regarding the effector $CD8^+$ T-cell subset, the higher apoptotic rate observed in PDT decreases after treatment, and the same was observed in antigenic-stimulation assay. Despite the lower frequency of effector $CD8^+$ T cell observed in PCC, the low apoptotic rates of these cells seem to be favorable to the healing of these patients. We showed changes of the $CD8^+$ T-cell frequencies, from treatment to clinical cure, pointing to these cells are implicated in the healing process. **Conclusion:** Our results strengthen the role of effector $CD8^+$ T cells in the healing process of CL patients as well as show that the apoptosis could modulate the rates of these cells compromising an effective host's immune response against L. braziliensis. The new approach of evaluating patients during treatment proved to be of utmost importance for understanding the immune response in the healing process of human cutaneous leishmaniasis. **Financial Support:** Capes, Cnpq, IOC-FIOCRUZ,IPEC-FIOCRUZ, Flow Cytometry core Facility – IOC.

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CHARACTERIZATION OF A MURINE MODEL (TNFRP55-/-) FOR STUDY OF CHRONIC CUTANEOUS LESIONS BY LEISHMANIA BRAZILIENSIS INFECTION

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A chronic skin manifestation of leishmaniasis is caused by an exaggerated cellular immune response. Lesions in nasal mucosa and cartilage, months or years after an initial skin lesion, cause mutilation and morbidity in affected individuals. There is no murine model for the study of mucocutaneous leishmaniasis. However, data from our group showed that TNFRp55$^{-/-}$ mice, when infected with L. major, develop chronic lesions, which are not progressive as in the BALB/c classic susceptible strain, but there aren't mucosal lesions. Based on this information the aim of this study is the characterization of chronic infection by L. braziliensis in TNFRp55$^{-/-}$ mice. Wild-type (C57BL/6) and TNFRp55$^{-/-}$ mice were inoculated in the ear with L. braziliensis (1x10^{6} parasites) and lesions were followed for 15 weeks. In the chronic phase of infection (15 weeks) samples from the site of infection were collected and processed for, quantification of parasites and analysis of the inflammatory infiltrate. Our previous results show that lesions in TNFRp55$^{-/-}$ mice were chronic non-progressive lesions, did not ulcerate, and persisted for more than 20 weeks of infection. Interestingly, these animals can control parasitism similar to wild-type mice at 15 weeks of infection.

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CHARACTERIZATION OF IMMUNE RESPONSE IN EXPERIMENTAL MURINE AFRICAN TRYPANOSOMIASIS TREATED WITH BENZNIDAZOLE

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Introduction: African Trypanosomiasis (AT) is a neglected disease caused by the protozoan Trypanosoma brucei subspecies to human and animals in Sub-Saharan Africa. There are some efforts in order to produce new medications for AT in the absence of an effective vaccine. The medications currently in use are quite toxic, and some of them have reported resistances (Br. J. Pharmacol. 152:1155–1171, 2007). Therefore, there is a dire need to discover novel molecules less toxic and more effective against this disease. Benznidazole (BNZ) is an antichagasic of the nitroimidazole family. This molecule is the standart of treatment for Chagas disease, and was considered for mucocutaneous leishmaniasis. The mechanism of action is still poorly understood, being generally accepted that the main mechanism is the production of reactive nitrogen species that damage nucleic acids (Br. J. Cancer 50:291-303, 1984). Being highly liposoluble, BNZ crosses the blood brain barrier with ease, making it a good candidate for treating second stage of AT. The objective of this work is to ascertain differences in immune response in the treatment of murine AT with BNZ.

Methods and results: 24 CD-1 strain mice were infected IP with a dose of 500 parasites per animal of the T.brucei brucei GVR35/1.6 strain. The infection was detected in all animals by stained thick smear in the 6th day post-infection (DPI). 12 animals were medicated per os with the pediatric dose of BNZ (10mg/kg SID) from the 12thDPI until the death of the first animal (22ndDPI), 12 were untreated. 12 animals from both groups were sacrificed, 6 on 14DPI and 6 on 26DPI, to collect serum for anti-T. brucei (a-Tbb) antibody ELISA and cytokine quantification (IL-4, IFN-g, TGF-b1 and NO), compared with healthy controls. Parasitemia and survival were measured constantly during the duration of the experiment. The results show no significant differences in parasitemia and survival in both groups ($p=0.441$ and $p=0.507$, respectively). As for a-Tbb IgG1, IgG2a, IgG3 and IFN-g, there were significant differences between the two groups (IgG1: $p(26DPI)<0.001$; IgG2a: $p(14DPI)<0.01$; $p(26DPI)<0.001$; IgG3: $p(14DPI)<0.05$; $p(26DPI)<0.001$; IFN-g: $p(14DPI)<0.01$).

Conclusion: The results show that although clinically BNZ is ineffective in treating AT, it tends to stimulate a Th-1 response with increased titers of a-Tbb IgG subclasses and IFN-g.

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COMPARATIVE STUDY OF PUMONARY INFLAMMATORY RESPONSE IN MURINE CAUSED BY YEASTS OR CONIDIA OF PARACOCCIDIOIDES BRASILIENSIS

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Introduction: Paracoccidioides brasiliensis (PB) causes a chronic granulomatous mycosis (PCM). Natural infection predominantly occurs by spore inhalation, and the temperature difference between the environment and the body stimulates the transformation of spores to yeast. As most PCM experimental studies use yeast and not conidia for infection they do not reflect the initial events of natural infection. Objectives: Compare the initial phase of the inflammatory response of murine PCM caused by yeasts or conidia of P. brasiliensis. **Methods and Results**: Male adult BALB/c, were infected by the intratracheal route with 0.5 $x10^6$ yeast (Y-INF) or conidia (C-INF) of PB. Controls received saline (SF) by the same route. The pulmonary index (PI), lung histopathology, cellularity of bronchoalveolar lavage (BAL) and leucocyte profile of mediastinal lymph node (MLN) were evaluated on days 1, 3, 6, 10, 14 and 28 post-infection (dpi). For statistical significance ($p<0.05$) ANOVA with Tukey post-test was used. In Y-INF mice PI increased gradually throughout the experimental period (0,66 ± 0,02, 1,38 ± 0,01, 1,54 ± 0,05, 1,61 ± 0,04, 2,39 ± 0,04 and 2,53 ± 0,05), while C-INF reached the maximum PI on the 10^{th} dpi , followed by a decline (0,78 ± 0,01, 1,03 ± 0,03, 1,43 ± 0,05, 1,8 ± 0,03, 1,08 ± 0,02 and 1,02 ± 0,03). BAL cellularity increased gradually in both groups, throughout the experiment and on the 10^{th} dpi Y-INF ($9,83x10^6$ ±0, 72) was higher than C-INF ($7,10x10^6$ ± 0,02). $CD3^+$ and $B220^+$ increased as of the 6^{th} dpi with a significant difference only on the 10^{th} dpi in Y-INF ($CD3^+$: $1,87x10^6$±0, 02 vs $0,82x10^6$±0,01, $B220^+$: $1,40x10^6$±0,02 vs $0,42x10^6$±0,01). C-INF (17, 33%±4,4) presented a higher rate of apoptosis compared to Y-INF (3,33%±0,4) and controls (1,66%±0,3). There was a decrease of MLN $CD3^+$ cells in both Y-INF (51,9%±4,3) and C-INF (42,93%±2,8) compared to controls (65%±2,9) with a gradual increase of $B220^+$ cells in C-INF (53,13% ± 1,9) compared to Y-INF (44,46% ± 4,9) and controls (30,96 ± 4,2%) as of the 10^{th} dpi. Histopathology of C-INF revealed extensive pulmonary inflammatory infiltrate with well-defined granulomas on 14^{th} and 28^{th} dpi while Y-INF had no well-defined granulomas. **Conclusion:** The analysis of the results suggests that the initial phase of murine infection by conidia of P. brasiliensis results in a more intense pulmonary inflammatory infiltrate than by yeast infection and can influence the late phase of the PCM.

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COMPUTATIONAL MODELING OF IMMUNE RESPONSE IN CHAGAS DISEASE

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INTRODUCTION: Chagas disease (CD), caused by Trypanosoma cruzi, is a public health problem that affects about four to six million Brazilians. In its chronic form, high mortality occurs between CD which develop heart disease (Circulation, 115:1101-1108, 2007). To understand the pathophysiology of CD is paramount understanding of the immune system (IS), which participates significantly in the process. The events of the IS processes are not fully understood needing to be tested in experimental models (J Bras Med 95:28-34, 2008). Computational modeling of IS is a research method that seeks to simulate the performance of the immune response in order to understand, more accurately, the basic principles of its operation. In this communication are presented results of a computational model (in silico research) IS human simulation of the phenomena involved in the pathophysiology of heart of CD, with emphasis on the interaction between pathogen and host. **METHODS AND RESULTS:** A review of the literature for defining the requirements for the preparation of artificial immune system for the study of interactions between pathogen / innate immunity and the inflammation / immune-mediated typical CD. The literature review provided subsidies for the construction of a computational model based on multi-agent systems – denominated AutoSimmune – using the framework Repast Simphony. Models were made of: (1) zones – bone marrow, circulation, lymph node, and thymus tissue (heart), (2) agents antigen (T. cruzi), antibody, and cells, and (3) diffusion of substances (cytokines). Initial assays performed to investigate CD events showed that the model presents behavior coherent with the current biomedical literature. The requirements for the investigation of the immune response in CD were also raised and are being implemented in AutoSimmune. **CONCLUSION:** In silico research is faster and cost effective when compared to studies in vitro and in vivo, no significant bioethical problems related to experiments with humans and animals. The proposed computational modeling aims the understanding of CD related to human inflammatory response in order to generate knowledge about this disease that will reflect in better quality of care to the population who suffers of this illness.

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COMPUTER MODELING OF THE NEUTROPHIL: PERSPECTIVES OF IN SILICO RESEARCH OF THE SEPSIS

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Introduction: Sepsis is a serious public health problem worldwide. The computational approach (in silico experimentation) has been used for the verification of hypotheses related to important aspects in the study of sepsis in the areas of immunology. We seek to implement in this study a system for computational modeling of the immune system (IS) in order to perform in silico experiments in sepsis pathophysiology. **Methods and Results:** AutoSimmune, a computational simulation system based on multi-agent systems (MAS), was built using the Repast Symphony framework and is based on the definition of "individual aspects, relating to the agents in such a way as to permit emergence of the collective aspects" (Hübner et al., 2011) . Important IS elements could be modeled and implements such as: (1) agents – antigen, antibody, bacteria, viruses and cells; (2) zones – tissue, lymph node, circulation, bone marrow, thymus, air way tissue and kidney tissue; and (3) diffusion of substances (cytokines). Among the simulated cells are neutrophils, modeled on AutoSimmune with the following characteristics: The neutrophil agent follows the signaling substance PK1 (stress factor released by tissues that are suffering damage due to infection or immune response), circling to find the site of infection. The neutrophil agent searches for cells that are emitting PK1 (stressed cells and / or infected), then exerts phagocytosis. The simulation includes the inflammatory process, dead cells, pathogens, and antigen-antibody complexes. When the neutrophil lifetime ends, the agent undergoes apoptosis. The first experiments performed to investigate autoimmune events showed that the model presents behavior in accordance with the current biomedical literature (Possi et al., 2011; Silva et al., 2012). The study is currently focused on mapping the principal aspects of the immune response in sepsis to gram negative bacteria (Enterobacteriaceae). **Conclusion:** The preliminary results point to a context in which stimulation of the IS using MAS would allow for the individual modeling of the agents and the emergence of the systemic behavior of sepsis. Therefore, in silico experimentation of sepsis, using AutoSimmune, may help in the pathophysiological knowledge of this disease.

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CONTRASTING CONTROL OF CD8+ T LYMPHOCYTES IN VIVO PRIMING BY DENDRITIC CELLS EXPOSED TO TRYPANOSOMA CRUZI OR TO A HUMAN ADENOVIRUS TYPE 5 VACCINE

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Introduction: Infection by the protozoan parasite Trypanosoma cruzi, the etiologic agent of Chagas disease, stimulates a delayed $CD8^+$ T cell-mediated immune response characterized by cells with a proapototic phenotype and high expression of the death receptor CD95. Those cells are unable to completely clear infection and allow host´s death or the establishment of a chronic phase affecting millions of individuals in the Americas. In contrast, previous or simultaneous administration of an adenoviral vaccine expressing the immunodominant antigen of T. cruzi (AdASP-2) rapidly expand highly viable $CD8^+$ T cells expressing low levels of CD95 which promote parasite control and host´s cure. The basis of such differences was investigated in this study. More precisely, we tested the hypothesis that dendritic cells (DC) exposed to T. cruzi or AdASP-2 provide distinct signals that lead to different activation and phenotype of $CD8^+$ T cells. **Methods and Results**: Bone marrow derived DC (BMDC) were exposed to T. cruzi and/or AdASP-2 and studied in their phenotype and antigen presenting function in vitro and in vivo. BMDC exposed to T. cruzi or AdASP-2 upregulate molecules MHC I and II, CD40, CD80 and CD86. In both cases, the CD8 epitopes are processed through the cytosolic pathway, as indicated by TAP dependence. Most relevant was the fact that BMDC exposed to T. cruzi or AdASP-2 activated effector specific $CD8^+$ T cells in vitro albeit at different levels (AdASP-2>T. cruzi). Experiments using co-exposed BMDC suggested that neither T. cruzi nor AdASP-2 interfere with the specific CD8 activation capacity of each other. To compare the activation of naïve cells in vivo, BMDC exposed to T. cruzi or AdASP-2 were stimulated with LPS, pulsed with SIINFEKL peptide and transferred into mice that previously received naïve OTI cells. Five days after immunization, we observed that BMDC exposed or not to AdSP-2 induced OTI cells to proliferate and present an effector phenotype (CD44 high CD62L low CD95 low). These cells were also capable to produce IL-2, TNF and IFN-γ when restimulated ex vivo. In sharp contrast, T. cruzi-exposed BMDC did not activate naïve OTI cells and all splenic T cells upregulated CD95 expression on a CD4 dependent manner. **Conclusion**: We confirmed our initial hypothesis that DC exposed to T. cruzi or AdASP-2 vaccine provide different signals to $CD8^+$ T lymphocytes by respectively altering or not the in vivo priming. **Financial Support**: CNPq, INCTV, FAPESP.

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CONTRIBUTION OF C5A AND PLATELETS IN THE PATHOGENESIS OF EXPERIMENTAL CEREBRAL MALARIA

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Introduction: Cerebral malaria (CM) is a severe form of the disease that may result, in part, from an overt inflammatory response during infection by Plasmodium falciparum. The Complement System plays an important role in immune response, leading to inflammation, endothelial activation, opsonization and coagulation, processes which have been implicated in CM pathogenesis [Cell. Microbiol., 12 (8): 1036-45, 2010]. In addition, several studies have shown the thrombocytopenia in experimental cerebral malaria and in human severe malaria (Am. J. Trop. Med. Hyg., 66: 686-91, 2002; Mem. Inst. Oswaldo Cruz, 106: 52-63, 2011). The aim of our study was to investigate the role of Complement system and platelets in the pathogenesis induced by Plasmodium berghei ANKA (PbA), an experimental model of cerebral malaria (ECM). **Methods and Results**: C57BL/6 mice were infected with 5 x 10^5 PbA-parasitized erythrocytes, and the course of infection and survival were evaluated periodically. The thrombocytopenia was assessed by number of platelets in the blood of PbA infected mice on 3, 5 and 6 days post infection (dpi). Brain homogenates of mice with CM, infected mice and non-infected mice were analyzed for C5a levels by ELISA. CM was confirmed by histological examination of cerebral pathology: cerebral cortex with intense hemorrhage, and condensed hyperchromatic neurons, especially around the hemorrhagic focus, and intravascular leukocyte accumulation on the 5 and 6 dpi, respectively. The platelets analysis confirmed the thrombocytopenia in PbA infected mice on 6 dpi. C57BL/6 mice had higher brain C5a levels (969±334 pg/mL), particularly on day 6 compared with non-infected mice (314±94 pg/mL). **Conclusion:** This study suggests that there is a correlation between the C5a increase and decrease of the number of platelets in ECM. These data provide evidence implicating C5a and the platelets levels in the pathogenesis of ECM.

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CONTRIBUTION OF P2X7 RECEPTOR IN IMMUNE RESPONSE DURING CHRONIC TOXOPLASMOSIS

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Introduction: The purinergic receptor P2X7 is activated by extracellular ATP and is involved in several physiological and pathological events. P2X7 receptor was described to participate in the immune response against different intracellular pathogens such as Leishmania amazonensis, Chlamydia and the acute phase of toxoplasmosis. Toxoplasma gondii is a protozoa parasite that infect homoeothermic host, and may cause serious impairment or death of immunocompromised individuals such as HIV patients, transplanted patients and pregnant women. In this work we evaluated if P2X7 receptor could contributes for the immune response during the chronic phase of toxoplasmosis.

Methods and Results: We used female C57BL/6 and P2X7 knockout ($P2X7^{-/-}$) mice 6 - 8 weeks old, orally infected with 5 or 10 cysts of Me-49 strain T. gondii, and the survival was monitored. We observed that with 5 cysts all $P2X7^{-/-}$ mice succumbed 8 week after infection, while all WT mice survived. In a different infected groups animals were euthanized after 30 days and we analyzed the effect of infection on two chock organs. We quantified ALT and AST enzymes in the liver, and found higher levels of both enzymes in WT mice when compared with $P2X7^{-/-}$ (5.6 ± 0.6, n=8; 1 ± 0.2, n=8, to ALT and 1.8 ± 0.4, n=8; 0.8 ± 0.1, n=8, to AST, in WT and $P2X7^{-/-}$, respectively). We observed a larger number of cyst in brain of $P2X7^{-/-}$ animals when compared with WT, (22 ± 8 and 2 ± 0.6, n=6 to $P2X7^{-/-}$ and WT mice respectively. In addition, we found increased levels of IL-12 in brain extracts of both WT and P2X7-/- animals, Although the secretion was lower in brain of P2X7-/- mice (1.1 ± 0.06, n= 8; 0.8 ± 0.05, n= 6, to WT and P2X7-/- , respectively). The same effect also was observed in animals infected with 10 cysts of T. gondii. This factor could be the important contribution of P2X7 receptor during chronic toxoplasmosis

Conclusion: The P2X7 receptor participates in immune response against T. gondii during chronic toxoplasmosis.

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CONVENTIONAL CD4+ T CELLS PROMOTE ICOS-DEPENDENT POLYCLONAL B CELL ACTIVATION DURING BLOOD-STAGE PLASMODIUM CHABAUDI AS MALARIA

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Text: B cell and antibody response are crucial for protective immunity to blood-stage malaria infections. The early B cell response to Plasmodium chabaudi results in intense production of parasite-specific low-affinity IgM and IgG and autoantibodies.

Methods and Results: According to our results, in the acute phase of the disease, T-B cell cooperation through the MHC class II molecules is essential for B cell activation, proliferation and for antibody production. The $CD4^+$ T cell population involved in the polyclonal B cell activation shows a huge increase of ICOS and OX40 molecules. $CD4^+$ T cells from acute infection also showed concomitantly expression of CXCR4 and CXCR5. However, with time, the levels of CXCR5 and PD1 on the $CD4^+$ T cells increase, showing characteristics of T_{FH} cells. Moreover, PD1 expression is higher on germinal center T_{FH} than T_{FH} GL7. Our in vitro assays show that T cells from the acute infection are potent helpers for naïve B cells in the presence of iRBC. The IgM and IgG production contribute not only to the uptake of the iRBC exposed to phagocytes, but also to the uptake of BCG mycobacterium. In addition, our results show that during the acute infection $CD4^+ICOS^{HIGH}$ T cells are responsible for polyclonal B cell activation in vitro in the presence of iRBC.

Conclusions: Our results suggest that the cooperation between conventional $CD4^+$ T cells and B cells has a central role in the polyclonal antibody response to P. chabaudi.

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CROSSTALKING BETWEEN DENDRITIC CELLS AND NEUTROPHILS: A PROTECTIVE EFFECT MEDIATED THROUGH TLR9 DURING VISCERAL LEISHMANIA INFECTION

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The protozoan Leishmania infantum is the causative agent of visceral leishmaniasis (VL) in Brazil and South America, causing high morbidity/mortality. The resistance against VL is related to the development of cellular immune response. During infection, the dendritic cells (DCs) recognize antigenic products through Toll-like Receptors (TLRs) and then orchestrate the cellular recruitment and immune response development. Among several TLRs, it has been showed that TLR9 is related to resistance to cutaneous leishmaniasis. In the present study, our aim was to determinate the role of TLR9 during L. infantum infection. Our results demonstrated that TLR9 is up-regulated during in vitro and in vivo L. infantum infection. TLR9 is critical for protective immunity against L. infantum, since $TLR9^{-/-}$ mice infected were more susceptible to infection, displaying high amounts of parasites in spleen and liver, at 4^{th} and 6^{th}weeks post-infection. Phenotyping the leukocytes into the spleen, $TLR9^{-/-}$ mice presented reduced neutrophils when compared to WT. Likewise, imunohistochemistry analyses showed the reduced of $7/4^{+}$ cells (specific to neutrophils) staining into the $TLR\text{-}9^{-/-}$ liver. The neutrophil migration failure is not associated to their stage of activation impaired, but due the reduced levels of KC and MIP-2 (neutrophil chemoatracttant) produced into into the spleen cells culture from infected $TLR9^{-/-}$. Furthermore, DCs from $TLR9^{-/-}$ presented a semi-mature stage during in vitro and in vivo L. infantum infection. Interestingly, the DC ability to produce the neutrophil chemotact mediators (KC and MIP-2) was reduced by that derived from $TLR9^{-/-}$ mice, affecting neutrophil migration into Boyden chamber. Altogether, we demonstrated that TLR9 presents a critical role in the protective response against L.infantum through the mechanism dependent of crosstalk between neutrophil recruitment and DC activation.

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CRUCIAL ROLE OF PI3K-GAMMA FOR ANTIVIRAL AND INFLAMMATORY RESPONSES AGAINST INFLUENZA A INFECTION IN MICE

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Introduction: PI3Ks are central signaling enzymes, involved in cell growth, proliferation, survival and migration. Class IA PI3K are named PI3Kα, β or δ and are activated by tyrosine kinase receptors; class IB PI3K or PI3Kγ are mainly expressed on leukocytes and activated by GPCRs coupling. Influenza A virus causes a severe pulmonary disease that affects millions of people worldwide every year. PI3Kγ is involved in cell migration under inflammatory conditions. Our hypothesis was that PI3K-gamma might be involved in the recruitment of inflammatory cells during Influenza A infection and may contribute to lung damage and death in models of severe disease. **Methods and Results:** To address this question we infected C57/BL6 wild type (WT) or PI3Kγ knock out (KO) mice with the mouse adapted virus Influenza A/WSN/33 H1N1 and followed disease signs for 21 days. To our surprise, whereas 40% of WT mice succumbed to flu infection, 100% of KO mice died. To investigate the causes of this increased susceptibility, we euthanized WT and KO mice at 3, 5 and 7 days after infection. Neutrophil infiltration into lungs of KO mice was more intense than WT mice and the release of ROS by lung leukocytes and lung damage were increased in KO cells at day 7. The transmigration to the airways of T CD8+ lymphocytes, NK cells and resolving macrophages ($Gr1^{-}$ $F4/80^{med}$ $CD11b^{low}$) but not neutrophils, NKT cells, CD4+ T cells and gamma/delta T cells was reduced in KO mice when compared to WT. The airways leukocyte apoptosis induced by the infection was higher in KO mice at day 5, but in the lungs, increase in Caspase-3 cleavage upon infection was similar in WT and KO. Type I IFN (IFN-α4 and IFN-β1 mRNA levels) response induced by flu infection was completely abolished in lungs of KO mice. Reduction of effector cells against the virus - CD8+ T cells and NK cells – in KO mice led to increased viral loads in the lungs at day 7. The same phenotype was also found in KO mice infected with Influenza A strain A/PR/8 H1N1. **Conclusion:** PI3Kγ activation during Influenza A infection is necessary for an early antiviral response that promotes recruitment of effector cells against the infected cells and contributes to viral clearance. PI3Kγ activation is also important for neutrophil transmigration to the site of infection and resolution of inflammation that causes lung damage. **Financial support:** CNPq/FAPEMIG

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CYTOKINE EXPRESSION IN THE HEART OF MICE INFECTED WITH DIFFERENT FORMS INFECTIVE

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It is known that blood trypomastigotes (BT) and metacyclic trypomastigotes (MT) differ with regard to surface molecules. MT and BT infections are associated with distinct cytokine profile in the spleen during the acute phase of Chagas disease. Thus, the aim of this study was to assess the cytokine profile and histological changes in heart tissue of mice infected by different infectious forms during the acute phase of infection and assess whether this tissue display the same pattern observed in spleen. Five mice of each group (non-infected, infected with MT forms and infected with BT forms) were euthanized before infection (0) and at 7, 14, 28 and 42 days after infection (DAI) and the heart was removed for quantification of anti and pro-inflammatory cytokines mRNA levels by Real Time-PCR. For the cytokine IL-12 it was observed in both infected groups an increase of mRNA expression on 28th DAI, being this increase maintained at 42nd day only in BT group. The animals of the BT group showed an increase in mRNA expression for IFN-γ on 7th DAI and 28th DAI. On the other hand, in MT group it was observed an increased expression of mRNA for IFN-γ on 14th and 42th DAI. Thus, can be noted a decrease in the expression of IFN-γ on the peak of parasitemia at days 14 and 28 for BT and MT, respectively. Regarding the anti-inflammatory cytokines, there was an increased expression of mRNA for TGF-β in the MT group, thus demonstrating that in these animals appears to occur an immunoregulatory profile of cytokines in the heart, while the BT group showed an increase in IL-10 only at 28th DAI. Corroborating to these results morphometric analysis of cardiac inflammation on the BT group demonstrated the presence of inflammatory cells in the 7th DAI, returning to occur from the 28th DAI on. Otherwise, a morphometric analysis in the MT group shows inflammatory process only at the 28th DAI, however there was a reduction of the same at 42nd DAI. No significant differences were found in the process of collagen neoformation in the hearts of mice infected by MT or BT forms. In this sense, animals infected by MT forms were able to induce an immunoregulatory response in heart and a decrease in cardiac inflammation at the end of the acute phase, when the parasitemia has already come under control. Moreover, animals in the BT group failed to present this kind of response in early infection, which leads to an exacerbation of the heart inflammatory process. Supported by FAPEMIG and CNPq.

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CYTOKINE PRODUCTION FROM HUMAN MACROPHAGE LINEAGE THP-1 AND PBMC INFECTED WITH ALIVE OR HEAT–KILLED PREPARATIONS OF MYCOBACTERIUM BOVIS BCG MOREAU-RJ

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Introduction: Tuberculosis continues to cause great public health impact with high rates of mortality and the only prophylactic measure is BCG vaccine. BCG is also used to providing protection against the most serious forms of the disease, such as miliary tuberculosis and tuberculous meningoencephalitis in a population younger than 5 years old. Various studies have shown that BCG provides some protection against other mycobacterial infections, such as leprosy, as well as their effects on immunotherapy of several types of cancer, in particular the superficial urothelial bladder carcinoma, in type 1 diabetes and allergic processes. The present study aimed to evaluate the release of pro-inflammatory cytokines such as IL-1β, TNF-α, IL-6 in BCG (obtained in IVM medium-oral vaccine and Sauton medium-intradermal vaccine) infected human macrophage lineage (THP-1) and in human peripheral blood mononuclear cells (PBMC), and to compare alive and heat-killed mycobacteria in order to better understand the mechanisms involved in this protection and to improve future applications of BCG Moreau strain - RJ. Methods and Results: The THP-1 cells were cultured in RPMI 1640 with 10% inactivated fetal bovine serum with addition of PMA at 30 nM for differentiation of macrophages and incubated in 37 º C with 5% CO2. PBMC were cultured in RPMI 1640 with 10% human serum and incubated in 37 º C with 5% CO2. After 24 and 72 hours infection, supernatants were centrifuged and the cytokines were measured by ELISA. We observed a marked release of IL-1β, TNF-α and IL-6. Interestingly the heat-killed mycobacterias produced similar pattern of cytokines. The IFN-gamma production was observed in supernatants from PBMC. In THP-1 macrophages the IFN-gamma production was detected in BCG from IVM medium. Conclusion: Based in these results one can also speculate that the production of cytokines as TNF-α and IL-6 by dead bacilli occurs due to the adjuvant effect of mycobateria in producing antibodies as previously described (J. Adv. Tuberc. Res., v. 7: 130-148, 1956).

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CYTOTOXICITY IN THE PROGRESSION TO CLINICAL CURE OF HUMAN CUTANEOUS LEISHMANIASIS

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Introduction: The clinical course of human cutaneous leishmaniasis (CL) depends on the interaction between the parasite and the immune response of patient. It is well known that $CD8^+$ T lymphocytes plays an important role in this response, both being related to participation in protective immunity and regression of lesions, as well as in the maintenance of infection and tissue damage. NK cells, NKT cells and a cytotoxic subpopulation of $CD4^+$ T lymphocytes have participation in this immune response, however little is reported about the cytotoxic function of these cells in the immunopathogenesis of CL. Thus, in order to better understand the role of cytotoxicity in the evolution to clinical cure of CL, the aim of this study was to evaluate the frequency and cytotoxic activity of these cell populations, through immunophenotyping and evaluation of CD107a expression, in peripheral blood samples of patients with active disease; during and after antimonial treatment. **Methods and Results**: Flow cytometry was used in samples from three cohorts of CL patients and from healthy individuals. In vitro assays were also carried out to determine the cytotoxic behavior of these populations in the response to specific L. braziliensis antigens. In active CL we observed a lower frequency of $CD8^+$ and $CD4^+$ T lymphocytes. Frequencies of $CD8^+$ T lymphocytes were similar to healthy subjects during treatment and after recent clinical cure, while the frequency of $CD4^+$ T lymphocytes remained at lowest levels. Cytotoxic marker was more remarkable in $CD8^+$ and $CD4^+$ T lymphocytes and NKT cells in patients during treatment**.** However, these same cells showed impaired cytotoxic activity when exposed to specific antigens. NK and NKT cells are present at high levels in CL patients and did not return to normal levels with the recent cure**.**Analyzing subpopulations of NK and NKT cells, a $CD56^{dim}$ NK subpopulation was the most prevalent and shows a predominantly cytotoxic activity, regardless the presence of disease or treatment. A $CD4^+CD8^+$ NKT subpopulation seems to play an important cytotoxic role in the process of clinical cure of CL. **Conclusion**: These results indicate that cytotoxicity exerted by $CD8^+$ and $CD4^+$ T lymphocytes and NKT cells, along with the beneficial effects of therapy, assist in leading to the clinical cure of CL. In contrast, the circulating NK cells cytotoxicity did not appear to influence in this healing process.

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DEFICIENCY IN INVARIANT NATURAL KILLER T CELLS IMPAIRS ACUTE IMMUNE RESPONSE AGAINST PARACOCCIDIOIDES BRASILIENSIS

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Introduction: The fungus Paracoccidioides brasiliensis (Pb) is the etiological agent of Paracoccidioidomicosys (PCM); one of the most prevalent human systemic mycosis in South America. Although is well described that innate immunity plays an important role in host resistance against Pb infection the role of invariant Natural Killer T (iNKT) cells in this phenomenon remains unclear. **Objective:** evaluate the participation of iNKT cells in the acute inflammatory response during Pb infection. **Methods:** BALB/c WT or iNKT cells deficient ($J\alpha18^{-/-}$ KO) mice were infected via intra-tracheal inoculation of 10^6 Pb cells. Animals were euthanized 72h post-infection in order to determine the influx of inflammatory cells and the cytokines levels present in the pulmonary environment. **Results:** iNKT deficiency impaired the recruitment of inflammatory cells to the airways, in comparison to WT animals. The analysis of the bronchoalveolar lavage (BAL) revealed that the total number of cells was lower in $J\alpha18^{-/-}$ KO than in WT group (280 ± 37.2 vs 480 ± 77.2), especially in the context of mononuclear cells (100.9 ± 24.7 vs 177.1 ± 9.1). Paradoxically, the levels of IFN-γ (261.3 ± 136.6 vs 23.1 ± 9.9) and IL-17 (232.5 ± 67.6 vs 59.1 ± 22.8) were higher in $J\alpha18^{-/-}$ KO mice than in the WT groups. **Conclusions:** although preliminary, our results indicate that iNKT cells play an important role in the early phase of the immune response against Pb, suggesting that these cells can be implicated in host resistance against Pb infection. **Financial support:**FAPESP (2011/50256-6) and CAPES.

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DEGRANULATION ACTIVITY OF CD8+ T-LYMPHOCYTES FROM HTLV-1-INFECTED PATIENTS WITH HAM/TSP

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Introduction: Human T-cell leukemia virus type 1 (HTLV-1) is an exogenous retrovirus that infect preferentially CD4+ T cells and affects 15-20 million people worldwide. While the majority of HTLV-1 carriers remain asymptomatic lifelong, some develop adult T cell leukemia/lymphoma (ATL) and chronic inflammatory diseases like the neurodegenerative disorder known as HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP). Previous studies demonstrated that proviral load was increased in the peripheral blood from patients with HAM/TSP compared to HTLV-1 asymptomatic carriers (AC), suggesting an inefficient control of viral infection. Like the CD8+ T cells are crucial components of the immune response against viruses and has been demonstrated that CD107a (LAMP-1) expression is marker sensitive for the degranulation activity determination, this study evaluated the cell-mediated cytotoxicity of CD8+ T-lymphocytes by CD107a mobilization assay in 32 HTLV-1-infected individuals, comparing 14 individuals AC to 18 patients with HAM/TSP – 5 patients classified as HAM/TSP-PB (probable) and 13 as HAM/TSP-D (definitive) according Belem criteria (De Castro-Costa clinical classification). **Methods and Results:** Peripheral blood mononuclear cells (PBMCs) were obtained from by density gradient centrifugation over ficoll. PBMCs ($2x10^6$) were cultured in supplemented RPMI 1640 medium with anti-CD107a^{FITC} for 6 hours at 37ºC and 5% CO_2. Monensin (1µM) and Brefeldin A (1µM) were added in the culture at the last 5 hours. After the incubation, PBMCs were washed and stained with anti-CD3$^{APC\text{-}CY7}$, anti-CD8^{APC} and anti-INF-γ^{PE}. The cells were fixed in 2% paraformaldehyde and stored at 4ºC before flow-cytometric analysis. Statistical analysis was performed by kruskal-wallis non-parametric test. In contrast to previously reported findings about the increase spontaneous degranulation and INF-γ production in patients with HAM/TSP, was not observed significant difference between the CD107a expression in $CD8^+$ T cells from HAM/TSP and AC patients. In addition, the expression of IL-15, which induced INF-γ production and degranulation, was enhanced on surface of CD14+ cells in all patients. **Conclusions:** These results provided evidence that spontaneous degranulation is not a distinguishing feature of $CD8^+$ T cells from patients with HAM/TSP. Further studies will be conducted in an attempt to clarify these questions.

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DETECTION OF ANTIBODY TO PURPUREOCILLIUM LILACINUM BY IMMUNOFLUORESCENT ASSAY AND FLOW CYTOMETRY IN SERUM OF INFECTED C57BL/6 MICE.

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FIOCRUZ, RIO DE JANEIRO - RJ - BRASIL.

Purpureocillium lilacinum (Thom) is an emerging opportunistic pathogen in immunocompromised individuals. Also, the incidence of infections in immunocompetent hosts is increasing. The diagnosis of P. lilacinum infection has been based on morphological features and histology.The change in fungal infections has emphasized the need to develop better diagnostic and to recognize this increasingly large group of potential fungal pathogens. The aim of our study is to evaluate the antibody response against P. lilacinum by an indirect immunofluorescence test and by flow cytometry approaches and their association with clinical progression of the disease using immunocompetent and immunosuppressed mice models.

Methodology: Strains–The strain used throughout the study was originally isolated from a human subcutaneous infection, and was grown on PDA at 25^{o}C for 12 days in order to obtain conidia.

Mice-Male C57BL/6 mice, aged 6-7 weeks and weighing approximately 21g, were used. To induce immunosuppression, mice received 5 mg/Kg of dexamethasone administered ad libitum in the drinking water. Blood sample collection was performed by cardiac puncture and sera were stored at -70^{o}C for later serological evaluation.

IFA-5 ml of 4 x 10^{7}conidia of P. lilacinum was placed on glass microscope slides and allowed to air dry. To each dried spot, 5 ml of mouse sera were added at two-fold serial dilutions (starting at 20 up to 160) in PBS containing 2% BSA (PBS-BSA) and incubated in a moist chamber. The slides were washed and incubated as before with FITC-IgG and after, analyzed under a Zeiss Colibri fluorescence microscope.

FCM-Sera titrated at 1:500 and 1:1,000 were incubated with 2.5 x 105 conidia l of PBS containing 10% PBS-FCS. After, samples weremdiluted in 50 incubated with FITC- IgG, FITC-IgG1, or FITC-IgG2a, or FITC- IgG2b. In order to evaluate serum reactivity by FCM, we applied the so-called integrated MFI (iMFI), a validated metric approach that is able to combine both the number of conidia stained with the specific antibody, and the amount of antibodies per conidia.

Conclusions-We showed, that is possible to detect, using IFA and FCM, high titers of total IgG antibody, and its isotypes by FCM, in sera of mice infected with P. lilacinum. These techniques are sensitive tools to study the humoral immune response against this fungus.

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DETECTION OF ARGINASE ACTIVITY IN DOGS WITH VISCERAL LEISHMANIASIS

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UNESP - FMVA, ARAÇATUBA - SP - BRASIL.

Introduction: The canine Visceral Leishmaniasis (VL) is a serious public health problem because infected animals are powerful transmitters of the parasite to humans through the vector. Leishmania is an obligate intracellular parasite that lives and replicates predominantly in macrophages. Depending on the balance of two inducible enzymes, nitric oxide synthase 2 and arginase 1, macrophages can kill the parasite or allow its growth. These two enzymes utilize a common substrate, L-arginine, and competitively regulate cytokine type 1 and 2. The metabolism of L-arginine appears to be an important regulator of the immune response may lead to T cell low response or promote growth of the parasite in the host. The progression of canine disease is accompanied by failure in cellular immunity with reduced circulating lymphocytes and cytokines that suppress the function of macrophages, suppression of T cells is well documented, but the mechanisms that lead to failure in the immune response are poorly understood.

Methods and Results: The city of Araçatuba is considered to be an endemic region for canine VL. Twenty dogs aged from 2 to 4 years, males and females, of non-defined breed and of different weights, were serum positive for L. (L.) chagasi by indirect ELISA. They were symptomatic and showing at least three clinical signs of canine VL. A control group of eighteen healthy dogs, males and females, serum negative for L. (L.) chagasi, by indirect ELISA were included in the study. Serum were used for quantification of the arginase activity. Quantification of samples was performed with a calibration curve in increasing amounts of urea.The results were compared using the Mann-Whitney test, with significance level of 5%. It was observed a arginase activity increased in the serum from dogs with visceral leishmaniasis compared to healthy ($p=0,0452$). Mean and standard deviation of the control animals were ($5,81 \pm 1,73$) and infected dogs were ($8,91 \pm 5, 24$).

Conclusions: This result indicates that the immune suppression seen in dogs with visceral leishmaniasis may be related to increased activity of arginase 1 in the serum of these dogs. Knowledge of this study may be useful in the design of immunotherapeutic drugs.

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DETECTION OF CD-68, IFN-G AND IL-10 IN SAMPLES OF PATIENTS INFECTED BY UTERINE CERVICE BY HUMAN PAPILLOMAVIRUS (HPV)

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Introduction: The cervical cancer is associated with persistent infection by Human papillomavirus (HPV). The viral clearance counts on the participation of Th1 response profile and the cytokines produced by these cells, among them the IFN-g, as well as the presence of macrophages, characterized by the presence of the CD-68 marker. The inhibition of IFN-g production by some viral genes or immunosuppressive cytokines such as IL-10, affect the macrophage activation and compromises the viral clearance. The IL-10 produced by keratinocytes infected by HPV or cells with regulatory profile makes this microenvironment immunosuppressed favoring the phenotypic change of macrophage pro-inflammatory (M1) for the macrophage associated to tumor IL-10-secreting (M2). Thus, this study aimed to detect the CD-68 marker, IL-10 and IFN-g cytokines in the stroma of the uterine cervix of patients with and without histopathological changes, infected or not infected by HPV and, therefore associates them with histopathological findings. **Methods and Results:** For the reactions of Immunohistochemistry (IHC) we used 45 biopsies of uterine cervix in patients submitted to detection of HPV DNA by real-time PCR and histopathology analyses. IHC was performed with antigen retrieval by moist heat and detection system LSAB+Sys HRP (DAKO®). For the survey of CD-68 marker and IL-10 and IFN-g cytokines we used the antibodies anti -CD-68 (DAKO, clone KP1), anti-IL-10 (anti-IL10 Invitrogen clone: 945A2A5) and respectively anti- IFN-g (eBioscience, clone: MD-1). This study was approved by the CEP UFMS (n° 87527/2012). We observed a progressive increase of the CD68 marker and the IFN-g cytokine in different degrees of lesions, predominating, however, in large quantities among the samples with high-grade lesion / HSIL (57.5% and respectively 51.6%). Regarding the frequency of IL-10, we observed its expression in large quantities, among HSIL samples (66.7%). **Conclusion:** According to our results we observed a progressive increase of macrophages and IFN-g reflecting the presence of Th1 immune response in high-grade lesions. However, the presence of IL-10 in large quantities among HSIL samples can alter the immune state in the microenvironment infected by the virus and enable the progression of the lesion.

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DETECTION OF DETECTION OF TGF-B, IL-10 AND IFN-Y IN UTERINE CERVIX OF PATIENTS INFECTED BY HUMAN PAPILLOMAVIRUS (HPV)

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Introduction: One of the factors which lead to the persistence of Human papillomavirus (HPV) and the subsequent progression to the cervical cancer is the lack of the Th1 cytokine production, especially IFN-γ. Such failure can be influenced by the presence of immunosuppressive cells, capable of inhibiting the Th1 response profile necessary for the viral clearance. Such cells play their immunosuppressive capacity by the secretion of various cytokines. Among them TGF-β and IL-10 are highlighted. Based on the above, this study aimed to detect the IFN-γ , TGF-β and IL-10 expression in the uterine cervix stroma of patients with and without pathological changes, infected or not infected by HPV and therefore to associate them to histopathological findings and viral load in order to understand better the infection immune response**. Methods and Results:** For the reactions of immunohistochemistry (IHC) we used the uterine cervix biopsies of patients undergoing HPV DNA detection by real-time PCR and histopathology analysis. The IHC reaction was performed with antigen retrieval by moist heat and detection system LSAB+Sys HRP (DAKO®). We used 74 samples for the detection of IFN-γ (anti- IFN-γ eBioscience, clone: MD-1), 77 for TGF-β (TGF-β Spring Bioscience, ref: E11264) and 76 for IL-10 (anti- IL10 Invitrogen clone 945A2A5). This study was approved by the CEP/UFMS (n° 87527/2012).There was a high frequency of IFN-γ in HPV-positive samples (46.1%) and with high viral load (48.3%). This cytokine expression prevailed in large quantities in low-grade lesion samples (57.2%). We found a higher frequency of TGF-β in large quantities in the HPV-positive samples (84.8%) and high viral load (86.9%). Its expression was predominant in large quantities in the high-grade lesions samples (HSIL) and carcinoma (91.2%). For the detection of IL-10, we also observed its higher frequency in a large amount in HPV-positive samples (83.6%) and with high viral load (83.9%). The expression of this cytokine in large quantity predominated in HSIL and carcinoma samples (88.0%). **Conclusion:** The reduction of IFN-γ and the predominance of TGF-β and IL-10 in the HSIL samples, indicates that there is a deficit of Th1 response profile and the probable presence of immunosuppressive cells, enabling this microenvironment favorable for the development of cervical cancer.

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DETECTION OF IGG ANTI-LEISHMANIA ANTIGEN BY FLOW CYTOMETRY

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Introduction: Cutaneous leishmaniasis (CL) caused by Leishmania braziliensis is characterized by the presence of one or more ulcerated lesions with elevated borders. CL patients develop severe inflammatory response and the presence of mononuclear cells infiltrating lesion site. The contribution of humoral immune response to protection, dissemination or immunopathological mechanisms is still controversial and not well understood. Thus, our goal is to develop a new serological technique using polystyrene microspheres sensitized with soluble L. braziliensis antigen (SLA) for the detection of IgG antibodies in the serum of patients with CL by flow cytometry. **Methods and Results**: We conducted sensitization of microspheres with SLA at a concentration of 1mg/ml in carbonate-bicarbonate buffer pH 9.6 and incubated for 1 hour in the dark at room temperature. After incubation of the microspheres with 27 serum or plasma samples from CL patients, 10 samples of serum or plasma of healthy individuals and 9 sera or plasmas of individuals with Chagas disease, the detection was done with anti-IgG labeled with Fluorescein isothiocyanate (FITC) diluted 1:500 in 1x PBS at 37°C and incubated for 30 minutes in the dark. The reading was performed on a flow cytometer (FACS Canto II). To validate the assay it was carried out a comparison between the ELISA and the test developed by us. The flow cytometry test showed 100% sensitivity and 50% specificity, while the ELISA showed 78% sensitivity and 90% specificity. **Conclusion:** The flow cytometry test developed by us for IgG detection is more sensitive than the ELISA, but less specific.

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DETECTION OF INTERLEUKIN-17 AND INTERLEUKIN-23 IN KAPOSI'S SARCOMA

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Introduction: Human herpesvirus 8 (HHV-8) is the etiologic agent of all Kaposi's sarcoma (KS) forms, including KS-AIDS, classic KS, endemic KS and iatrogenic KS. HHV-8 infection alone appears to be insufficient for the development of KS. The progression of KS relies also on some degree of host immune dysfunction. Multiple factors are related to KS pathogenesis, thus the aim of this work was to evaluate the expression of interleukin (IL)-17 and IL-23 in KS-AIDS and classic KS patients. **Methods and Results**: This study included 13 patients who presented skin lesions characteristics of KS, with confirmed histopathology. The samples were obtained from the archives of the Department of Pathology at the Federal University of Rio Grande do Norte and the University of São Paulo. From the 13 samples analyzed (12 male and one female) nine (69.2%) were infected with HIV (KS-AIDS) and four (15.4%) presented KS-classic. The immunohistochemic assays for the detection of the IL-23 and IL-17 were realized using monoclonal antibodies anti-IL-23 (Abcam) or anti-IL-17 (Bioscience). The KS lesions were classified histologically in patch-stage KS (54%), plaque-stage KS (23%) and nodular-stage KS (23%). The immunostaining was positive in 61.5% (8/13) of cases for IL-23 and in 30.8% (4/13) for IL-17, whereas 23% (3/13) of the samples presented positivity for both cytokines. From the positive IL-23 and IL-17 samples, two were classified as nodular-stage KS and one patch-stage KS, being two cases of KS-AIDS and one case of KS-classic. In all these three cases, the immunostaining in histological sections was strong and difuse, especially on nodular stage. **Conclusion**: The IL-23/IL-17 axis plays an important role in the development of chronic inflammation. In this study, we detected two cytokines, IL-17 and IL-23, particularly in nodular-stage KS, characterized by visible masses that showed a dominance of spindle cells and immune cell infiltrates.

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DETECTION OF TGF-B, IL-10 AND COEXPRESSION OF CD25/FOXP3 ON UTERINE CERVIX SAMPLES IN PATIENTS INFECTED BY HUMAN PAPILLOVAVIRUS (HPV)

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Introduction: The progression of the infection by Human papillomavirus (HPV) has been associated to the regulatory T cells (Treg). Such cells are characterized by the molecule expression CD25+ in high quantity and the Foxp3 transcription factor. These cells can perform their immunosuppressive capacity by the secretion of various cytokines which play an important role in the negative regulation of the immune response, such as TGF-β and IL-10. Thus, this study aimed to detect the coexpression of CD25/Foxp3 and the presence of TGF-β and IL-10 in the uterine cervix stroma of patients with and without pathological changes, infected or not infected by HPV and therefore associates them to histopathological findings and viral load. **Methods and Results**: For the reactions of immunohistochemistry (IHC) we used the uterine cervix biopsies of patients submitted for HPV DNA detection by real-time PCR and histopathology. The reactions were performed with antigen retrieval by moist heat and detection system LSAB+Sys HRP (DAKO®), for the simple staining and Vectastain kit (VECTOR ®) for double staining. We used 77 samples for the detection of TGF-β (anti-TGF-β Spring Bioscience), 76 for IL-10 (anti-IL10 Invitrogen, clone 945A2A5) and 74 for CD25/FOXP3 (anti-human IL-2R/CD25 eBioscience , clone: B-B10 and anti-Foxp3 eBioscience, clone: 236A/E7). This study was approved by the CEP/UFMS (n° 87527/2012). We observed a higher frequency of TGF-β in large quantities in the HPV-positive samples (84.8%) and high viral load (86.9%). The detection of IL-10 in large amount was also higher in HPV-positive samples (83.6%) and with high viral load (83.9%). Regarding the histopathological findings, the TGF-β and IL-10 expression was predominant in large quantities, with high-grade lesion (HSIL) and carcinoma samples (CA) respectively (91.2% and 88.0%). In relation to the coexpression of CD25/FOXP3 we observed the predominance, in large quantities, in the HPV-positive samples (66.7%) and with high viral load (66.1%). In the histopathology findings we observed the predominance, in large quantities between HSIL and CA samples (70.9%). **Conclusion:** Based on the high coexpression of CD25/Foxp3 and the presence of TGF-β and IL-10 in the stroma of HSIL and CA samples, we suggest that the presence of Treg in this microenvironment, inhibiting a proper effector response, makes it favourable for the development of cervical neoplasia.

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DETERMINATION OF EXPERIMENTAL MODEL OF FOOTPAD INFECTION IN MICE INDUCED BY PARACOCCIDIOIDES BRASILIENSIS

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Paracocidioides brasiliensis is the etiological agent of paracoccidioidomycosis (PCM), an endemic systemic mycosis in Latin America, especially in Brazil, which has the highest number of cases. The PCM is regulated by a Th1/Th2 pattern of immune response. The activation of Th2 type cells is associated with severe and progressive disease, whereas Th1 cells, as well as inflammatory factors associated with this pattern are related to increased resistance to infection of the host. The objective was to establish an experimental model of footpad infection in mice induced by P. brasiliensis. Balb/c mice were infected via footpad (i.pl) and first was evaluated the optimal inocullum concentration. To this the mice were infected with different inocula concentrations, $1x10^6$, $5x10^6$ and $1x10^7$ cells/30µL. Then, using the optimal inocullum concentration the mice were euthanized at 3^{th}, 7^{th} 10^{th}, 15^{th} and 30^{th} post infection. Analysis of lesion and measurement of edema was performed daily. Feet, lung, spleen, liver, blood, bone marrow and popliteal lymph node were removed for determination of fungal burden. In addition, feet, spleen, liver and lung were also used for analysis of myeloperoxidase (MPO) and N-acetylglucosaminidase (NAG) activity, which are indirect measures of neutrophils, macrophages content, respectively. The optimal inocullum concentration was $5x10^6$, wherewith was observed low fungal burden associated with high levels of MPO and NAG activity. Furthermore, in the early stages of infection, were observed increase in foot edema and high levels of myeloperoxidase and N-acetylglucosaminidase activity compared to the control. Thirty days post infection, the level NAG remained high, however, there was a decrease in the levels of MPO, but both still remained higher than the control. The fungal burden in the foot remained higher than the control during whole infection period. No colonies were recovered in other organs analyzed. The results confirm the Th1 inflammatory profile in disease resolution.

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DIFFERENT STRAIN OF TRYPANOSOMA CRUZI ALTER CYTOKINE AND CELLULAR RESPONSE

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Introduction The dog presents, in chronic phase, cardiac abnormalities and congestive heart failure with features of the human heart, as indicated by echocardiograph and electrocardiograph. The infectivity and cardiac lesion depends of T. cruzi strain morphology. So we aim to evaluate the immunological alterations during infection with strains from different DTUs (T. cruzi I or II).

Methods Ten mongrel dogs, 4 months old, obtained from the kennel at UFOP, Brazil. Of this, 7 dogs were inoculated intraperitoneally with Y strain (T. cruzi II, n=03) or Colombian strain (T. cruzi I, n=04). Age-matched uninfected dogs were used as controls (NI, n=03). During experimental protocol, blood was collected at the following times: before infection (T0); 9 days post-infection (T1); 1 month pi (T2); 2 mpi (T3); 3 mpi (T4); and 8 mpi (T5). For haemathologycal analysis, the quantification overall leukocytes in blood was obtained using Auto Hematology. Whole blood leukocyte was marked with antibodies of CD3, CD4, CD8, B cell and CD14. Culture of peripheral blood mononuclear cells was done at times T1, T3, T4 and T5, these cultures were stimulated with epimastigote antigen corresponding strain infecting animals. Subsequently, the cell populations were identified by specific surface molecules anti-CD4 or anti-CD8 and intracytoplasmatic cytokines (IFN-γ and IL-4).

Results Animals infected with Y strain presented leucopenia (6300±529), lymphopenia (2224±562), neutropenia (3647±8,8) and monocytopenia (231±32) at T1. Meanwhile, Col group have leukocytosis in T1 (12975±536) compared to NI group and reduction of monocyte at T3 (306±57) and T4 (232±70) compared to T0 (602±155). In ex-vivo analysis, $TCD4^+$ and $TCD8^+$ lymphocytes were reduced at T1 (1814±63; 1059±107); and T4 (1705±332; 1321±374) in infection with Y strain. However, only in T4 the number of T lymphocytes and $CD14^+$ Monocyte (10±2) were decrease in Col group. In relation to the results of blood culture, it was observed an increase in the $TCD4^+IFN\text{-}\gamma^+$ lymphocyte at T3 in Y group (2,09±0,2) compared to NI (0,94±0,05). Besides this, the percentage of $TCD8^+IL\text{-}4^+$ lymphocyte increased in the same group at T3 (2,5±0,2) and T5 (4,2±1,6). It was not observed any difference in the Col group.

Conclusion These results indicate that infection with Y strain alters the population of leukocytes and the cytokine production in the peripheral blood earlier than infection with Colombian strain.

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DIFFERENTIAL PROFILE OF SPECIFIC IGG AVIDITY TO BRUCELLA ABORTUS ANTIGENIC MARKERS IN INFECTED AND VACCINATED COWS

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Introduction: Brucella abortus is a Gram-negative intracellular bacterium that causes infectious abortion in food-producing animals and chronic infection in humans. The characterization of specific IgG avidity has been used to discriminate acute from chronic infections. Recently, we developed an avidity-ELISA for serological distinction between B. abortus infected and vaccinated cows. In this study we evaluated the profile of specific IgG avidity to different B. abortus antigenic markers by avidity-immunoblot assays in sera from infected and vaccinated cattle.

Methods and Results: Serum samples of three groups of cattle were obtained: GI, 30 naturally infected cows; GII, 30 S19-vaccinated heifers; and GIII, 30 nonvaccinatedseronegative cows. B. abortusS19 antigen was obtained by Triton X-114 extraction (hydrophilic phase) and submitted to polyacrylamide gel electrophoresis (SDS-PAGE). Serum samples of each group were analyzed by immunoblot and avidity-immunoblotusing 8M urea. Both assays employedanti-bovine IgG/peroxidase or protein A/peroxidase as detection reagents. The avidity index (AI) to different polypeptide bands was calculated and bands with AI>50% were considered reactive to high avidity IgGand those with AI<50% to low avidity IgGantibodies.

The electrophoretic profile of B. abortusTX-114 antigen revealed a broad spectrum of polypeptides(10 to 79 kDa),with differential immunoreactivity between GI and GII, regardless of the conjugates used. High avidity IgG antibodies were detected in sera of GI by both conjugates, but protein A/peroxidase was more able to detect low avidity IgG antibodies in sera of GII. Five major antigenic components (24,28,35,47 and 50 kDa) were predominantly recognized by high avidity IgG antibodies in sera of GI, in addition to three minor antigenic components (10,12 and 17 kDa) that were recognized exclusively by sera of this group. These antigens can be considered specific markers to exclude vaccinal response and to characterize B. abortus infection in cattle.

Conclusion: The evaluation of specific IgG avidity to different B. abortus antigenic markers can be considered an additional toll for serological distinction in response to infection and vaccination in bovine brucellosis.

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E. COLI TYPE VI SECRETION SYSTEM MEDIATES IMMUNE RESPONSE EVASION BY DECREASING LIPID BODY BIOGENESIS

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Introduction: The type VI secretion system (T6SS) is a gram-negative export pathway that has the ability to translocate protein effectors into largest cell types. The intracellular multiplication protein F (IcmF) is a component of the T6SS inner membrane and it is associated to inhibition of phagosome-lysosome fusion in macrophages and intracellular multiplication. In the linage Escherichia coli SEPT 362, the protein is associated to biofilms synthesis, flagella expression, adhesion and invasion of epithelial cells and intracellular macrophage survival. In order to understand the mechanisms of intracellular macrophage survival associated to IcmF, the present work aimed to investigate the lipid immunomodulatory pathway mediated by IcmF in mice macrophage, analyzing lipid body biogenesis in this conditions. Lipid bodies can synthesize inflammatory lipids, such as prostaglandins and leukotriens, and therefore formation of lipid bodies can be a cellular activation marker into a diversity of models.

Methods and Results: The linage SEPT 362, a mutant with IcmF gene deletion (**Δ**IcmF) and its deletion complement (**Δ**IcmFC) strain were used in the infection of mice macrophages (1:10). 24 hours after infection, the macrophages were fixed and processed to oil red staining to visualize and quantify lipid body biogenesis. SEPT 362 infected cells had a significantly decrease number of lipid bodies compared to IcmF deleted strain **Δ**IcmF, but similar with the deletion complement **Δ**IcmFC.

Conclusion: Taken together our data suggests the linage SEPT362 shows mechanisms that decrease macrophage activation by reducing the formation of lipid body, which may be associated with evasion of the immune system, and type VI secretion system plays an important role in this process.

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EARLY AND LATE ACUTE LUNG INJURY AND THEIR ASSOCIATION WITH DISTAL ORGAN DAMAGE IN MURINE MALARIA

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Introduction: Severe malaria is characterized by cerebral oedema, acute lung injury (ALI) and multiple organ dysfunctions, however, the mechanisms of lung and distal organ damage need to be better clarified.

Methods and Results: C57BL/6 mice were injected intraperitoneally with 5×10^6 Plasmodium berghei ANKA-infected erythrocytes or saline. Mechanical parameters were evaluated by constant flow ventilator. Parasitemia levels were low at day 1 post-infection (3.32 ± 0.29) and no changes in quantity of Evans blue dye recovered from brain tissue were observed. Symptoms of cerebral malaria (SHIRPA protocol) such as impaired locomotor activity, high parasitemia (19.33 ± 2.40) and Evans blue accumulation in brain tissue were only observed up to 5 days postinfection (d1: 0,35 ± 0,24; d5: 4,21± 1,32). P. berghei infected mice presented greater number of areas with alveolar collapse (d1: 30,44 ± 1,50; d5: 11,78 ± 1,53), neutrophil infiltration (d1: 10,82 ± 0.69; d5: 5,68 ± 0,37) and interstitial oedema (d1: 0,84 ± 0,08; d5: 0,56 ± 0,10) associated with increased static lung elastance (d1: 46,38 ± 4,27; d5: 38,78 ± 2,78), resistive pressure (d1: 0,71 ± 0,32; d5: 0,53 ± 0,06), and viscoelastic/ inhomogeneous pressure (d1: 1,20 ± 0,16; d5: 1,01), which was more severe at day 1 than day 5. It was also observed pRBC in lung parenquima, probably due to adhesion to ICAM-1 expressed in endothelial cells. Lung TNF-a and CXCL1 levels were higher at day 5 (0,004 ± 0,002 and 0,005 ± 0,001, respectively) compared to day 1 (0,00 ± 0,00 and 0,002 ± 0,001, respectively). In parallel, histological analysis of the brains of P. berghei-injected mice exhibited cortical oedema, glial cell swelling, and congested capillaries, with erythrocytes adhered to the endothelium, at days 1 and 5. The hearts of P. berghei-infected mice demonstrated interstitial oedema of the myocardium, which was more marked at day 5 than day 1. In the liver, swollen hepatocytes and a greater number of Kupffer cells containing malarial pigment grains were observed at days 1 and 5, which were concentrated in centrilobular or portal areas. Kidney damage, as inflammatory cell infiltration, was more severe at day 5 compared to day 1.

Conclusion: Our results suggest that ALI develops prior to the onset of cerebral malaria symptoms. Later during the course of infection, the established systemic inflammatory response increases distal organ damage.

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EFFECT OF ANTIVIRAL THERAPY WITH PEGYLATED INTERFERON-ALPHA PLUS RIBAVIRIN ON THE EXPRESSION OF AUTOIMMUNITY BIOMARKERS IN CHRONIC HEPATITIS C

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Introduction: Chronic hepatitis C is the main cause of cirrhosis, hepatocellular carcinoma and hepatic transplantation worldwide. The chronic infection caused by the hepatitis C virus (HCV) promotes a complex dysfunction in B-lymphocytes, stimulating the production of non-organ-specific-autoantibodies (NOSA) and cryoglobulins. The role of these autoimmune markers in the immunopathology of hepatitis C and in their importance to predict the success of the antiviral therapy is controversial. The aim of this work was to evaluate the effect of antiviral therapy on the expression of these autoimmune biomarkers in chronic hepatitis C patients after treatment.**Methods and Results:** Twenty-nine untreated patients were enrolled in this study and investigated for the presence of NOSA (ASMA, ANA, rheumatoid factor, thyroid peroxidase and thyroglobulin antibodies) and cryoglobulinemia before treatment with pegylated interferon-α (IFN-α) plus ribavirin and at 12 and 24 week post treatment. Twenty-one individuals had mild or moderate liver necroinflammatory activity (A1 or A2, respectively) and 15 patients had advanced fibrosis (F3 - F4). Rheumatoid factor, ASMA and cryoglobulinemia were the most prevalent autoimmune biomarkers in untreated patients with a prevalence of 62% (18/29), 52% (15/29) and 46% (13/28), respectively. Antiviral therapy decreased cryoglobulin seropositivity after 12 and 24 weeks of treatment (P = 0.026 and P = 0.013, respectively). Both cryoglobulinemia and ASMA were associated with advanced fibrosis (P = 0.003 and P = 0.017, respectively). The presence of ANA or rheumatoid factor was not associated with liver necroinflammatory activity or fibrosis, and their presence was not influenced by antiviral therapy. **Conclusions**: Our results suggest that ASMA and cryoglobulinemia are associated with chronic hepatitis C severity, but the presence of NOSA or serum cryoglobulin do not allow predicting the outcome of the antiviral therapy.

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EFFECT OF BREAST MILK FROM SCHISTOSSOMOTIC MICE IN THE ACTIVATION AND PROLIFERATION OF LYMPHOCYTES

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INTRODUCTION: Schistosomotic maternal infections are common in endemic areas. Exposure to parasite components in utero and through breastfeeding modulates the immune response to homologous antigens. In regard to heterologous antigens, it was observed that adult offspring mice, previously breastfed in schistosomotic mothers, showed an enhancement of the anti-OVA antibodies and IL-2 production. This study aimed to evaluate activation and lymphoproliferation of immature and functional splenocytes in the presence of breast milk from infected mothers and non-infected mothers with or without Soluble Eggs Antigen (SEA). **METHODS AND RESULT**: For this, infected and non-infected Swiss Webster female mice (60 days) were mated. After the delivery, the female were milked. The spleens from non-infected mother offspring were collected on 7th days and six weeks old. The splenocytes were cultured in the presence of culture medium, milk from S. mansoni infected mothers, milk from non-infected mothers with or without SEA. After 24 hours, the cells were labeled-doubly with anti-CD3, anti-CD28, anti-CD154 and anti-CD71 monoclonal antibodies fluorescent and analyzed by FACS. There was more percentage of CD3+/CD71+ cells in the culture of mature cell than immature cell, but without statistical difference among the in vitro stimulus. However, the frequency of CD3+ cells expressing CD28+ on immature culture was higher in response to milk from non-infected mothers, but not in mature cells. Differently, the mature CD3+CD154+CD28+ cells were in high frequency upon the stimulus of the milk from mothers infected or non-infected mothers plus SEA**. CONCLUSIONS**: Then, the breastfeeding improved the activation of T lymphocyte in early life, while milk from schistosomotic mother and SEA induced activation on mature T lymphocyte. These results corroborate the immune-stimulatory potential of the breastfeeding in schistosomotic mothers. **FINANCIAL SUPPORT:** Pró-Reitoria para Assuntos de Pesquisa e Pós-Graduação (Propesq-UFPE)

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EFFECT OF CLOSTRIDIUM DIFFICILE TOXINS A AND B OVER ADENOSINE RECEPTOR EXPRESSION IN INTESTINAL EPITHELIAL CELLS IN VIVO AND IN VITRO

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Introduction: Clostridium difficile is recognized to be a nosocomial pathogen that causes intestinal inflammation, epithelial barrier disruption and diarrhea. Adenosine production is increased under inflammatory situations. The adenosine receptor A2b (A2bR) is the most expressed receptor in the human and mice intestine.

Methods and Results: We investigated whether the adenosine receptors and cytokine expression, such as IL-6 and IL-8, could be affected by toxin A (TcdA) and toxin B (TcdB) intoxication in vitro and in vivo, and the effect of A2bR antagonist (PSB603), in the same parameters. HCT-8 cells received TcdA or TcdB in different concentrations (0.1, 1, 10 and 100ng/ml) for 6, 12 and 24hours. For the in vivo studies, we used a murine cecal loop model intoxicated only with TcdA for the same time points and isolated the cecum epithelial cell. We also used a murine model of C.difficile (strain VPI10463) infection (CDI) and the cecum epithelial cells were removed on day 3 and day 7 after infection. We demonstrated that TcdA/TcdB intoxication was able to upregulate the A2bR, A2aR and A1R gene expression, as well as IL-6 and IL-8, in vitro , independent of toxins concentrations. TcdA and C.difficile infection had similar effect in vivo. PSB603 reduced the adenosine A2b, A2a and A1 receptors expression and caused decrease of IL-6 and increase of IL-8 gene expression in HCT-8 intoxicated cells.

Conclusion: Clostridium difficile toxins affect adenosine receptor, as well as cytokine expression and this action may be related to their severe inflammatory effect.

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EFFECT OF HYPOXIA ON MACROPHAGE INFECTION BY TRYPANOSOMA CRUZI

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Introduction: Cells of the immune system are subject to various physiological and pathophysiological conditions, including tissue hypoxia. Chagas disease is caused by the protozoan Trypanosoma cruzi. There is both clinical and experimental evidence that platelet aggregates in the cardiac microcirculation and hypoxic changes are associated with injury of the myocardium. However, the role of hypoxia in infection of macrophages by T. cruzi has not been investigated. **Objectives:** In the present study, we compared the effect of 2% oxygen tension (hypoxia) with a normal tension of 21% oxygen (normoxia) on macrophage infection by the protozoan parasite Trypanosoma cruzi. **Methodology:** Primary mouse macrophages were obtained from normal BALB/c mice by peritoneal lavage 4 days after injection of thyoglicolate. Cells were maintained in the culture plates were then placed in a gas-tight modular chamber. The chamber was gassed at a flow rate until the saturation point using certified gases containing O_2, CO_2 and N_2 (White Martins, Campinas, SP, Brazil) and placed in a 37°C temperature-controlled incubator. In all experiments, exposure of cells to 2% O_2, 7% CO_2 and balanced N_2 is referred to as hypoxia and exposure of cells to 21% O_2, 7% CO_2 and balanced N_2 is referred to as normoxia. **Results:** Here we show that peritoneal macrophages maintained in a hypoxic condition showed a significant decrease on NO and ROS production and on parasite replication. Hypoxia diminishes the efferocytosis in a time-dependent manner and led to a deficient T. cruzi replication in response to putrescine addition. Peritoneal macrophages maintained in a hypoxic condition and stimulated by apoptotic cells showed changes on cytokine production profile, with increased production of TNF-α and IL-1β, without affecting production of TGF-β. **Conclusion:** We suggest that TNF-α is an important factor that contributes to the decrease of T. cruzi replication. Taken together, our results indicate that efferocytosis modulates T. cruzi replication and production of cytokines, even under hypoxic conditions. **Financial support**: CNPq, FAPERJ, INCT

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EFFECT OF IFNG ON CARDIOMYOCYTES CULTURES INFECTED BY TRYPANOSOMA CRUZI STRAIN SYLVIO X10/4

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Introduction: Infection with Trypanosoma cruzi causes a chronic and progressive cardiac inflammation in 30% of individuals. Host genetic factors as well as the parasite strain may lead to differences in the infection and signaling of infected cardiomyocytes. IFNγ is an important cytokine to control the infection and its effect over cardiomyocytes is poorly studied. In this sense, our study search to identify differences in the response of cardiomyocytes from C3H/HePAS and C57BL/6 mice during infection by the Sylvio X10/4 strain parasite. C3H/HePAS mice infected develop a strong cardiopathy, whereas C57BL/6 do not show cardiac damage in chronic phase of the infection. **Methods and Results:** Cardiomyocytes from C3H/HePAS and C57BL/6 neonatal mice were isolated. The cells were maintained in culture with medium alone, or treated with IFNγ and infected with trypomastigotes form. After 24 and 48 hours of infection, the cultures were fixed and stained for the analysis of the infection or, RNA was extracted, converted into cDNA and quantified the gene expression for Toll-like receptors, cytokines and chemokines. **Results:** Our results show in the first graph that infection by the Sylvio X10/4 parasite is similar to both strains of mice, with or without treatment with IFNγ. Next, we avaluated the gene expression of receptors involved in the recognition of T. cruzi. Toll 2 gene was increased in cardiomyocytes from the C3H/HePAS while cardiomyocytes from the C57Bl/6 had increase the toll 2 and 9. As the chemokines expression, the infection minimally increased gene expression of chemokines CCL4 and CCL5, but CXCL10 was widely expressed in both cardiomyocytes. Regarding cytokines, TNFα and IL-6 gene were more expressed on both strain mice after 48 hours of infection, and further when treated with IFNγ. In addition, the cardiomyocytes C57Bl/6 had significants increases when treated with IFNγ in comparison with cardiomyocytes C3H/HePAS. In relation the TGFβ expression, both the strain did not have change in the gene expression. **Conclusions:** Even though the infection had been similar in both strains, it is possible that subtle differences in gene expression of proinflammatory cytokines and chemokines induced by IFNγ are responsible for maintenance or elimination of the parasite in the cardiac tissue.

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EFFECT OF PLECTRANTHUS AMBOINICUS (LOUR.) SPRENG (LAMIACEAE) ESSENTIAL OIL USING AS MARKER THE EXOTOXIN A EXPRESSION OF THE PSEUDOMONAS AERUGINOSA.

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Introduction: Plectranthus amboinicus (Lour.) Spreg (Lamiaceae), popularly known as “malvarisco”, is an aromatic herb cultivated all over the North and Northeast regions of Brazil. It is widely used in Brazilian folk medicine for several therapeutic purposes. Pseudomonas aeruginosa is a Gram-negative, opportunistic, and pathogenic. Among the virulence factors, the Pseudomonas Exotocin A (PE) is the most toxic substance, significantly affecting the protein synthesis of the host cells. Several studies have shown that PE to be toxic to murine and PMNs and to inhibit phagocytosis and killing of P. aeruginosa by PMNs in vitro. Furthermore, this toxin could result in inflammation, with ocular damage consistent in mice and corneal ulceration and cell death in rabbits, for e.g. This present work proposed to investigate the antimicrobial activity of the essential oil against P.aeruginosa standard strains, through of subinhibitory and inhibitory concentrations, using as marker the PE quantification. **Methods and results:** The oil essential of the P. amboinicus, was obtained by hydrodistillation for Clevenger and the chemical analysis was performed for CG/MS. The standard strain (ATCC 27853) was obtained from the Instituto Oswaldo Cruz, Brazil. The determination of the minimal inhibitory concentration (MIC) was performed in a previous study. The detection on production of exotocin was evaluated by Western blot and ELISA. Carvacrol is the major constituent identified in the oil essential (98%). The exotoxin detection by Western blot revealed that there was a decrease in production these toxin when bacterial cells were treated with inhibitory concentrations (4 x MIC, 2 x MIC and MIC), compared with the control (no treatment), and showed the similar action commonly used antibiotic (ciprofloxacin). ELISA analysis showed complementary in the results obtained in Western blot. It was observed that there were decreases in the amount of the PE detected ($p<0.001$), verified by reduction density optical rates in the samples. **Conclusion:** The results showed antimicrobial activity of the essential oil of P. amboinicus front of the standard strains of P. aeruginosa verified by reducing the production of exotocina A. Thus, it is necessary other studies to evaluate a possible direct action of the essential oil of this important virulence factor as a perspective for mechanism of action elucidation. **Financial support:** This research was financially supported by the CNPq and FUNCAP/PPSUS.

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EFFECT OF THALIDOMIDA TREATMENT ON BRUCELLA ABORTUS CLEARANCE AND VACCINE

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Introduction and Objectives: Brucella is a gram-negative, facultative intracellular pathogen that causes undulant fever, endocarditis, arthritis and osteomyelitis in humans. In livestock, it causes abortion and infertility which results in serious economic losses. The immune response against Brucella involves manly TH1 cytokines and CD8+ T cells. In this context, many studies have shown that Thalidomide is able to induce high production of IFN-, proliferation and cytokine production, mainly by CD8+ T lymphocytes. In accordance, our previous works showed that treatment with high concentrations of thalidomide is able to improve immune response against Brucella, increasing cytotoxicity, CD8+ T cells, Nitric Oxide and IFN- production. Thus, the aim of this study was to evaluate different protocols of treatment and the potential of thalidomide to protect IFN- KO mice and to improve the protection induced by RB51vaccination.

Methods and Results: Firstly, C57BL/6 mice were treated with Thalidomide at 150mg/kg/day during seven days and infected with B. abortus (S2308) after one or ten days. The PBS treated mice were used as control. A week after the infection, the bacterial load was determined in the spleen. After that, the bacterial load was evaluated in treated mice with 30 or 50 mg/kg/day and infected after ten days. The ability of Thalidomide treated IFN- knockout mice to survive to B. abortus infection also were determined. Finally, the bacterial count was determined in treated and RB51 immunized mice.

The results have shown a significant reduction of bacterial number in mice infected ten days after treatment (150mg/Kg/day). A reduction was also observed when mice were treated with 30 or 50mg/kg/day. Nonetheless, thalidomide treatment wasn't able to improve the survival of IFN- knockout mice. Finally, the protection induced by RB51 vaccine was slightly improved in thalidomide treated mice.

Conclusion: Thalidomide is able to potentiate the clearance of Brucella abortus and has potential to improve the RB51 vaccine.

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EFFECTS OF ALANYL-GLUTAMINE ON NF-KB AND IL-8 RESPONSES OF INTESTINAL EPITHELIAL CELLS

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Introduction: Glutamine is an amino acid that plays many roles of biological relevance, including cell proliferation, cytoprotection and immunomodulation. Despite its importance, low solubility and aqueous stability of glutamine compound diminish therapeutic use of glutamine. Alanyl-glutamine (Ala-Gln) is a dipeptide with greater stability and it has been shown to maintain gut barrier function. In this context, we aimed to evaluate cell proliferation and innate immune response induced by Ala-Gln on intestinal epithelial cells. **Methods and Results:** Rat intestinal epithelial cells (IEC-6) were seeded in 96-well plates at $2,5x10^4$ cells/well for proliferation assay. At each time point, WST-1 reagent was added to the wells, followed by measurement at spectrophotometer (450nm). Ala-Gln from 0.3 to 30mM was tested for proliferation assay at 24 hours and the half maximal effective concentration (EC_{50}) was used for other time points and mRNA analysis (1mM). Ala-Gln was added to the wells and, after 12, 24 and 48 hours, cell proliferation was evaluated. For mRNA analysis, cells were seeded in 12-well plates and incubated with Ala-Gln. After each time point, mRNA was extracted, cDNA was synthesed and Real Time-PCR was performed. Transcription levels of NF-kB and IL-8 genes were quantified at 6 and 12 hours after treatment. Statistical analysis was performed with ANOVA and Bonferroni test. All Ala-Gln concentrations (0.3, 1, 3, 10 and 30mM) increased proliferation significantly ($p<0.05$) at 24 hours time point. Ala-Gln at 1mM was chosen for time-course evaluation as it shows effect of 50% (EC50). Ala-Gln increased NF-kB transcript levels at 12 hours time point and did not alter IL-8 transcription. **Conclusion:** Ala-Gln supplementation promoted benefits on cell proliferation of intestinal epithelial cells. Increased NF-kB can be associated to anti-apoptotic pathways and growth factors depending on its substratum of activation. Thus, the increased transcription of NF-kB gene may lead to proliferative effects.

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EFFECTS OF BETA-CAROTENE ON NF-KB AND IL-8 RESPONSE OF INTESTINAL EPITHELIAL CELLS

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Introduction: Beta-carotene is an important precursor on vitamin A synthesis pathway, which is an essential micronutrient for growth and cell differentiation. Considering its chemical structure and experimental data, it has been proposed that beta-carotene has antioxidant properties. However, in vitro studies have shown its oxidative effects based on analysis of biomarkers. The objective of this study was to evaluate the role of beta-carotene on cell proliferation and innate immune response (NF-kB and IL-8) of intestinal epithelial cells. **Methods and Results:** Rat intestinal epithelial cells (IEC-6) were seeded in 96-well plates at $2,5x10^4$ cells/well and cultured for 24 hours. Initially, beta-carotene from 0.3 to 30 mM was added and after 24 hours, cell proliferation was evaluated by adding WST-1 reagent, following measurement at spectrophotometer. Using the same protocol, beta-carotene at 8 mM was tested at 12, 24 and 48 hours time point. For mRNA analysis, cells were seeded in 12-well plates and treated with beta-carotene with 8 mM, followed by mRNA extraction, cDNA synthesis and RT-qPCR. Transcription levels of NF-kB and IL-8 genes were quantified at 6 and 12 hours after treatment. Statistical analysis was performed with ANOVA and Bonferroni test. Beta-carotene at 30 mM was able to improve cell proliferation only after 24 hours of treatment ($p<0.05$). Using linear regression, 8 microM was the concentration utilized in the following experiments. Besides not improving cell proliferation at 12, 24 or 48 hours time point, beta-carotene at 8 mM increased NF-kB gene transcription at 12 hours ($p<0.05$). IL-8 gene transcription was 6-fold increased at 6 hours after treatment ($p<0.05$). **Conclusion:** Our results suggest the involvement of increased levels of IL-8 and NF-kB in the pro-oxidative effects of beta-carotene, which may be explained by its conversion to retinoic acid. As beta-carotene molecular mechanism has not been clearly elucidated, these data could help to clarify some of its phenomenon.

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ENDOTOXIN-INDUCED ROS-DEPENDENT CELL DAMAGE IS CRITICALLY DEPENDENT ON TRPM ION CHANNELS ACTIVITY

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Introduction: During endotoxemia-induced severe inflammation, it has been observed an increased generation of reactive oxygen species (ROS). ROS modulate the activity of the transient receptor potential melastatin 4 (TRPM4) and 7 (TRPM7) ion channels, to promote deleterious effects on cells. In this sense, TRPM4 has been involved in H_2O_2-induced epithelial cell death, and TRPM7 has been linked to neuronal death induced by nutrient deprivation-derived oxidative stress. Thus, our objective was to study the participation of TRPM4 and TRPM7 ion channels in the LPS-induced endothelial and neuronal death respectively.

Methods and Results: We used two different models: primary human endothelial cells (ECs, >99% pure) as a model of systemic inflammation and hippocampal primary rat neurons (HPNs, >95% pure) as a model of central nervous system inflammation. Both preparations were cultured in presence or absence of the bacterial endotoxin, 20 mg/mL lipopolysaccharide (LPS) for 48 h. Data are expressed as mean±SD. N=3-6. Our results showed that ECs and HPNs exposed to LPS exhibited a ROS-dependent increase in cell death (>63±5% and >50±3% of LPS-induced cell death compared to maximal death, respectively). In addition, ECs and HPNs exposed to LPS showed an increased intracellular ROS levels (>9.1±1-fold and >7.4±0.7-fold of ROS increase compared to basal ROS level, respectively). Noteworthily, suppression of TRPM4 expression using a siRNA protects ECs against LPS challenge (>65±6% decreasing of LPS-induced EC death). Likewise, HPNs transfected with a siRNA against TRPM7 were resistant to the deleterious effect of LPS (>90±5% decreasing of LPS-induced neuronal).

Conclusion: ECs and HPNs exposed to LPS exhibit an increase in cell death through a ROS-dependent mechanism. Suppression of the expression of the ROS-modulated TRPM4 and TRPM7 ion channels was efficient to protect endothelial and neuronal cells to LPS injury, respectively.

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ENRICHED ENVIRONMENT AND AGING EXACERBATE CLINICAL OUTCOMES OF ANTIBODY-ENHANCED DENGUE DISEASE AND GLUCOCORTICOIDS REDUCES THESE EFFECTS IN YOUNG BUT NOT AGED MICE

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Introduction: We previously demonstrated in young mice that in comparison with animals raised in an impoverished environment (IE), animals from an enriched environment (EE) show more severe dengue disease, associated with an increased expansion of memory T target cells. Because active older adults show less functional decline in T cell adaptive immunity, we hypothesized that aged mice from EE would show higher mortality than mice from IE.

Methods and Results: To test this hypothesis, we administered serial i.p. injections of anti-DENV2 hyperimmune serum, followed 24 h later by DENV3 (genotype III) infected brain homogenate (AED). Control mice (n=5) received equal volumes of uninfected brain homogenate. The presence of virus or viral antigens was indirectly detected by real-time quantitative RT-PCR and immunohistochemistry. Compared to infected IE, EE mice independent of age showed higher mortality and more intense clinical signs. After AED infections, EE young (n=16, EY) and aged (n=8, EA) animals showed earlier and more intense clinical signs including dyspnea, tremor, hunched posture, ruffled fur, immobility, preterminal paralysis, shock, and deaths compared to IE young (n=18, IY) and aged (n=8, IA) animals. Kaplan–Meier analysis showed significant differences in the survival probability curves of the experimental groups (IA vs IY, log-rank test, $p = 0.022$; EA vs EY, log-rank test, $p = 0.011$; EA vs IA; log-rank test, $p = 0.011$; EY vs IY log-rank test, $p = 0.016$) and 100% of deaths in EA, 75% in IA, 44% in EY, and 17% in IY. 100% of young animals (EY and IY) that did not receive glucocorticoids died or reduced burrowing to below 40%, earlier than all other groups with corticoids. Deaths in EY started earlier than in IY in the mice with no corticoids (EY vs IY log-rank test, $p = 0.0025$, Fig. 2E), and all animals of the uninfected group survived. As compared to young animals from both enriched and impoverished environments, glucocorticoid injection had no significant effects in aged mice independent of the environment.

Conclusion: We suggest that the higher mortality rate in aged mice associated with a reduction of glucocorticoid effects may be explained by reduction of low-affinity glucocorticoid receptors previously described in aged rats. Because we did not assess glucocorticoid receptors in the present work and do not know the systemic distribution of those receptors in aged mice, this question deserves further investigation.

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ENZYME IMMUNOASSAY USING MONOCLONAL ANTIBODIES RAISED AGAINST NEOSPORA CANINUM PURIFIED ORGANELLES TO DETECT SPECIFIC IGG IN SERA OF INFECTED CATTLE

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Introduction: Neospora caninum, the etiological agent of neosporosis, is an obligate intracellular parasite with worldwide distribution. It was discovered in the late 80s and since 1990 studies shown the relationship of this parasitic disease with abortions in cattle herds. This fact causes considerable impact on livestock, requiring an accurate diagnosis of neosporosis in such animals. Several studies using monoclonal antibodies have been developed as a marker of the stage of infection of various diseases. Thus, this study aimed to check the kinetics of antibody reactivity to the different organelles of N. caninum in infected cattle.

Methods and Results: Seronegative calves were experimentally infected with tachyzoites and challenged after 270 days. Blood was collected from the animals every 30 days over one year and used for analysis of the anti-N. caninum presence specific to different soluble antigens (NLA and NcESA). Capture ELISAs were performed with monoclonal antibodies 20D2 (rhoptry), 3A5 (vacuole) and 10B10 (vacuole). Overall our results showed that animals presented initial antibody reactivity after 30 days of infection, however, IgG levels decreased and stayed negative to low until the reinfection, which induced a high specific IgG occurring a peak (300 days). Antigens captured by 10B10 and 3A5 MAbs presented a very similar profile to crude soluble antigens of the parasite, with a moderate to low initial reactivity and an intense IgG peak after 30 days of the second infection. Interestingly, 20D2 MAb, which recognizes an epitope within NcROP4 protein, was only recognized after reinfection.

Conclusion: Our data show the potential application of organelle specific mAb's in the diagnosis of N. caninum infection, with a possibility to identify the stage of infection in cattle.

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EOSINOPHILS PHENOTYPES AS ANTIGEN-PRESENTING CELLS IN TOXOCARA CANIS INFECTED MICE

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Introduction: In vitro studies indicate that eosinophils kill multicellular helminthes by a variety of mechanisms, although their role as effectors of host defense against these organisms has been challenged by observations that depletion of eosinophils in vivo fails to alter worm burdens in mice infected with trematodeo or nematode. Toxocara canis infection results in pulmonary inflammation, systemic eosinophilia, increased serum IgE levels and induction of a Th2 type of immune response. Several studies have demonstrated roles for eosinophils during innate and adaptive immune responses to helminthes infections. T. canis infection induced in pulmonary inflammation characterized by the infiltration of lymphocytes, neutrophils and principally eosinophils. To present antigen, eosinophils should provide co-stimulatory signals for lymphocytes. As B7 molecules CD80 and CD86 and related B7 homolog's are especially significant on APCs for delivering requisite co-stimulatory signals to lymphocytes, B7 molecules on eosinophils have been studied.The aim of this study was to determine CD80 and CD86 molecules expression on eosinophils from peripheral blood in infected mice with T. canis after 18 days pos infection, and evaluated the leukocytes number in the blood. **Methods:** Mice were divided into control group (C) and infected mice group (Tc). The Tc group received 500 eggs/mice/gavage after 18 days pos infection were euthanasia and was collected the cells from blood for count number and CD80, CD86 e MHC II expression determination. **Results**: The results showed that blood eosinophilia after 18 days pos infection when compared with C group. The CD80, CD86 e MHC II expression on eosinophils increased in mice infected when compared with the control group. However, the CD80 e MHC II expression on eosinophils were biggest than CD86. **Conclusion:** Finally, our dates suggested that during infection by T. canis eosinophils can be phenotypes for antigen presentation cell (APC), increased the co-stimulatory molecules expression as MHC II, possible contribution for immune response amplification in the infected mice with T. canis.

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EPIGENETIC AND PHENOTIPIC ALTERATIONS IN DENDRITIC CELLS DURING SEVERE SEPSIS AND SEPSIS-INDUCED IMMUNOSUPPRESSION.

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INTRODUCTION: Sepsis is a systemic inflammatory response syndrome against the infection which can develop in immunosuppression sepsis-induced. Actually, several inflammatory dysfunctions have been described in immune cellular system which could be responsible to impair the immune response towards the secondary infection. The PPAR-g is a lipid-activated nuclear receptor which it participates in inflammation, lipid metabolism and cellular differentiation. Previous studies have shown the role of PPAR-g in acute sepsis besides their effects in the immunosuppression sepsis-induced still unclear. Our aims were evaluated the epigenetic and phenotype changes in dendritic cells (DC) from post-septic mice and assessed the effects of PPAR-g on DC functions. **METHODS AND RESULTS:** Post-septic mice were susceptible against M. bovis BCG and pulmonary DC from septic mice has lower CD86 and MHC class II, higher number of lipid droplets and inhibition of several inflammatory, chromatin modification and lipid metabolism gene expression. Additionally, BMDC from post-septic mice showed inducible microRNA expression and chromatin alterations when compared with BMDC from Sham mice. Infected-BMDC from septic mice exhibited an immature profile and positive shift to anti-inflammatory cytokines production. Also, the BCG-infected post-septic BMDC showed inhibition of inflammatory and lipid metabolism genes, increased microRNA expression and chromatin alterations when compared to infected-BMDC from Sham mice. PPAR-γ flanked mice in CD11c cells were more susceptible to severe sepsis. Activation of PPAR-γ in infected-BMDC from post-septic mice reduced lipid droplets formation and phagocytosis but not the cytokines production when compared with infected-BMDC from Sham mice.**CONCLUSION:** After severe sepsis, epigenetic and phenotypic changes modulate dendritic cells functions and it may contribute to sepsis-induced immunosuppression. The understanding of PPAR-γ could be important to development of new therapy in immunosuppression sepsis-associated and long-term inflammatory diseases. **SUPPORT**: CNPq; FAPERJ, CAPES/PDSE.

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ESSENTIAL ROLE OF CD81 IN B CELL ACTIVATION BY DENGUE VIRUS

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Introduction: Dengue virus (DENV) belongs to the Flaviviridae family and comprises four serotypes, named DENV1-4. Dengue infection is associated to a spectrum of clinical manifestations, ranging from mild symptoms to the severe forms of dengue, which seems to be associated to an exacerbated inflammatory response and antibody-dependent enhancement. Antibody-mediated response is essential to virus neutralization and infection control, but is also attributed to exacerbated infection and severe dengue. It has been recently demonstrated that DENV-infected patients present an increased activation of B cells, and we have previously demonstrated that culture of primary human B lymphocytes with different DENV serotypes induces increased immunoglobulin response. However, the role of direct virus infection and the receptors involved in this activation were not addressed yet. CD81 is part of the B cell co-receptor complex and plays an important role in Hepatitis C virus (HCV) infection, another member of the family. The aim of this study was to investigate whether human B cells are productively infected by DENV and to evaluate the influence of CD81 in activation, adsorption and subsequent DENV replication.

Methods and Results: Purified human B lymphocytes were cultured with CD81 neutralizing antibody and infected with DENV-2 at a multiplicity of infection (MOI) of 1. The activation of B cells was analyzed by measuring IgM secretion by ELISA and qRT-PCR was used by viral quantification in the adsorption and replication assays. We observed that DENV induced and increased secretion of IgM after 10 to 15 days of culture, which was abolished in cells treated with anti-CD81 antibody. qRT-PCR of the B cells cultured with DENV demonstrated that the virus is internalized and secreted, but the amount of virus detected in B cells of all donors after 24, 48 and 72 h post-infection was not altered, indicating that DENV was unable to productively replicate in these cells. In addition, anti-CD81 treatment did not influence on virus adsorption and internalization.

Conclusion: These results suggest DENV interaction with CD81 in the surface of B cell is essential to induce cell activation, but it is not associated to virus adsorption and internalization. The interaction of CD81 and viral proteins may be associated to polyclonal activation of B cells and their knowledge might provide a better understanding of the pathophysiology of dengue.

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EVALUATION OF HUMAN MONOCYTES FUNCTION FROM HIV+ PATIENTS IN RESPONSE TO MYCOBACTERIUM TUBERCULOSIS IN VITRO INFECTION

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Introduction: Monocytes are key cells on immune response against intracellular pathogens, which include different mechanisms of pathogen control or elimination. However, cells from immunocompromised hosts, such as HIV+ infected individuals, may not be able to control infection. Thus, the aim of this work was to evaluate with a simplified method the main functional activity of human monocytes from healthy donors and HIV+ patients against Mycobacterium tuberculosis infection.

Methods and Results: PBMC from healthy individuals were obtained using Ficoll-Paque protocol. Monocytes were separated by CD14+ microbeads and then infected with Mycobacterium tuberculosis (H37Rv). Phagocytosis and bacterial growth control were evaluated by resazurin reduction test and analyzed in fluorimeter. Plasma and cell culture supernatants collected after 24h in culture were used to measurement of cytokines by a Multiplex platform. Reactive oxygen species (ROS) production by cells stimulated with PMA or heat-killed M. tuberculosis were measured by chemiluminescence using Luminol. We observed a higher phagocytic ability of healthy individuals compared to HIV+ patients and a tendency for lower microbicidal activity of monocytes from HIV+ patients. We also observed a higher concentration of IL-6, TNF-α, IL-1β, IFN-γ, IFN-α2, IL-4, IL-5, IL-10, IP-10 and MCP-1 in the plasma of infected patients compared to control subjects. After M. tuberculosis in vitro infection, the major cytokines produced by monocytes were IL-6, TNF-α and IL-1β, followed by IL-10. The chemokines RANTES and MCP-1 were moderately stimulated, in both HIV+ patients and control individuals. Moreover, HIV+ patients treated with HAART showed a trend of higher ROS production compared to HIV+ patients without treatment. We found a negative correlation between the number T CD4+ cells/mm^3 and IL-6, TNF-α, IP-10 and ROS production after M. tuberculosis infection in HIV+ patients, in which the lower the number of T CD4+ cells, the higher the concentration of cytokine and ROS. On the other hand, a positive correlation was found between viremia and the production of IL-6, TNF-α, IP-10 and ROS in which the higher the viremia, the higher the production of cytokines and ROS.

Conclusion: Our results showed the importance of these biomarkers associated with disease progression in our patients and may be chosen as a target for future studies.

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EVALUATION OF MYELOID CELL LINE FUNCTION ON INFLAMMATORY RESPONSE IN HTLV-1 INFECTION

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Introduction: The human T lymphotropic virus type 1 (HTLV-1) infects T cells inducing lymphocyte proliferation and activation that are associated to pathology. Very little is known about monocyte/macrophage function in HTLV-1 infection. We evaluate the monocyte subpopulations and expression of some co-stimulatory molecules in this infection, furthermore the ability of HTLV-1 infected macrophage to kill an intracellular parasite, and cytokine/chemokine production by these cells. **Methods and Results**: Participants of this study include 15 HTLV-1 infected individuals and 10 healthy subjects. The frequencies of three monocyte subsets (classical, intermediate and non-classical monocytes) and the expression of co-stimulatory molecules were measured by flow cytometry. After isolation of mononuclear cells by density gradient centrifugation, 6 days monocytes-derived macrophages were infected with L. braziliensis stationary phase promastigotes at a 5:1 ratio or stimulated with LPS (100 ng/mL). The percentage of infected macrophages and the number of amastigotes per 100 cells were evaluated. Determination of cytokines and chemokines were performed by ELISA in supernatants of these cells after 48 hours of infection or stimulation with LPS. There were no significant difference in the frequency of the three monocyte subsets and in the expression of co-stimulatory molecules between HTLV-1 infected subjects and healthy controls groups. Macrophages from HTLV-1 infected subjects were infected with L. braziliensis in the same proportion that macrophages from healthy individuals, and both groups had the same ability to kill this parasite. Nevertheless, macrophages from individuals infected with HTLV-1 produced more TNF-α, CXCL9 and CCL5 than macrophages from healthy subjects when infected with L. braziliensis ($p<0,05$). **Conclusion**: The high production of chemokines and TNF-α by myeloid cell line suggests an important role in the pathogenesis of HTLV-1. There is dissociation between the production of cytokines/chemokines and killing ability in HTLV-1 infection. Financial support: CNPq, CAPES, NIH and INCT-DT.

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EVALUATION OF PLECTRANTHUS AMBOINICUS (LOUR.) SPRENG IN THE INFLAMMATION IN MURINE MODEL OF METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS SKIN ABSCESSES

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Introduction: Methicillin-resistant Staphylococcus aureus (MRSA) comprises more than 50% of nosocomial bacterial infections in some countries, including the United States and Brazil. Plectranthus amboinicus (Lour.) Spreng (Lamiaceae) is distributed throughout tropical Africa, Asia, Australia, and the Americas. P. amboinicus has been a topic of studies because it is rich in metabolites with potential antimicrobial and anti-inflammatory activitie. For this reason, we proposed the present study to investigate the therapeutic effects of extracts from the leaves of P. amboinicus in MRSA infected mice. **Methods and Results:** The hydroalcoholic extract (HE) was obtained by macerating fresh leaves with 70% ethanol. The fraction F4 was obtained by successive chromatographic fractionations of ethanol extract. The bacterial suspension of MRSA was adjusted to match the 0.5 McFarland standard and 0.2 mL was inoculated subcutaneously into the back of previously shaved male Swiss mice. The control suspension consisted of BHI broth containing 2% Cytodex. Seven groups of 6 animals in each group were tested. The control group consisted of 4 animals. Two doses of the HE extract, the fraction F4 (250 and 500mg/kg/dose) or vancomycin (10 and 20mg/kg/dose) were given intraperitoneally at 3 and 12h after infection. Abscess diameters were measured after 72h of infection. The animals were then sacrificed, and subcutaneous abscesses were excised. A small fragment of the injured tissue was removed, fixed in 10% formalin, and submitted for histopathological analysis. The parameters of inflammation were evaluated and graded on a 0–3 point scale. The HE at 500mg/kg/dose and vancomycin at 20mg/kg/dose proved to be efficient in resolving the abscesses, compared to the untreated animals ($p<0.05$). The fraction F4 at 500mg/kg/dose proved to be the most effective treatment ($p<0.001$). **Conclusion**: Some anti-inflammatory effects of P. amboinicus extracts are described in the literature, including the blockage of NF-kB activation, which would consequently reduce the production of proinflammatory cytokines. To the best of our knowledge, this is the first report demonstrating the efficacy of P. amboinicus in treating a subcutaneous abscess model caused by MRSA. **Financial support:** This research was supported financially by the CNPq and Funcap/PPSUS.

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EVALUATION OF PROFILE CYTOKINES PRODUCED IN INFECTED-MICE WITH LARVAE (L3) OF TOXOCARA CANIS

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Introduction: Visceral larva migrans (VLM) is a syndrome with high prevalence in developing countries reaching 54% in Brazil. It is characterized by the migration and retention of larval helminths in tissues from accidental hosts, and the nematode Toxocara canis is the main etiological agent. The mechanisms by which infection by T. canis modulates the immune response are poorly understood, especially the profile of cytokines produced, as they vary from tissue parasitized and time of infection. There is an urgent need for new drugs and effective vaccines to control this syndrome. Thus, studies may broaden the knowledge on the immune response against this parasite and aid in the development of control strategies for this syndrome. The aim of this study was to evaluate the cellular profile and cytokine produced by mice experimentally infected with larvae (L3) of T. canis. **Methodology**: We used BALB / c mice weighing 15 to 18 kg divided into two groups: Control and Tc (infected with 500ovos/animal orally). After 18 days of infection were assessed cell profiles (eosinophils) and the level of IL-4 and IL-5 in blood and lungs of infected animals. **Results:** There was an increase in the level of cytokines in infected animals compared to the control group in the blood and lungs 18 days after infection, which comes against the increased number of eosinophils in the same period, featuring a Th2 response profile shown in helminth infections. **Conclusion**: It is concluded that infection with larvae of T. canis induces an anti-inflammatory profile mediated by Th2 cells with increased IL-4 and IL-5.

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EVALUATION OF THE CD4+CD25+FOXP3+ T CELLS IN THE HEPATITIS C TREATMENT FOLLOW UP

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Introduction: Hepatitis C virus (HCV) causes chronic infection in most infected individuals. However, the mechanisms that promote HCV persistence are not completely known. Among them, quantitative and functional deficiency of T regulatory cells has been reported in chronic hepatitis C patients that present extra hepatic manifestations of autoimmunity. The aim of this work, was to investigate the frequency of $CD4^+CD25^+FoxP3^+$ Treg cells in Brazilian HCV patients with and without manifestations of autoimmunity, before and after antiviral treatment with alpha-interferon plus ribavirin. **Methods and Results:** Twenty-three patients (11 men and 12 women) chronically infected with HCV genotype 1 and 20 control healthy individual (10 men and 10 women) were included in this study. Treg cell frequency was determined by flow citometry. The presence of serum cryoglobulin was probed by both cryoprecipitation and gel-diffusion. Non-organ-specific-autoantibodies (NOSA, ANA and ASMA) were investigated by indirect fluorescent antibody test, whereas rheumatoid factor was tested by nephelometry. Ten out of 23 patients treated with PEG-IFN plus ribavirin were followed up 12 weeks post treatment. Five (50%) subjects achieved a complete early virologic response (cEVR) demonstrated by normal ALT levels and undetectable HCV-RNA, while five patients were null responders (NR). Patients that were seropositive for cryoglobulinemia or NOSA had a lower frequency of $CD4^+CD25^+FoxP3^+$ Treg cells in comparison with the patients without these extra hepatic manifestations of autoimmunity (P= 0.011 and P= 0.006, respectively). The frequency of Treg cells in HCV patients, before and after antiviral treatment and in the controls was similar (1.13%; 1.04% e 1.11%, respectively; $P > 0.05$). However, patients presenting cEVR had a higher $CD4^+CD25^+FoxP3^+$ T cell frequency than the null responders (1.6% and 0.72%, respectively; $P = 0.008$). There was a strong negative correlation between the $CD4^+CD25^+FoxP3^+$ T cell frequency and blood HCV load in the NR patients ($P = 0.009$; $r = -0,79$). **Conclusion**: Our results suggest that Treg cells are involved in the extra hepatic manifestations of autoimmunity in patients chronically infected with HCV.

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EVALUATION OF THE INFLAMMATORY PROFILE AFTER ORAL INFECTION BY NEOSPORA CANINUM

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Introduction: Neospora caninum is an intracellular parasite with worldwide distribution and a broad rage of hosts, causing abortions in livestock, consequently, genering losses in the economy. However, the mechanisms involved in this host-parasite interaction remains unclear.To date, experimental models that assess host responses against the parasite are usually based on parenteral inoculations, but it is important to understand the mechanisms involved in resistance/susceptibility to infection mimicking at most the natural conditions. Thus, we aimed to evaluate the profile of the cellular and humoral immune responses in mice infected orally with N.caninum.

Methods and Results: Female C57BL/6 mice were orally infected by gavage with $3x10^7$ live tachyzoites and were euthanized at 7, 14 and 21 days post-infection (p.i.) and the serum, brains, lungs, livers and gut sections was colected and subject to histological analysis,cytokine quantification, parasite DNA quantification and specific IgG antibody detection. Histological analysis revealed distinct patterns of inflammatory reactions in the analyzed tissues. We noted severe diffuse inflammation was present in livers, lungs and central nervous systems (CNS) at 7, 14 and 21 days p.i., respectively. Parasitism of CNS was so evident at 21 days p.i. and could be visualized by HE staining of the tissues.The gut was the primary site of infection and increased expression of IFN-g was observed 14 days p.i. in the duodenum, proximal and distal jejunum, while increased IL-10 production was mainly detected in the duodenum and proximal jejunum at 21 p.i..Thus, the expression of IFN-g increased progressively in the central nervous system since 7 days post-infection, while IL-10 expression was observed only at 21 days p.i.. The levels of IgG and sub-class were quantified by ELISA and we observed crescent levels of IgG in the serum samples, which were mainly composed of IgG2a, while IgG1 was almost undetected, suggesting a predominance of a Th1 immune response in these animals, differently of that usually observed in parenteral protocols.

Conclusion: Oral infection with N. caninum induces fast colonization and inflammation of different tissues. However, in that sense, we can infer that the experimental model here in proposed present a promising tool for the study of the immunopathogenesis induced by neosporosis, since it can closely mimic tissue damage seen in domestic animals and wildlife in a laboratory model of infection.

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EVALUATION OF THE MACROFAGE ACTIVITY IN DIFERENT STRAINS OF MICE INFECTED BY RHIZOPUS SP.EVALUATION OF THE MACROFAGE ACTIVITY IN DIFERENT STRAINS OF MICE INFECTED BY RHIZOPUS SP.

AMANDA RIBEIRO DOS SANTOS[1]; DÉBORA DE FÁTIMA ALMEIDA[2]; ANGELA CAROLINA FINATO[3]; BÁRBARA CASELLA AMORIM[4]; THAÍS FERNANDA DE CAMPOS FRAGA-SILVA[5]; JAMES VENTURINI[6]; OLAVO SPERANZA DE ARRUDA[7].

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Introduction. Mucormycosis is a severe fungical infection, caused by fungus from the order of mucorales. The genus Rhizopus is responsible for 70% of the cases. It is the third most common fungus invasive in patients immunocompromised, with mortality rates over 90%. Despite the importance of the disease, only a few researches approach the immunological aspect involved in Rhizopus-host. Because of the difficulties in performing researches in humans, we aimed to study some strain of mice which one would be an appropriated murine experimental model.

Material and methods. Females BALB/c, C57Bl/6 and Swiss mice, 6-8 weeks old, were infected through the tail vein with $3x10^4$ spores of Rhizopus sp/mouse. The control group was composed by mice which were submitted to the same procedure, but inoculated only with sterile saline solution. After 7, 14 e 30 days of the fungus inoculation, the mice were sacrificed and studied as follows: samples of spleen, brain, liver, lung and kidney were submitted to microbiological analyses; adherent peritoneal cells were cultivated and stimulated, or not, with heat-killed spore of Rhizopus and lipopolysaccharide. After 24 hours, the cells were submitted to determination of peroxide hydrogen (H_20_2) and the supernatants were submitted to nitric oxide (NO) dosages.

Result. BALB/c, C57Bl/6 and Swiss mice were susceptible to fungal infection. Besides, all strains showed a reduction in the total fungal load after the 7th day of the inoculation. On the other hand, the infection progressed differently in the three strains: C57BL/6 mice showed the highest fungal load on day 7, BALB/c mice on day 14 and Swiss mice on day 30. We observed no correlation between fungal load, production of H_2O_2 and NO.

Conclusions. The results support that different murine strains show different response to the infection by Rhizopus and that C57Bl/6 mice is the most appropriate to study the early events of the disease and the Swiss mice to study the later events

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EVALUATION OF THE MECHANISMS OF LESION REDCUTION IN LEISHMANIA MAJOR-INFECTED MICE TREATED WITH TOTAL OR PURIFIED BONE MARROW CELLS: A POTENTIAL NEW CELL THERAPY FOR MUCOCUTANEOUS LEISHMANIASIS

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Leishmaniasis is a disease caused by parasites of the genus Leishmania. We have previously shown that when infected with L. major, TNFR1 KO mice develop chronic lesions and can control parasite growth at the site of infection when compared to WT mice, but maintain the intense inflammatory infiltrate. Objective: the aim of this study was to characterize the chronic lesions of TNFR1 KO mice infected with L. major and analyze the effects of using purified cells from bone marrow (PBMC) as a cell therapy for these lesions. WT and TNFR1 KO mice were inoculated with L.major on the footpad and the course of infection was followed for 15 weeks. The immunological aspects of the infection were analyzed at different time-points after infection and after treatment with PBMC. Results: We found higher expression of IFN-γ and IL-10 in lesions of TNFR1 KO mice infected and treated with PBMC when compared to non-treated mice. We also observed higher expression of IL-17 in lesions of TNFR1 KO mice infected and treated with cells than in non-treated mice. However, we could not find any difference in the expression of TNF-α, TGF-β, FoxP3, iNOS and arginase I in lesions of TNFR1 KO infected and treated with PBMC. The persistence of the lesions in TNFR1 KO is correlated with an intense inflammatory infiltrate with higher percentage of $Ly6G^+$ cells e T $CD8^+$ lymphocytes and lower number of apoptotic cells at the site of infection in these mice when compared with WT mice. TNFR1 KO mice infected with L.major were treated with PBMC during the chronic phase of infection and 4 weeks later the lesions were analyzed. Treatment with PBMC significantly reduced the chronic lesions of TNFR1 KO. Analyses of the donor cells in the lesions showed that 7 days after treatment these cells are differentiating into dendritic cells ($CD11c^+MHCII^+$). Conclusion: The treatment with PBMC can ameliorate the chronic lesions during L.major infection most likely via reduction of the inflammatory infiltrate.

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EVALUATION OF THE RECRUITMENT OF EOSINOPHILS AFTER TREATMENT WITH EXTRACT OF HARPAGOPHYTUM PROCUMBENS DURING THE EXPERIMENTAL VLMS

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Introdution: The helminthic parasites are able to develop several mechanisms to modulate and manipulate the immune response of the host. Among these mechanisms is the suppression of cellular immune response and stimulation of the humoral immune response, which allows them to promote their permanence, for decades until, in the host. The Visceral Larva Migrans (VLM) is of a Syndrome worldwide occurrence. Toxocara canis is the etiologic agent of VLMS. In the human becomes infected by ingesting eggs which develop into larvae in gastrointestinal tract and then penetrate through the wall invading various organs. Presence of antigen-secreting eggs in tissue initiates immunological response of Th2-type that is marked by eosinophilia, high levels of IgE. Harpagophytum procumbens (Hp) has been used as treatment for a variety of illnesses, including, arthritis and diseases of the digestive tract. The present study examined the role of H. procumbens in eosinophlis recruitment and interleucina-5 (IL-5) serum levels and integrin CD11b expression on the blood cells after treatment with Hp. **Methodology**: We used Balb/c mice divided into groups: control, Tc and Tc+Hp. Infected animals treated or not received 500 eggs/animal T. canis by gavage. After 18 days of infection the animals were euthanized and the fluid was extracted for further evaluation of eosinophlis/mm^3. The level of IL-5 was determined by ELISA from plasma samples in period described. Subsequently we evaluated the expression of molecule CD11b on eosinophils blood by flow cytometry. **Resultds**: Our results showed decreased expression of the integrin CD11b and IL-5 levels in treated animals as compared to those infected only. These results reflect the reduction of eosinophils in the blood in the same period. **Conclusion:** Thus we suggest, Hp natural extract may modulate the immune responses during the VLMS by interfering with the migration of EO decreasing the IL-5 levels and can down modulate this CD11b expression in these compartments analyzed.

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EVALUATION OF URIC ACID EFFECTS IN P. AERUGINOSA INFECTION ON NEUTROPHILS

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Introduction: Patients with sepsis present elevated serum total antioxidant capacity and uric acid (UA) is the principal contributor for this index. However, the actual pathophysiologic role of UA in critically ill patients is still a matter of debate (Crit Care. 10:R36, 2006). UA is oxidized to urate hydroperoxide by myeloperoxidase (MPO) in a situation that mimics the oxidative burst by inflammatory cells. Urate hydroperoxide can oxidize proteins susceptible to redox modulation and alter their functions (J. Biol. Chem. 286:12901-12911, 2011). Therefore, we hypothesized that urate or its oxidation product urate hydroperoxide would modulate the killing activity of neutrophils against Pseudomonas aeruginosa, a Gram-negative bacterium that causes opportunistic infections in hospital bed.

Methods and Results: human HL-60 cells were differentiated into neutrophils by incubating it for 5 days with 1.3% dimethylsulfoxide (DMSO). Neutrophils ($2x10^6$ cells/well) were incubated for 3 h at 37°C with P. aeruginosa 14 ($2x10^7$ cells/well) with increasing concentrations of UA (0; 0.2; 0.5 and 2.0 mM). The suspension was used to evaluate bacterial viability by colony forming unit (CFU) counting. UA (1 and 2 mM) prevented bactericidal activity of neutrophils.The supernatant was collected and subjected to measurement of cytokines. Quantification of IL-1β and TNF-α assay was performed by Enzyme-Linked Immunosorbent Assay (ELISA) according to the manufacturer's recommendations (R&D systems). The incubation of neutrophil with P. aeruginosa induced IL-1β and TNF-α release by 111.5 ± 4.9 pg/mL and 55.2 ± 5.9 pg/mL, respectively. UA prevented cytokines increase in all concentrations. At 100 µM UA the IL-1β and TNF-α levels were 83.5 ± 4.8 pg/mL and 17.6 ± 2.2 pg/mL.

Conclusion: these data indicate that urate can disrupt neutrophils bactericidal activity and down regulate cytokines release by these cells. Thus, this propitiates a new perspective about UA effect in opportunistic bacterial infection.

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EVIDENCE OF HETEROGENEITY IN VIRULENCE AMONG STRAINS BELONGED TO DIFFERENT PHYLOGENETIC SUBLINEAGES OF MYCOBACTERIUM TUBERCULOSIS BEIJING FAMILY

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Introduction: Tuberculosis (TB), caused by Mycobacterium tuberculosis (MTB), continues to kill 2 million people worldwide annually. Some genetic lineages of MTB, like the Beijing genotype family, are a cause of special concern because of growing prevalence in different regions of the world, suggesting that these strains are more competitive compared with other genotype strains. Recent studies demonstrated that Beijing strains isolated in the regions of rising prevalence belonged predominantly to the modern sublineage. These strains, but not ancestral counterparts, demonstrated a high degree of clonal expansion and were associated with an increased ability to spread and cause disease. These data suggest that the strains of recently evolved Beijing sublineages could display increased virulence. To address this issue, we measured virulence of four clinical MTB isolates belonged to modern Beijing sublineage and three ancestral strains, isolated in Brazil and Mozambique, compared with the virulence of reference H37Rv MTB strain. **Methods and Results:** Differentiation of ancestral and modern Beijing sublineages was based on a series of genetic markers, e.g., insertions of IS6110 into NTF region, RD deletions and specific SNPs. MTB virulence was measured in C57BL/6 mice, intratracheally infected with low (100 bacilli) or intermediate (2500 baciilli) doses of each strain, through the evaluation of animal survival curves, histopathology and CFU quantification. Additionally, we evaluated production of cytokines by the lung cells ex vivo. The obtained results demonstrated that the modern Beijing strains were more virulent than the ancestral strains. Two modern strains displayed hypervirulent phenotypes, characterized by increased mortality rates, higher levels of bacterial growth in lungs and dissemination to spleen and liver. The most striking difference was in the observed histopathology. The hypervirulent strains caused extensive pulmonary consolidation, aggravated by neutrophilic pneumonia and necrotic areas, detected 28 d after infection, whereas other strains caused typical granulomatous inflammation. Severe inflammation was associated with increased production of proinflammatory mediators by lung cells: cytokines (IFN-g, IL-17, IL-6, IL-1b, TNF-a), neutrophil-recruiting chemokines (MIP-2, KC), and matrix metalloproteinases (MMP-2, MMP-9). **Conclusion:** Beijing strains of modern sublineages are more likely to display increased virulence than the ancient strains.

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EXPRESSION AND FUNCTIONAL ACTIVITY OF PD-1/PD-L1 IN LYMPHOCYTES FROM C3H/HEPAS MICE CHRONICALLY INFECTED WITH TRYPANOSOMA CRUZI SYLVIO X10/4 PARASITES

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Introduction: C3H/HePAS mice infected with T. cruzi Sylvio X10/4 parasites develop a chronic cardiomyopathy similar to that observed in humans. Both parasite and host elements contribute to the incomplete elimination of T. cruzi, and, among the late ones, an inefficiency of the effector mechanisms of the adaptive immunity due to exhaustion induced by the persistent stimulation of T lymphocytes. In this context, senescence of $CD4^+$ T cells, as well as loss of the effector ability of $CD8^+$ T cells can occur by interaction of inhibitory molecules PD-1 and PD-L1 expressed in these cells. Thus, we assessed the expression of PD-1 and PD-L1 in lymphocytes of Sylvio X10/4 chronically infected C3H/HePAS mice, and evaluated the importance of these molecules for the in vitro activation of lymphocytes from these animals.

Methods and Results: Heart pathology progression was observed in the course of infection. Relative to the control group, an increase in the total number of cells in the spleen of chronically infected mice, as well as an increase in the number of $CD4^+$, $CD8^+$ and B lymphocytes ($CD19^+$) were observed. Moreover, the spleen of chronic mice showed an increase of activated $CD4^+$ cells, as well as a significant increase in PD-L1 expression by $CD4^+$ T lymphocytes. In contrast, there were no significant differences in the expression of PD-L1 in other lymphocyte populations, and no increase in the expression of PD-1 in any splenic lymphocyte type. In the heart of chronically infected animals, significant augment was observed in the expression of PD-L1 relative to controls, as assessed via qPCR. To evaluate the possible inhibitory effect of PD-L1, splenocytes from chronically infected animals and controls were stimulated in vitro with T. cruzi antigen in the presence or absence of inhibitory anti-PD-L1 antibody. After 72h of stimulation, in vitro proliferation of $CD4^+$ T cells was increased by PD-L1 blockade. However, there was no significant increase in the secretion of IFNg in the presence of anti-PD-L1.

Conclusion: The increased expression of PD-L1 seen in the heart and spleen of chronically infected animals raises the possibility that T lymphocyte activity might be inhibited via interaction of PD-1/PD-L1 molecules, a process that could hinder control of the parasite at the inflammatory site, allowing its persistence.

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EXPRESSION IN LIVER TISSUE AND PERIPHERAL BLOOD OF MIR-33A AND MIR-122 IN INDIVIDUALS INFECTED BY HCV

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Introduction: MicroRNAs (miRs) are small highly conserved noncoding RNAs that regulate biological processes. The miR-122 and miR-33a are described as lipid metabolism regulators. Hepatitis C Virus (HCV) infection leads to the expression of genes involved in biosynthesis and transport of lipids resulting in the stimulation of the lipid metabolism, creating a favorable environment for virus replication. To our knowledge, there are not any reports linking miR-33a and miR-122 expression to the lipid profile in HCV infection. In the present study, we evaluated the expression levels of miRs 33a and 122 on the liver tissue and peripheral blood. **Material and Results**: 67 patients were enrolled in this study (55 infected by HCV-1 and 12 infected by HCV-3a). miRNAs were isolated from blood samples and liver biopsies. Expression levels of miR-33a and miR-122 were evaluated using specific primers and probes. Reactions were carried out on 7500 Fast Real-Time PCR (Applied Biosystems, Foster City, CA, USA). Samples were tested in triplicate and RNU44 was used as endogenous control. The expression values are presented as fold change (2^{-ddCT}). miR-33a expression was higher in the tissue compared to that found in the blood either to HCV-1 ($p = 0.0036$) and to HCV-3 ($p = 0.023$). In addition, in individuals infected by HCV-3a, a negative correlation was observed between miR-33a expression and cholesterol levels ($r = -0.6304$, $P = 0.028$, $R2 = 0.3975$). miR-122 expression was higher in blood than in tissue, regardless of the viral genotype. For genotype 3, there was a negative correlation between serum levels of miR-122 and viral load ($r = -0.8933$; $p < 0.0001$, $r^2 = 0.7979$). **Conclusion**: The higher expression of miR-122 in blood is associated with injury to liver cells. The negative correlation between expression levels of miR-122 in the liver and the level of HCV RNA-3a confirms that the release of this miR into the blood is a consequence of cell damage caused by HCV. A strong negative correlation between serum cholesterol and the liver expression of miR-33a was observed only in HCV-3a agreeing with the fact that lipid metabolism is important for the maintenance of this infection due to viral replication needs for lipids.

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EXPRESSION OF INDUCIBLE NITRIC OXIDE SYNTHASE IN RESPONSE TO STIMULATION BY HYPHAE AND YEAST FORM OF CANDIDA ALBICANS.

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Introduction: Since this is an important public health problem, associated with increased mortality in immunocompromised patients, infections caused by Candida albicans, is a field to be explored. Relevant aspect of the pathogenesis of this fungus is its ability to switch between different morphologies. Accordingly, the objectives of this research were outlined in order to understand how these morphological changes are reflective in the immune response, specifically in the expression of inducible nitric oxide synthase (iNOS), the enzyme responsible for the synthesis of nitric oxide, an important mediator of the response of the host against microorganisms. Methods and Results**:** We used male rats of Wistar strain (n=12), aged 90-120 days. Alveolar macrophages (AM) were obtained after tracheotomy by bronchoalveolar lavage. Performed cell isolation were established four systems in vitro: Negative Control (NC) composed of MA in culture; Positive Control (PC), added to lipopolysaccharide and two test systems: 1. (Y) MA added in yeast fungus and 2. (H) MA added in the hyphae fungus. After 24 hours of incubation, RNA extraction was performed with subsequent reverse transcription and cDNA amplification by specifically iNOS real-time PCR. The quantification of iNOS was obtained from the absolute expression. To construct the standard curve, serial dilutions were made DNA, the concentrations from 1fg to 1ng. The reaction efficiency was determined according to the formula $e=(10^{1/slope}) -1$. Statistical analysis used Mann-Whitney and the results were expressed as median± Std. Error. The efficiency of the rection was 96,46%. Were detected higher levels of expression in the PC (9,368±1,228) when compared to the NC (0,00190±0,00689) $p<0,05$. Both systems tests Y (0,0148±0,00433) and H (0,0307±0,0151) also showed higher levels of expression when compared to NC, $p<0,05$. However no differences were found between the test systems Y and H, $p>0,05$. Conclusion: The real-time PCR system developed in this study for the iNOS detection, showed excellent performance. Both systems stimulated with fungal showed higher levels of expression when compared to the NC, thus indicating the effective participation of this enzyme in immune responses involving Candida albicans. However no difference was detected between the different morphologies analyzed, suggesting that the change of morphology does not influence the process of macrophage activation and consequent response microbicidal.

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EXPRESSION OF TLR2 AND TLR4 AND CYTOKINE PRODUCTION IN PATIENTS WITH VISCERAL LEISHMANIASIS PRE AND POST TREATMENT

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Introduction: Visceral leishmaniasis (VL) is an emerging public health problem, with 500,000 new cases per year. Toll-like receptors (TLRs) play an important role microbial recognition and adaptative immune response development. Our objective was to evaluate in patients pre and post-treatment the expression of TLâ€‹R2 and TLR4 and correlate with production of IL-10 and TNF-α. **Methods and results**: Were evaluated 13 patients pre-treatment, 12 post-treatment and 16 healthy subjects. Analysis of the expression of TLR2 and TLR4 on CD3 and CD14 cells was performed by flow cytometry. Detection of IL-10 and TNF-α in the supernatants of PBMCs stimulated with agonists of TLR2 (PGN) and TLR4 (LPS) was performed by CBA. In patients pre-treatment there was a lower percentage of expression of TLR2 (90,9±10,7) and TLR4 (29,3±29,7) in CD14 compared to controls (98,7±1,5 and 62,7±32,9) and higher percentage of expression of TLR2 (23,8±20,9) and TLR4 (4,3±6,1) in CD3 compared to post-treatment (4,5±4,4 and 1±1,5) and controls (0,6±0,8 and 1,3±2,5). Patients with active VL produced more TNF-α compared to post-treatment and controls, and this production was related to TLR2. After treatment, the percentage of expression of TLR4 in CD14 (22,6±8,9) was lower than in controls and the percentage of TLR2 expression in CD3 was higher than in controls. After treatment there was a reduction in TNF-α production compare to pre-treatment. There was no difference in IL-10 production between pre-treatment and post-treatment, however when PBMC were stimulated with LPS and PGN, the levels of IL-10 have increased, showing that TLR2 and TLR4 are associated with this cytokine production. **Conclusion**: Together these results suggest the involvement of TLR in the cytokines production as well as in the pathogen clearance and pathological process.

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FISH OIL SUPPLEMENTATION AMELIORATES LEUKOPENIA AND THROMBOCYTOPENIA AND MODULATES NITRIC OXIDE AND TNF-γ PRODUCTION DURING THE ACUTE PHASE OF TRYPANOSOMA CRUZI INFECTION

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Introduction: Omega-3 polyunsaturated fatty acids (n-3 PUFA) supplementation has been reported to reduce inflammation and in some circumstances diminish infectious disease resistance in rodents. We investigated whether supplementation with fish oil, a source of n-3 PUFA, impaired host resistance against Trypanosoma cruzi, the causal agent of Chagas' disease. **Methods and Results**: C57BL/6 mice were supplemented (0.6% v/w) by gavage with phosphate buffer saline (PBS), corn oil (CO) or fish oil (FO) for 15 days prior to T. cruzi challenge and throughout the course of the infection. In this study, we observed an increase in spleen weight (PBS = 0.5 g ± 0.03 g, CO = 0.5 g ± 0.04 g vs FO = 0.6 g ± 0.03g, $p < 0.05$) and cellularity ($x10^6$) in the infected-FO supplemented mice (601 ± 1 vs PBS = 306 ± 41 and CO = 351 ± 41). Importantly, FO supplementation in infected mice improved platelets and leukocytes counts, decreased heart parasitism, decreased nitric oxide production in plasma (12.72 µM ± 0.98 vs 21.22 µM ± 3.01, on day 7 post infection) and cardiac tissue (12.62 µM ± 4.98 vs 29.4 µM ± 5.4, on day 12 post infection, $p < 0.01$), and increased TNF-α production by spleen cells stimulated with parasite antigen (743 ± 92.6 vs 359,5 ± 88.1 $p < 0.05$, on day 7 post infection). **Conclusion:** These data suggest the potential of FO supplementation to attenuate the clinical course of T. cruzi infection and further studies are warranted.

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FUNCTIONAL ANALYSIS OF MIR-135B AND MIR-130B EXPRESSION IN THE LUNG OF MYCOBACTERIUM TUBERCULOSIS-INFECTED MICE.

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Introduction: Tuberculosis (TB) is an infectious disease caused by Mycobacterium tuberculosis (Mtb), remaining as the second leading cause of death from infectious diseases worldwide. The immune response in TB has shown a Th1 pattern with IFNγ production, wich have been described as microRNA target in dendritic cells, NK cells and $CD4^+$ T cells after different stimulus. Mature microRNAs are derived from a long primary transcript, from genome, which undergoes a series of maturation stages processed by endonucleases, until they become mature, inhibiting translation of mRNA target. Indeed, some studies have demonstrated the differential microRNA expression in the peripheral blood of TB infected patients, suggesting that it could be a biomarker of disease. However, there is a lack of information about the involvement of these microRNAs during the inflammatory process in the Mtb-infected lungs.**Methods and results:** To perform the functional analysis of microRNAs in lungs of Mtb-infected mice by microarray data**,** BALB/c mice were infected by intranasal route (i.n.) using 100 µl of suspension containing $1x10^5$ bacilli of Mtb strain H37Rv. After 30 and 60 days of infection, total lung RNA of infected and control mice were extracted. The identification of microRNAs was accomplished through Agilent miRNA microarray system and analyzed by GeneSpring GX11.5 software. We identified 27 and 24 microRNAs differentially expressed on 30 and 60 days, respectively, which were confirmed by qPCR analysis. Using specific prediction programs, we found direct interactions of some microRNAs with mRNA Peli-1, that participate in innate immunity activation and down-regulate the $CD4^+$ proliferation. **Conclusions:** Our data described the differential expression of microRNAs in lungs of Mtb-infected mice and its possible interference on $CD4^+$ proliferation due to the interaction with Peli-1. In this way, our study will contribute to the understanding of the role of microRNAs expressed in the lung of Mtb-infected mice.

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GAIN- AND LOSS-OF-FUNCTION APPROACHES IN PULMONARY PARACOCCIDIOIDOMYCOSIS USING FOXP3-GFP REGULATORY T CELLS

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Introduction: Paracoccidioidomycosis (PCM), the most relevant systemic mycosis in Latin America, is a granulomatous disease caused by the dimorphic fungus Paracoccidioides brasiliensis. Although the involvement of regulatory T (Treg) cells in suppressing immunity against PCM has been previously demonstrated, the mechanisms through which this occurs remain incompletely understood. In this work, we used Foxp3-GFP transgenic mice in order to track the development, location and function of Foxp3-expressing Treg cells in the acute and chronic stages of PCM.

Methods and Results: Animals were intratracheally infected with 10^6 yeast cells and examined at 2 and 10 weeks post-infection. The phenotype and activity of Treg cells were characterized in the lesions at both post-infection periods by flow cytometry. Treg cell ablation after anti-CD25 injection led to dramatically reduced fungal burden in the lungs and limited dissemination to other target organs, as shown by colony forming units assays. Furthermore, histopathologic examination of lung sections revealed diminished tissue pathology both in early and late stages of infection. Disease severity was also evaluated following adoptive cell transfer into $Rag1^{-/-}$ mice. After cell sorting, different groups of animals received naïve $CD4^+$T cells, Treg cells, or both cell populations prior to infection with the fungus and the course of acute and chronic PCM was compared. In line with the findings for Foxp3-GFP knockin mice, we demonstrated that P. brasiliensis infection in $Rag1^{-/-}$ mice was more efficiently controlled in the absence of Treg cells.

Conclusion: These data clearly demonstrate that Treg cells negatively impact the course of PCM and help to elucidate some of the immunoregulatory phenomena associated with this important deep mycosis.

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GROWTH OF THE FUNGUS PARACOCCIDIOIDES BRASILIENSIS AFTER INTERACTION WITH HUMAN DENDRITIC CELLS.

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Introduction: Paracoccidioidomycosis (PCM) is a systemic mycosis, whose etiologic agent is the dimorphic fungus Paracoccidioides brasiliensis (Pb). Phagocytic cells play an essential role in the immune response against this fungus, acting as effector cells through fungistatic and fungicidal activities and as modulators of the innate and adaptative response. Specifically in relation to the effector functions , human monocytes and neutrophils and murine macrophages exert fungicidal activity against Pb after activation with the cytokines IFN-γ, TNF-α, GM-CSF and IL-15. This activity is mediated by products of oxidative metabolism, mainly H_2O_2. Dendritic cells (DCs) are essential for early recognition of microorganisms, as well as to instruct adaptive immune response. This second role has been studied in paracoccidioidomycosis. However, similarly to neutrophils, monocytes and macrophages, these cells may play an important role as effector cells. Differences in the ability of DCs to kill P. brasiliensis, may result in differences in the spread of the fungus during its migration from the periphery to secondary lymphoid organs. The aim of this study was to evaluate whether human DCs exert fungicidal activity against Pb and the involvement of H_2O_2 in this process. Furthermore, the effect of IFN-γ and TNF-α was evaluated**. Methods and Results:** Immature DCs derived from differentiation of human monocytes obtained from healthy donors and cultured with GM-CSF and IL-4 (7 days) were activated for 18 h with IFN-γ, TNF-α , challenged with Pb18 (virulent) or Pb265 (avirulent strain) for 4 h, and evaluated for fungicidal activity and H_2O_2 production. We found that DCs do not exert fungicidal activity against Pb, even after activation with cytokines. Instead, after interaction with DCs, a significant growth of the fungus was detected, which was higher for Pb18. The inability of these cells to kill Pb and instead allow its growth, is in agreement with the results of H_2O_2production whose levels were very low, even after DCs activation with cytokines. **Conclusion:** The results have important implications for the immunopathogenesis of PCM. Pb growth within the DCs probably represents an escape mechanism of this fungus and can result in the spread of infection as well as in the inability of DCs to induce an adaptive immune response against this pathogen.

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HAART ALTERS INFLAMMATION LINKED TO PLATELET CYTOKINES IN HIV-1 INFECTED PATIENTS

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Introduction: Platelets have inflammatory activities beyond haemostasis. For instance they can respond to infectious injury, including human immunodefiency virus (HIV), by secreting adapted panels of cytokines, but to date, the participation of activated platelets in immune activation and inflammation during HIV infection, remains under-considered. Yet, platelets are the major source of circulating sCD40L, a master immune activator which is upregulated upon HIV infection, and contributes to persistant inflammation observed in HIV-1 patients. Therefore, we aimed to assess, in vitro the participation of platelets in inflammation in HIV-1 infected patients, either receiving, or not, highly active antiretroviral therapy (HAART).

Methods and Results: 41 HIV-positive patients without co-infection, who had been receiving stable HAART for at least one year and 30 HIV-positive patients who did not receive HAART were recruited. 40 healthy donors were recruited as negative control. Soluble proteins of interest were quantitated by Luminex® technology in plasmas samples and in platelet-free and platelet-rich plasma samples, stimulated or not with thrombin receptor-activating peptide (TRAP)-SFLLRN peptide. We observed that plasma levels of several platelet-associated inflammatory molecules i.e. sCD62P, RANTES, GRO-α and sCD40L were significantly increased in HIV-positive patients (respectively 82.3, 44.1, 1.2 and 5.4 ng/ml vs. 28, 19.7, 0.7 and 2.3 ng/ml for healthy donor) which is in favor of the participation of platelets to the systemic activation during HIV infection. Interestingly platelets displayed different responses to TRAP according to the HIV status of patients and whether HAART was implemented. Platelets from HAART-treated patients, and even more platelets from untreated patients, released significantly more sCD40L and GRO-α, spontaneously and upon TRAP-stimulation. However, upon TRAP-stimulation, platelets from HAART-treated patients released significantly less RANTES and sCD62P (respectively 7.9 and 25.7 ng/ml for HAART-treated patients vs 47.5 and 36.8 ng/ml for healthy donnors).

Conclusion: This suggests a hyper-responsive status of platelets during HIV infection; which may be dampened upon HAART. Thus, our data support the use of molecules targeting a platelet-inflammatory role, in combination with anti-retroviral molecules.

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HEMATOGENOUS CANDIDIASIS IN C57BL/6 MICE DISSEMINATES TO THE BRAIN

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Introduction: Candida albicans is an opportunistic pathogen that is still an important cause of mucocutaneous and systemic infections in immunocompromised hosts. A higher risk of dissemination to the central nervous system (CNS) is observed in neonates in comparison to older patients. The present study was designed to establish if a hematogenous candidiasis in adult C57BL/6 mice spreads to the brain.

Methods and Results: Adult female C57BL/6 mice were infected by intravenous route with 5.10^6 viable C. albicans yeasts. The mice were euthanized 3, 7, 14, 21, 30 and 60 days (n = 6 animals/period) after infection to evaluate fungal loads in spleen, liver, lung, kidney and brain. Histopathological analysis was performed in brain samples to assess inflammation and fungus presence. Spleen cells were stimulated in vitro with heat-killed C. albicans. IFN-γ, TNF-α, IL-10 and IL-17 were quantified in the culture supernatants by ELISA. The results showed 87.5% of survival rate after 60 days post infection (p.i.). Fungal recovery in spleen, brain, liver and lungs was lower on day 30 p.i. ($p<0.05$) comparing to previous periods; but the fungus persisted in the kidneys. Fungi and inflammatory cell infiltration were observed in the brain from the 3rd to the 30th day of infection. Spontaneous release of IFN-γ and IL-10 by spleen cell culture was detected in the beginning of infection. Culture stimulation with heat-killed C. albicans increased production of IFN-γ ($p=<0.001$), IL-10 ($p=<0.001$) and IL-17 ($p=0.031$).

Conclusion: These data show that infection of C57BL/6 mice with C. albicans causes a systemic infection in which the fungus spreads to the CNS and the fungal clearance is associated with an elevated IL-17 production.

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HEME OXYGENASE-1 IN CANINE VISCERAL LEISHMANIASIS AND CORRELATION WITH OXIDATIVE STRESS IN DOGS

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Introduction: Heme oxygenase-1 (HO-1) is a stress inducible enzyme with antioxidant capacity which main function in the organism is to metabolize heme, an end product of hemoglobin. Besides, its immunoregulatory function has also been studied in both innate and adaptive immune responses, contributing to resistance or susceptibility to many pathologic conditions. Considering that the oxidative stress is a pathologic condition of canine visceral leishmaniosis (CVL) which could also contribute to immune deficiency of infected animals, and that HO-1 affects immune response, this study aimed to measure the plasma levels of HO-1 in moderate and very severe stages of CVL and correlate its levels with markers of oxidative stress.

Methods and Results: To this end, 30 healthy dogs and 53 with CVL were selected. Based on clinical examination, dogs with leishmaniosis were classified according to LeishVet Consensus in moderate (n=31) or very severe (n=22) stages of the disease. HO-1, bilirubin, lipid peroxidation (thiobarbituric acid reactive substances - TBARS) and total glutathione were determined using commercial reagents; total antioxidant capacity (TAC) and total oxidant capacity (TOC) were measured using ABTS cation reduction and xylenol orange methods, respectively. Statistical analysis was performed using Kruskal-Wallis or ANOVA tests followed by Dunn's or Tukey post hoc test respectively and correlations were performed using Spearman test. Results were considered statistically significant when $P<0.05$. Plasma levels of HO-1 were lower in dogs with CVL ($p < 0.0001$), with reduction of the enzyme level dependent on the disease stage. Dogs with very severe disease (3.84 ± 2.43 ng/mL) showed the lowest levels, followed by dogs in moderate stage (5.92 ± 1.68 ng/mL) compared to the control group (8.31 ± 2.3 ng/mL). Considering all dogs, the levels of HO-1 were correlated with markers of oxidative stress, such as a positive correlation with TOC ($p = 0.0244$; $r = 0.2598$) and a negative correlation with TBARS ($p = 0.0335$; $r = -0.2459$) and GSH ($p = 0.0097$; $r = -0.4254$). No correlation was observed between HO-1 and total bilirubin or TAC.

Conclusion: Heme oxygenase-1 is altered in canine visceral leishmaniasis with reduction of enzyme levels dependent of the disease stage, which is probably associated with the higher oxidative stress observed in the final stage of leishmaniosis.

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HISTOPATHOLOGICAL AND PARASITEMIA EVALUATION IN DIFFERENT MICE TISSUES INFECTED WITH THE Y STRAIN OF T. CRUZI AND TREATED WITH BENZONIDAZOL AND / OR BBI

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Currently, the proteases have been selected as a target in antichagasic drug development, since they are directly involved in the parasite survival and replication during the Chagas disease development. In addition, some studies have shown that protease inhibitors have trypanocidal activity and reduce the inflammation and tissue injury. Knowing that the immune system mobilization, in Chagas disease, is important in parasite load reducing, but contributes to the onset of clinical manifestations, is necessary to seek a compound capable of not only eliminate the parasite, but also to reduce the inflammation. In this way, the aim of this study was to evaluate, during the acute phase, parasitemia and the inflammatory process in heart and skeletal muscle of mice infected with Y strain of T. cruzi and treated with BBI, a Glycine max peptide (soybean) capable of inhibiting serine proteases, associated or not with Benzonidazol (BZ). When evaluating the parasitemia curve, it was observed that the animals that received treatment with BZ in parallel with BBI presented lower patent period, and after negativation of the parasitemia, relapse wasn't observed as well, same result was observed in animals treated only with BZ. Although the animals treated with BBI didn't show negative results for parasitemia on the 20th day, they showed lower parasitemia curve and lower peak of parasitemia when compared to untreated animals (CI) (CI: 663800 ± 106360 ; BBI: 296000 ± 162997) . A significant reduction in cardiac inflammation was observed in animals infected and treated when compared to untreated infected group, regardless of treatment (CNI: 60,73 ± 9,624; CI: 173,7 ± 24,18; BZ: 69,08 ± 5,859; BBI: 71,53 ± 3,220; BZ/BBI: 74,92 ± 8,601) , and this same result was observed in skeletal muscle (CNI: 22,15 ± 0,7762 ; CI: 75,00 ± 6,557; BZ: 28,28 ± 10,43; BBI: 38,28 ± 7,455; BZ/BBI: 55,38 ± 8,343). These preliminary results indicate that treatment with BBI and BZ in parallel has the potential to reduce parasitemia so as to maintain the heart and skeletal muscle with normal histological pattern. Furthermore, treatment with BBI until the 10th day post-infection showed to be able to control the tissue inflammatory process, which has great impact on characteristic lesions observed in the chronic phase.

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HTLV-1 INFECTED THYMIC EPITHELIAL CELLS TRANSMIT THE VIRUS TO CD4+ LYMPHOCYTES

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Human T Lymphotropic Virus type 1 (HTLV-1) can lead to development of lymphoma and leukemia (ATL) or to commitment of muscular/nervous systems (HAM/TSP). It remains unknown what triggers these diseases. Although the virus has T lymphocyte tropism, it can infect other cells by cell contact and cell-free virus. Activated T lymphocytes re-circulate in lymphoid organs, including the thymus, where the thymic epithelial cells (TEC) interact with recirculating cells. Concerning that, infected lymphocytes might transmit the virus to TEC that can infect novel lymphocytes. By flow cytometry we observed in TEC the HTLV-1 entry molecules expression: GLUT-1 (59±8) and NRP 1 (59±22) (n=5). By immunofluorescence using anti-HTLV-1 mAbs, we showed TEC infected from direct contact with cell lines derived from ATL (C91/PL) and HAM/TSP (CIB) patients after 24h (n=4). We use the filtered supernatant (SN) from 72 hours of culture from CEM (non-infected), CIB or C91/PL and put in contact with TEC for 1.5h, then changed the medium. By confocal microscopy, HTLV-1$^+$ TEC were observed after 24h. After 10 dpi, virtually all the cells in culture were infected, showing that the virus can spread through the culture. A microarray assay (n=3) comparing TEC treated with C91/PL or CEM SN, 315 genes differentially expressed (DE). Comparing TEC treated with CIB or C91/PL, 271 genes DE. Only 18 genes were DE comparing TEC treated with CEM or CIB SN. The most enriched processes were anti-apoptotic, immune and inflammation according to Gene Ontology. The results revealed an increase of chemokine and adhesion molecules expression confirmed by RT-PCRq (n=4). No difference in non-infected lymphocyte attraction was observed in a Transwell migration model where we put the HTLV-1$^+$ TEC on the bottom then add CEM on the upper chamber to migrate (n=3). The expression of HLA class I (77±22; 80±6) and class II (22±17; 34±9), GLUT-1 and NRP-1 did not change after 24h or 10 dpi. There is no difference in cell proliferation by CFSE dilution. By immunofluorescence we show that in co-culture, HTLV-1$^+$ TEC can infect lymphocytes as soon as 24h (n=3). We cultivated the newly infected lymphocytes for 10 dpi and measure the entry receptors and migration molecules expression, apparently with no change (n=3). Together these results show that TEC can be infected by HTLV-1+ lymphocytes and cell-free virus infect other TEC and convey the virus to T lymphocytes.

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HUMAN MILK FOR THE DIAGNOSIS OF ACUTE TOXOPLASMOSIS

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Introduction: Toxoplasmosis is a zoonosis caused by an intracellular parasite, Toxoplasma gondii that infects different hosts including up to a third of the world's human population and can cause severe damage to the fetus in congenital infection. Maternal breastfeeding is the most natural and safe way to feed a newborn, with ability to provide a good development of the infant's immune system. All immunoglobulin isotypes and their subclasses are present in human breast milk, mainly in colostrums, with systemic or local source. There are several evidence that breastfeeding protects the infant against a wide range of diseases, but few efforts have been directed to identifying the protector action of human milk against parasitic infections, including with T. gondii. Due to the importance of breastfeeding in protecting the newborn against innumerous infections and to the high prevalence of toxoplasmosis, this study was conducted in order to detect and evaluate the presence of specific T. gondii IgG, IgM and IgA antibodies in paired serum and colostrum samples. **Methods and Results:** The study was carried out on 289 puerperal women from Clinical Hospital of Federal University of Uberlândia (mean age 24.8 years, range 14 – 43 years). ELISA immunoassays showed positivity for IgG, IgM and IgA anti-T. gondii, respectively, for 136 (47.0%), 20 (6.9%), 8 (2.8%) in serum samples and 133 (46.0%), 23 (7.9%), 8 (2.8%) in colostrum samples. Also, it was observed significant correlation rates between anti-T. gondii antibodies levels in serum and colostrum samples. Immunoblotting assays showed that it is possible to detect IgG, IgM and IgA antibodies specific directed to various antigens of T. gondii in serum and human colostrums. Seric IgG recognizes more antigen fractions than IgM and IgA, in contrast with colostrum, which has IgA recognizing more antigenic components than IgG and IgM isotypes. **Conclusions:** Our results showed that is possible to diagnose toxoplasmosis using human milk, a noninvasive way to obtain biological samples, and that the immunoglobulin isotypes from colostrum may be protective against T. gondii active infection by oral route during neonatal period.

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HYPERACTIVATION OF M1 AND M2 MACROPHAGES CAN BE ASSOCIATED WITH FAILURE OF THE IMMUNE RESPONSE IN HIV+ PATIENTS

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1,2,3,5,8.FMRP/USP, RIBEIRÃO PRETO - SP - BRASIL; 4,6,9.FCFRP/USP, RIBEIRÃO PRETO - SP - BRASIL; 7.FUNDAÇÃO HEMOCENTRO DE RIBEIRÃO PRETO, RIBEIRÃO PRETO - SP - BRASIL.

Introduction: Even 30 years past from recognition of the Acquired Immunodeficiency Syndrome (AIDS) as a worldwide epidemy caused by Human Immunodeficiency Virus (HIV), many questions still remains unanswered. Besides of huge CD4+ T cells commitment, classical or alternatively activated macrophages derived from HIV+ patients exhibit phenotypic changes, what can be associated with the immune impairment, characteristic of these patients. Our aim was to evaluate the immune response developed by macrophages obtained from HIV+ patients to different PAMPs. This project was approved by ethical committee FMRP-USP and Fundação Hemocentro de Ribeirão Preto. All clinical samples were obtained from HIV+ treatment-naive patients, assisted at HC-FMRP-USP. Control group was recruited from blood-donors of Blood Bank of Ribeirão Preto – FMRP-USP.

Methods and Results: Mononuclear CD14+ cells were isolated from peripheral blood samples and plated using M1 or M2 driven medium. After six days in culture, LPS or β-Glucan were added to cells during 24 hours and the supernatant was collected. Cytokines and chemokines from cell culture and plasma samples were quantified. Nitric oxide was quantified by Griess reaction. Analysis of mRNA expression was performed to confirm cell differentiation. The results shown were obtained from samples of 7 patients and 22 blood-donors. The purity of plated cells was upper than 93%, confirmed by flow cytometry. Our preliminary data have shown higher levels of IFN-ɣ (10,01±2,01), IL-12 (p70) (6,36±2,19), IL-1β (1±0,24), TNF-α (15,73±5,01), IL-10 (21,41±15,95) and CXCL10 (1815±516,4) on plasma samples from HIV+ patients. Similar results were found in cell supernatant. Interestingly, nitric oxide was not detected on supernatant of cells stimulated with PAMPs, indicating that this might not to be the main cellular mechanism to immune response against fungi and bacteria in humans. In parallel, the NOS-2 and Arg-1 expression are not good markers to confirm MDM polarization in humans. The CCL3 expression seems to be better indicator of cell activation under M1 conditions.

Conclusion: Together, our results indicated an inflammatory disorder caused by HIV infection, which may be associated with prolonged macrophage activation on M1 pattern, resulting on exhaustion of the immune response against pathogens and allowing the occurrence of opportunistic infections.

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HYPERVIRULENT MYCOBACTERIA INDUCE DIFFERENT PROFILES OF IMMUNE RESPONSE IN THE LUNGS DURING SEVERE TUBERCULOSIS

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Introduction: Tuberculosis is a public health problem. One-third of the world population is infected with Mycobacteria tuberculosis (Mtb) and 10% of them develop the disease. Our research group has shown that hypervirulent mycobacteria strains (MP287/03 – M. bovis (Mbv); Beijing 1471 – Mtb) induce distinct patterns of immune response in mice. Highly proinflammatory response is observed in mice infected with Beijing 1471 Mtb, mainly characterized by production of IL-1β, IL-6, TNF-α, IFN-g and IL-17 in the lungs, while mice infected by MP287/03 Mbv secrete IL-10 but low amounts of proinflammatory cytokines. **Method and Results**: In this study, we characterized by flow cytometry the cellular infiltrates in the lungs of mice infected intratracheally with MP287/03, Beijing 1471 and H37Rv strains. On day 28 of infection, higher $CD4^+$ T cell frequency and cell number per lungs were observed in mice infected with Beijing 1471 Mtb in comparison with mice infected with MP287/03 Mbv and H37Rv Mtb. No difference in these parameters was observed for $CD8^+$ T cells and $CD19^+$ B cells. The percentage of $CD62L^{low}$ $CD4^+$ T cells was higher in mice infected with Beijing 1471 and MP287/03 strains than H37Rv strain. Furthermore, Beijing 1471 Mtb induced high expression of CD69 in $CD44^+CD4^+$ T cells, while MP287/03 Mbv did not. Additionally, we observed an increase of the interstitial macrophage population ($F480^+CD11C^-$) in mice infected with Beijing 1471 and MP287/03 strains. Moreover, Beijing 1471 Mtb also induced an increase in frequency and cell numbers of inflammatory monocytes ($CD11b^+Ly6C^+F4/80^-LyCG^-$), whereas MP287/03 Mbv induced an increase of non-inflammatory monocytes ($CD11b^+Ly6C^+F4/80^-LyCG^-$). **Conclusion:** These data are consistent with previous results in which Beijing 1471 Mtb induced high levels of proinflammatory cytokines, whereas MP287/03 Mbv induced low proinflammatory response. Thus, the extremely high and low induction of proinflammatory cytokines observed in mice infected with Beijing 1471 and MP287/03 strains was characterized by recruitment of inflammatory and non-inflammatory monocytes, respectively, which may reflect in the activation or not of $CD4^+$ T cells.

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IFN-Γ-INDUCED PRIMING MAINTAINS LONG-TERM STRAIN-TRANSCENDING IMMUNITY AGAINST BLOOD-STAGE PLASMODIUM CHABAUDI MALARIA

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Introduction: The mechanism by which protective immunity to Plasmodium is typically lost in the absence of continued exposure to this parasite has yet to be fully elucidated. It has been recently shown that IFN-γ produced during human and murine acute malaria primes the immune response to TLR agonists. Herein, we investigated whether IFN-γ-induced priming is important to maintain long-term protective immunity against P. chabaudi AS malaria.

Methods and Results: On day 60 postinfection, C57BL/6 mice still had chronic parasitemia and efficiently controlled homologous and heterologous (AJ strain) challenge. The spleens of the chronic mice showed augmented numbers of effector/effector memory $CD4^+$ (T_E/T_{EM}) cells, which is associated with increased levels of IFN-γ-induced priming, i.e., high expression of IFN-inducible genes and TLR hyperresponsiveness. After parasite elimination, 200 days postinfection, IFN-γ-induced priming was no longer detected and protective immunity to heterologous challenge was mostly lost with >70% mortality. Spontaneously cured mice had high serum levels of parasite-specific IgG, but T_E/T_{EM} cell numbers, parasite-driven $CD4^+$ T cell proliferation and IFN-g production were similar to non-infected controls. Remarkably, the priming of cured mice with low doses of IFN-γ rescued TLR hyperresponsiveness and the capacity to control heterologous challenge, increasing the T_{EM} cell population and restoring the $CD4^+$ T cell responses to parasites. The contribution of TLR signaling to the $CD4^+$ T cell responses in chronic mice was supported by data obtained in mice lacking the MyD88 adaptor.

Conclusions: These results indicate that IFN-γ-induced priming is required to maintain protective immunity against P. chabaudi and aid in establishing the molecular basis of strain-transcending immunity in human malaria.

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IFNG +874T/A POLYMORPHISM AND CYTOKINE LEVELS IN INDIVIDUALS NATURAL EXPOSED TO MALARIA IN BRAZIL

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Introduction: Interferon-γ (IFN-γ), encoded by IFNG, is a key mediator of anti-parasitic immune effectors mechanism and has been suggested to play an important role in both protection and pathogenesis of malaria. Regarding the importance of interferon-gamma (IFN-gamma) in malaria and the functional role of IFNG +874T/A single nucleotide polymorphism (SNP) in the IFN-γ production, the present study aims to investigate the relationship of this polymorphism in clinical malaria and also the influence of SNP in the circulating levels of IFN-γ in individuals from malaria endemic area. **Methods and Results:** A total of 626 individuals, 278 malaria-exposed and 348 control subjects non-exposed were screened for IFNG+874T/A SNP. DNA samples extracted from blood were used to investigate +874T/A polymorphism in IFNG gene by ARMS-PCR and IFN-γ plasma concentration was measured by luminex. We compared allelic and genotypic frequencies of IFNG gene (+874 T/A) between malaria-exposed and control groups and the associations with, clinical malaria, and number of previous malaria episodes. Firstly, the allele frequencies were similar in both groups, with A allele being significantly more frequent than T allele ($P<0.05$). However, malaria-exposed group showed that homozygous for the variant allele AA represents 52.9% of the group, while in control group the most frequent genotype was the heterozygous AT (44.8%; OR=1.99, $P<0.001$) The control group also presented higher frequency of wild TT genotype (18%) than malaria-exposed individuals (7.9%; OR=3.24, $P<0.001$). Furthermore, in malaria-exposed group, the frequency of TT alleles were higher in individuals that had >6 previous malaria episodes (11.9%; OR=2.84, $P<0.026$). Finally, in malaria-exposed individuals high IFN-gamma levels were observed in malaria infected (191.1±491.6 pg/ml) when compared to non-infected individuals (94.3±371.3 pg/ml). However no differences in the cytokine levels were observed among the alleles or genotypes studied. **Conclusion:** The presence of polymorphism in IFNG +874 does not seem to have influence and is not a determining factor for clinical malaria and IFN-γ secretion.

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IGE ANTIBODIES AGAINST MAJOR STRUCTURAL AND NON-STRUCTURAL HEPATITIS C VIRUS (HCV) ANTIGENS IN CHRONIC HEPATITIS C PATIENTS

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Introduction: Chronic hepatitis C is characterized by important dysfunction of both T and B-lymphocytes, which has been mainly represented by deficient cellular immune response against HCV and autoimmunity, respectively. However, the immune response of IgE antibodies to HCV core and non-structural antigens is still unknown. In this work, we investigate the presence of IgE antibodies against major HCV antigens in chronic hepatitis C patients. **Methods and Results:** Were investigated 13 untreated patients that were seropositive for anti-HCV IgG antibodies and HCV-RNA, 12 chronically infected with HCV genotype 1 and one infected with HCV genotype 2. Their blood HCV load varied from 19 x 10^3 to 4.8 x 10^6 IU/mL, whereas their total serum IgE varied from 258 IU/mL to 1,094 IU/mL. Anti-HCV IgE antibodies were investigated using amplified IgE immunoblotting (anti-human IgE MoAb, biotinylated anti-mouse IgG antibody and alkaline-phosphatase avidin conjugate) performed on nitrocellulose strips containing recombinant HCV antigens Core1, Core2, Helicase (part of NS3 with helicase activity), NS3 (containing protease and helicase activity), NS4 and NS5 (recomLine HCV IgG strips, Mikrogen, Germany). To assess antibody specificity, control tests were performed with sera from individuals without HCV infection that had similar total IgE levels, and without human serum. IgE antibodies against HCV core and NS3 protein, including helicase, were detected in all patients. However, three patients were seronegative for NS5 IgE antibodies and weak or negative reactions for NS4 IgE antibodies were observed in four subjects. Weak reaction with core HCV antigen was detected in sera from control individuals having high total IgE.**Conclusion:** Patients chronically infected with hepatitis C virus produce IgE antibodies against the same HCV antigens that are recognized by IgG antibodies. The participation of these antibodies in hepatitis C pathogenesis or in infection control still needs investigation.

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IGG LEVELS IN SHEEP ARTIFICIALLY INFECTED WITH HAEMONCHUS CONTORTUS AND TREATED WITH POINCINELLA PYRAMIDALIS

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Haemonchus contortus is a gastrointestinal nematode of ruminants such as sheep and goats, having a great worldwide importance for causing great economic losses. The uses of conventional anthelmintics have leading the resistance to combat this nematode. Thus, strategies in search of new substances are being used in an attempt to resolve this problem. In this study, it was evaluated the aqueous extract of Poincinella pyramidalis (popularly known as “catingueira” or “pau-de–rato”). In order to verify the production of immunoglobulin G (IgG) and possible reduction in parasite burden in sheep artificially infected with H. contorturs, three groups of five sheep were used. Two of them (G2 and G3) were orally artificially infected with, about 10,000 infective larvae (L3) of the parasite, while G1 was the negative control group. The administrations of plant extract occurred after 45 days of infection in the group of infected animals G3 and G1, using 100mg/mL per Kg. The three groups were monitored during 90 days by assessment of parasitological egg counts per gram of feces (EPG) and by evaluating of the IgG serum levels (indirect ELISA). It was observed that the G3 group had a low production of IgG and this decrease can be related to increased parasite burden seen in feces of animals under the P. pyramidalis stimulus when compared with the group G1and G2. This result shows that in this experimental conditions, the plant extract has a low capacity to protect the animal against the parasite. More researches are needed to assess this approach. Finantial support: CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior**)**

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IGG1 ANTIBODY RESPONSE TO DOSR AND RPF MYCOBACTERIUM TUBERCULOSIS ANTIGENS IN TB PATIENTS BEFORE AND AFTER CHEMOTHERAPY

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Introduction: Tuberculosis (TB) affects millions of people worldwide every year. Detection of specific antibodies may represent a valuable tool in the diagnosis of tuberculosis. Mycobacterium tuberculosis is capable of showing phase specific gene expression. The induction of DosR regulon genes, associated with latency, and resuscitation-promoting factors (Rpfs), which are small bacterial proteins that promote proliferation of dormant mycobacteria, appears to be relevant in the host immune response to M. tuberculosis. Previoulsy, our group demonstrated that IgG1 antibodies for the immunodominant M. tuberculosis antigens ESAT-6 and CFP-10, and against the DosR antigen 16kDa were higher in active TB and lower after six months of successful anti-TB chemotherapy (Intern. Immunol. 9:775-782, 2010). **Methods and Results:** In this study, levels of serum IgG1 antibodies against four DosR antigens (Rv1733, Rv1737, Rv2029, Rv2628), two Rpfs antigens (Rv0867 and RV2389), and two antigens associated with viable and metabolically active bacilli (Rv0717, and ESAT-6/CFP-10 fusion protein) were measured through ELISA in 34 active pulmonary TB patients (0M-TB), in 46 patients after 1–3 months of treatment, in 20 patients who had completed 6 months of chemotherapy (6M-TB), and in 6 patients after one year of treatment. The control group consisted of 25 BCG vaccinated healthy individuals. Elevated levels of IgG1 antibodies against ESAT-6/CFP-10, Rv0717, Rv1733, Rv2029, Rv2628 and Rv0867 were detected in OM-TB in comparison to healthy controls ($p<0,001$). IgG1 levels against Rv0717 and Rv1733 were higher after 1-3 months of chemotherapy in comparison to 0M-TB or 6M-TB. The IgG1 response to ESAT-6/CFP-10, Rv2029, Rv2628 and Rv0867 was reduced to control levels after 6 months of anti-TB chemotherapy ($p<0,01$). After one year of treatment, the IgG1 antibody response to all antigens studied was similar between TB patients and healthy controls. **Conclusions:** These results suggest that detecting IgG1 antibodies against ESAT-6/CFP-10, Rv0717, Rv1733, Rv2029, Rv2628 and Rv0867 may represent an additional tool in the diagnosis of active TB. The increase in serum levels of IgG1 antibodies against Rv0717 and Rv1733 during chemotherapy-induced bacterial killing may represent useful TB disease biomarker monitoring treatment outcomes.

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IL-2 AND IL-10 INFLUENCE IN ASSOCIATION SCHISTOSOMIASIS AND BACTERIAL TRANSLOCATION / SEPSIS IN MICE SPLENECTOMY UNDERWENT.

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Introduction: The S. mansoni comprises one of the most potent Th2 immunomodulator and is well known a promoting bacterial diseases factor due to the parasite interaction with gram-positive. Studies demonstrated that severe sepsis was not developed in the first days after total splenectomy. In contrast, occurrence of an Overwhelming Post-Splenectomy Infection (OPSI) was also reported, characterized as a fulminant sepsis. Thus, it is uncertain whether schistosomiasis may contribute to the bacterial translocation (BT). Hence, the aim of this study was to analyze the development of sepsis by determining alterations in Th1 and Th2 levels between schitosomotic mice in the cronic phase and without infection or not subjected to splenectomy. **Methods:** A 35 days old Swiss Webster female mice were infected with 50 cercariae (n=24) and the same number of rodent were set in the control group. Ninety days after cercarial exposure, 08 schistosomotic and 08 control mice were underwent conventional splenectomy. In the other hand, 08 other mice of both groups were underwent Sham. Egg counting was evaluated by using Kato-Katz method at 45th and 97th days of infection. At the 100th day, all mice were anesthetized and euthanatized. Peripheral blood, fragments of mesenteric lymph nodes, spleen and liver were sampled for the BT analysis. Multiplex assay was performed with blood drawn by cardiac puncture for the dose levels of serum IL-2 and IL-10. **Results**: Enterobacteria were found in the spleen, liver and blood for all infected splenectomized mice as well as 62.5% in the spleen of infected mice in the control group. The eggs number were greater at 45th day than at 97th day of infection in all subgroups. IL-1b level of was greater in splenectomized subgroup when compared to control and sham subgroup. There was no difference of IL-2 levels in comparisons between and within groups, ie, there is no effect of the procedure or infection. Difference in production of IL-10 was not observed when comparing the three groups infected. There was no difference when comparing the mean between uninfected. In the comparison between infected and uninfected intra-group, there was no difference in the procedure and splenectomized groups, with a higher level of IL-10 in the infected group. **Conclusion**: The level of IL-2 and IL-10 is observed that there is not an effect of the splenectomy or infection.

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IL-27 ENHANCES LEISHMANIA AMAZONENSIS INFECTION VIA THE DS-RNA DEPENDENT KINASE (PKR) AND IL-10 PRODUCTION.

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1,2,3,11.LABORATORY ON THYMUS RESEARCH. OSWALDO CRUZ INSTITUTE, FIOCRUZ, RIO DE JANEIRO - RJ - BRASIL; 4,5,6,10.LABORATORY OF MOLECULAR PARASITOLOGY, INSTITUTE OF BIOPHYSICS, FEDERAL UNIVERSITY OF RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL; 7,9.LABORATORY OF IMMUNOBIOLOGY OF LEISHMANIASIS, FEDERAL UNIVERSITY OF RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL; 8.INFLAMMATORY GENES LABORATORY, INSTITUTE OF BIOLOGICAL SCIENCES, FEDERAL UNIVERSITY OF MINAS GERAIS, BELO HORIZONTE - MG - BRASIL.

Introduction: Leishmania is a protozoan parasite that replicates within macrophages after vertebrate infection by the sandfly vector. The infection control basically depends on the robust development of a specific Th1 response against the parasite, and Interleukin (IL)-27 has been described as a cytokine able to participate in the early steps of Th1 commitment. Here, we address the direct function of IL-27 on the intracellular replication of L. amazonensis, and we describe that this cytokine promotes L. amazonensis replication in both human and murine primary macrophages.

Methods and Results: We found that IL-27 mRNA expression is increased in macrophages exposed to Leishmania (n = 3; EBI3 2,33 fold control ± 0,04, p28 12,92 fold control ±1,37), and that IL-27 treatment is able to induce the Protein Kinase R (PKR) activation in macrophages by detecting its phosphorilation in Western Blottings(n = 3). We also observed that the PKR blockage, with pharmacological inhibitor (n = 5; IL-27 1,79 fold control ± 0,09; IL-27+PKRi 1,15 fold control ± 0,17), and PKR gene deletion, in PKR-KO mice (n = 4; WT+IL-27 2,25 fold control ± 0,17; PKR-KO+IL-27 1,01 fold control ± 0,19), abrogate the intracellular Leishmania enhancement driven by IL-27. In addition, we show that PKR regulates the IL-27-induced IL-10 mRNA expression(n = 3; WT+IL-27 2,58 fold control ± 0,15; PKR-KO 0,28 fold control ± 0,01) and the blockage of IL-10 receptor on the surface of Leishmania-infected macrophages with neutralizing antibodies abolished the IL-27-mediated enhancement of Leishmania growth(n = 5; IL-27 1,95 fold control ± 0,31; IL-27+anti-IL10R 1,12 ± 0,18). We further demonstrate that L. amazonensis-induced expression of IL-27 depends on Type I Interferon signaling and the engagement of TLR2 by assessing the mRNA expression of IL-27 subunits in infected WT, TLR2-KO and IFNR1-KO macrophages.

Conclusion: Our results place IL-27 as an important molecule in L. amazonensis replication inside the mammalian host cells and strongly suggest that it also contributes to intracellular replication of this parasite, revealing unexpected aspects of the biology of this cytokine. Taken together, our results indicate that IL-27-driven PKR activation and IL-10 induction merit additional studies in other experimental/natural models involving other Leishmania species, or even other pathogenic protozoa with intracellular growth.

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IL-27-PRODUCING MACROPHAGES PREVENT TRYPANOSOMA CRUZI INDUCED-MYOCARDITIS BY HAMPERING TH1 LYMPHOCYTES VIA TR1 CELLS

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INTRODUCTION AND OBJETIVE: IL-27 is a heterodimeric cytokine produced by macrophages and dendritic cells known to induce IL-10-producing Tr1 cells and to regulate Th1, Th2, and Th17 lymphocytes, depending on the underlying disease. Because the infection caused by Trypanosoma cruzi normally induces myocarditis mirrored by an outstanding migration of Th1 cells to the heart tissue, we analyzed the regulatory role of IL-27 in this inflammatory condition. **RESULTS:** We firstly verified that IL-27 was promptly induced by in vitro T. cruzi-infected spleen cells. To induce myocarditis coordinated by Th1 lymphocytes, we polarized lymphocytes to a Th1 pattern by infecting mice in the absence of Th17-related molecules (IL-17R$^{-/-}$, IL-23$^{-/-}$, and IL-6$^{-/-}$ mice). As expected, an impressive cardiac inflammation and damage was observed in the absence of Th17-related molecules, leading IL-17R$^{-/-}$, IL-23$^{-/-}$, and IL-6$^{-/-}$mice to the premature death, notably by inducing an exuberant Th1 migration to the heart tissue via CXCL9 and CXCL10 chemokines. To explore the mechanisms by which IL-27 controls T. cruzi-induced myocarditis, we found a striking recruitment of IL-27-producing macrophages to the heart tissue mediated by increased levels of CCL3 and CCL4 chemokines in the absence of Th17-associated molecules. To gain further insights into the receptors required to IL-27 production, we observed that bone marrow-derived macrophages from TLR4$^{-/-}$, TLR9$^{-/-}$, and NLRP3$^{-/-}$ mice completely abolished IL-27 production after in vitro T. cruzi infection, while TLR2 was dispensable. To verify how IL-27 prevents Th1 lymphocytes, we observed that IL-27 produced by macrophages induced IL-10-producing Tr1 cells after T. cruzi infection. We next assessed whether IL-27 was correlated to cardiac protection during Chagas Disease. We observed augmented serum levels of IL-27 in either patients with indeterminate (asymptomatic) form or mild cardiac form, whereas patients with moderate or severe cardiomyopathy were poor producers of IL-27. **CONCLUSION:** Here, we described a novel regulatory mechanism developed by IL-27-producing macrophages in the control of T. cruzi-induced myocarditis. IL-27-producing macrophages induced IL-10-producing Tr1 cells, thus suppressing inflammatory processes caused by Th1 lymphocytes, the bona fide culprits of Chagas Disease. **FINANCIAL SUPPORT:** CAPES, CNPq, and FAPESP.

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IL-27P28 IS PRODUCED DURING LEISHMANIA INFANTUM INFECTION

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Leishmania infantum is a protozoan parasite which causes visceral leishmaniasis (VL), a non-healing and life-threatening disease endemic in Brazil. The immune response against this parasite is cell-mediated, i.e. IFN-g plays a major role through activation of microbicidal mechanisms in infected macrophages. On the other hand, IL-10 produced by several cell types seems to suppress the immunity against this parasite through inducing anti-inflammatory mechanisms, which leads to disease susceptibility. IL-27, formed by IL-27p28 and EBi3 subunits, is a novel cytokine that has a role in regulating the immune response through several mechanisms, such as inducing IL-10 production by T CD4+ lymphocytes. Concerning the importance of studying mechanisms of susceptibility to VL, we investigated IL-27 production during the disease. We found that VL-patients (n=25) strongly produce IL-27 compared with asymptomatic (n=25), or control individuals (n=25). In a murine model, C57BL/6 and Balb/c mice were infected with 10^7 stationary growth phase promastigotes, and 6 weeks post infection we observed high amounts of parasites in Balb/c mice, confirming the susceptibility difference of these mice strains. We also observed that both mice strains produce high level of IL-27p28 during the infection. Bone marrow-derived dendritic cells and macrophages, obtained through differentiation with GM-CSF and L929 supernatant, respectively, and further infected with a MOI of 5, up regulate the mRNA expression of il-27p28, analyzed by RT-PCR, and also its protein, analyzed by ELISA, independently of the strain. Finally, splenocytes from infected mice cultured with L. infantum crude antigen increased the IL-27p28 production compared to unstimulated cells, showing an antigen-dependent induction of this cytokine. Altogether, our results showed that IL-27p28 is produced during VL and may play a role in regulating the host immune response against L. infantum infection.

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IL-33 REGULATES INFLAMMATORY RESPONSE IN INDIVIDUALS WITH CUTANEOUS LEISHMANIASIS

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INTRODUCTION: IL-33 is a cytokine belonging to the superfamily IL-1 that binds specifically to the receptor ST2 and induces Th2 immune response. In recent studies, it was reported a protective role of IL-33 in atherosclerosis, obesity, type 2 diabetes and toxoplasmosis through the Th1/Th2 balance. Cutaneous leishmaniasis (CL) is characterized by a strong inflammatory response with high levels of IFN-γ and TNF production resulting in macrophages activation and parasite destruction. However, in CL, the higher the inflammatory response is, the increased tissue damage and disease severity occurs. Conversely, subjects with subclinical infection by L. braziliensis produce lower concentrations of IFN-γ and TNF and are able to resolve the infection without any disease expression. It is believed that they present a balanced Th1/Th2 immune response and that this response may be beneficial for individuals infected by L. braziliensis. The aim of this study was to determine the role of IL-33 in modulating the immune response of patients with CL.

METHODS AND RESULTS: Peripheral blood mononuclear cells (PBMC) and biopsies from the edge of the lesion were obtained from CL patients and cultured in presence of soluble Leishmania antigen (SLA), in the presence or absence of recombinant IL-33. After 72 hours we measured IL-33, IL-5, IL-13 and IL-1β by ELISA and IL-2, IL-4, IL-6, IL-10, TNF, IFN-γ, IL-17A by Cytometric Bead Array (CBA) by flow cytometry, on supernatants of these cultures. CL patients did not produce IL-33, IL-2, IL-4 and IL-17A in response to the SLA. However, high concentrations of TNF, IFN-γ, IL-6 and IL-1β were observed in supernatants from SLA-stimulated PBMC cultures. Supernatants from non-stimulated biopsy cultures had also high concentrations of IL-1β and IL-6. Addition of exogenous IL-33 down-regulated IL-1β, IL-6 and TNF levels in PBMC and in biopsy cultures, and increased production of IL-5 and IL-13 in PBMC cultures.

CONCLUSION: This study shows for the first time a regulatory role of IL-33 in inflammatory response in CL patients.

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IL-6 AND IL-10 PRODUCTION TRIGGERED BY PLASMODIUM VIVAX INFECTION IS DEPENDENT ON THE PARASITE LOAD

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Introduction: Recent studies have been shown that the inflammatory process, including the cytokine production, and the intense activation of innate immune responses are greater in the malaria caused by Plasmodium vivax than other species. Here, we examined the levels of circulating cytokines and their interaction during acute malaria. **Methods and Results:** Blood samples were collected from P. vivax-infected patients at admission and from healthy donors. Circulating cytokine levels were measure by Cytometric Bead Assay or ELISA. P. vivax infection triggered the production of both inflammatory and regulatory cytokines. Levels of IL-6, IL-8, IFN-g, IL-5 and IL-10 were higher in P. vivax-infected patients than in healthy donors. On the other hand, malaria patients produced lower levels of TNF-a, IL-12 and IL-2 than healthy individuals. While the levels of IL-10 and IL-6 were found independent on the number of malaria episodes, higher levels of these cytokines were seen in patients with higher parasite load. **Conclusion:** A mixed pattern of pro-inflammatory and regulatory cytokines is produced in P. vivax malaria. Analysis of cytokine network suggests that IL-10 and IL-6 are a robust axis in malaria patients and that this interaction seems to be dependent on the parasite burden.

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IL-6, IL-17 AND IL-23 PRODUCTION INDUCES HOST RESISTANCE TO PARACOCCIDIOIDES BRASILIENSIS

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Introduction: Paracoccidioides brasiliensis (Pb), a thermally dimorphic fungus, is the causative agent of paracoccidioidomycosis (PCM), one of the most frequent systemic mycosis that affects the rural population in Latin America. Th17 cells are an arm of the immune system that enhances host protection against several infections, including mycosis. To better understand the mechanisms which are involved in resistance to P. brasiliensis infection we evaluated the role of IL-6, IL-17 and IL-23 during the PCM experimental. **Methods and results:** At first, we realized intravenous infection in C57BL/6 (WT) mice with $1x10^6$ yeast forms of Pb18, a highly virulent Pb strain. At 7 days post infection, we measured by ELISA the IL-6 (1900 ± 805 pg/ml x g^{-1}), IL-17 (2956 ± 1160 pg/ml x g^{-1}) and IL-23 (1432 ± 756 pg/ml x g^{-1}) secretion in the lung of mice (n=5), which was statistically higher compared to uninfected mice (n=3). Subsequently we infected IL-$6^{-/-}$, IL-$17R^{-/-}$ and IL-$23^{-/-}$ mice under the same conditions mentioned above and we verified that the infection induced increased colony forming units (CFU) recoveries from the lung, liver and spleen of knockouts mice compared with WT mice (n=5). Using silver staining, we proved that the absence of IL-6, IL-17R and IL-23 impaired the control of the fungal replication, due the increased amount of yeast observed at lung from knockouts mice in relation to WT mice (n=5). Histopathological analysis showed that IL-6 and IL-17 contribute to compact granulomas formation due to adequate production of reticulin fibers. By immunohistochemistry, we verified that the deficiency of IL-6 or IL-17R was accompanied of disorganized $CD4^+$ T cell infiltration at lung. The absence of IL-6, IL-17R or IL-23 resulted in lower production of IFN-γ and IL-10 compared with WT lung at 15 and 30 days post infection. Additionally, the frequency of $CD3^+CD4^+IL\text{-}17^+$ cells, as well as IL-17 production, was decreased in IL-$6^{-/-}$ or IL-$23^{-/-}$ mice when compared with WT mice at 15 and 30 days post infection (n=4). This was associated with an impaired neutrophils ($Ly6G^+CD11b^+$) and macrophages ($F4/80^+CD11c^-$) lung recruitment analyzed by flow cytometry in lung from IL-$6^{-/-}$, IL-$17R^{-/-}$ and IL-$23^{-/-}$ mice in relation to WT mice. **Conclusion:** Taken together, these results demonstrate that IL-6, IL-17 and IL-23 contribute to control of experimental Pb-infection through an efficient granulomatous organization.

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IL-6-MEDIATED IMPAIRMENT OF REGULATORY T CELLS DURING INTESTINAL INFLAMMATION INDUCED AFTER PARASITE INFECTION

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Introduction: In sites of high antigen exposure, such as the gastrointestinal tract, sophisticated mechanisms of immune regulation are essential for maintaining the tolerance to inoculum antigens and, simultaneously, allowing the development of immunity against pathogens. In this context, the oral infection with Toxoplasma gondii leads to an intense intestinal inflammation in susceptible mice, which succumb to the infection due to the collapse of Tregs and the breakdown of tolerance to the microbiota. Here, we evaluated the mechanisms involved in these events, focusing on the role of IL-6. **Methods and Results:** Susceptible C57BL/6 and resistant BALB/c mice were orally infected with 40 cists of T. gondii. At day 8 post-infection, we detected a significant reduction on CD3+CD4+Foxp3+ Treg cells in the Lamina Propria (LP), mesenteric lymph nodes (MLN) and spleens of C57BL/6 mice compared to BALB/c mice. The protective role of Tregs in the resistant mice was confirmed after the treatment with anti-CD25 antibody (PC61): the PC61-treated BALB/c mice became susceptible to the infection. Associated with the collapse of Tregs, C57BL/6 infected mice also exhibited a progressive increase on IL-6 production, whose highest levels (day 8) coincided with the translocation of intestinal bacteria to the MLN. Therefore, we supposed that IL-6 could be involved in the Treg collapse, followed by increased inflammation, tissue damage and bacterial translocation found during the disease. To evaluate this, spleen cells were infected in vitro with T. gondii and treated with recombinant IL-6. The in vitro infection reduced the frequency of Tregs and the addition of IL-6 to the cultures accentuated the collapse of Tregs. In parallel, we found that IL-6-/- mice were resistant to the infection, presented higher Treg frequency and no bacterial translocation compared to C57BL/6 wild type mice. After the Treg depletion, IL-6-/- mice became susceptible to the infection and exhibited increased intestinal inflammation associated with higher levels of IFN-γ. **Conclusion:** Taken together, our data show that the IL-6 production induced during T. gondii infection is responsible for the impairment of Treg population resulting in tissue damage, bacterial translocation and the susceptibility to the immune-mediated pathology. Further experiments are undergoing to evaluate the mechanisms of IL-6 induction by the microbiota and by the parasite during the disease.

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IL-9 IS INDUCED DURING INTESTINAL INFLAMMATION TRIGGERED BY TOXOPLASMA GONDII INFECTION

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Introduction and Objective: The oral infection with Toxoplasma gondii induces an intense intestinal inflammation due to the exacerbated Th1 response. IL-9 is a cytokine that can act as negative or positive regulator of immune response. Recent studies revealed that mainly T cells generated in the presence of the cytokines TGF-β and IL-4 called Th9 cells producing IL-9. However, the involvement of the IL-9 and Th9 cells in infection immunity by intracellular pathogen has not been investigated. In this work, we evaluated role of IL-9 and Th9 cells in model of the inflammation intestinal induced by T. gondii. **Material and methods:** For this, susceptible (C57BL/6) and resistant (BALB/c) mice were orally infected with 100 cysts T. gondii (strain ME49) for detection of IL-9 and Th9 cells in spleen and mesenteric lymph nodes (MLN). We verified that T. gondii infection induces the production of IL-9 on both the lineages of mice during disease progression. At day 7 of infection, an intense intestinal inflammation is observed in C57BL/6 but not BALB/c mice. C57BL/6 mice exhibited a higher frequency of Th9 ($CD3^+CD4^+IL\text{-}9^+$) cells in the spleen and MLN compared to BALB/c mice. Additionally, we found an increased frequency of Th1 ($CD3^+CD4^+IFN\text{-}gamma^+$) cells in both susceptible and resistant mice during the toxoplasmosis progression. However, whereas C57BL/6 mice exhibited an increased frequency of Th9 and Th17 ($CD3^+CD4^+IL\text{-}17A^+$) cells, BALB/c showed a similar frequency these cells. **Conclusion:** Taken together, these data suggest that IL-9 plays an important role during development of intestinal inflammation induced by T. gondii infection. Further studies are undergoing to evaluate the mechanisms induction of Th9 and IL-9 and the relationship of these cells with susceptibility to T. gondii infection. Understanding of this mechanism will help development of new control strategies for the disease. **Financial support:** FAPESP, CNPQ and CAPES.

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IL1-B INFLUENCE IN ASSOCIATION SCHISTOSOMIASIS AND BACTERIAL TRANSLOCATION / SEPSIS IN MICE SPLENECTOMY UNDERWENT.

CARLOS ROBERTO WEBER; JOSÉ REBELO NETO; MARIA HELENA MADRUGA LIMA RIBEIRO; SANDY BEATRIZ GOMES DA SILBA; CLARISSA ATAÍDE DE ARAÚJO; CÉLIA MARIA MACHADO BARBOSA DE CASTRO.

UFPE, RECIFE - PE - BRASIL.

Introduction: Helminths are known as host immune response regulators. The Schistosoma mansoni comprises one of the most potent Th2 immunomodulator in Brazil, being responsible for the higher rates of infection. In addition, schistosomiasis is well known a promoting bacterial diseases factor due to the parasite interaction with gram-positive and anaerobic bacteria. Some studies have demonstrated that severe sepsis was not developed in the first days after total splenectomy. In contrast, occurrence of an Overwhelming Post-Splenectomy Infection (OPSI) was also reported, characterized as a fulminant sepsis. Thus, it is uncertain whether schistosomiasis may contribute to the bacterial translocation (BT) and sepsis or whether the immune response is protected against such process. Hence, the aim of this study was to analyze the development of sepsis by determining alterations in Th1 and Th2 levels between schitosomotic mice in the cronic phase and without infection or not subjected to splenectomy. **Methods:** A 35 days old Swiss Webster female mice were infected with 50 cercariae (n=24) and the same number of rodent were set in the control group. Ninety days after cercarial exposure, 08 schistosomotic and 08 control mice were underwent conventional splenectomy. In the other hand, 08 other mice of both groups were underwent Sham. Egg counting was evaluated by using Kato-Katz method. At the 100th day, all mice were anesthetized and euthanatized. Peripheral blood, fragments of mesenteric lymph nodes, spleen and liver were sampled for the BT analysis. Fecal samples from the middle of the small intestine were collected for the microbial analysis. Multiplex assay was performed with blood drawn by cardiac puncture for the dose levels of serum IL-1β. **Results**: All splenectomized group developed sepsis. E. coli was the most prevalent bacteria found in fecal analysis and BT. Enterobacteria were found in the spleen, liver and blood for all infected splenectomized mice as well as 62.5% in the spleen of infected mice in the control group. IL-1b level of was greater ($p < 0.05$) in splenectomized subgroup when compared to control and sham subgroup. There was no difference when comparing the control and sham subgroups. Comparing IL-1b average among uninfected, there was no difference ($p = 0.264$). In the infected group was higher levels of IL-1b that in the non-infected**. Conclusion**: The level of IL-1b is observed that there is an effect of the procedure and infection.

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IMMUNE RESPONSE TO LUTZOMYIA INTERMEDIA SALIVA IN INDIVIDUALS FROM A CUTANEOUS LEISHMANIASIS ENDEMIC AREA

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Introduction: Sand fly saliva contains a variety of pharmacologic agents, such as anticoagulants, vasodilators, immunomodulatory and anti-inflammatory molecules. Differently from others parasite/vectors interactions, prior immunization with the L. intermedia saliva, one of the vectors of Leishmania braziliensis in Brazil, enhancement of Leishmania infection, in mice. In addition, patients with active ulcers displayed higher levels of anti-L. intermedia saliva antibodies when compared with individuals with sub-clinical L. braziliensis infection, suggesting that exposure to sand flies saliva influences the outcome of L. braziliensis infection. In the present work we characterize the immune response against L. intermedia saliva in residents of L. braziliensis endemic area. **Methods and Results:** Participants in the present study included 264 individuals living in the endemic area of Corte de Pedra, Bahia, were evaluated regarding serology and cellular immune response to L. intermedia saliva. Anti-L. intermedia saliva antibodies was found in 150 (56.8%) subjects and a positive serology was associated with male gender ($p<0.05$) and with home arrival after 16h ($p=0.01$). Moreover, there was a predominance of IgG1 and IgG4 Immunoglobulin subclass. Cytokines and chemokine productions were determined by ELISA in supernatants of peripheral blood mononuclear cells (PBMC). Individuals naturally exposed to L. intermedia bites displayed higher concentrations of IL-10, IL-13 and IFN-γ compared to controls whereas TNF levels were similar in both groups. In addition, subjects exposed to sand fly Saliva secrete high levels CXCL-9 and CCL-2 than controls. Furthermore, the main sources of IL-10-secreting cells are CD4+, including CD25+ and Foxp3+ subsets. **Conclusion:** This type of immune response, with high IL-10 and IL-13 concentrations, may favor the L. braziliensis infection and facilitate the proliferation of the parasite co-inoculated with the saliva.

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IMMUNIZATION WITH RPB27 REDUCES THE LEVELS OF PULMONARY FIBROSIS CAUSED BY THE INFLAMMATORY RESPONSE AGAINST P. BRASILIENSIS

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Introduction: The paracoccidioidomycosis (PCM) is a systemic mycosis, whose host response to the infectious agent usually consists in a chronic granulomatous inflammatory process. This condition causes lesions that impair lung function and lead to chronic pulmonary insufficiency, resulting from fibrosis development, sequel disabling feature of the disease. The rPb27 protein has been studied for prophylactic and therapeutic treatment against PCM. Previous studies from our laboratory have shown rPb27 protective effect against PCM. However, these studies have not described that rPb27 immunization prevent fibrosis formation.

Methods: This study compared the levels of fibrosis resulting from infection by P. brasiliensis. For this, the animals were immunized with rPb27 and subsequently infected with a virulent strain of P. brasiliensis (Pb18). Were analyzed fungal burden (CFU), granulomatous response and levels of pulmonary fibrosis 90 days after challenge infection. Immunized animals (Pb27), when compared to other infected groups had a reduction in areas of inflammatory infiltrate and pulmonary fibrosis.

Conclusion: In conclusion, the Immunization with Pb27r was effective to modulate the inflammatory response, decreasing levels of fibrosis, thus, Pb27r immunization decreases and disabling sequelae generated by infection with P. brasiliensis.

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IMMUNOENDOCRINE INTERACTIONS DURING LYMPHOCYTE MIGRATION IN HUMAN CHAGAS DISEASE ARE RELATED TO SEVERE CARDIOPATHY

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Introduction: Chagas Disease remains a public health issue in Americas. In the pathological processes seen in patients, we found changes in imunoneuroendocrine interactions and an imbalance in lymphocyte migration to inflammatory sites. We evaluated T lymphocyte migratory responses from chagasic patients with different forms of cardiopathy, correlating these events to immunoendocrine alterations that occur during chronic disease. We observed that a pro-inflammatory profile was more expressed in parallel with the severity of disease, as well as an imbalance on Hypothalamus-Pituitary-Adrenal axis. Also in parallel, we found an enhanced migratory response in infected subjects. As miRNAs are known to be involved in migratory responses, we also investigated their presence in serum. These results suggest that endocrine disturbances, correlated to an inflammatory profile, may contribute to increase migratory potential of T lymphocytes to inflammatory sites and myocarditis.

Methods and results: Chronic chagasic patients were grouped into INDETERMINATE **IND** (n=18), MODERATE **MOD** (n=18) and SEVERE **SEV** (n=15) cardiopathy degrees, and CONTROL **CT** donors (n=7). By ELISA assays we observed that pro-inflammatory molecules such as IFN-γ (13 pg/ml SEV x 5 pg/ml CT), TNF-α (37 pg/ml SEV x 3 pg/ml CT), IL-17(45 pg/ml SEV x n.d. CT) were higher expressed during chronic disease, and it was directly related to cardiopathy degrees, as well as an imbalance on Hypothalamus-Pituitary-Adrenal axis, where a decreasing of DHEA hormone leads to dirturbances on circulating cortisol/DHEA ratio (3.5 AU SEV x 1.2 AU CT). We also observed by in vitro Transwell migration, an enhance on migratory response over fibronectin, CXCL12, fibronectin + CXCL12 and fibronectin + TNF-α as well as with Cortisol and DHEA pre- treatment in different concentrations (preliminary results). By Agilent Bioanalyzer™ assays, we also found miRNA expression in all subjects (preliminary results).

Conclusion: These results indicate that immunoendocrine disturbances, correlated to a systemic inflammatory profile, may also contribute to enhance migratory potential of T lymphocytes to inflammatory sites, including the heart tissue, being thus involved in the cardiopathy seen in this disease.

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IMMUNOLOGICAL RESPONSES OF THE MANGROVE OYSTERS CRASSOSTREA GASAR NATURALLY INFECTED BY PERKINSUS SP. IN THE MAMANGUAPE ESTUARY, PARAÍBA STATE (NORTHEASTERN, BRAZIL).

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Introduction: The protozoan Perkinsus spp. affects commercial bivalves worldwide and causes mortalities. Representative of this genus, including the modifiable P. marinus was recently found in Brazil. Nevertheless little is known about its impact on the host. The aim of this study was to evaluate the effect of Perkinsus sp. infection on the immunological responses of the cultured oysters Crassostrea gasar. **Methods and Results:** Adult oysters were collected in December 2011, March, May, August and October 2012 (N=182) at the Mamanguape River estuary. The Perkinsus sp. prevalence and infection intensity (0- uninfected to 4- maximum parasite burden) were evaluated in the gills by Ray's Fluid Thioglycollate Medium (RFTM) assay. The hemocytic parameters were analyzed by flow citometry: Total hemocyte (THC) and differential (DHC) count, cell mortality, phagocytic capacity and reactive oxygen species (ROS) production. The agglutination titter was used to estimate the level of plasma lectins. Histological sections of oysters were prepared to assess the impact Perkinsus sp. on tissues. The prevalence of Perkinsus sp. was the highest (93.3%) seen so far in oysters from Brazil, although the intensity was moderate (1.9 ± 0.07; Mean ± SE). The parasite infection did not modulate lectins. In contrast, some immunological parameters were impaired by the parasite infection: the phagocytic capacity (0: 15.9%; 4: 9.3%), the ROS production (0: 101.3 a.u.; 4: 71.2 a.u.) and the hyalinocytes percentage (0: 50.4%; 4: 43%), decreased; while cell mortality (0: 4.2%; 4: 7.5%) and the Blast-like cell percentage (0: 32.8%; 4: 43.5%) and THC (0: 1.1×10^6; 4: 2×10^6 cels ml^{-1}) increased. Perkinsus sp. infected mainly epithelia of digestive organs, mostly with light infection. Host was able to defend from this parasite invasion, since there was an hemocytic infiltration and parasites were found engulfed by hemocytes. Schizonts of Perkinsus sp. were also observed in cases of heavy intensity of infection, which indicates the capacity of the parasite to divide and proliferate in host tissues. **Conclusion:** Despite the high impairment of the oysters C. gasar immune system caused by the Perkinsus sp. infection, the host seems to be able to properly defends itself and prevent serious tissue damages. On the other hand, Perkinsus sp. succeeds in proliferate but only in heavy infection intensities. **Financial Suport:** CAPES and CNPq (Brazil); CNRS (France)

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IN PULMONARY PARACOCCIDIOIDOMYCOSIS, IDO EXERTS A PROTECTIVE EFFECT TO SUSCEPTIBLE MICE BUT A DELETERIOUS EFFECT TO RESISTANT MICE.

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Introduction: In pulmonary paracoccidioidomycosis, the regulatory mechanisms mediated by innate and cellular immunity are still unclear. It is known that indoleamine 2,3-dioxygenase (IDO), an IFN-g-induced enzyme which catalyzes the tryptophan metabolism, can control pathogen growth due to tryptophan starvation and inflammation by its immunosuppressive effects on innate and adaptive immunity. Our previous studies demonstrated that P. brasiliensis infected mice showed increased IDO expression, controlling fungal growth but suppressing T cell responses of resistant (A/J) and susceptible (B10.A) mice to P. brasiliensis infection. The aim of this study was to further characterize the role of IDO in the behavior of pulmonary dendritic cells and the expansion of T cell subpopulations. **Methods and Results:** B10.A and A/J mice were treated with 1-methyl-DL-tryptophan (1MT, an IDO inhibitor) or left untreated, and intratracheally infected with yeasts. Our data demonstrated that in both mouse strains IDO activity reduced the migration of IL-6^+ DCs, but increased the numbers of TGF-b^+ and IL-12^+ DCs in A/J and B10.A mice, respectively. IDO expression also reduced the migration of IL-17^+ $CD4^+$ and $CD8^+$ T cells concomitantly with an increased influx of Treg cells to the lungs of B10.A and A/J mice. **Conclusion:** Furthermore, mortality and histopathological studies revealed that IDO exerted a protective effect on B10.A mice due to its inhibitory activity on the pro-inflammatory response associated with their susceptible pattern, but a deleterious effect on A/J mice due to its enhancing effect on the tolerogenic activity of DCs and the expansion of Treg cells, which delayed the expression of protective T cell immunity.

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IN VIVO AND IN VITRO ROLE OF IGM AND IGG PARASITE-SPECIFIC ANTIBODIES IN THE INTERACTION OF T. CRUZI SYLVIO X10/4 PARASITES WITH CELLS OF THE MONOCYTIC LINEAGE

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Introduction: Mononuclear phagocytes are known to play a key role in control of T. cruzi infection. Interaction of T. cruzi trypomastigotes with antibody and/or complement facilitates their uptake by macrophages. Previous studies demonstrated the importance of specific IgG in blood clearance of T. cruzi, promoting the uptake by mononuclear phagocytes. Yet, the role of specific IgM in parasite removal has not been assessed. In this study we evaluated the in vivo and in vitro role of IgM in clearance of Sylvio x10/4 parasites, a myotropic T. cruzi clone which in normoimune mice yields no patent parasitemia.

Methods and Results: Trypomastigotes isolated from cell cultures or from the blood of infected RAG2KO mice, disappear from the circulation one hour after inoculated iv in C57BL/6 mice. Analysis of these mice 24 hours after inoculation, revealed most parasite RNA and viable parasites in the liver and spleen, a result that suggests parasites removal, considering the recognized myotropism of the Sylvio X10/4 clone. In vivo depletion of macrophages/DCs by treatment with clodronate liposomes extends the time in the circulation of the parasite, demonstrating the importance of macrophages in the spontaneous clearance of this parasite. Given this spontaneous exit we decided to study the immune removal of Sylvio X10/4 parasites on day 6 of infection, when subpatent parasitemia shows a significant increase. For this, C57BL/6 mice were inoculated iv, at day 4 pi, with either normal mouse serum (NMS), serum from C57BL/6 chronically-infected mice (B6-IMS) and serum of $CD28^{-/-}$ chronically-infected mice (CD28KO-IMS; which contains exclusively specific IgM; Scand. J. Immunol 66:297-368, 2007), the subpatent parasitemia levels evaluated in subsequent days by LIT assay. Increased removal of blood parasites was found in mice treated with IMS compared to mice treated with NMS. Nonetheless, B6-IMS was more efficient than CD28KO-IMS. Concordant results were observed in vitro studying Sylvio X10/4 parasites invasion of macrophages derived from bone marrow or elicited in the peritoneum, where the intracellular amastigote number was found to increase not only in the presence of B6-IMS, but also in the presence of immune sera containing exclusively IgM antibodies.

Conclusion: Macrophages are essential elements for T. cruzi parasite clearance, an activity enhanced not only by specific IgG antibodies, but also by those of the IgM class.

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INCREASED FREQUENCY OF CD4 AND CD8 REGULATORY T CELLS IN INDIVIDUALS UNDER 15 YEARS WITH MULTIBACILLARY LEPROSY

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Introduction: Leprosy is a chronic disease, caused by Mycobacterium leprae, which poses a serious public health problem worldwide. The high incidence in children under 15 years old in Ceará state, Brazil, reflects the difficulty of its control. The spectrum of clinical manifestations is associated with the immune response developed, with the Th1 and Th2 responses being related to the paucibacillary (PB) and multibacillary (MB) forms, respectively. Regulatory T cells (Tregs), which can suppress Th1 and Th2 response, have received special attention in the literature and have been associated with development of chronic infections. However, their role in leprosy in individuals under 15 years old has not been elucidated yet. We evaluated the frequency of $CD4^{+}CD25^{high}FOXP3^{+}$, $CD8^{+}CD25^{high}FOXP3^{+}$, $CD4^{+}CD25^{high}FOXP3^{high}$ and $CD8^{+}CD25^{high}FOXP3^{high}$ cells in leprosy patients and household contacts (HHC), both cases under 15 years old.

Methods and results: Peripheral blood mononuclear cells from 12 patients and 17 HHC were cultured for 72 hours with anti-CD3 and anti-CD28 (activators) associated with total sonicated fraction of M. leprae. After culture, Tregs frequency was measured by flow cytometry. The unpaired t-test was used to compare two variables and Pearson's test for correlation analyses. Cells stimulated by activators and antigen from MB patients showed Treg frequency almost two times that of the HHC: $CD4^{+}CD25^{high}FOXP3^{+}$ (21.93+8.43% vs. 13.79+8.19%; p= 0.0500), $CD4^{+}CD25^{high}FOXP3^{high}$ (10.33+5.69% vs. 5.57+4.03%; p= 0.0362), $CD8^{+}CD25^{high}FOXP3^{+}$ (13.88+9.19% vs. 6.18+5.56%; p= 0.0230) and $CD8^{+}CD25^{high}FOXP3^{high}$ (5.36+4.17% vs. 2.23+2.68%; p= 0.0461). These findings were not observed between PB patients and HHC. Furthermore, the FOXP3 MFI in Tregs was higher in MB patients than in the HHC: $CD4^{+}CD25^{high}FOXP3^{+}$ (578.2+115.3 vs. 428+129.9; p=0.0208), $CD4^{+}CD25^{high}FOXP3^{high}$ (1098+141.7 vs. 879.2+193.3; p=0.0198), $CD8^{+}CD25^{high}FOXP3^{+}$ (521.2+110.9 vs. 371.2+134.7; p=0.0236) and $CD8^{+}CD25^{high}FOXP3^{high}$ (1088+223.5 vs. 870.5+220.9; p= 0.0511). The frequency of all Tregs evaluated was positively correlated with the bacillary index and number of lesions in patients.

Conclusion: For the first time, we have demonstrated that MB patients under 15 years old have greater $CD4^{+}$ and $CD8^{+}$ Treg frequencies and these correlate with clinical and laboratorial aspects of disease. These findings suggest the involvement of these cells in the perpetuation of M. leprae infection.

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INFLAMMATION AND ANGIOGENESIS DURING ACUTE TRYPANOSOMA CRUZI INFECTION IN C57BL/6 MICE

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Introduction: Trypanosoma cruzi infection induces, especially in heart tissue, an inflammatory process mediated by various inflammatory cells and cytokines. These inflammatory cells and mediators are directly or indirectly associated with angiogenesis. Vascular endothelial growth factor (VEGF), angiopoetins 1 and 2 (Ang-1 and Ang-2) are some of the important angiogenic proteins. Since angiogenesis is an essential process in physiological and pathological conditions, we focussed our study on the involvement of T. cruzi on inflammatory angiogenesis.

Methods and results: In our study, C57BL/6 mice (n=10) were infected with two different strains (Colombian and Y) of T. cruzi (100 parasites). Blood and heart samples were collected during acute phase of infection to evaluate angiogenesis and inflammation by following methods: immunoassays (CCL2, CCL5 and TNF-α), conventional histology and real time-PCR (CCL2, CCL5, TNF-α, VEGF, Ang-1 and Ang-2; in fold expression). Morphometric analysis of heart in mice infected with Colombian strain showed increased inflammatory cells (312.7±23.34) when compared with mice infected with Y strain (246.4±15.39) and not infected (NI) (210.4±9.27). Colombian infected mice also demonstrated increased CCL5 (306.7±40.96) and TNF-α (5.69±0.51) expression in heart when compared with Y infected mice CCL5 (7.11±5.9) and TNF-α (2.32±0.44). Similar pattern for CCL5 production was found in plasma from Colombian (1194pg/ml±73.40) and Y infected mice (738.3pg/ml±51.8). Although there was increased inflammatory mediator levels in plasma and heart by both strains, particularly in heart, the expression of angiogenic proteins like VEGF (Colombian: 0.17±0.027; Y: 0.43±0.09; NI: 0.91±0.14), Ang-1 (Colombian: 0.60±0.12; NI: 2.8±0.55) and Ang-2 (Colombian: 0.33±0.08; NI: 1.30±0.1486) was reduced in infected mice.

Conclusion: Therefore, our current findings suggest that persistent intracellular T. cruzi (heart tissue) trigger a down regulation in expression of angiogenic inflammatory mediators in contrast with a high inflammatory proteins production. Together, these data put forth the involvement of T. cruzi in regulating neovascularization/angiogenesis during infection.

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INFLAMMATION VERSUS MICROPARTICLES WITH ARCHAEAL DNA IN CHAGAS' DISEASE

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Chagas` disease is caused by Trypanosoma cruzi infection, with around 30% percent of infected individuals developing heart failure (HF) and myocarditis; the remaining individuals stay asymptomatic, in the so-called indeterminate form (IF). Antigens and DNA from T.cruzi are scarce, not explaining the intensity of this myocarditis. Circulating microparticles (MPs) were described in idiopathic dilated cardiomyopathy in association with myocardial dysfunction. Previously, we found MPs in chagasic patients, with two ultrastructural morphologies: one electron dense (ED) predominantly in the IF group and other electron lucent (EL), predominantly in HF form. Both MPs have double external waved membrane, reminding archaea.

In this work we investigated if chagasic individuals have different amount of ED and EL MPs than normal hearts (NH) and if these MPs present archaeal DNA which could be related with different outcomes in chagasic patients.

Material and methods: Fragments of chagasic endomyocardial biopsy from patients studied at electron microscopy in 80's were revised: 6 HF and 5 IF and compared with myocardial fragments from NH (from donors). ARCH915 probe linked to 10nm colloidal gold detected archaeal DNA by in situ hybridization technique. Mean numbers of ED and EL MPs, and archaeal DNA positive dots in and outside MPs were counted.

Results: MPs with archaeal DNA were present in all cases. MPs in chagasic groups had lower numbers of archaeal DNA inside (HF=0.11; IF=0.2 and NH=0.34 positive dots/μm^2), but higher numbers outside MPs/ μm^2 (HF 8.40, IF 9.6 and NH 2.4), $p<0.001$.There was a higher amount of EL/μm^2 in HF than IF group (0.07 vs 0.04) p=0.09 and a lower number of ED/μm^2 (0.01 vs 0.07) p=0.006. Focusing on the morphology, intra EL archaeal DNA dots/ μm^2 were lower in HF than IF (0.15 vs 0.78) p=0.03 without difference in ED (0.04 vs0.45) p=0.2. A significant correlation between archaeal dots intra EL vs extra EL in HF (0.69, p=0.0040), but not with intra ED.

Conclusion: Chagas' disease patients present EL MPs carrying archaeal DNA particles that apparently release these DNA particles to the extracellular. Larger numbers of EL are present in HF individuals, suggesting a role of them in development of myocarditis and myocardial dilatation. MPs present in the myocardium of donors are associated with low values of extracellular archaeal DNA, and may have origin from ischemic intestine, a frequent complication in agonic period.

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INFLAMMATORY AND STRUCTURAL CHANGES IN SPLEEN OF NATURALLY INFECTED DOGS BY LEISHMANIA INFANTUM

JOSÉ CLAUDIO CARNEIRO; ADAM LEAL LIMA; GLAUCO JONAS SANTOS; MARCIO GOMES ALENCAR-ARARIPE; DIANA CELIA NUNES-PINHEIRO.

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS VETERINÁRIAS, FORTALEZA - CE - BRASIL.

Introduction: Canine visceral leishmaniasis (CVL) is a chronic infectious disease characterized by impairment of the immune system impeding the return to homeostasis. Mechanisms involving both innate immunity as acquired immunity are compromised by the exacerbated response to protozoan. These changes have been associated with inflammatory process, including increased release of chemokines and proinflammatory cytokines, may cause structural and functional damage in lymphoid organs and trigger the onset of clinical signs characteristic of the disease. This work evaluated the splenic immune-inflammatory and structural changes of naturally infected dogs by Leishmania infantum.

Methods and Results: This study was approved by the Ethics Committee for Animal Use of the State University of Ceará, Protocol No. 08622833-1. Ten adult and symptomatic dogs were used, varying in age and weight, selected by immunochromatographic dipstick test (DPP) and enzyme-linked immunosorbent assay (ELISA), were considered positive with ELISA cut-offs ≥ 1:40. All animals were clinically evaluated and, after the euthanasia procedure, the spleens were dissected and weighed to determine the relative weight. Spleen fragments were collected and subjected to histology procedures (H&E) and visualized for optical microscopy (40x). The same samples were subjected to Congo red stained and visualized in polarized light microscopy. The findings were expressed as percentage. It was observed increased relative weight of spleen in all animals (100%) and splenic nodular hyperplasia in eight animals (80%). Moderate inflammatory infiltrate, hyperplasia and hypertrophy of red and white pulps, thickening of the capsule and congestion were observed in all animals (100%). In addition, megakaryocytes and plasmocytes were observed (80%). Amyloidosis was confirmed, in the same animals that showed splenic nodular hyperplasia, by the analysis of samples stained with Congo red in polarized light microscopy (40x).

Conclusion: It follows that the spleen of naturally infected dogs by L. infantum has histological changes consistent with immune-inflammatory response, demonstrating the functional impairment of the same. Further studies may be realized to evaluate the association among the visceral leishmaniasis, splenic amiloidosis and splenic nodular hyperplasia.

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INFLAMMATORY CYTOKINES IN CHILDREN WITH INVASIVE BACTERIAL INFECTION IN NORTHEAST BRAZIL

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Introduction: invasive bacterial infection such as pneumonia and sepsis are major public health problems worldwide. Recognition of bacterial components by the innate immune system is essential to provide early detection of the pathogen and consequently eliminate infection. Systemic Inflammatory Response Syndrome occuring in patients with sepsis is caractherized by high blood levels of pro-inflammatory cytokines such as TNF-α, IL-1β, IL-6, IL-18, MCP-1 (CCL-2), MIP-1α (CCL3), MIP-1β (CCL4). Biological responses induced by theses pro-inflammatory cytokines are widely diverse and perform important role in sepsis, once they determine the extent of tissue damage and prognosis. The aim of this study was to analyze the innate immune response through measurement of inflammatory cytokine levels in children with different clinical diagnosis (skin abscess, pneumonia or sepsis). **Methods and Results:** a prospective cross-sectional study study was perfomed between August 2012 and May 2013. Children were enrolled if older than 28 days and younger than 14 years with a clinical diagnosis of skin abscess, pneumonia or sepsis (based on internacional guidelines of sepsis of Surviving Sepsis Campaign, 2012) within 48 hours of hospital admission to Instituto de Medicina Integral Prof. Fernando Figueira (IMIP). Exclusion criteria was a previous diagnosis of primary or secondary immunodeficiency. Blood levels of cyotkines were measured using BD™ CBA – Human inflammatory kit (IL-1β, IL-6, IL-8, IL-10, IL-12p70, IL-17a and TNF-α) through flow cytometry. With non-parametric statistical tests, each cytokine had concentrations compared for differences between groups (Mann Whitney U test). Out of 45 children evaluated, 12 (26,7%) had skin abscess, 15 (33,3%) pneumonia and 18 (40%) sepsis. IL-10 was the only cytokine to show significant differences between the group of children with pneumonia when compared to children with skin abscess ($p = 0.021$). IL-8, IL-10 and IL-17a concentrations were higher in children with sepsis when compared to those with skin abscess (IL-8, $p=0,005$; IL-10: $p=0,015$; e IL-17a: $p =0.017$). There were no differences on blood levels of cytokines of children with clinical diagnosis of pneumonia and sepsis.**Conclusion**: the study demonstrated a clear distinction in the inflammatory response of localized versus systemic infection in children with respect to blood levels of IL-8, IL-10 and IL-17a.

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INFLUENCE OF AGING AND ENVIRONMENT ON ANTIBODY-ENHANCED DENGUE DISEASE T CELL RESPONSE IN ALBINO SWISS IMMUNOCOMPETENT MURINE MODEL

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Introduction: In the present study, we tested the influences of aging and EE on dengue disease pathological features in a murine model of AED serial infections in adult female albino Swiss mice.

Methods and Results: To test this hypothesis, we administered serial i.p. injections of anti-DENV2 hyperimmune serum, followed 24 h later by DENV3 (genotype III) infected brain homogenate. Control mice (n=5) received equal volumes of uninfected brain homogenate. The presence of virus or viral antigens was indirectly detected by real-time quantitative RT-PCR and immunohistochemistry. Compared to the impoverished environmental (IE, young n=18 and aged n=8) groups, larger infiltrates with dominant T-lymphoid (CD-3 positive) cells were detected in liver, near the efferent perivein and in lungs, near the peribronchial spaces in enriched environmental (EE, young n=16 and aged n=8) animals with immunopositive T (CD-3, CD4, CD-8) and B (CD-20) lymphocytes in spleen and kidneys of aged infected mice. These pathological changes were virtually absent in the uninfected control group. Consistent with an inflammatory hypothesis, our findings demonstrate striking differences between infected and control animals in the spleen white pulp, independent of age and environment, with a significant lymphoid hyperplasia in infected animals. Aging and a sedentary lifestyle have been associated with a decline in the normal functioning of the immune system that may contribute to the increased outcomes of infection seen in the elderly. Regular exercise, as observed in EE mice, has been associated with lower numbers of exhausted/senescent T cells, increased T cell proliferative capacity, lower circulatory levels of inflammatory cytokines, increased neutrophil phagocytic activity, lowered inflammatory response to bacterial challenge, and greater NK-cell cytotoxic activity, indicating that habitual exercise can regulate the immune system and delay the onset of immunosenescence.

Conclusion: Because our findings demonstrate higher mortality rate in EE aged mice associated with an increased T cell response we suggest that aging and environment exacerbate the inflammatory response in antibody-enhanced DENGUE disease in albino Swiss immunocompetent murine model.

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INFLUENCE OF ANTIFUNGAL TREATMENT ON THE FUNCTION OF THE PERIPHERAL BLOOD MONOCYTES FROM PARACOCCIDIOIDOMYCOSIS PATIENTS WITH THE CHRONIC FORM.

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Introduction. Paracoccidioidomycosis (PCM) is systemic and granulomatous mycosis, caused by thermal dimorphic fungi of the Paracoccidioides brasiliensis complex. The main clinical forms are acute / subacute (FA), which generally affects organs of the mononuclear phagocytic system, and chronic (FC), that predominates in the lungs and in the mucosa of the upper airdigestive tract. Although the antifungal therapy is efficacious, the frequency of sequelae is high, especially in lungs and adrenal. Monocytes are a heterogeneous population of cells with different phenotypic and functional aspects playing distinct roles in the inflammatory process and fibrogenesis. The present study aimed to determine the functional in vitro activity of monocytes from PCM patients (PMC-p). **Patients and Methods.** A total of 23 patients with the chronic form of PCM were studied: 11 non-treated patients (NT-p) and 12 patients in apparent cure (AC-p), a patient presented apparent cure when clinical cure and negative specific antibodies serum levels were reached, and these conditions persisted for a least one year after the end of therapy. We also evaluated 7 healthy individuals as the control group (GC). Monocytes from peripheral blood mononuclear cells were cultured and stimulated, or not, with lipopolysaccharide (LPS) and P. brasiliensis exoantigen from Pb113 strain (PbAg) for 24 hours. The supernatants were submitted to analyses of inflammatory (TNF-α, IL-6, IL-10, IL-12p70 and IL-1β) and fibrotic (TGF-β and basic fibroblast growth factor - bFGF) mediators. The averages among the groups were compared by ANOVA and significance was set up at $p \leq 0.05$. **Results.** AgPb–stimulated monocytes from NT-p and Ac-p produced higher levels of IL-1β and TNF-α than the CG. The spontaneous production of TNF-α was higher in both NT-p and AC-p groups than in the CG. The production of TGF-β1 and bFGF by AgPb-stimulated monocytes was higher in the NT-p than in the AC-p and CG. IL-10 and IL-12p70 were not detected in the supernatants**. Conclusions.** Similar production of proinflammatory cytokines by specific antigen-stimulated monocytes from PCM-p, before antifungal treatment and after reaching AC, suggests persistent inflammatory response to P.brasiliensis. In addition, AgPb-stimulated TGF-β and bFGF production by NT-p suggests that the monocytes already reach the lung tissue presenting a pro-fibrotic profile.

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INFLUENCE OF HYPOINSULINEMIA-HYPERGLYCEMIA (HH) CONDITION ON THE TISSUE EXPRESSION OF TOLL-LIKE RECEPTORS 2 AND 4 AND DECTIN-1 DURING MURINE DERMATOPHYTE INFECTION

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Introduction: Diabetes mellitus (DM) is a complex metabolic disease that affects several physiological and immunological mechanisms; as a consequence these patients present abnormalities, including several fungal infections. Considering that the tissue of diabetes-induced mice infected by Trichophyton mentagrophytes play different responses to the fungal, the aim of the present study was to quantify the expression of Toll-Like Receptors (TLR) 2 and 4 and Dectin-1 in hypoinsulinemic-hyperglicemic (HH)-induced mice during the early phase of the dermatophytic infection.

Methods & Results: In the present study we employed alloxan-induced mice that mimick the HH condition, a hallmark of type I DM. Thus, alloxan HH-induced Swiss mice were inoculated into the footpad with 10^7Trichophyton mentagrophytes microconidia (HHTM Group). Non-infected HH-mice (HH), only infected-mice (TM), non-infected and free-HH mice (CTRL) were used as control groups. Mice were evaluated at 24 and 48 hours and 7 days after the fungal inoculation. Fungal load and mRNA expression of TLR-2, TLR-4 and Dectin-1 were determined in the footpad, spleen and kidney. The averages among the groups were compared by ANOVA and significance was set up at $p<0.05$. In the footpad, the fungal load was similar among the groups. At 24 and 48 hours after the infection, the expression of TLR-2, TLR-4 and dectin-1 were higher in the TM group than the control group; on day 7 there was a higher expression of dectin-1 in the TM group than the control group. In the HHTM group, we observed lower levels of expression of TLR-2, TLR-4 and Dectin-1 in the footpad at 48th hour than the TM group. In the spleen, the TM group presented higher fungal load than HH-TM group at 24th hour; at 48th hour, this picture changed: the HH-TM group showed higher fungal load than the TM group. Despite of these findings, there was no statistic difference in the spleen tissue expression of the receptors among the groups. In the kidney, the HHTM group showed lower fungal load and lesser expression of TLR-2 and TLR-4 than the TM group only at 24th hour.

Conclusions: Our results suggest that the delay of fungal clearance observed both in human and experimental models could be associated with the early alterations in the tissue expression of TLR-2, -4 and Dectin-1. In this context, these receptors are important targets in the development of new immunotherapeutic treatments.

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INFLUENCE OF VIRAL LOAD "BLIPS" ON CD8+ T CELL ACTIVATION AND MICROBIAL TRANSLOCATION IN HIV ELITE CONTROLLERS

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Introduction: HIV elite controllers are a rare population of HIV-infected subjects, who have persistently undetectable plasma viral load, in the absence of antiretroviral therapy. However, a subgroup of elite controllers presents episodes of detectable viral load (blips). The influence of these blips in immune activation and microbial translocation in these individuals are not clear.

Methods and Results: A total of 30 HIV-1 infected subjects were recruited from Hospital Evandro Chagas and 10 HIV uninfected individuals also were included in this study as controls, all subjects gave written informed consent. The HIV infected individuals were stratified into four groups: Elite Controllers (EC, n=7) with VL <80 copies/mL in all determinations; 2) Ebbing Elite Controllers (EEC, n=5) with transient viremia (blips); Viremic Controllers (VC, n=7) with plasma viremia <5,000 copies/mL and Non-controllers (NC, n=11). Plasma HIV-1 viral loads were measured over time using nucleic acid sequence based amplification (NASBA) system, and the Versant HIV-1 3.0 RNA assay. The median time of viral load follow-up was 9 years (IQRs: 8-11). A cross-sectional evaluation of immune activation and microbial translocation was performed. CD8 T cell activation was evaluated by measuring the percent of CD38+HLA-DR+CD8+ T cells. While in EC, the level of activated CD8+ T was no significantly higher than in HIV uninfected participants, EEC presented higher values than HIV-uninfected (P = 0.0027); VC and NC also presented higher rates of CD8 activation than HIV-uninfected individuals. The microbial translocation was assayed by the level of sCD14 in plasma, using ELISA assay-sCD14 Quantikine. The median of concentration of sCD14 in plasma of HIV uninfected, EC and VC did not differ. However, EEC showed a higher level of microbial translocation than uninfected subjects and the other controllers. As expected NC presented higher level of sCD14 than HIV negative group (P = 0.0378).

Conclusion: Elite controllers with transient viremia showed higher levels of immune activation and microbial translocation than EC without blips, suggesting that these episodes of increased viremia have an impact in immune activation and microbial translocation.

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INHIBITION OF CASPASE-8 ACTIVITY PROMOTES A HOST PROTECTIVE TH-2 MEDIATED IMMUNITY TO LEISHMANIA MAJOR INFECTION

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Introduction

Resistance to Leishmaniasis depends on Th1 (IFN-γ) cytokine, but T cell death may affect Th1 responses to parasite infection. T cells undergo apoptosis upon interaction between Fas Ligand (FasL) and Fas, followed by caspase-8 activation. We investigated how apoptosis mediated by death receptor Fas and caspase-8 affects cytokine responses and immunity to Leishmania major parasites.

Methodos and Results

To block T cell apoptosis, we employed mice expressing a T-cell restricted transgene for viral FLICE/caspase-8 inhibitory protein (vFLIP). C57BL/6 (WT) and vFLIP mice (n= 4-7 mice/group) were infected in hind footpads with L. major LV39, and injected i.p. in the course of infection with anti-IL-4 mAb or control IgG mAb, as approved by Institutional Ethics Committee. Cells from lymphoid organs were cultured with L. major antigen, and analyzed for apoptosis and cytokine expression. Splenic CD4 T cells undergo Fas-mediated apoptosis and blockade of FasL increased IFN-γ and IL-4 cytokine responses to L. major antigens. Inhibition of caspase-8 signaling in vFLIP mice also enhanced both Th1 and Th2 (IL-4, IL-13) cytokine responses to L. major infection, even in the Th1-prone C57BL/6 background. Despite an exacerbated Th2 response, vFLIP mice controlled L. major infection, with reduced lesions and lower parasite loads compared to WT mice. Moreover, injection of anti-IL-4 mAb in infected vFLIP mice disrupted control of parasite infection.

Conclusion

Therefore, blockade of caspase-8 activity in T cells improves immunity to L. major infection, by promoting a Th1 response along with protective Th2 cytokines.

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INHIBITION OF PDE4 DURING STREPTOCOCCUS PNEUMONIAE INFECTION REDUCES INFLAMMATORY RESPONSES AND LUNG INJURY IN MICE

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Introduction: Pneumonia, caused most frequently by Streptococcus pneumoniae, is a major global health problem and is a leading cause of morbidity and mortality worldwide.The inflammatory responses that follow microbial infections control dissemination of bacteria but may also cause tissue damage and mortality. Drugs such as phosphodiesterase 4 (PDE4) inhibitors decrease inflammatory responses effectively. Therefore, our question was if the anti-inflammatory action of PDE4 inhibitors interferes with the ability of the murine host to deal with infection.

Methods and Results: Male Balb/C mice (n=6) were infected intranasally with S. pneumoniae serotype 3 (ATCC 6303, 10^4 CFU) and treated with Rolipram (ROL, 6 mg/kg, 1 h before infection) or vehicle (V). After 24 hours, the peak of inflammation as described, mice were killed to assess inflammation and bacteria counts. ROL treatment decreased neutrophil recruitment in lungs and in airways, and decreased levels of cytokines in BALF at 24 h. Number of bacteria in BALF and lethality were similar in ROL and V treated animals. However, ROL-treated animals showed better lung pathology scores than V treated animals. In order to see if the combination of ROL with antibiotics (Ab) used in the clinics given in a later time after infection improves disease outcome, a higher inoculum of S. pneumoniae was used, 10^5 CFU and the treatment with Ceftriaxone (10mg/kg) and ROL started only after 60 hours of infection. After 96 hours, mice were killed and neutrophil infiltration to the airways and lungs and bacteria counts were assessed. The delayed treatment with either Ab or ROL did not prevent neutrophil infiltration, whereas neutrophil numbers returned to basal with combined treatment. The bacteria counts were diminished by Ab treatment, but not ROL; with the combined treatment bacteria counts reached even lower levels than in Ab treated mice.

Conclusion: In this study, we show that inflammation was an important determinant of morbidity after infection with S. pneumoniae as seen by the large neutrophil influx and cytokine production during infection. Pretreatment of mice with Rolipram decreased several parameters of the inflammatory response, ameliorates histological score, without interfering with bacterial load or survival suggesting that partial blockade of pulmonary inflammation may be beneficial for the murine host, mainly when combined to standard antibiotics treatment.

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INHIBITION OF T-CELL APOPTOSIS REPROGRAM PARASITE-PERMISSIVE M2 MACROPHAGES INTO EFFECTOR M1 PHENOTYPE IN MICE INFECTED WITH TRYPANOSOMA CRUZI

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FEDERAL UNIVERSITY OF RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL.

Introduction

In experimental Chagas disease, T cells undergo activation and apoptosis in the course of infection with Trypanosoma cruzi, negatively affecting immune responses. Moreover, macrophages phagocytosing apoptotic cells promote intracellular infection. We hypothesized that phagocytosis of apoptotic cells can affect macrophage phenotype and change the ability to kill parasites, a distinctive feature of “classically activated” M1 macrophages. By contrast, “alternatively ativated” M2a and M2b macrophages participate in healing and regulatory processes.

Methods and Results

To study macrophage phenotype, we infected BALB/c mice i.p. with T.cruzi clone Dm28c and collected peritoneal exudates to analyze macrophage markers by flow cytometry and cytokines in peritoneal fluid. We also activated splenic T cells from infected mice with anti-CD3 in the presence of zVAD or control vehicle (DMSO) for i.p. injection in infected mice, approved by Institutional Ethics Committee. First, we evaluated the functional phenotype of macrophages from T. cruzi-infected mice by using IL-12 and MGL-1, as M1 and M2-macrophage markers. Whereas macrophages from normal mice expressed almost exclusively MGL-1, both IL-12 and MGL-1 defined distinct subsets of macrophages from infected mice. A mix of M1 and M2 macrophages at a 1:1 ratio was detected during acute T. cruzi infection. In addition, macrophages from normal mice were more permissive to parasite infection in vitro. To investigate how macrophages react to apoptotic cells during T. cruzi infection, we injected infected mice with T cells undergoing activation-induced cell death upon stimulation with anti-CD3. Upon inhibition of apoptosis with zVAD, injected T cells induced a shift in macrophage phenotype, by increasing about 3 times the M1/M2 ratio in peritoneal macrophages from infected mice. In addition, treatment with T cells induced a broad modulation of soluble factors in peritoneal fluid. Interestingly, activated T cells treated with zVAD, but not DMSO induced downregulation of M2a marker CCL17 and M2b marker CCL1, as well as IL-10 and IL-13 in peritoneal fluid, but induced IL-12 expression and improved NO production by peritoneal exudates cells.

Conclusion

Therefore, prevention of apoptosis in T lymphocytes helped macrophages to control infection, by a functional reprogramming of macrophages from parasite-permissive M2 to effector M1 phenotype.

Support: FAPERJ and CNPq.

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INTERPLAY BETWEEN HELICOBACTER PYLORI INFECTION AND POLYMORPHISM 14PB INSERTION / DELETION OF THE GENE HLA-G

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Introduction: The subversion of immune responses that Helicobacter pylori (H. pylori) use to escape immune surveillance and to establish persistent infection has been poorly understood. Human leukocyte antigen-G (HLA-G) is a non-classical HLA class Ib molecule shown to exhibit immunomodulatory function in a wide range of immune-based disorders. Recent reports indicate that the 14-bp deletion/insertion polymorphism in exon 8 of the 3'UTR region of the HLA-G gene influences the HLA-G mRNA stability and isoform splicing patterns, thus modulating the levels of HLA-G expression. In the context of viral infections, the expression of HLA-G is a complex process modulated by many factors such as HLA-G polymorphism, stage of infection, which may contribute to an immunological environment affecting the outcome of infection. On the other hand, neither was found about the relationship of this polymorphism in bacterial infections, specifically those related to H. pylori. The aim was to study the 14-bp deletion/insertion polymorphism in exon 8 of the 3'UTR region of the HLA-G gene and relationship with the H. pylori infection.

Methods and Results: The present study analyzed by conventional PCR the 14-bp insertion/deletion polymorphism of exon 8 HLA-G gene in 87 patients (H. pylori positive - Hp+= 68 and H. pylori negative - Hp- = 19) and 190 healthy controls. Our study found that the +14bp allele significantly increased proportions in the HP+ group when compared with healthy controls (Hp+= 52.20% and controls= 40.26%, p-value = 0.02). Moreover, we observed that the protective effect of the +14bp allele is exerted only when the polymorphism is present in homozygosis (Hp + = 22.05% and controls = 16.31%, p- value = 0.46). When evaluated the +14bp homozygosis and -14bp homozygosis in Hp+ group we observed that -14bp/-14bp were markedly increased in the group (+14bp/+14bp = 22.05% and -14bp/-14bp = 26.47%, p-value = 0.46). These outcomes suggest that the -14/-14bp genotype may be considered as a risk factor that is involved clinical implications in the H. pylori infection.

Conclusion: In conclusion, the data of our study suggests that these markers could be involved in mechanisms escape in H. pylori infection. However, would be interesting increase the number patients to evaluate more carefully evaluate this association.

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INTRACARDIAC EDEMA PROPAGATED VIA THE MAST CELL- KALLIKREIN-KININ SYSTEM FUELS TRYPANOSOMA CRUZI INFECTION, CONTRIBUTING TO CHRONIC MYOCARDITIS AND HEART FIBROSIS

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Introduction: In a recent article (Scharfstein et al., 2013) we suggested that extracellular trypomastigotes released from disrupted "pseudocysts" may evoke intermittent flares of trans-endothelium plasma-leakage in the chronically infected heart through the activation of the kallikrein-kinin system (KKS). Here we tested the hypothesis that T. cruzi may exploit the fact that bradykinin (BK) and des-Arg-BK are proteolytically released in inflammatory exudates to invade cardiovascular cells through the signaling of cognate bradykinin (B2R or B1R) receptors **Methods and Results:** Guided by echocardiography, Dm28c tissue-culture derived trypomastigotes (TCTs) were injected in the left ventricle of C57BL/6 (WT), $B2R^{-/-}$ mice, mast cell deficient (B6-KitW-sh/W-sh), or WT mice pretreated with a single dose of HOE-140 (B2R antagonist), B9858 (B1R antagonist), and FXII inhibitor (blocker of contact phase/KKS activation). WE then measured (i) intracardiac leakage of TRITC-dextran (detected by confocal microscopy) (ii) parasite DNA in heart tissues (qPCR at 3 d.p.i) (iii) myocarditis and fibrosis at 30 d p.i. Our data showed that Dm28c TCTs evoked intracardiac edema in WT mice but not in $B2R^{-/-}$ mice nor in those either pretreated with HOE-140, B9858 or FXIIa inhibitor. Strikingly, the intracardiac parasitism was markedly reduced in mice pretreated with each of these drugs. We then checked whether the heart parasitism was coupled to mast cell/KKS activation, reminiscent of intravital microscopy findings described in the accompanying abstract (Nascimento C. et al.). Indeed, we found that the intracardiac levels of T. cruzi DNA were drastically reduced in the heart of mast cell-deficient mice or in WT mice pretreated with cromoglycate, a mast cell stabilizer. Finally, we interrogated whether KKS blockade at early stages of heart infection could have cardioprotective effects in the long term. In keeping with our working hypothesis, histopathological analysis (30 d.p.i.) revealed that blockade of KKS activation at the onset of intracardiac infection nearly abolished chagasic myocarditis and collagen deposition. **Conclusions**: By restoring microcirculatory homeostasis in the chagasic heart, drugs that efficiently target the mast cell/KKS pathway may limit the parasite ability to efficiently infect cardiovascular cells via GPCRs, thereby sparing the myocardium from excessive inflammation and fibrosis.

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INVESTIGATION OF NK CELL ALTERATIONS IN HIV-1+ INFECTED PATIENTS

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Introduction: The acquired immunodeficiency syndrome is caused by human immunodeficiency virus (HIV) and is associated with leading causes of death worldwide. In HIV infection, several immune dysfunctions associated with natural killer (NK) cells have been reported which increase the susceptibility of infected individuals to opportunistic infections. The objective of our work is to characterize the phenotype and function of NK cells from a group of Brazilian HIV-1 infected patients who have not started antiretroviral therapy.

Methods and Results: Control samples were obtained from healthy blood donors (n=7). Plasma levels of proinflammatory cytokines were increased in HIV-1 infected individuals (n=7) as determined by Multiplex Detection Platform with a significant increase of TNF-α (11.18 ± 4.47 pg/mL) compared to the control group (4.9 ± 2.5 pg/mL). Peripheral blood mononuclear cells were isolated from individuals by density gradient and NK cell frequency was determined by flow cytometry. NK cell ($CD3^-CD56^+$) frequency was diminished in HIV-1 patients (7.15% ± 1.28) compared to control (13.10% ± 3.78). NK cell subpopulations determined according to CD56 and CD16 expression were also diminished in HIV-1 patients. Otherwise, the NK cell subpopulation characterized as non-functional ($CD56^-$ $CD16^+$) was increased in HIV-1 patients. The NK cell function was evaluated by LDH cytotoxicity assay after magnetic separation using $CD56^+$ MicroBeads. NK cell from HIV-1 infected people showed lower percentage of cytotoxicity compared to control when analyzed in different effector:target cell ratio. We also quantified cytokines in the supernatant of the cytotoxicity assay by Multiplex Detection Platform. TNF-α production by NK cells was decreased in HIV-1 infected individuals, despite it was not statistically significant, it could be correlated to the lower cytotoxicity in this group. Interestingly, production of the chemokine RANTES was also lower in the HIV-1^+ group and could be a result of evasion mechanisms of the virus.

Conclusion: Despite of having $CD4^+$T cells above 300 cells/mm^3, HIV-1^+ patients analyzed had showed impaired NK cell function that could contribute to their state of immunosupression. Therefore, our future perspective is to investigate the relationship of NK cell alterations with epigenetic changes in genes related to immune response.

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LEUKOTRIENE B4 MODULATES P2X7 RECEPTOR-MEDIATED LEISHMANIA AMAZONENSIS ELIMINATION IN MURINE MACROPHAGES

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Objectives. Extracellular ATP (eATP) has been reported as an important signaling molecule through its coupling to P2X7 receptor. Once activated, the P2X7 receptor promotes several intracellular mechanisms such as apoptosis, and cytokines release such asIL-1b. Our group has shown that eATP was able to eliminate Leishmania amazonensis (Chaves et al, Microbes Infect., 11, 842, 2009). It has been studied that LTB_4 is able to reduce parasitic load of infected macrophages (Serezani et al, J Immunol, 177, 320, 2006) and others works have demonstrated that the P2X7 receptor can induce PLA_2 activation and arachidonic acid mobilization (Alzola et al, J Biol Chem, 273, 30298, 1998; Balboa et al, J Biol Chem, 274, 36764, 1999). Thus, we investigated whether eATP through P2X7 receptor could induce the LTB_4 release and parasite elimination.

Methods. We used peritoneal macrophages of balb/c, Sv129, 5-$LO^{-/-}$, C57Bl/6 or P2X7receptor$^{-/-}$ mice infected with L. amazonensis at 10:1 ratio. These were tested for parasitic load and LTB_4 production in the presence of 500 µM ATP for 30 min at 37°C. For estimate parasitic load, macrophages were fixed and stained with Panotic kit after 24 h for analysis by optical microscope. LTB_4 production quantification was evaluated by EIA. The graphs were generated using the GraphPad Prism 5.0.

Results. We have shown that ATP-treatment decreased parasite burden in macrophages from WT mice (difference between means 0.8833 ± 0.1596 infection index) while ATP had no effect in infected macrophages from P2X7 receptor-deficient mice. Furthermore, our data showed that ATP was efficient to induce LTB_4 release in infected macrophages(47 ± 22 pg/ml) only from WT animals. In addition, when accessed the importance of 5-LO in L. amazonensis elimination, we found that ATP was not able to decrease the parasitic load in macrophages from 5-$LO^{-/-}$ mice (-0.333 ± 0.1876). In addition, the use of specific inhibitor of 5-LO before treatment with ATP completely inhibited the ATP-induced parasitic elimination. Then we found that macrophages from 5-$LO^{-/-}$ mice are able to eliminate L. amazonensis in the presence of exogenous LTB_4 (0.9972 ± 0.3231) and macrophages P2X7$R^{-/-}$ are also capable of eliminating Leishmania when incubated with ionomycin, an inducer of LTB_4 production (0.6167 ± 0.09379). Finally, we also demonstrated that ATP was not able to reduce parasitic load when the cells were pre-treated with BLT1R receptor antagonist (CP105696), demonstrating the importance of BLT1R signaling, Therefore, these data suggest the involvement of LTB_4/BLT1 in the elimination of L. amazonensis via activation of P2X7 receptor by eATP.

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LIPID BODIES WITHIN THE PARASITE TRYPANOSOMA CRUZI ARE ABLE TO PRODUCE PROSTAGLANDIN E2

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Introduction: Lipid bodies (LBs) are complex organelles, rich in lipids and delimited only by a monolayer of phospholipids (Protoplasma, 249(3):541-585, 2012). LBs are present in all eukaryotic cells, being sites for synthesis of inflammatory mediators in cells from the immune system (Mediators of Inflam., 2012:1-11, 2012). Accumulation of LBs in the cytoplasm of pathogens in response to interactions with host cells has been attracting great interest, but the meaning of this accumulation is not yet understood. In this study, we investigated the formation of LBs in the intracellular parasite Trypanosoma cruzi, during different situations of interaction with host cells (macrophages) and the potential role of these organelles as sites of inflammatory mediators. **Methods and Results:** LBs were identified in both tripomastigotes and amastigotes forms of the parasite after staining with different lipid probes (Osmium tetroxide, BODIPY and Oil red O). Stimulation of trypomastigotes from culture with fatty acids (arachidonic acid (AA), oleic acid (OA)) induced significant formation of LBs (Mean ± SEM: 4.67 ± 0.208 in control and 8.19 ± 0.19 in stimulated with 7.5 µM of AA, n = 6) and PGE_2 (Mean ± SEM: 16.02 pg/mL ± 3.76 in control and 101.5 pg/mL ± 10.52 in stimulated with 1.5 µM of AA, n ≥ 3) in a mechanism dose- and time-dependent. Transmission Electron Microscopy (TEM) analyses of infected macrophages showed that LBs within the parasite were significantly larger and more electron-dense in cells infected in vivo (heart macrophages) compared to LBs formed in response to the in vitro infection (peritoneal macrophages) (0.03 μm^2 ± 0.00 in vitro and 0.04 μm^2 ± 0.00 in vivo, n = 90 and 66 LBs analyzed, respectively). Animal ethical approval number: # 57/11- 4 (CEUA-FIOCRUZ). **Conclusions:** Our study demonstrates that LBs within the parasite Trypanosoma cruzi are organelles able to respond to stimuli and to produce PGE_2, with potential roles during the infection process and maintenance of the parasite in the host cell. These findings bring a new focus on LBs and help to understand the functional capabilities of these organelles during inflammatory responses induced by infectious diseases. **Financial support:** CAPES, CNPq and Fapemig.

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LIPOSSOMAL TRIVALENT ANTIMONY AS AN EFFICIENT DELIVERY SYSTEM FOR TREATMENT OF MURINE VISCERAL LEISHMANIASIS

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Introduction: The first therapeutic approach for leishmaniasis consisted in the administration of trivalent antimony (SbIII), which was later abolished due to its high toxicity. The employment of a drug delivery system could result in reduced dosage and consequently, diminished toxicity associated to SbIII, enabling its reinclusion as an efficient therapy against leishmaniasis. This study evaluated the utilization of liposome-encapsulated SbIII and its association with ascorbic acid (AA) for the treatment of murine visceral leishmaniasis.

Methods and results: Liposome-encapsulated SbIII composed of diestearoylphosphatidylcholine and cholesterol (5:4) was obtained. Characterization of the liposomal formulation revealed size, polydispersity index and encapsulation efficiency of 222.5 nm, 0.214 and 15%, respectively, for a formulation containing 4 mg SbIII/mL. BALB/c mice were intravenously inoculated with 1 x 10^7 Leishmania infantum promastigotes. After six weeks, animals were treated intraperitoneally, as a single dose, with: (1) phosphate buffer, (2) SbIII at 9 mg Sb/kg, (3) empty liposomes, (4) liposomal SbIII at 9 mg Sb/kg, (5) AA at 300 mg/kg, (6) association of AA (300 mg/kg) with SbIII at 9 mg Sb/kg or (7) association of AA (300 mg/kg) with liposomal SbIII at 9 mg Sb/kg. After ten days, parasite load was evaluated through the limiting dilution technique. Immunophenotyping of spleen cells was performed using flow cytometry. Histopathological examinations of the liver, kidney and heart were also conducted. After treatment with liposomal SbIII, 47%, 33% and 47% reduction in parasite load in the liver, spleen and bone marrow was observed, respectively. Parallel administration of AA with either free SbIII or liposomal SbIII did not interfere with SbIII activity. No reduction in parasite load was observed after treatment with free SbIII. No significant alteration in the profile of spleen cells by flow citometry was observed. Histopathological analyses demonstrated that the co-administration of AA with liposomal SbIII was able to preserve the integrity of the hepatic and kidney tissues.

Conclusion: Our results allowed concluding that liposome-encapsulated SbIII represents a therapeutic alternative able to reduce parasite burden with the potential to eliminate parasitism at the bone marrow. Co-administration of AA and liposomal SbIII reduced toxicity of SbIII to liver and kidney tissues.

Fapemig, LGqA/DEGEO, Rede Mineira de Bioterismo.

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LOWER GRANZYME B EXPRESSION IN GAMMA DELTA T-CELL OF PATIENTS WITH HTLV-1-ASSOCIATED MYELOPATHY

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Introduction: Human T-cell lymphotropic virus type 1 (HTLV-1) is an etiologic agent of adult T-cell leukemia and HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP). HAM/TSP is a chronic inflammatory disease whose symptom is the loss of motor movement in response to spinal marrow cells destruction by an inflammatory reaction involving T lymphocytes. HTLV-1 preferentially infects T $CD4^+$ lymphocytes. The risk of HAM/TSP disease is positively correlated with the magnitude of the proviral load in the blood. The efficient immune response seems to be important to control viral replication, and it is also associated to the development of the disease. Thus, the individual immune response induced by HTLV-1 infection is directly linked to the susceptibility or the resistance to develop HTLV-1-associated diseases. γδ T cells are increasingly recognized as having important functional roles in a range of diseases, such as infection, autoimmunity and cancer. Therefore, we investigated the relationship between HTLV-1 infection and γδ T lymphocytes during HAM/TSP disease. Methods and Results: This study compared the phenotype of gamma delta T cells of health donors with HTLV-1 carriers by flow cytometry. We demonstrated that the percentage of γδ T cell population was reduced in HAM/TSP patients. However, no difference was observed in T γδ cells subtypes: $Vγ9d2^+$ and $Vγ9d2^{neg}$. Recent reports have shown that CD27 play a critical role in the development and functioning of γδ T cells, but the expression of CD27 did not modify in γδ T cells of HTLV-1 carriers. We analyzed the expression of Granzyme B and CD16 on γδ T cells. No difference was observed in CD16 expression, but we demonstrated an important reduction of Granzyme B expression (in about 50%) in γδ T cells obtained from HAM/TSP patients. Conclusion: The results suggested that the reduction of Granzyme B expression may impair the γδ cytotoxicity activity, thereby increasing the proviral load and the development of HAM/TSP. Financial support: FAPERJ, CNPq and CAPES.

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LYMPHOCYTE ACTIVATION PROFILE IS INFLUENCED BY THE METHOD OF CELL CULTURE: WHOLE BLOOD ASSAY VERSUS PERIPHERAL BLOOD MONONUCLEAR CELLS

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Introduction: Visceral Leishmaniasis (VL) is characterized by lack of activation of peripheral blood mononuclear cells (PBMC) after Soluble Leishmania Antigen (SLA) stimulation and with impaired Th1 cytokine production. Those patients present a lack of response to the Leishmania skin test, which is reversed after successful treatment with production of IFN-γ by PBMC. However, recent study, using whole blood (WB) assay, showed the presence of IFN-γ after SLA stimulation during active disease. The aim of this work was to compare the activation profile of PBMC and WB in the same set of patients. **Methods and Results:** We evaluated 3 groups: symptomatic VL, recovered VL and endemic control (EC) group. Lymphocytes CD4 and CD8, as well as, CD69 for recent activation and CD25 for late activation were determined by flow cytometry. No specific activation in $CD4^+CD25^+$ lymphocytes after SLA stimulation was observed in any of the WB assay groups ($p>0.05$). However, in the PBMC assay, recovered VL and the EC group had an increased frequency of these cells after SLA stimuli ($p=0.0004$ and $p=0.0084$, respectively). Similarly, no specific activation in $CD4^+CD69^+$ cells using WB assay was observed ($p>0.05$), but in PBMC there was specific activation (Recovered: $p=0.0203$, EC: $p=0.0015$). In WB culture we only observed stimuli specific activation in $CD8^+CD25^+$ cells in EC group ($p=0.0461$). Recovered VL and EC group had specific activation in $CD8^+CD25^+$ cells ($p=0.0156$ and $p=0.0003$, respectively) when PBMC were used. There was no difference in the frequency of $CD8^+CD69^+$ cells, using WB assay from any of the groups ($p>0.05$). Recovered VL ($p=0.0156$) had increased frequency of $CD8^+CD69^+$ cells when PBMC were used. **Conclusion:** Similar to production of IFN-γ by active VL patients, WB and PBMC culture showed important differences in the activation of CD4 and CD8 cells in response to SLA antigen. Therefore, there are important differences using this two methods and this should be evaluated in subsequent studies. Support: National Institutes of Health AI-30639 and CAPES.

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M1 MACROPHAGE POLARIZATION CONFERS RESISTANCE TO PLAGUE

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Mus spretus SEG/Pas (SEG) mice contrast with laboratory mice by their exceptional resistance to bubonic plague. We previously reported that SEG early immune response against Y. pestis involves a faster recruitment of monocytes/macrophages than that of susceptible C57BL/6 (B6) mice. We established here that SEG macrophages were more potent than B6 macrophages at killing internalized Y. pestis, producing bactericidal nitric oxide, and resisting Y. pestis-induced apoptosis. Furthermore, SEG macrophages responded to Y. pestis by producing high levels of pro-inflammatory cytokines (TNFa, IL-6 and KC), and low levels of anti-inflammatory cytokines (IL-10 and G-CSF), whereas the opposite response was observed in B6 macrophages. Therefore, exposure to Y. pestis induced an M1 response in SEG macrophages and an M2 response in B6 macrophages. These M1 and M2 polarizations were observed in peritoneal and splenic macrophages, indicating that they were not tissue-restricted. They were also observed in macrophages derived in vitro from bone-marrow, showing that M1/M2 polarization did not require in-vivo signals. The B6 macrophages were not intrinsically unable to display an M1 profile, because a prior GM-CSF treatment allowed B6 bone-marrow-derived macrophages exposed to Y. pestis to produce an M1 cytokine profile and resistance to Y. pestis-induced apoptosis comparable to SEG. M1 polarized B6 macrophages transferred to susceptible B6 mice significantly increased their survival to a Y. pestis infection. Therefore, the capacity to mount an M1-polarized macrophage response is a key mechanism to resist plague.

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MAST CELLS ARE KEYS CELLS IN THE RESISTANCE TO ORAL INFECTION WITH TOXOPLASMA GONDII

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Introduction: Important functions of mast cells as effectors cells during innate and acquired immunity have been reported. Toxoplasma gondii is an intracellular protozoan parasite which infects around 1 billion people worldwide. T. gondii is known to elicit a strong Th1 response and to induce high levels of IL-12 and IFN-g. Two routes of infection - oral and intraperitoneal - are commonly used in mice model, the oral infection being the natural route. Due to the predominant localization of mast cells in mucosa, it appeared relevant to investigate the role of these cells in the innate immune response after oral infection with T. gondii.

Methods and Results: Genetically mast cell-deficient mice (W/W^v) and control mice ($^{+/+}$) were orally infected with 5 cysts of ME49 strain. The mortality was evaluated daily during 40 days after infection. At different time, spleen cells from W/W^v and $^{+/+}$ mice were plated in 24 well plates and IL-12p40/IL-23, IFN-g, IL-10 and IL-18 evaluated by ELISA. Our data showed that mortality was accelerated in infected W/W^v mice when compared with $^{+/+}$ mice. That is in accord with the delayed Th1 response observed in infected W/W^v. Bone marrow-derived mast cells (BMMCs) were generated from C57BL/6 and $MyD88^{-/-}$ mice by cultured for 4 weeks as previously described. BMMCs were stimulated with LPS or Toxoplasma lysate (Ag) and IL-6 evaluated by ELISA. BMMCs from C57BL/6, but not from $MyD88^{-/-}$, were able to recognize Ag, suggesting the requirement of MyD88 in the recognition of the pathogen. The role of mast cells during infection with T. gondii was confirmed when adoptive transfer of BMMCs generated from $^{+/+}$ mice was performed in W/W^v. W/W^v mice after reconstitution showed similar levels of cytokines when compared to $^{+/+}$ mice indicating that W/W^v mice retrieved their capacity to establish an early Th1 response after T. gondii infection.

Conclusion: According to our data mast cells may be the first cells engaged in the immune response during the oral infection with T. gondii.

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MEMORY CD4+T CELLS IN SCHISTOSOMIASIS PATIENS WITH PERIPORTAL FIBROSIS

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Introduction: Schistosomiasis is a serious parasitic disease affecting ~200 million people worldwide. Approximately 5 % of individuals infected with S. mansoni progress to periportal fibrosis. The mechanism underlying this pathology is still not well understood. Memory T cells are able to mount a recall response to antigens and may be involved in the pathology of schistosomiasis. The aim of this study was to evaluate the profile of memory T lymphocytes in schistosomiasis patients with different degrees of periportal fibrosis.

Methods and Results: Twenty-seven patients were enrolled in the study to date. Periportal fibrosis was classified using abdominal USG according to the WHO criteria. The lymphocytes were obtained from peripheral blood mononuclear cells (PBMC) and classified into central memory T cells (T_{CM}; $CD4^+CCR7^+CD45RA^-$), effector memory T cells (T_{EM};$CD4^+CCR7^-CD45RA^-$) and naïve T cells ($CD4^+CCR7^+CD45RA^+$). Cytokine expression in T cells was evaluated using flow cytometry. . **Results:** The frequency of T_{CM} cells in the group of individuals without fibrosis [median (min.-max.) = 40 % (38 – 54.6 %)] was higher than in the group with moderate to severe fibrosis [34 % (27.2 - 49 %; p=0.03)]. The frequency of T_{EM} cells was also higher in individuals without fibrosis [11 % (5.6 - 13.4 %)] than in those with moderate to severe form of the disease (7.4 % [4.0 – 10.3 %]; p=0.05) and those with incipient fibrosis [5.1 (2.8 – 8.5 %); p=0.005]. Moreover, there was a positive correlation between the frequency of T_{EM} cells and T cells expressing TNF in the group with moderate to severe fibrosis (r=0.9, p=0.012). The frequency of T cells expressing TGF-β was positively correlated with T_{EM} cells from individuals with incipient fibrosis (r=0.7; p=0.04).

Conclusion: In individuals without periportal fibrosis a high frequency of T_{CM} and T_{EM} cells was observed, suggesting these cells may be protective. On the other hand, the positive correlation between the frequency of T_{EM} cells and T cells expressing TNF and TGF-β is controversial, and indicates that as an important source of inflammatory and wound healing-related cytokines, the T_{EM} cells could be associated to periportal fibrosis development.

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MESENCHYMAL STROMAL CELLS MODULATE THE ANTIPROTOZOAL ACTIVITY OF MACROPHAGES

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Introduction: Leishmaniasis is endemic in 98 countries and Chagas disease continues to expand beyond its traditional range of tropical and subtropical zones. These neglected diseases remain a major health problem, mainly by the lack of available vaccines and a few drugs for therapy. Mesenchymal stromal cells (MSCs) have been used in research and therapeutic studies for years. Despite this, little is known about the immunoregulatory effects of MSCs on protozoan infection. In this study we evaluated the infection of MSCs by Leishmania amazonensis and Trypanosoma cruzi and its influence on nitric oxide (NO) production and trypanocidal and leishmanicidal activity of macrophages in vitro. **Methods and Results:** MSCs were isolated from abdominal fat of BALB/c mice and characterized by differentiation into osteogenic and adipogenic lineages. MSC-conditioned media (CM) was prepared by plating 3 x 10^4 cells in 1 ml of DMEM 10% for 24 hours. To evaluate the infection of MSC by parasites, MSCs were incubated with L. amazonensis or T. cruzi (ratio 10 parasites/cell) and the infectivity determined by the presence of amastigote forms of parasites. The influence of MSCs on the leishmanicidal activity of macrophages was determined by quantifying the number of Leishmania released by infected macrophages, while the trypanocidal activity by the determination of the percentage of infected cells and the number of amastigotes/300 macrophages examined by panoptic staining method. For this, cells were infected and stimulated in vitro with LPS plus IFN-g in the presence of medium, supernatant of MSC (CM) or aminoguanidine during 48 h. The NO production by activated MSCs and by macrophages stimulated with LPS/IFN-g in the presence of MSCs' conditioned medium (CM) and/or aminoguanidine, was assessed by Griess. It was found that the supernatant of MSCs caused significant decrease in NO production and inhibited the leishmanicidal activity in 61.6% with respect to the LPS/IFN-g stimulated cells. Currently we are investigating the effects of CM of MSCs on the trypanocidal activity of macrophages. **Conclusion:**Our results suggest that MSCs may serve as reservoirs for Leishmania and Trypanosoma, as well as negatively modulate the parasiticidal activity of macrophages against these protozoans.

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MICROGLIAL ACTIVATION DURING EXPERIMENTAL CEREBRAL MALARIA COULD BE ASSOCIATED TO LONG-LASTING COGNITIVE IMPAIRMENT

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Introduction: Microglial cells activation is necessary and beneficial in response to injury or disease and communication between neurons and microglia is essential for maintaining homeostasis in the central nervous system (CNS) during both physiological and inflammatory conditions. However, excessive or prolonged activation can have deleterious effects on brain function and behavior. Our group showed previously the development of cognitive impairment in mice due to experimental cerebral malaria. The aim of this work was to evaluate the microglial activation during different models of malaria infection in order to correlate to cognitive decline. **Methodology**: C57BL/6 mice were infected with 10^6 Plasmodium berghei ANKA (PbA), Plasmodium berghei NK65 (PbNK65) or Plasmodium chabaudi (Pch) parasited red blood cells. Control groups received the same amount of non-infected red blood cells (RBC). Mice underwent to a clinical score and chloroquine (25 mg/kg b.w.) was orally given at the first signs of cerebral malaria (CM) in PbA infected mice; despite no signs of CM, Pch and PbNK65-infected mice were submitted to the same therapeutical approach. On day 3, 6, 7, 10 and 15 mice were euthanized and the brain removed and fixed to cryosection procedures. Brain slices (30 µm) underwent to immunohistochemistry assay to Iba-1 (microglial marker) and Fluoro Jade B (neuronal degeneration marker) and were evaluated fluorescence microscopy. In other experiment, cognitive function of animals recovered from parasitic disease by the treatment with chloroquine was accessed by habituation to the open-field task (day 15-16 post-infection). **Results**: We observed an increased immunoreactivity for Iba-1 on day 6 and 7 post-infection on hippocampal CA1, dentate gyrus and cortex regions of PbA-infected mice. PbA infected mice also presented positive fluorescence to Fluoro Jade B on day 7-post infection. Pch and PbNK65 mice presented lower microglial activation and Fluoro Jade B staining. No signals of microglial activation or neuronal degeneration were observed on day 10 to 15 post-infection in infected mice treated with chloroquine. As expected, only PbA mice recovered from parasitic disease presented cognitive impairment. **Conclusion**: taken together, these data suggest that microglial activation during experimental cerebral malaria could contribute to neuronal damage and consequent cognitive impairment.

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MICROVESICLES (MVS) RELEASING FROM GIARDIA INTESTINALIS MODULATE THE PARASITE-HOST CELL INTERACTION

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Introduction: Giardia intestinalis, is an aetiological agent of giardiasis, an infection that induces a loss of epithelial barrier function and functional injuries of the enterocyte, producing diarrhoea and other symptoms (Curr Opin Infect Dis 2003 16 (5) 453-460. Recently the descriptions of MVs are widely detected in various biological fluids and eukaryotic cells (Reviewed in J.Cell Biol 2013 Feb18; 200(4):373-83. We have studied if G. intestinalis is releasing MVs during the interaction with mammalians host. Our objective is to analyze the role of MVs from G. intestinalis at the pathogenesis of giardiasis.

Methods and results: Purified MVs were obtained through ultracentrifugation at 100.000 g x 1.5 h, quantified by flow cytometry analysis and used in different essays. Proteomic analyses were performed by 1D-LC-MS/MS. We have demonstrated a high production of MVs in response of pH changes and different environmental conditions during the course of the infection. We also observed that MVs from G. intestinalis increase two times the adhesion of the parasite to the Caco cell in a dose dependent way [from $2{,}4.10^5 \pm 0{,}41$ to $4{,}76.10^5 \pm 0{,}12$ parasites/ml ($P < 0.05$)]. Moreover less than half attachment of G. intestinalis treated with MBCD (a lipid raft inhibitor) to Caco cells, suggested a membrane plasmatic compromised of G. intestinalis at the MVs formation [from $1{,}5.104 \pm 0{,}2$ to $6{,}6.103 \pm 0{,}6$ MVs/ml ($P < 0.05$)]. MVs from G. intestinalis are captured by dendritic cells at early times and proteomic analyses of MVs are indicating putative virulence factors mediating the parasite – host cell interaction.

Conclusions: G. intestinalis is releasing MVs during the life cycle. The origin of the MVs could be involved with lipid rafts from the plasmatic membrane of the trophozoites. G. intestinalis use the MVs to increase the adhesion to eucariotic cell. Our results are helping to understand the role of MVs at the pathogenesis of giardiasis.

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MODULATION OF ADAPTIVE IMMUNITY WITH TOLL LIKE RECEPTORS DURING THE INFESTATION WITH RHIPICEPHALUS SANGUINEUS TICKS

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Introduction: Rhipicephalus sanguineus are hematophagous arthropods that parasitize and transmit a variety of infectious agents to domestic animals. The ticks also secrete a diversity of pharmacologically active molecules that modulate both innate and acquired host immune responses in their favor, allowing successful feeding. Among the mechanisms used by ticks to evade the protective response of their hosts, our group has shown that R. sanguineus tick-infested mice present a mixed Th1/Th2 response with a predominantly Th2 response, and that the saliva of ticks can suppress the function of various immune cells, including macrophages, lymphocytes, and modulate the maturation, migration and function of dendritic cells (DCs). We also observed that tick saliva induces a high expression of TLR-2 receptor on mice DCs surface. Toll-like (TLRs) are key components of the innate immune system that detect microbial infection. Recently, it has been shown that TLRs are also dedicated to the control of adaptive immunity by orchestrating the responses of different cell populations including T and B cells. The present work evaluates the role of tick infestation with R. sanguineus in the modulation of the adaptive immune response mediated by TLRs.

Methods and Results: We evaluated the expression of TLR1, 2, 4, 5, 6,and 9, by flow cytometry, on T and B cells from the spleen and lymph nodes on days 3 and 7 of twice tick-infested mice (n=5). Our results showed that the expression of TLR4 and 6 molecules on B cells was significantly reduced ($p<0.05$, t test) on the tick-infested group; on the other hand TLR5 and 9 were increased on T and B cells ($p<0.05$) when compared with the non-infested group (sham). This experiment was repeated three times.

Conclusions: These results suggest that one mechanism by which the saliva of R. sanguineus modulates the host immune response possibly is through TLRs, and can contribute to new perspectives for the control of ticks. The next step will be to analyze if the tick infestation can modulate the function of lymphocytes stimulated with TLRs ligands (proliferation; activation; cytokines production).

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MOLECULAR AND IMMUNOLOGICAL TOOLS TO CYTOKINES STUDIES IN NEOTROPICAL SAIMIRI SCIUREUS MONKEYS

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Introduction: Malaria remains a major public health problem and the development of a vaccine has been a major research priority. Primates of the genus Saimiri and Aotus are recommended by World Health Organization for vaccine trials, because they are capable of being infected by human plasmodial species. Although non-human primates offer advantages for the study of malaria, one limitation is the lack of immunological tools to assess their immune response. The present study focused on the development and comparative use of molecular and immunological methods to evaluate the cellular immune response in S. sciureus.

Methodology and Results: Blood samples from nine clinically healthy Saimiri monkeys breeding in the Department of Primatology, Fiocruz, Rio de Janeiro, Brazil were collected via femoral venipuncture. Mononuclear cells were isolated by density gradient centrifugation and then cultured for 6, 12, 18, 24, 48, 72 and 96 hours in presence of PMA/Ionomycin. The detection of supernatant levels of cytokines was done by BD Cytometric Bead Array Human and non-human primate Th1/Th2 Cytokine Kit (TNF, IFNg, IL2, IL4, IL6 and IL10), Bio-Plex Pro Human Cytokine Th1/Th2 Assay (IL2, IL4, IL5, IL10, IL12, IL13, GMCSF, TNFα and IFNg), ELISA and ELISPOT for IFNg (Mab GZ-4/ Mab 7-B6-1 Mabtech, B27/4SB3 Pharmingen); IL4 (8D4-8/ MP4-25D2 Pharmingen), IL5 (TRFK5/ JES1-5A10 Pharmingen), TNFα (MAb1/Mab11 Pharmingen), IL10 (JES3-9D7, JES3-12G8 Pharmingen; 945A5D11/945A5A10 Biosource), IL13 (Mab IL13-I /Mab IL13-II Mabtech). Gene expression analysis was done by Applied Biosystems Real Time PCR TaqMan Gene Expression Assays (IFNα, IFNβ, IFNg, IL10, IL12, IL13, IL15, IL16, IL17, IL18, IL1, IL2, IL3, IL4, IL5, IL6, IL8, IL9, LTA and TNF). By using both Human and non-Human CBA, it was possible to detect only IL2 cytokine. The same result was observed by using Bio-plex where only IL2 was detected. Only the couple of Mabtech antibodies allow the dosage of IFNg and IL4 cytokines by ELISA and ELISPOT. Real Time PCR allows the gene expression analysis of 12 out 20 cytokines tested (IFN-g, LTA, IL6, IL5, IL4, TNF, IL2, IL10, IL12, IL1, IFNβ, IL17).

Conclusion: Real Time PCR TaqMan Gene Expression Assays allowed us to analyze a larger number of cytokines that play an essential role during parasite infection, being therefore considered the better tools to evaluate the cellular immune response in Saimiri sciureus monkeys.

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MONOCYTES SUBSETS IN LIVER PATHOLOGY IN HUMAN SCHISTOSOMIASIS

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Introduction: About 5 to 10% of individuals infected with Schistosoma mansoni progress to periportal fibrosis. This pathology is predominantly caused by the host immune response to parasite egg antigens and studies have emphasized the role monocyte in the progression of fibrosis in experimental models. The aim of this study was to evaluate monocytes subsets in schistosomiasis patients with periportal fibrosis.

Methods and Results: Forty patients were enrolled in this study. Periportal fibrosis was classified using abdominal USG according to the WHO criteria. Monocytes were obtained from peripheral blood mononuclear cells (PBMC) and classified into: classical ($CD14^{++}CD16^{-}$), intermediate ($CD14^{++}CD16^{+}$) and nonclassical ($CD14^{+}CD16^{++}$) subtypes using flow cytometry technique. The expression of HLA-DR in intermediate monocytes from subjects with moderate to severe fibrosis was higher [median (min-max) of MFI= 1265 (186-1666)] compared to individuals without fibrosis [MFI= 208 (79.2-2756; $p<0.05$)]. The expression of TGF-β in the three monocytes subsets was higher in individuals with moderate to severe fibrosis [Classical: 55.5 (24.1-81.8), Intermediate: 64.7 (22.4-115), Nonclassical: 28.5 (13.7-44.2)] compared to individuals without fibrosis [Classical: 26.2 (8.9-46.2), Intermediate: 25.6 (12.2-52.8), Nonclassical: 13.4 (7.6-27.7)]. Similarly, the expression of TNF was also higher in all three subsets of monocytes in individuals with moderate to severe fibrosis [Classical: 244 (47.4-324), Intermediate: 368 (73.1-413), Nonclassical: 85.6 (24.3-123)] compared to individuals without fibrosis [Classical: 20.5 (4.1-108), Intermediate: 48.7 (14.7-125), Nonclassical: 25.6 (8-112)]. The expression of IL-12, however, was higher in the classical and nonclassical monocytes of individuals without fibrosis [416 (57.2-716); 422 (28.4-624), respectively] compared to individuals with moderate to severe fibrosis [117.5 (81-584); 84.8 (37.5-550), respectively].

Conclusion: The monocytes from individuals with moderate to severe fibrosis are more activated and express higher levels of proinflammatory and profibrotic cytokines than individuals without fibrosis. Moreover, they express low levels of anti-fibrotic cytokines, suggesting they participate in the immunopathogenesis of periportal fibrosis in human schistosomiasis.

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MONONUCLEAR CELLS GENE EXPRESSION PROFILING SHOW CHARACTERISTIC INFLAMMATORY RESPONSE IN SEPTIC PATIENTS AND DISTINGUISHES PATIENTS OUTCOMES BASED ON OXIDATIVE STRESS REGULATION

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Introduction

Sepsis is defined as a systemic inflammatory response secondary to a proven or suspected infection. Mechanisms governing this inflammatory response have been shown to be complex and dynamic, involving cross-talking among diverse signaling pathways. However, current knowledge on mechanisms underlying sepsis is far from providing a complete picture of the syndrome, justifying additional efforts that might add to this scenario. Microarray-based expression profiling is a powerful approach for the investigation of complex clinical conditions such as sepsis: the analysis of gene transcription at the genome level potentially avoids results derived from biased assumptions.

Methods and Results

In this study we investigate whole-genome gene expression profiles of mononuclear cells from survivor and non-survivor septic patients. Blood samples were collected at the time of sepsis diagnosis and seven days later, allowing us to evaluate the role of biological processes or genes possibly involved in patient recovery. Aiming to circumvent, at least partially, the heterogeneity of septic patients we included only patients admitted with sepsis caused by community-acquired pneumonia. Global gene expression profiling allowed us to characterize early sepsis, as compared to healthy individuals. Our results corroborate literature reports on inflammation response in the early stages of sepsis but highlight great heterogeneity in gene expression during sepsis progress. Additionally, global gene expression in the early stage was also able to distinguish severe sepsis from septic shock and correlated with patient outcome. Differences in oxidative stress seem to be associated with clinical outcome, since significant differences in the expression profile of related genes were observed between survivors and non-survivors at the time of patient enrollment.

Conclusions

Our results substantiate current knowledge supporting that sepsis syndrome development is indeed multifaceted. Although the initial infection of enrolled patients was pneumonia, seven days later gene expression profiles seemed to be characteristic of each patient, common gene expression changes distinguishing survivors from non-survivors. This result could be associated with the underlying health status of each one of them, with complications due to sepsis itself as well as with distinct timing for response to treatment.

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MYCOBACTERIUM BOVIS BCG TRIGGERS M-TOR PATHWAYS THROUGH TLR2-DEPENDENT MECHANISM, INCREASING PPARΓ EXPRESSION/ACTIVATION

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Introduction and Aim: PPARγ is a member of the lipid-activated nuclear receptor superfamily and plays a recognized role in the transcriptional regulation of cellular proliferation, differentiation and inflammation, in addition to metabolic regulation of lipids and glucose. Recent studies indicate a remarkable action of this receptor with infection by intracellular pathogens, contributing to the escape mechanism of the parasites on host responses. Our group recently demonstrated that the host response to Mycobacterium bovis BCG infection is related to the regulation of PPARγ expression through TLR-2-dependent mechanisms, resulting in the modulation of host cell lipid metabolism and immune responses. In this present study, we focus on elucidate the cell signaling pathways involved in the PPARγ activation induced by M. bovis BCG.

Methods and Results: HEK 293T cells were transfected with different constructs containing TLRs, co-receptors and PPARγ genes, and they were infected with M. bovis BCG. The Mycobacterium was able to induce an expressive NF-κB activation and IL-8 production in TLR2-transfected cells, by luciferase and ELISA assays, respectively. Furthermore, by the western blot analysis, only in cells transfected with TLR-2 plasmid was observed the increase of PPARγ expression. Interestingly, the bacteria induce FABP4 production, a target gene of PPARγ, suggesting that this nuclear receptor has not only been expressed, but it has been activated. Moreover, BCG activated the mTOR pathway in TLR2-transfected cells, inducing the phosphorylation of mTOR, S6 Kinase and 4E-BP1.

Conclusions: The above set of results shows that the Mycobacterium bovis BCG can play a key role on manipulating the intracellular signaling system of host, stimulating the PPARγ expression through TLR2-dependent mechanisms. Thus, a better understanding of cell signaling pathways triggered by this pathogen, it may represent a potential therapeutic target.

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MYD88-DEFICIENT MICE DEVELOP HIGHER CD8+ T CELL INFILTRATE AND MORE SEVERE MYOCARDITIS THAN WT CONTROLS DURING THE ACUTE PHASE OF INFECTION WITH TRYPANOSOMA CRUZI.

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Introduction: It is known that MyD88-deficient mice are particularly susceptible to infection with T. cruzi. However, the pathogenesis of myocarditis during T. cruzi infection in this strain of mice was not evaluated until now. Here we investigated CD4+ and CD8+ T cell migration to the heart during the acute phase of infection of T. cruzi in MyD88-KO and B6 (WT) mice. We also compared electrocardiographic (ECG) measurements, the levels of CK-MB enzyme in the sera and parasite load in the cardiac tissue between these strains.

Methods and results: Groups of 6-8-week-old males of the C57BL/6 (WT) and MyD88-KO strains were infected i.p. with 2x10e3 blood stage trypomastigote of the Y strain of T. cruzi. At day 14 p.i. ECG were performed, sera collected and spleens and hearts were removed for flow cytometry analysis, as well as quantification of the parasite load by q-PCR, as previously described. All procedures were approved by the UFRJ Animal Care and Use Committee (CEUA-CCS). We found that effector CD4+ T cells are diminished in the heart of MyD88-KO, compared to WT mice, reflecting their lower numbers in MyD88-KO spleens. On the other hand, effector CD8+ T cells are significantly augmented in the MyD88-deficient myocardium, although equal numbers of these cells are found in the spleens of WT and KO mice. Parasitic load and CK-MB levels are significantly increased in MyD88-KO infected mice and correlate to ECG alterations observed in MyD88-KO infected mice. Data are representative of three independent experiments and considered statistically significant when $p<0,05$.

Conclusion: These findings define a key role for MyD88 signaling in controlling heart tissue parasitism. More over, higher parasite load in MyD88-KO mice results in extended host tissue damage possibly due to the effector CD8+ T cell infiltration, wich is increased in the heart of these mice and might lead to the observed more severe myocarditis.

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NANOSTRUCTURED IMMUNOSENSOR FOR LEISHMANIA INFANTUM

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Introduction: Visceral leishmaniasis (VL) is among the main neglected diseases around the World, with an estimated incidence of 500,000 cases per year and endemic in 65 countries. A chronic, debilitating and ocasionally fatal disease, caused by protozoa of the genus Leishmania, with an anthroponotic species (L. donovani) occurring in Asia and Africa and a zoonotic species (L. infantum) occurring in the Mediterranean region and South America, where it was previously called L. chagasi. The techniques employed for the detection of Leishmania infantum in biological amstras are invasive, time consuming, and have problems regarding sensitivity and specificity. The detection of specific antibodies to Leishmania sp and effective diagnostics of VL is of fundamental importance to the efficient and appropriate treatment of the disease and quality of life of the patient, becoming a challenge for the scientific community. **Material and Methods:** Exploring the properties of nanomaterials, an immunosensor sensitive, rapid and selective was developed for diagnosis of VL. Nafion polymer was used to modify the electrode surface of quartz-crystal, acting as a platform for linked-gold nanoparticles on gold surface of the piezoelectric crystal. The L. infantum (rLci2B-NH6) recombinant antigen was previously immobilized on self-assembled monolayers (SAM) on the surface of gold nanoparticles, and then added to the nafion film. **Results:** The immunosensor was able to detect anti-L infantum antibodies at different dilutions of the serum sample, showing good linearity r = -0.98899 (p <0.0001n = 4), with a low relative error = 5 %, **Conclusions:** The results obtained indicate that it maybe a promising alternative tool for the diagnosis of VL, being able to distinguish positive and negative canine serum to L. infantum.

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NATURAL KILLER CELLS IN EXPOSED UNINFECTED SUBJECTS EXPRESS A HIGH FREQUENCY OF NK CELLS ACTIVATION / INHIBITION RECEPTORS

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Introduction. Natural killer cells (NK) are important effectors in the early response to infection and there is increasing recognition of their role as regulators of the adaptative imune response in addtion to the containment of infection through cytokine production and the killing of infected cells. NK cells is regulated by complex interations of germ-line enconded activating and inhibition receptors. Although the role of NK cells receptors in HIV-1 is not clear, it is possible that they participate in mechanisms of resistence to HIV-1 exposed uninfected (EU) subjects. **Aim**. To evaluate the frequency of activation and inhibition NK cell receptors in EU subjects. **Methods**. EU subjects and HIV-1-infected (HIV) partner (n=15/each) were from Instituto de Infectologia Emilio Ribas of São Paulo-Brazil. Blood samples were collected in EDTA tubes, next at immunophenotyping of the NK cells subtypes were accessed by flow cytometry. **Results**. Were observed increased in $CD56^{bright}$ cells expressing 4 markers (CD94, NKG2A, NKG2C, NKG2D) in EU subjects when compared to HIV or healthy controls (HC) groups. In $CD56^{dim}$ cells this increase was higher in HIV subjects. **Conclusion**. These data suggest that NK cells subtypes have different expression profiles of activation and inhibition receptors, and they may represent an important mechanism of resistance for HIV-1 infection.

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NATURALLY ACQUIRED IMMUNE RESPONSE AGAINST RECOMBINANT CHIMERAS OF PLASMODIUM VIVAX MSP-1 AND RBP-1 IN INDIVIDUALS FROM BRAZILIAN AMAZON.

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Introduction: Malaria is one of the most prevalent parasitic diseases in the world. In Brazil, Plasmodium vivax account for more than 80% of cases. The search for vaccines against P. vivax is poorly explored and the numbers of proteins antigenically relevant are restricted. However, the production of recombinant chimeric proteins expressing different epitopes of antigenic targets of parasite became a recent approach in P. vivax vaccine research. Thus, in the present work we evaluated the reactivity of IgG antibodies and the profile of IgG subclasses from individuals naturally exposed to malaria against two chimeric proteins representing different epitopes of P.vivax. **Methods and Results:** Two proteins were expressed: CH27, containing the C-terminal region of Merozoite Surface Protein 1 (PvMSP-1.19) and five T-Cell promiscuous epitopes; and CH28, containing the Reticulocyte Binding Protein 1 ($PvRBP\text{-}1_{431\ -\ 748}$) and three T-Cell epitopes. Plasma samples of 263 naturally exposed individuals were screened by ELISA to detect IgG antibodies and IgG subclass against CH27 and CH28. Firstly, we observed that both the CH27 and CH28 are naturally immunogenic, being recognized by 49,4% and 47,1% of studied population respectively. The frequencies and RIs were similar for the both chimeras (P=0,78). The reactivity index (RI) of IgG antibodies ranged from 0,07 to 12,3 in response against CH27 and 0,21 to 7,30 against CH28. However, the RIs against CH28 were associated with time of exposure (p<0,0001) and number of past malaria infections (p<0,05). Whereas, the response against CH27 was inversely correlated with the months since the last infection (p<0, 0001) and directly associated with the number of malaria episodes during the collection year (p<0,05), indicating a association between high antibody levels and a recent infection. Finally, the frequency response IgG subclasses for these proteins showed a predominance of IgG1 for CH27 and CH28, with 90% and 73.3% respectively, while IgG2, IgG3 and IgG4 reactivity was low. **Conclusion**: Thus, our data suggest that CH27 and CH28 appears to have retained their structural identity in the context of specific immune response and are also naturally immunogenic in populations living in endemic areas. Moreover, the predominance of cytophilic IgG1 response and the cumulative IgG response could also suggest a potential role in protective immunity, however further studies are necessary to confirm these chimeras as a vaccine candidates.

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NEW STRAIN OF PROTEUS SP. POTENTIATES LTC4 EXPRESSION IN LUNG INFLAMMATORY RESPONSE INDUCED BY LPS.

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Introduction: P. mirabilis is a gram negative bacillus belonging to the family Enterobacteriaceae, described as an etiologic agent in various infections. **Objectives**: Evaluate the pulmonary inflammatory response in mice infected with different strains of Proteus sp.

Methods and results: Groups of 6-8 male C57Bl/6 mice (20-25g) were infected with a suspension containing 10^{-1} CFU of P. mirabilis ATCC 25933, NCDC 2059-70 (40 µl, i.n.) or with 10^{-1} CFU of Proteus sp. (40 µl, i.n.) isolated from the lungs of mice in routine screening of the Control Laboratory of the Health Central Animal ICB/USP (CAM strain). Twenty-four hours after infection, mice were given LPS (E. coli – Sigma, i.n.). After a further period of 24 hours, mice were euthanized with overdose of anesthetic (150 mg/kg) and the bronchoalveolar lavage fluid was collected to evaluate total and differential cellular infiltration in lung. The administration of LPS increased 48x the total cells in bronchoalveolar lavage fluid, when compared to control mice. Similar result was obtained in mice infected with ATCC (50x). The influx of inflammatory cells into bronchoalveolar lavage fluid was higher in mice infected with ATCC (60%) plus LPS than the group infected with only ATCC. The CAM strain induced significant cell infiltration (89x), in bronchoalveolar lavage fluid, when compared to animal control; the administration of LPS in mice infected with the CAM strain increased cellular infiltration in 101%, when compared to mice that were infected with CAM strain. Histological analysis of cell infiltrate in lung tissue showed that both infection with ATCC (168%) and CAM strain (225%) induced increased cell influx into the lung, when compared to control group. After LPS administration, mice infected with the CAM strain showed increased lung inflammatory infiltrate (25%) when compared with the ATCC strain. The administration of LPS increased 94% PGE2 production when compared to control group and this increase has not been altered in any of the experimental groups. LTB4 production was no different between the groups, however levels of LTC4 were increased (405%) only in groups infected with the CAM strain.

Discussion/Conclusion: Administration of LPS induced a higher lung inflammation response in mice infected with P. mirabilis CAM strain than in mice infected with P. mirabilis ATCC strain and this phenomenon is modulate by leukotriene expression.

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NOD2/RIP2 SIGNALING INDUCES TH1 IMMUNE RESPONSE AND HELPS TO CONTROL LEISHMANIA INFANTUM INFECTION

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During visceral leishmaniasis the Th1 profile is classically associated with protection. Recent data also demonstrated a positive correlation between Th17-related cytokines and resistance to VL. The activation of innate immunity pattern recognition receptors is essential for the induction of adaptative immune response through the antigen-presenting cells (APC) activation and soluble mediators release (i.e. cytokines, chemokines and lipid mediators). Thus, we aimed to determine the role of NOD1/NOD2/RIP2 signaling in the induction of adaptative immune response against Leishmania infantum. Our data demonstrate that L. infantum-infected macrophages up regulate the nod1 (2 times) and nod2 (1.7 times) mRNA expression. Besides, the absence of Nod2 and Rip2 on the infected macrophages or dendritic cells reflects on inhibition of the total functional expression of MHC class II, CD40, TH17-polarizing cytokines (TGF-β, IL-23, IL-1β, IL-6), Th1-polarizing cytokine (IL-12) and also inos expression (p

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OPPORTUNISTIC INFECTION IN CYSTIC FIBROSIS: ROLE OF MACROPHAGES ON INTERACTION WITH BURKHOLDERIA CEPACIA

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Introduction: Cystic fibrosis is an inherited disease characterized by extensive dysfunction of exocrine glands, resulting in a wide range of clinical manifestations and complications. On the airways, this condition allows continuous infections by opportunistic microorganisms, among which the multiresistant bacteria Burkholderia cepacia (BC). In fact, little is known about the mechanisms that govern the interaction between the bacteria and the host cells. Our group has evaluated the role of macrophages in response to infection by BC, showing the response of RAW 264.7 peritoneal cells after interaction with the microorganism. However, this strain does not characterize the cell type involved in the process of airway infection. So, in this study we used AMJ2-C11 cells, a strain of alveolar macrophages. **Methods and Results:** All experiments were performed at least three times and the results are presented as the mean and standard deviations. ELISA tests were used to assess the cytokine production. The colorimetric method using Griess reagent was utilizing to indirectly evaluate the production of nitric oxide (NO). When both alveolar and peritoneal macrophages cultures were infected with BC at multiplicity of infection (MOI) of 1:1, significant production of TNF was found in the supernatants (1053±41pg/mL and 2236±25pg/mL, respectively) compared with uninfected controls (non-detectable levels). However, while positive control IFN-g/LPS induces the production of NO by both lineages (approximately 55±5uM by alveolar and 66±3uM by peritoneal), infected cultures did not release NO on supernatants, even when used MOI of 10:1, 1:1 or 1:10. Interestingly, BC-infected cultures express iNOS identically to cultures treated with IFN-g/LPS. Furthermore, peritoneal macrophages treated with heat-inactivated bacteria produced NO similarly to those IFN-g/LPS-treated cultures (104±1uM). **Conclusion:** Taken together, our results demonstrate that alveolar macrophages AMJ2-C11 respond similarly to peritoneal macrophage RAW264.7 after infection by BC and this microorganism is able to modulate NO production in both strains.

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OUTSTANDING INFLAMMATORY RECRUITMENT MEDIATED BY NOD2 SIGNALING IS DELETERIOUS TO HOST DURING N. CANINUM INFECTION

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Neospora caninum is an intracellular parasite which infects a wide range of warm-blooded hosts worldwide. The innate immune response against this parasite is crucial to the host resistance, however it is unclear. It was previously demonstrated that MyD88$^{-/-}$ and TLR2$^{-/-}$ mice rapidly succumb to the N. caninum infection, but IFN- and IL-12 production was not abolished, indicating that additional PRRs are required to parasite recognition. NLRs are intracellular receptors identified as sensors of PAMPs and efficient inductors of inflammatory response against several intracellular pathogens. Firstly, we found that N. caninum is able to induce NOD2 expression on macrophages, which encouraged us to evaluate the role of Nod2 in the host response against N. caninum infection. For that purpose, Nod2$^{-/-}$ and WT mice were infected with N. caninum tachyzoites, and acute phase parasitism, inflammatory cell migration and cytokine production in target organs (pancreas, lungs, and liver) of these mice were assessed. We observed that Nod2$^{-/-}$ mice exhibited higher parasite burden in all organs compared to WT mice. Furthermore, inflammatory cellular migration was impaired in all organs and peritoneal exudates of Nod2$^{-/-}$mice, as compared to WT. Additionally, we verified that macrophages from Nod2$^{-/-}$ mice induced lower proinflammatory cytokine production and higher IL-10 production. This lower proinflammatory production in Nod2$^{-/-}$ mice was related to the impaired p38 and ERK phosphorylation. Strikingly, Nod2$^{-/-}$ mice were more resistant than WT mice. Although Nod2 receptor has been able to control the parasite growth by inducing an outstanding inflammatory recruitment to the organs infected by N. caninum, we proposed that this inflammatory milieu may contribute to the pathogenesis and mortality during this infection. Financial support: CNPq, FAPESP, CAPES

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OXLDL CO-LOCALIZES WITH LIPOPROTEIN FROM MYCOPLASMAS IN ELECTRON LUCENT MICROPARTICLES IN VULNERABLE PLAQUES

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Microparticles seem to contribute to vessel inflammation and coagulation, participating in the pathogenesis of plaque vulnerability. Atherosclerosis is related with inflammation by oxidized LDL (oxLDL). Previously we found large numbers of Mycoplasma pneumoniae (Mp) antigens and Electron Lucent microparticles (EL) in vulnerable plaques (VP). As mycoplasmal lipoprotein is known to cause exacerbated inflammation, here we search for a relationship between EL microparticles, oxLDL and Mp lipoproteins and VP.

Methods: By using immunohistochemistry at electron microscopy we counted the mean numbers of EL, oxLDL and Mp antigens inside and outside EL, in three groups of coronary arteries: VP (n= 13, obtained from atherotomy), stable plaques (SP) (n= 7, from ischemic heart disease receptors) and normal arteries (NA) (n= 7, from dilated cardiomyopathy receptors). Double colloidal immunogold particles (5nm for anti-oxLDL and 15nm for anti-Mp) allowed the simultaneous localization of both antigens, and counting the mean number of positive dots/ μm^2 in 7 photos 50.000x of each case.

Results: There was a significant higher amount EL microparticles in VP than in NA group. In VP, there was higher amount of positive dots for both oxLDL and Mp antigens inside EL than in the other two groups. Double immunoelectron microscopy showed that Mp and oxLDL antigens intra EL co-localized on lipidic particles, the quantity of both antigens were highly correlated (R= 0.99, p<0.001). A larger amount of oxLDL and Mp antigens extra EL were seen in VP.

Mean numbers/μm^2 of Electron lucent microparticles (EL), Mycoplasma pneumoniae (Mp) antigens and oxLDL in and outside EL, in vulnerable (VP) and stable plaques (SP) and normal arteries (NA).

	EL	Mp in EL	Mp out EL	LDL in EL	LDL out EL
VP	2.0 (1.1)	0.8 (0.7)	3.2 (3.1)	1.8 (1.4)	7 (3.7)
SP	0.8 (0.6)	0.0 (0.0)	0.2 (0.2)	0.3 (0.3)	4.3 (4.6)
NA	0.7 (0.5)	0.2 (0.4)	0.9 (1.0)	0.2 (0.2)	3.2 (2.1)
TT VPxSP	0.02	0.008	0.02	0.01	0.19
TT VPxNA	0.009	0.06	0.08	0.008	0.03
	0.64	0.17	0.1	0.39	0.57

Conclusion: Vulnerable plaques are rich in EL, which contains lipidic particles positive for both oxLDL and Mp antigens, which favors a role for EL microparticles to oxidize Mp lipoproteins, leading to aggravation of vascular inflammation and development of VP. These oxLDL linked to Mp are possibly released to the extracellular medium.

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PARTICIPATION OF TLR-2 AND TLR-6 IN THE SKIN FIBROBLASTS IMMUNE RESPONSE DURING EXPERIMENTAL INFECTION BY LEISHMANIA (LEISHMANIA) AMAZONENSIS

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Introduction: The initial moments of infection by protozoa of the genus Leishmania are crucial to the evolution of the disease and involve the host and parasite factors, such surface molecules, parasite species and genetic background. Several studies have shown the involvement of TLRs in response to Leishmania infection. We have demonstrated that the absence of TLR-2 induce a lower susceptibility to infection by L. (L.) amazonensis, which controlling the parasite load and cellular profile alterations of the inflammatory infiltrate at the site of infection, however, the immune response developed during infection is not known. To identify the profile of response and participation of resident cells in modulation and recruitment of inflammatory cells in the absence of TLR-2, we evaluated the production of inflammatory mediators by skin fibroblasts (SF) in the early stages of infection.

Methods and Results: TLR-2 and TLR-6 deficient mice were inoculated in the ear with 10^5 L. (L.) amazonensis promastigotes. After 1, 7, 15 and 30 days of infection, the analysis the cellular profile was performed by light and electron microscopy and the production of inflammatory mediators evaluated by flow cytometry. Our results showed that the absence of TLR-2 induced a distinct cellular response, effective in reducing the parasite load and infection control. On the other hand, the absence of TLR-6 did not affect the infection, demonstrating that this receptor is not directly involved in infection by L. (L.) amazonensis. Furthermore, it was observed that the SF on site of inoculation are capable of responding to infection by producing cytokines such as IL-4, IL-10, TGF-b, IL-12 and IFN - g, contributing to the initial response to infection. In TLR-2 deficient mice were found significant increase in production of IL-4 and IFN-g by SF on the first day of infection different from that observed in WT mice, which showed high production of IL-4 only after 30 days of infection. These results may suggest the likely participation of fibroblasts in the intense recruitment of eosinophils in the initial moments of infection in the absence of TLR-2.

Conclusion: Modulation of the TLR-2 may be a crucial factor for the development of a more efficient immune response in controlling infection by L. (L.) amazonensis, and a pathway in the search for alternatives in the development of new therapies for the treatment of leishmaniasis.

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PATTERN OF IMMUNE RESPONSE IN ORAL/INTRAGASTRIC TRYPANOSOMA CRUZI INFECTION.

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Introduction: Nowadays Trypanosoma cruzi oral transmission through ingestion of contamined food promotes several outbreaks of Chagas' disease in Brazil and other Latin America countries (Int J Parasitol 39:615-23, 2009; Clin Infect Dis 54(6):845-52, 2012). Few reports address carditis and immune response in this model of infection. The aim of this study is to evaluate parasitologic, target organs and immunologic features following T. cruzi oral/intragastric infection. **Methods and Results:** BALB/c mice were infected with $5x10^4$ trypomastigotes (Tulahuén strain) through intraperitoneal (IP), intragastric (IG) or oral (IO) route. IP presented higher parasitemia and mortality (n=20) than IG and IO (0%, 45% and 20% of living mice, respectively, after 31 dpi). Interestingly, IO group showed higher mortality than IG. Histopathological analysis of the heart (n=2-5) presented microinfiltrates that increased in size through time (9, 12 and 15 dpi), and amastigotes nests were detected in IP, IG and IO. Moreover, the liver (n=3-6) has shown mild lesions in both IG and IO groups, and moderate/severe in lesions in the IP group after 9 dpi. By 25 dpi, IG and IO also presented moderate/severe lesions. Multiplex analysis of cytokines (n=2-6) revealed that IP group presented higher type 1 cytokines (IFN-γ, 2328.2pg/mL±355.5; TNF-α, 118.2pg/mL±42.8) serum levels than IG/IO (IFN-γ, 328.2pg/mL±75.4/194.7pg/mL±23.8; TNF-α, 10.4pg/mL±3.9/26.1pg/mL±3.3). Interestingly, in IG and IO groups, IL-17 production (4.3pg/mL ±0.9/12.2pg/mL±8.8) was higher than IP (0.8pg/mL±0.5). Next, we analyzed secondary lymphoid tissues (uninfected: n=9-11; IP: n=3-8; IG: n=3-8). IP group presented higher hyperplasia in the subcutaneous lymph nodes (uninfected=$19.5x10^6$±2.9 cells; IP=$57.6x10^6$±9.1 cells; IG=$31.8x10^6$±3.2 cells) with increased $CD8^+/CD19^+$ population, similarly to the spleen (uninfected=$239.1x10^6$±49.7 cells; IP=$776.3x10^6$±180.9 cells; IG=$307.5x10^6$±80.3 cells) with increased $CD4^+/CD8^+/CD19^+$ population earlier than IG. Mesenteric lymph nodes and Peyer patches (PP) analysis demonstrated that both tissues showed hypoplasia and depleted $CD19^+$ cells (PP:uninfected=$1.5x10^6$±0.4 cells; IP=0.5 x 10^6±0.2 cells; IG=$0.8x10^6$±0.1 cells). **Conclusion:** Our results suggest that IG/IO infection affects parasitemia, mortality and commitment of target organs, triggering alterations in serum cytokines levels and secondary lymphoid organs dynamics when compared with IP.

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PERIPHERAL BLOOD MONOCYTE SUBSETS IN PARACOCCIDIOIDES BRASILIENSIS INFECTED MICE

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Introduction: Paracoccidioidomycosis (PCM) is a systemic mycosis caused by fungi of the Paracoccidioides brasiliensis complex. The control of PCM depends of an effective cellular immune response of the host. Therefore, it is possible that the distribution of different subsets of monocyte is changed in PCM. The monocytes comprise a heterogeneous population of immune cells with different functions. At least two subsets of these cells have been described according to the Ly6C (Gr1) cell surface marker expression. The $Ly6C^{high}$ monocytes are well known cells that act during the early inflammatory response; the $Ly6C^{neg}$ monocytes, although functionally less known, act in the blood vessel patrolling and in the tissue homeostasis. The present study was aimed to determine the distribution of peripheral blood monocytes subsets in a murine model of pulmonary PCM.

Methods and Results: BALB/c mice, male, 8-12 week-old, were infected with 10^6 P. brasiliensis yeasts by the intratraqueal route and evaluated after 4 and 8 weeks post-infection (pi). Naive mice inoculated with sterile saline solution were used as control group (CG). The animals were submitted to histological evaluations and recovery of viable fungi from of spleen, liver, lungs and kidneys. Peripheral blood samples were collected into EDTA tubes and were immunolabelled using antibodies anti-CD45, -CD115 and -Ly6C. At 4^{th} weeks pi, P. brasiliensis-inoculated mice showed intense multifocal pulmonary granulomatous lesions constituted of viable and non-viable fungi, epithelioid cells and giant cells; mild disposition of collagen fibers were observed surrounding the granulomas. At 8^{th} weeks pi, the area was increased and the granulomas were larger than the 4^{th} weeks ones and an intense collagen deposition were observed in the periphery of the granulomas. Spleen, liver and kidneys showed no inflammatory lesions or fungal cells. Infected mice revealed an increased percentage of $Ly6C^{neg}$ monocytes in the peripheral blood.

Conclusion: The pulmonary infection by P. brasiliensis induced high percentage of $Ly6C^{neg}$ monocytes. Considering that its corresponding human monocyte subtype, $CD14^+CD16^+$, is elevated in patients with hepatic fibrosis, our results suggest this subset is involved in the progress of the disease and in the pulmonary fibrosis induction.

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PHAGOCYTOSIS OF LEISHMANIA AMAZONENSIS BY MONONUCLEAR PHAGOCYTES DERIVED FROM B-1 LYMPHOCYTES

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Leishmaniasis is caused by Leishmania parasites which mainly infect macrophages. The promastigote stage of Leishmania is internalized by phagocytic cells and then transformed into amastigotes which characterize intracellular stage where the parasite can proliferate. B-1 cells are a subpopulation of B cells resident in the peritoneal and pleural cavities in mice. These cells are able to differentiate in vitro into mononuclear phagocyte-like cells with phagocytic properties. B-1 cells phagocyte by activity of several receptors such as mannose receptor (MR), Fc gamma receptors (FcγRs) and third complement receptor (CR3). Leishmania binds to the same receptors in macrophage cells. Nevertheless, the role of B-1 cells in the phagocytosis of Leishmania has not yet been clarified. This study aimed to investigate the phagocytosis of L. amazonensis by mononuclear phagocytes derived from B-1 lymphocytes in vitro and in vivo. In this report we demonstrated that phagocytes derived from B-1 cells were able to internalize in vitro promastigotes of L. amazonensis. The internalized promastigotes became amastigotes. Our results showed that phagocytic index was higher in phagocytes derived from B-1 (3209±363), compared to peritoneal macrophages (2065±350) and bone-marrow-derived macrophages (979.4±81.1); ($p < 0.001$). Results also showed that phagocytic index was higher in phagocytes derived from B-1 after 24 hours of infection (3896±202), compared to 8 hours (601.0±60.2) and 16 hours (3209±363); ($p < 0.001$). Besides, L. amazonensis stimulated TNF-alpha production by B-1 cells. TNF-alpha amounts were higher in B-1 cells that phagocytosed L. amazonensis, as compared with B-1 control (cells non-stimulated with parasites). The in vivo phagocytic ability of B-1 cells was also evaluated. Flow cytometry analyses were performed to evaluate the peritoneal B-1 cells-L. amazonensis interaction. Parasites were marked with stable intra-cytoplasmic fluorocrome, 5,6-carboxyfluorescein diacetate succinimidyl ester (CFSE) and subsequently inoculated in peritoneal cavities of mice. After 8, 16 or 24 h peritoneal cells were collected and marked with anti-CD19 and anti-CD23. We found out that many CD19+ CD23- cells were positive for FITC, which we attributed to phagocytosis of the parasite by B-1 cells. This is the first report that B-1 cells can internalizeLeishmania species.

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PHENOTYPIC AND FUNCTIONAL ANALYSIS OF CD4+FOXP3+ REGULATORY T CELLS (TREG) IN THE EARLY PHASE OF MURINE INFECTION WITH TRYPANOSOMA CRUZI

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Introduction: We previously observed that in T. cruzi-infected mice (Y strain) depletion of T_{REGs} by anti-CD25 mAb treatment does not affect acute parasitemia and heart pathology, suggesting that T_{REGs} do not regulate the early anti-T. cruzi response. Using $FoxP3^{+}GFP^{+}$ mice, we now studied the phenotypic changes in spleen T_{REGs} along the early infection with Sylvio X10/4 T. cruzi parasites and evaluated their suppressive activity.

Methods and Results: Peak spleen cellularity and $CD4^{+}$ cell number occurred on the 11th day post-infection (d.p.i.). No major changes in T_{REG} number were observed from the 4-18 d.p.i., despite an increase in large T_{REG} cells. Regarding expression (MFI) of different markers by T_{REG}, we observed a slight increase in FoxP3 at 7-14 d.p.i., a strong progressive increase in CD25 expression that peaked at 14 d.p.i., the appearance of a small CTLA-4^{HIGH} population, and a late increase in GITR expression. Besides, we observed increases in ICOS in the last days analyzed and increased expression of Fas (11 and 18 d.p.i) and FasL (18 d.p.i.). In addition, CD69 suffered a slight persistent augment, and no changes were observed in CD127 and OX40 expression. Regarding the expression of these markers on the $CD4^{+}FoxP3^{-}$ population, the most relevant changes after infection were a brief small increase in CD25, a persisting increase in Fas with presence of a small Fas^{HIGH} subset, a slight persistent augment in FasL from 11th d.p.i, a persisting increase in ICOS, an increase of OX40 from 7-11 d.p.i., an increase in CTLA-4 on the 7 and 14 d.p.i. and a late augment of GITR. According to their suppressive activity upon the anti-CD3-induced proliferation of $CD4^{+}FoxP3^{-}$ splenocytes and upon IFN-g production by total splenocytes, there were no major differences between T_{REGs} cells from control and 7d-infected mice. Moreover, responding 7d-$CD4^{+}FoxP3^{-}$ splenocytes showed similar susceptibility to suppression by control and 7d-T_{REGs}.

Conclusion: We demonstrate that during the early infection by T. cruzi T_{REGs} maintain their suppressive activity with increase in expression of some markers and responding $CD4^{+}$ cells do not become resistant to suppression.

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PI3KGAMMA IS ESSENTIAL FOR RESISTANCE AGAINST TRYPANOSOMA CRUZI INFECTION: POSSIBLE ROLE IN MACROPHAGES FUNCTION

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INTRODUCTION. The phosphatidylinositol-3-kinases (PI3Ks) are a family of lipid kinases that plays a crucial role in several cellular processes including survival and proliferation. PI3Kgamma is a member activated by G protein coupled receptors (GPCR), involved in the signaling of chemotactic factors and leukocytes migration and activation. In this study, we evaluated the role of PI3Kgamma during experimental infection by the protozoan parasite T. cruzi, a great model of cardiac inflammation. **RESULTS.** We observed that T. cruzi infection causes an increase in the PI3Kgamma expression and activation in the heart mice tissue. Although there is no difference in the blood parasitemia between WT and PI3Kgamma$^{-/-}$ mice, all PI3Kgamma deficient mice died until day 25 after infection, whereas the WT remained alive after 30 days. PI3Kgamma$^{-/-}$ mice also showed greater inflammation, parasitism and lesion in the heart tissue. Interestingly, in the absence of PI3Kgamma, the heart tissue express higher levels of iNOS enzyme after infection compared with WT mice, but the expression of arginase I (ARG1), that also consume the amino acid arginine thus impairing the nitric oxide (NO) production, is also higher compared with WT mice. As consequence, the knockout mice produce less amount of NO in heart tissue. In vitro stimulated macrophages from PI3Kgamma$^{-/-}$ mice with T. cruzi plus IFN-gamma fail to produce NO and to kill the intracellular parasite compared with macrophage from WT mice. Corroborating these data, the addition of ARG1 inhibitor (BEC) to macrophage culture restores the ability of PI3Kgamma$^{-/-}$ macrophages to produce NO. **CONCLUSION.** These results indicate that PI3Kgamma is not involved in the leukocyte migration during T. cruzi heart infection, but it probably helps in the microbicidal mechanisms of macrophage by mediation of NO production, which is important to kill intracellular parasite.

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PLASMA FROM DENGUE-INFECTED PATIENTS INCREASE ENDOTHELIAL CELL PERMEABILITY DEPENDING ON MIF

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FIOCRUZ, RIO DE JANEIRO - RJ - BRASIL.

Introduction: Dengue is considered today the main human arboviral disease in the world, infecting millions of people and causing thousands of deaths annually. Dengue syndrome range from self-limited febrile illness to life-threatening disease accompanied by severe bleeding and shock. Thrombocytopenia and increased vascular permeability are often associated in both, mild and severe dengue syndromes. However, the factors that lead to significant increase in vascular permeability in dengue infection and the mechanisms underlying this process are not fully understood. Thus, this study aimed to investigate the ability of soluble factors in blood plasma from patients at different stages of dengue to induce endothelial activation and consequent permeability increase. Methods and Results: Plasma samples were collected from 35 dengue virus serotype 1-infected patients with mild, mild plus warning signs (WS) or severe dengue syndrome (approved by CEP IPEC##016/2010). Endothelial cells (HMEC-1) monolayers were stimulated with 10% plasma from dengue-infected patients and the permeability evaluated using a Transwell assay at 2h. Cell viability was assessed by MTT assay at the end of each experimental point. We observed that samples from patients with dengue induced increased permeability in endothelial cells monolayers compared to plasma from healthy volunteers (111±72 for control vs 355±198, 561±451 or 763±279 for mild, WS or severe, respectively; $p<0.05$, $n=5$), especially those from severe dengue patients ($p=0.0498$ compared to mild). By investigating the possible plasma cytokines responsible for permeability increase in our model, we measured the levels of TNF-α, IL-6, VEGF and MIF through ELISA. No detectable levels of TNF-α were observed in dengue samples compared to controls. VEGF was increased only in samples from patients with dengue plus warning signs and IL-6 in severe dengue patients($p<0.05$). MIF levels were increased in all dengue patients being higher in patients with severe dengue. To assess the role played by MIF in plasma from dengue patients induced endothelium permeability, we incubated HMEC-1 with plasma in the presence of the MIF antagonist ISO-1. Treatment with ISO-1 significantly reduced the permeability triggered by plasma from patients with dengue. Our results point MIF as an important effector in severe dengue-associated increase in vascular permeability.

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POLYAMINES AND PROSTAGLANDIN E2 BIOSYNTHETIC PATHWAY SUPPRESSES INFLAMMATORY RESPONSE IN DIFFUSE CUTANEOUS LEISHMANIASIS

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Diffuse Cutaneous Leishmaniasis (DCL) is a rare clinical manifestation of tegumentary leishmaniasis caused by Leishmania amazonensis. It is characterized by an inefficient parasite-specific cellular response and heavily parasited macrophages. It has been demonstrated “in vitro” that murine macrophage infection by L. amazonensis increases arginase I, TGF-β and PGE2 contributing to parasite proliferation enhancement. However, the relevance of these mediators for DCL pathogenesis remains unknown. Here, we evaluate systemic and local expression of inflammatory mediators in DCL patients and the role of arginase inhibition in vitro.

Plasma from 12 active DCL and 29 Localized Cutaneous Leishmaniasis (LCL) patients were evaluated for arginase I, TGF-β1, PGE2, TNF-α, IL-12 and MCP-1 levels by ELISA. Whereas Arginase I (11200 pg/ml ± 2388), TGF-β (45573 pg/ml ± 9.303) and PGE2 (1495 pg/ml ± 468) were increased, TNF- α (3.3 pg/ml ± 1.2), IL-12 (24.5 pg/ml ± 2.9) and MCP-1(22 pg/ml ± 9.5) were decreased in DCL plasma when compared with LCL (1458 pg/ml ± 206, Arg I; 5792 pg/ml ± 560, TGF-β; 975 pg/ml ± 60, PGE2; 6.8 pg/ml ± 1.7, TNF- α; 360 pg/ml ± 94, IL-12; 40 pg/ml ± 8.2, MCP-1) supporting an anti-inflammatory immune profile in DCL patients. Moreover, immunohistochemistry analysis confirmed arginase I and cicloxigenase 2 expression were higher in DCL lesions than LCL lesions. Besides, linear regression analysis showed that spermine (r=0.6) and spermidine (r= 0.64) synthase mRNA levels were associated with arginase mRNA levels in DCL and LCL lesions. In order to know if arginase could alter Leishmania infection, human monocyte-derived macrophages from health volunteers were infected with L. amazonensis and treated with NOHA (arginase inhibitor). The infectivity index 72 h post infection in the group treated with NOHA ($6,3 \times 10^3 \pm 2,4X10^3$) was lower than unstimulated (UNST) infected group ($6,8x\ 10^7 \pm 6,6x\ 10^6$). Interestingly, arginase inhibition with NOHA decreased TGF-β (83 pg/ml ± 1.9) and PGE2 (201 pg/ml ± 13) and increased TNF- α (12 pg/ml ± 1.7) and IL-12 (16 pg/ml ± 1.6) production when compared with the UNST group (83 pg/ml ± 1.9, TGF- β; 517 pg/ml ± 26, PGE2; 4 pg/ml ± 0.6, TNF- α; 2.5 pg/ml ± 0.2, IL-12).Our data suggest that arginase, PGE and TGF might be implicated in the inability of DCL patients to mount an efficient immune response against L. amazonensis.

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POTENTIAL IMMUNOMODULATORY AND ANTIVIRAL ACTIVITIES OF UNCARIA TOMENTOSA USING A MODEL OF HUMAN ENDOTHELIAL CELLS CONTINUOUS LINES INFECTED WITH DENGUE VIRUS-2.

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Introduction: Infection with Dengue virus (DENV) in Brazil is characterized as an endemic disease, with periodic epidemics. The Phytotherapy is considered an alternative for the immunomodulation of the immune system through the dynamic regulation of signaling molecules such as cytokines, influencing in their effector functions on cells and humoral components. However, so far, the phytotherapy is not used for the treatment of dengue. The aim of this study was to investigate if a compound derived from a medicinal plant, could modulate the innate immune response associated with immune responses in DENV infection using an in vitro infection model with human strain continuous endothelial cells (HMEC-1). Methods and Results: After target cell infection by DENV-2, cultures were treated with the alkaloid compound originated from Uncaria tomentosa (UTFA) and tested at different concentrations. The rate of cell infection was established after incubation for virus-specific immunofluorescence and flow cytometry (24-48h). Nonstructural viral proteins (NS1) were detected in the cell culture supernatants as were the cytokines IL-8 and MIF, all determined by ELISA assays. Antiviral effects of UTFA were observed as compared to untreated cells in concentrations that reduced the viral NS1detection (ratios = D.O./control D.O.): DENV=1,6±1,5 vs. DENV+1µg/mL UTFA=1,3±1,3; 10µg/mL DENV=1,6=±1,5 vs. DENV+10µg/mL UTFA=1,3±1,3. Immunomodulatory effects were shown by reduced IL-8 secretion (pg/mL): DENV=1210±965 vs. DENV+1µg/mL UTFA=746±502; 10µg/mLDENV=1210±965 vs. DENV+10µg/mLUTFA=728±500 in infected and treated HMEC-1, respectively. Values represented are: average ± standard deviation. Conclusion: These preliminary results show antiviral and immunomodulatory activities of UTFA in endothelial cells, opening new perspectives for studies on the mechanisms of action of this compound studied here and its active constituents.

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PPAR GAMMA ACTIVATION IMPAIRS IFN GAMMA PRODUCTION AND AUGMENT MICE SUSCEPTIBILITY TO TOXOPLASMA GONDII INFECTION

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Introduction: Peroxisome proliferator-activated receptor gamma (PPARγ) is a transcription factor expressed in T cells, macrophages, dendritic cells and epithelial cells. It is a receptor for endogenous lipid molecules and a molecular target for drugs against type 2 diabetes, like Pioglitazone, that activate PPARγ and also can induce a down regulation of inflammatory processes. Toxoplasma gondii infection induces a robust Th1 inflammatory response in C57BL/6 mice, similar to Inflammatory Bowel Disease (IBD) with excessive IFN-γ, TNF and nitric oxide (NO) production. The role of PPARγ in the gut inflammation caused by T. gondii and its possible association with modulation of immune response is still unclear. Then, the aim of this work was to evaluate the role of activation of PPARγ during the T. gondii infection. **Methods and Results:** C57BL/6 mice were orally infected with T. gondii, ME-49 strain. The transcripts of PPARγ were evaluated at 0, 4, 6 and 8 days post-infection (pi) by real time PCR (qPCR) of ileum and liver on mice inoculated with 40 cysts. The expression of PPARγ after infection was also assessed by immunohistochemistry (IHC). One group of animals infected with 5 cysts was treated orally with PPARγ agonist (Pioglitazone, PIO) for evaluation of disease clinical score, food and water intake besides mortality assessment. In addition, spleen cells from naïve mice were infected and/or treated with PIO in vitro for NO, IFNγ and IL-10 assessment in the supernatant. The results showed that there were no differences in PPARγ transcripts in C57BL/6 mice after infection, but IHC reveled an apparent augmented expression of PPARγ by ileum enterocytes on day 8 pi. In vitro infection and treatment with PIO led to PPARγ activation and reduced IFN-γ production after 72h of culture while there were no difference on NO and IL-10 production by T. gondii infected splenocytes. Moreover, in vivo treatment with PIO at 40mg/Kg/day for 6 days pi led to increased clinical score at days 6 and 11 pi together with elevated mortality after T. gondii infection. **Conclusion:** These data indicate that activation of PPARγ might restrain the Th1 inflammatory responses required for T. gondii control and augment susceptibility to the infection, suggesting that this receptor may be essential for gut homeostasis during toxoplasmosis. These results lead us to better understand the modulation of inflammatory process involved on this infection and provide basis for future approaches aimed at controlling exacerbated gut inflammation.

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PRION PROTEIN EXPRESSION IN HTLV-1-INFECTED PATIENTS

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Introduction: Human T lymphotropic virus type 1 (HTLV-1) is an oncogenic retrovirus that causes adult T-cell leukemia/lymphoma and a chronic progressive neurodegenerative disease called tropical spastic paraparesis or HTLV-1-associated myelopathy (HAM/TSP). Although HAM/TSP affects less than 5% of infected patients, the disease has a major impact in patient's life, since it is characterized by paraparesis associated with spasticity, hyperreflexia and Babinski signs in the lower extremities, which can also lead to incapability to walk in extreme cases. Urinary incontinence and sexual dysfunction are also described in HAM/TSP patients. It has been described that the cellular isoform of the prion protein (PrPc) and HIV coexpression decrease virion production. Roberts et al. suggested that PrPc can be used as a biomarker of HIV-associated neurocognitive impairment and neuroinflammation (2010). The aim of this study was to evaluate the PrPc levels in T cells obtained from HTLV-1 infected individuals. Methods and results: Flow cytometry and western blot were used to analyze PrPc expression in T cells from healthy or HTLV-1-infected donors, and lineage cells. We compared the PrPc expression in permanently infected lineage cells MT-2 and C91, $CD4^+$ T lymphocytes of healthy donors and Jurkat cells. Infected cell lineage has significantly lower expression of PrPc (MT-2 71% ± 25,96 (n=6); C91 55%±7,53 (n=3) than uninfected cells ($CD4^+$ 91%±12,94 (n=14); Jurkat 92%±3,58(n=4)). In order to evaluate whether the reduction in PrPc expression is due to HTLV-1 infection, healthy donors' PBMC were incubated with MT-2 cells, and then the PrPc expression was analyzed by flow cytometer. After 24h, no difference was observed in the PrPc expression between control cells and infected lymphocytes. However, the PrPc expression could be altered later. Moreover, the expression of PrPc in T lymphocytes from HTLV-1-infected individuals was evaluated. The PrPc expression in HTLV-1 patients was significantly lower than in T cells from healthy donors. Asymptomatic and HAM/TSP patients presented lower expression of PrPc; however, the percentage of $CD4^+PrPc^+$ cells from HAM/TSP patients (65%) was lower than $CD4^+PrPc^+$ cells from asymptomatic patients (78%). Conclusion: The cellular isoform of prion protein seems to be altered by HTLV-1 infection, but more studies are still necessary to relate this phenotype with HAM/TSP progression.

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PROBIOTICS PREVENT THE BACTERIA TRANSLOCATION AND DECREASES THE ILEITIS THAT DEVELOPS IN ORAL TOXOPLASMA GONDII INFECTION

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Abstract

Introduction: Toxoplasma infection serves as a trigger for inflammatory pathology caused by intestinal bacteria, which interferes in the interaction between microbiota and intestinal mucosal immune system and results in mucosal inflammation. Probiotic treatment may recover the commensal bacteria and normalize the host-microbiota interaction. Oral infection with T. gondii in certain mouse strains induces ileitis which lesions resemble to those of human Crohn's disease. In the present study, C57BL/6 mice were treated with Lactobacillus casei or Lactobacillus acidophilus before and during Toxoplasma gondii infection in order to evaluate immunological parameters and bacteria systemic translocation.

Methods and results: Animals treated with L. casei or L. acidophilus one day before and 8 days after oral infection with 30 ME-49 T. gondii cysts survived longer and presented 12 times reduction in parasite burden in distal jejunum. L. casei-treatment decreased macrophage activity (76.3 ± 1.02 vs 47.6 ± 0.04 $p > 0.05$, n= 4). In addition, none of the treatments were able to prevent Paneth cell loss and do not interfere in IgA^+ cell numbers in the small intestine in T. gondii-infection, however, L. casei avoid the goblet cell loss in the ileum compared to non-treated mice (653.5 ± 77.3 vs. 436 ± 58.5 $p > 0.05$, n=3). Microbiological culture of organs, blood and feces indicated that treatment with L. casei or L. acidophilus prevents bacterial translocation of intestinal lumen to liver, lung and blood. The qPCR analysis revealed that treatment with L. acidophilus decreased IFN-γ and TNF-α mRNA expression in the ileum induced by T. gondii infection, and L.casei increased Foxp3 and IL-10 expression.

Conclusion: The results demonstrated the ability of probiotics to control the inflammatory immune response and reduce mortality caused by ileitis.

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PROMINENT ROLE FOR T CELL-DERIVED TUMOUR NECROSIS FACTOR FOR SUSTAINED CONTROL OF MYCOBACTERIUM TUBERCULOSIS INFECTION

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Introduction: Tumour Necrosis factor (TNF) is critical for host control of M.tuberculosis and is produced by multiple cell types including macrophages, neutrophils, dendritic cells, lymphocytes and NK cells as well as by cells of non-hematopoietic origin. But the precise role of TNF derived from innate and adaptative immune responses during tuberculosis infection remains unclear and requires investigation.

Methods: Myeloid versus T-cell-derived TNF function in tuberculosis was investigated using mice with cell-type-specific TNF deletion.

Results: Mice deficient for TNF expression in myeloid cells displayed early, transient susceptibility to M.tuberculosis. But TNF release by lung infiltrating CD4+ and CD8+ T cells is not compromised by the absence of TNF from myeloid origin and controlled chronic infection. Strikingly, deficient TNF expression in T-cells resulted in early control but in susceptibility and mortality during chronic infection with increased pulmonary pathology. TNF inactivation in both macrophages/neutrophils and T-cells rendered mice critically susceptible to infection and reconstitutes the phenotype of complete TNF deficiency, indicating that myeloid and T-cells are the primary TNF sources collaborating for host control of tuberculosis.

Conclusions: Thus, while TNF from myeloid cells mediates early immune function and a redundant role in controlling mycobacteria replication, T-cell derived TNF is essential for long-term control of chronic infection in a non-redundant fashion during chronic tuberculosis infection.

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PROSTAGLANDIN F2ALPHA PRODUCTION IN LIPID BODIES FROM LEISHMANIA INFANTUM CHAGASI IS A CRITICAL VIRULENCE FACTOR

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Text: Lipid bodies (LB) are cytoplasmic organelles involved in eicosanoid production in leukocytes. Eicosanoids as prostaglandins (PG) have been implicated in the immune response control. Parasites such as Leishmania are also capable of producing PGs, but the role of parasite LBs in biosynthesis of PGs has not yet been investigated.

Methods and Results: In this work, we studied the dynamics of LB formation and PG release from Leishmania infantum chagasi. Using light and electron microscopy techniques, we described here the cellular arrangement and abundance of LBs during development of the protozoan L. i. chagasi. In this regard, a virulent metacyclic state of Leishmania displayed more LBs as well as expressed high levels of PGF2α synthase (PGFS) compared to others developmental stages. Moreover, PGFS was localized in the parasite LBs and the addition of exogenous arachdonic acid to procyclic Leishmania cultures increased parasite LBs formation and PGF2α release. During macrophage infection with L. i. chagasi, LBs were restricted to parasites inside the parasitophorous vacuoles (PV). Notwithstanding, Leishmania infection upregulated COX-2 expression but this was not followed by PGF2α release by macrophages. We detected PGF2α receptor (FP) on the Leishmania PV surface by immunogold electron microscopy and the blockage of this receptor with AL8810, a selective antagonist of FP, dramatically hampered Leishmania infection suggesting that PGF2α should be important to parasite infectivity. In addition, we detected high levels of PGF2α in patients (n=54) with visceral leishmaniasis (VL) compared to endemic controls with negative (n=31) or positive (n=21) DTH reactions, which were reversed after anti-parasite treatment.

Conclusion: Overall these results suggest that PGF2α production by L. i. chagasi is a critical virulence factor. The data demonstrate novel functions for LBs in the eicosanoid metabolism in Leishmania, with possible implications for interactions with the surrounding cell host immune microenvironment.

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PROTECTIVE EFFECT OF ALPHA LIPOIC ACID AGAINST SEPSIS-INDUCED OXIDATIVE STRESS IN RAT BRAIN

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Introduction: Neurological pathophysiological mechanisms of sepsis involving oxidative stress. Alpha lipoic acid (LA), a potent antioxidant is able to cross the blood brain barrier. As important enzyme cofactor and cellular energy metabolism, coupled with its ability to regenerate antioxidant enzymes, allows it to be used in the clinic as adjuvant and some diseases. We aimed to determine the use of AL in oxidative damage and neutrophil infiltration in rat brain 12 and 24 h after induction of sepsis model by cecal ligation and puncture (CLP).

Methods and Results: Male Wistar rats (250-350g) were subjected to CLP model, with sham control. Groups divided into sham + saline, sham + AL, CLP and CLP + AL (200 mg / kg orally with single administration after CLP) n = 10. Twelve and twenty-four hours, were euthanized, removed hippocampus, striatum, cerebellum, cortex and prefrontal cortex, assessed lipid peroxidation by TBARS, damage to proteins by protein carbonylation, myeloperoxidase activity (MPO) and the formation of nitrite and nitrate. Data analyzed by ANOVA with post hoc Tukey test with $P < 0.05$. 12 h compared with the CLP group, CLP + AL group showed a reduction in lipid peroxidation in the striatum in protein carbonylation in the cortex and hippocampus, the MPO activity in the striatum and hippocampus and decreased formation of nitrite and nitrate in the hippocampus and cortex. While no difference was observed in 24 to TBARS however found a decrease in protein carbonylation of damage to the CLP group compared to AL + CLP in the cerebellum, MPO in the striatum, hippocampus and prefrontal and hippocampus, cerebellum, prefrontal and for nitrite / nitrate.

Conclusion: AL may be important neuroprotective therapeutic agent in reducing oxidative stress in an animal model of sepsis.

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PROTEIN ACETYLATION BY ASPIRIN IN E. HISTOLYTICA REDUCES HEPATIC DAMAGE DURING AMEBIC LIVER ABSCESS FORMATION

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INTRODUCTION: During amebic liver abscess (ALA) development, an extensive tissue damage occurs, characterized by acute inflammation; the inflammatory host response is a major contributor to the establishment of hepatic damage during the ALA development (Infect Immun. 70:3208-15, 2002, Am J Pathol. 117:81-91, 1984). The host-parasite relationship is an absolute requirement for the development of tissue lesions, which result from the concerted action of many molecules or signaling pathways from both, the host and the parasite. Lysine acetylation crucially modulates protein function and affects signaling pathways, thereby altering cell fate and function (EMBO J. 19:1176-9, 2000). Therefore the aim of this work was to analyze the effect of proteins acetylation by aspirin, an acetylator agent, in Entamoeba histolytica and the consequences of this protein modification in the inflammatory host response during ALA development. METHODS AND RESULTS: Mesocrisetus auratus (n = 9) were infected with 1.5 x 10^6 aspirin-treated (1 mM) or non treated trophozoites. Twelve hours and seven days post-infection, animals were anesthetized and killed. Livers were removed, inspected for the presence of ALA, weighed and photographed and then treated for histological analysis. In aspirin treated-trophozoites, an increase in acetylated proteins was found, by western blot and confocal microscopy. Among strongly acetylated proteins, one of 42 kDa was confirmed to be actin. Therefore cellular functions depending on the actin cytoskeleton rearrangement, such as amebic movement and capping formation, were analyzed to determine their participation in amebic virulence/invasion. Both amebic movement and capping formation were heavily affected by aspirin. Hepatic damage was significantly reduced (73 %) in animals infected with aspirin-treated parasites in comparison with animals infected with non-treated or indomethacin-treated parasites. These results could be explained because virulence of E. histolytica trophozoites was severely affected by aspirin, thus inducing a less aggressive inflammatory response, and in consequence a dramatic reduction of ALA. CONCLUSIONS: Actin polymerization and function, affected by lysine acetylation induced by aspirin, are crucial elements in amebic virulence, making tropozoites a better target for the immune response . This work was supported by a CONACyT-México grant (No. 104108). LLC is supported by a CONACyT-México fellowship (No. 211715).

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PROTEOME PROFILING OF BIOPSIES FROM CUTANEOUS LEISHMANIASIS PATIENTS INFECTED BY L. BRAZILIENSIS

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Introduction: Proteomic technology has emerged as an important tool to discover biologic disease processes. Expression of proteins in a cell or tissue can be influenced by a variety of environmental stimuli. In the present study we describe changes in proteome profiles of biopsies from cutaneous leishmaniasis (CL) patients infected by L. brazliensis, and explore the contribution of these proteins to the pathogenesis of these diseases. **Methods:** The proteome profiles between lesions from CL patients and normal skin were compared by two-dimensional gel electrophoresis, MALDI mass spectrometry and database searches. Perform systemic analysis of the proteome profiles was also generated to visualize the molecular interaction networks and biological pathways affected by the identified proteins. **Results:** Total protein extracts obtained from each skin sample were separated by 2-DE. After image analysis in average, 1467 spots were detected in CL samples and 1443 in the normal skin samples. A total of 150 differentially expressed spots were excised from 2-DE gels from CL or N. Skin. Fifty-nine proteins were identified. Among them, 14 up or down regulated spots were identified in CL lesions, 27 spots were unique in LC patients and 18 were unique in N. Skin. These proteins were associated with biological regulation, including cell death (apoptosis), cell adhesion, cell cycle and defense response. We also detected an overlap in some biological process between CL and N. skin, showing the involvement of similar proteins. However, in most of the mechanisms affected we observed the expression of different proteins. To explore interactions between the identified proteins and proteins and genes that may be affected by them networks were generated. Five of the 41 proteins identified in CL biopsies were associated with the regulation of apoptosis. Subnetwork was generated to explore the interaction between these proteins with proteins that have an immunomodulatory role in molecular mechanisms. **Conclusion:** The comparative proteome profile data is novel since it has identified differentially expressed proteins between lesions from CL patients and N. Skin, which could contribute to understand the pathogenesis of disease.

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PURINERGIC RECEPTOR P2Y12 FUNCTIONAL ROLES IN HUMAN ISOLATED EOSINOPHILS AND IN THE SCHISTOSOMAL HOST RESPONSE

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Introduction: Identification of new target molecules through which eosinophils activate and secrete their stored proteins may be highly significant for our understanding about the pathophysiology of host immune responses to parasites and allergic inflammation. We have recently reported the expression of the purinergic P2Y12 receptor (P2Y12R) in human eosinophils (JACI 125:477-482, 2010). However its functional role in this cell type and involvement in eosinophilic inflammation are still unknown. In this work we investigated the functional roles of the P2Y12R in isolated human eosinophils and in a murine model of eosinophilic inflammation (Schistosoma mansoni).

Methods: We isolated eosinophils from blood of healthy donors by negative immunomagnetic selection. Eosinophil apoptosis and chemotaxis were evaluated by flow cytometry. Eosinophil cationic protein (ECP) and eosinophil peroxidase (EPO) measurements were assessed by colorimetric assays. In vivo, C57Bl/6mice were infected with 50 cercariae of S. mansoni and treated with a P2Y12R antagonist, clopidogrel (500µg/mL). Histopathological and biochemical analyses were performed in the liver. Blood eosinophilia and eosinophil count in the bone marrow were assessed after blood smears and cytospin analyses, respectively.

Results: Functionally, we found that ADP induced isolated human eosinophils to secrete cationic proteins, being the EPO secretion clearly dependent on the P2Y12R activation. In contrast ADP did not interfere with apoptosis or promoted eosinophil direct chemotaxis. In vivo, the P2Y12R blockage reduced the area of the hepatic granuloma (not treated: 5.47 ± 1.8 versus treated 4.2 ± 1.6 ($x10^4$ μm^2/granuloma), means ± EPM, N=5), promoted a suggestive reduction of the eosinophilic granuloma infiltration, as well as inhibited collagen deposition (not treated: 880 ± 7 versus treated 690 ± 6 (µg hydroxyproline/g liver), means ± EPM, N=5) and IL-13 production (reduction of 22%, N=5) in the liverwithout altering the parasite oviposition. Furthermore, the P2Y12R inhibition promoted blood eosinophilia (2-fold increase, N=5), whereas decreased the eosinophil count in the bone marrow (60% reduction, N=5).

Conclusion: Taken together our results suggest that the P2Y12R has an important role in the eosinophil activation and cationic protein secretion as well as in the establishment of the eosinophilic inflammation.

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REACTIVE OXYGEN SPECIES (ROS) IN TRYPANOSOMA CRUZI INFECTION

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Introduction: Reactive oxygen species (ROS) play microbicidal activity against Plasmodium, Leishmania and Toxoplasma infection. Recently, literature data displayed that mouse deficient in NADPH phagocyte oxidase (phox KO mice) succumbed precocious to infection with high virulent T. cruzi Y strain (Tc-II). Our study aimed to evaluate the role of ROS in experimental infection with T. cruzi Y and CL (Tc-VI) strains, which represent extremes of virulence and pathogenicity, and have different tissue tropisms. **Methods and Results:** Initially, we performed macrophage culture in the presence of trypomastigote forms of the parasite and displayed that T. cruzi induces ROS production in vitro. Subsequently, Swiss mice were infected with 1×10^4 blood trypomastigotes of Y and CL strains and treated with ROS inhibitors (N-acetylcysteine), or inducible nitric oxide synthase (iNOS) inhibitors (aminoguanidine) or both. The parasitemia, mortality, IgG production and cytokines (IL-17, IFN-γ, TNF-α) production were evaluated. Animals infected with the Y strain that had the simultaneous inhibition of ROS and NO showed precocious mortality (14 ° DAI) and higher parasitemia peak, when compared to NO inhibition group (25 ° DAI) was observed 100% of mortality, however the ROS inhibition group had 20% survival. When using the CL strain, no significant differences in parasitemia and mortality in different groups were observed. In attempt to elucidate the mechanisms involved in the ROS participation in resistance to infection we quantified the cytokines and IgG production in sera. Cytokine analysis showed higher IFN-γ production in the Y infected animals treated with ROS and NO inhibitors, when compared to other groups. High IFN-γ production can lead to deleterious effects to the host. Regarding the IL-17, TNF-α and IgG production, there was no difference among the groups of animals that had ROS or NO inhibited. **Conclusion:** The results indicate that reactive oxygen species helps to control experimental infection with T. cruzi.

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RECOMBINANTS ANTIGENS K39 AND K28 IN THE DIAGNOSIS OF CANINE VISCERAL LEISHMANIASIS BY ELISA ASSAY

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Introduction: Visceral Leishmaniasis (VL), also known as kala-azar, is an anthropozoonosis caused by protozoa of the genus Leishmania spp. that infect man and the dog. Serological tests, aiming for circulating antibodies are used as a tool in the diagnosis of clinical cases and in epidemiological surveys, and there is a wide variety of techniques and antigens employed. Among the diagnostic methods, the parasitological examination for direct detection of parasites has high specificity, but the sensitivity is low failing to detect cases where parasitism is low. This study aimed to compare the recombinant antigens K28 and K39, with respect to sensitivity and specificity, by performing indirect ELISA tests using serum from dogs naturally infected by Leishmania spp, confirmed by parasitological examination.

Methods and results: Were collected 44 blood samples by venipuncture from symptomatic dogs coming from CCZ - Center for Zoonosis Control of Araçatuba, São Paulo, Brazil. The dogs were submitted to thin needle aspiration of the popliteal lymph node. The smears were stained by Panoptic fast (Labor Clin ®) and examined under light microscope with 100X objective immersion. A total of 72.1% showed the presence of parasites. The blood was centrifuged to obtain serum for the test. ELISA test using rK28 antigen detected the presence of antibodies in two animals that the antigen rK39 failed to detect.

Conclusion: Preliminary results showed that the sensitivity of ELISA, with rK28 was observed higher than rk39 ELISA, suggesting that rK28 may be more sensitive than rK39.

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REGULATION OF LIPID METABOLISM DURING DENGUE VIRUS INFECTION IS INDEPENDENT OF MACROPHAGE MIGRATION INHIBITORY FACTOR

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1,2,6.FIOCRUZ, RIO DE JANEIRO - RJ - BRASIL; 3,4,5.UFRJ, RIO DE JANEIRO - RJ - BRASIL.

Introduction: Dengue virus (DENV) is responsible for the highest rates of disease and mortality among the members of the Flavivirus genus. We have recently demonstrated a role for lipid droplets (LD) – highly regulated, dynamic and functionally active organelles – in DENV infection with potential implications to viral replication (PLoS Pathog. 5(10):1-14, 2009). Although the molecular mechanisms involved in the physiopathology of DENV infection are still unknown, we have shown that macrophage migration inhibitory factor (MIF), stored in LD accumulated in leukocytes from patients with dengue, participates in the response to DENV infection and its pathogenesis (FASEB J. 24(1):218-28, 2010). Here, we investigate the role of MIF in lipid metabolism during DENV infection. **Methods and Results:** MIF was able to induce LD biogenesis in human monocytes, in similar levels as DENV2, when compared to control cells in vitro (from 5.8 ± 0.8 to 16.8 ± 1.4 LD/cell; n=3). To examine the involvement of MIF in lipid metabolism, we used a selective antagonist of MIF action, ISO-1, or a purified goat IgG against human MIF. After 24 h of infection with DENV2, human hepatoma (HepG2) cellular extracts were used for total RNA extraction for real-time PCR analyses. DENV-2 induced ADRP, LXRα and PPARγ gene transcription, but not FASN, ABCA1, PPARα, LXRβ and ACAT; when compared to mock-treated cells. Treatment with ISO-1 or α-hMIF did not affect the mRNA expression of these genes when compared to DENV2 infected cells. We also used recombinant human MIF-treated HepG2 cells to confirm these results. **Conclusion:** Taken together, our results indicate that DENV affects the lipid metabolism of the cells at transcriptional level. Moreover, MIF, secreted during DENV infection, is not able to regulate the expression of genes involved in this process, including ADRP and PPARγ. Further studies will be necessary to characterize the role of MIF in LD formation as well as the mechanisms involved in the release from these structures upon DENV infection. **Financial support:** CNPq, INCT-Dengue.

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RELATIVE QUANTIFICATION OF REGULATORY T CELLS IN PATIENTS WITH HEPATITIS C, SCHISTOSOMIASIS AND COMORBIDITY AND ASSOCIATION WITH HEPATIC FIBROSIS.

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Introduction: Hepatitis C and schistosomiasis are public health problems in Brazil and in worldwide. The main target of these two diseases is the liver tissue and damage may progress to chronicity. When these diseases occur in association, may lead the patients to develop cirrhosis and hepatocellular carcinoma more quickly. It is believed that regulatory T cells (Treg) are responsible to protect liver tissue damage caused by the strong cellular immune response against pathogens. Therefore, our objective was to correlate the fibrosis degrees/patterns found in patients with hepatitis C, hepatosplenic schistosomiasis (HSS) and in the comorbidity with the amount of Treg cells in peripheral blood (natural, induced and total). **Methods and results:** Patients with HSS (n=22), hepatitis C (n=25) or comorbidity (n=14), both sexes, aged up 18 to 65 years old, were selected from Clinical Hospital, Federal University of Pernambuco and a blood sample was collected for PBMCs separation (Ficoll-Hypaque Method). The cytometry technique was procedure through labeled cells with anti-$CD4^+$-APC, anti-$CD25^+$-FITC and anti-$FOXP3^+$-PE antibodies (BD-Biosciences) and the fluorescence samples were detected by BD FACScalibur flow cytometer and analyzed by the CellQuest PRO software. The statistical methods utilized for comparisons were Student t test and ANOVA, with p value < 0.05. The three types of Treg cells are present in large amounts in the disease with less morbidity, the HSS group, when comparing the relative amount of Treg cells among the three groups of patients ($p < 0.05$), which may indicate a protective effect of these cells in liver tissue. No differences was found when was compared the relative amount of Treg cells and the degree of hepatic fibrosis between the hepatitis C and comorbidity patients groups ($p > 0.05$). However, when was evaluated the fibrosis patterns between HSS and comorbidity groups, was observed a higher relative amount of induced and total Treg cells in the group with HSS in comparison with comorbidity group, with $p = 0.026$ and 0.040, respectively. **Conclusion:** These results show that the higher percentage of Treg cells appear to be associated with protection of liver tissue and hepatitis C may inhibit the expansion of the T reg number. However, further studies should be developed to elucidate the immunopathology role of Treg cells in front of these two diseases, separately or when occur in association.

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ROLE FOR GROUP V PHOSPHOLIPASE A2 IN LEISHMANIA MAJOR INFECTION IN MICE

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Introduction: Phospholipase A_2 (PLA_2) enzymes hydrolyze the sn-2 position of phospholipids, to release lysophospholipids and free fatty acids. Group V PLA_2 (PLA_2GV), a representative of the secretory PLA_2 family, is involved in a number of events related to innate immunity such as phagocytosis and killing of microorganisms as well as in adaptive immunity, such as allergic responses. Leishmaniasis is endemic in several countries, including Brazil, and in mice the susceptibility to infection is linked to the development of a Th2 response and the inability of the host to control parasite replication. Many of the PLA_2GV-modulated events are used by Leishmania major (L. major) parasites, so we hypothesized that PLA_2GV participates in the L. major infection

Methods and Results: We used cells and animals genetically deficient in PLA_2GV and their wild type (WT) Balb/c controls. Our results indicate the involvement of PLA_2GV in innate immune response by inhibiting production of IL-6 and TNF-α five hours after intraperitoneal L. major challenge (n=3). Although PLA_2GV is involved in phagocytosis of yeast and erythrocytes, it did not participate in the phagocytosis of L. major by peritoneal macrophages (n=3). It also did not participate in activation and cytokine synthesis by bone marrow derived dendritic cells (n=3). PLA_2GV-deficient mice developed smaller lesions associated with L. major infection, and increased numbers of $CD4^+$, $CD8^+$ and B cells in draining lymph nodes (n=3) when compared to WT mice. Ex vivo-stimulated draining lymph node T cells from PLA_2GV deficient mice showed reduced production of IL-4 and IL-13, but there was no difference in IFN-γ when compared to WT cells (n=3). IL-4 levels in the infected footpad were also reduced in PLA2GV deficient mice (n=2). Meanwhile, the parasite burden in the draining lymph node, as well as in infected paw was not altered (n=2).

Conclusion: Taken together our data suggest that PLA_2GV promotes the immunopathology associated with L. major infection in Balb/c mice.

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ROLE OF CD39 AND CD73 ECTO-ENZYMES IN RESIDENT MACROPHAGES INFECTED WITH LEISHMANIA AMAZONENSIS

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Introduction: Endogenous nucleotides produced by various group of cells under inflammatory conditions act as potential danger signal in vivo. Extracellular release of nucleotides such as ATP is brief and is rapidly cleaved to adenosine (ADO) by coordinated ectonucleotidasic activities of CD39 and CD73. Leishmania which are the obligate intracellular parasites of macrophages (MØ) are capable of modulating their host cells in order for them to survive and multiply. **Objectives:** In this current study, we investigated the effects of Leishmania amazonensis in infected resident MØ and the role of CD39 and CD73 in relation to purinergic receptor regulation in an infection in vitro. **Methods and Results:** Resident macrophages were harvested from naïve C57BL/6 mice and then rested for 72hrs of prior to infection by Leishmania amazonensis in a ratio of 1:3 and then analyzed by flow cytometry. Our findings demonstrated that in infected populations, MØ were characterized mainly by increased CD73 surface expressions. About 67% of MØ had both CD39CD73 expressing on their surfaces which was approximately twice as much as that of uninfected and LPS treated MØ. The percentage of cells expressing these enzmyes reduced further in uninfected(14%) and LPS treated MØ (8%) in 48hrs while maintaining higher expression in infected MØ. When we inhibited these extracellular enzymes in vitro by the use of inhibtors 4,4′-Diisothiocyanatostilbene-2,2′-disulfonic acid (DIDS) and alpha,beta-methyleneadenosine-5'-diphosphate (α,β MAD) at a concentration of 200µM respectively, we observed that within 24hrs, there was decreased parasitic infection to 50% by DIDS and 37% by α,β MAD. In 48hrs of infection, both of these inhibitors reduced parasitic infection to around 20%. Furthermore, we also evaluated the role of adenosine receptors in the function of parasitic survivability and infection and we found that inhibition of A2b receptors by MRS 1724 diminished both the percentage of infection, and amastigote number in both 24 and 48hrs of infection. **Conclusion**: Our results support that Leishmania amazonensis is capable of regulating CD39/CD73 pathway and adenosine receptors mainly A2b for their survivability inside MØ.

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ROLE OF CD40 IN HEMATOENCEPHALIC BARRIER PERMEABILITY, NEUTROPHIL INFILTRATION AND OXIDATIVE STRESS: IMPLICATION FOR BRAIN DAMAGE ASSOCIATED WITH SEPSIS IN RATS

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Introduction: Sepsis is a clinical condition resulting from the excessive inflammatory response of the host against an infectious agent. The brain is one of the targets associated with leading to the decline of mental processes, attention impaired, disorientation, delirium and coma. It has been seen that the permeability of the blood brain barrier (BBB) is associated with encephalopathy associated with sepsis allow cell infiltration and increased oxidative stress. Accordingly such events can be potentiated through the involvement of molecules that when activated perpetuate the inflammatory response and the breaking of the BBB and it is possible to postulate that the CD40 molecule may be involved by being under increased expression in microglia in inflammatory events occurring systemic. The aim of this study therefore is to evaluate the role of CD40 in the breakdown of the BBB, cell infiltration and oxidative damage in brain of rats with sepsis.

Methods and Results: Male Wistar rats were subjected to cecal ligation and puncture (CLP) to induce sepsis. The animals (n = 10) were divided into sham, CLP, CLP+1ng, 10ng and 100ng antiCD40 antibody(intracerebroventricularly) were killed 24 hours for assessment of oxidative damage in lipids (TBARS), damage to proteins by protein carbonylation, nitrite/nitrate, myeloperoxidase activity (MPO) and breakdown of the BBB. Data were evaluated by ANOVA and post hoc Tukey test with significance $p<0.05$. Our results show up in the most effective dose of 100ng/Kg antiCD40 showed a decrease in the breakdown of the BBB, MPO, NO and TBARS. In 1ng/Kg was effective only in the reduction of NO and 10ng/Kg was not effective in TBARS and carbonyl.

Conclusion: The modulation of the levels of CD40 may represent a potential therapeutic target in sepsis.

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ROLE OF HEME CYTOTOXICITY ON LEISHMANIA INFECTION

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Introduction: Visceral leishmaniaisis (VL) is a major public health problem worldwide. VL is highly associated with chronic inflammation and hematological manifestations, such as anemia, hemolysis and spontaneous bleeding. Given this scenario, mechanisms related to hemolysis and release of heme may be involved with the pathogenesis of VL. Heme is highly cytotoxicity to the host, and thought to participate in be the pathogenesis of infectious immune-mediated inflammatory conditions, i.e., malaria and sepsis. Besides heme has been demonstrated to sensitize macrophages to undergo necroptosis. Herein, we evaluated the role of heme in the infection by L. chagasi, the causative agent of VL cases in Brazil.

Methods and Results: Monocyte-derived macrophages (MDM) were infected in vitro with L. chagasi (LSH) and cultured with 30mM heme. The release of Lactate Dehydrogenase (LDH) was measured 12hours post treatment in culture supernatant. Intracellular reactive oxygen species (ROS) levels were measured 2hours post treatment with a fluorescent probe staining following analysis by flow cytometer. Heme induced LDH release (12.45±3.179, CTR vs. 96.13±17.6 Heme), and ROS generation (70.23±4.861, CTR vs. 115.5±9.534 Heme) in MDM and THP-1 cells, besides Annexin-V-PI staining showed the induction of a significant number of necrotic cells (6.747±4, CTR vs. 55.35±4.995 Heme). Interestingly, L. chagasi infection abrogated heme-induced cytotoxicity (96.13±17.6, Heme vs. 32.45±12.52 LSH+Heme). Futhermore, heme reduced macrophage parasite burden, while Necrostatin-1 (an inhibitor of necroptosis) treatment abrogated heme-induced parasite killing, and TNF/IL-6 release. Inhibitors of the signalling pathway related to necroptosis, such as Pentoxyfilin (TNF inhibitor) and SP600125 (JNK-inhibitor) reduced heme-induced cytotoxicity. In addition zVAD (caspase inhibitor) and zIETD (caspase-8 inhibitor) enhanced heme-induced cytotoxicity, Finally, we evaluated serum samples obtained from patients with VL (n=49) (62.25mM±5.739) and endemic controls (n=39) (23.86mM±2.273). from an endemic area in the Northeast of Brazil. Patients with VL presented higher concentrations of total heme in the serum compared to health individuals. **Conclusion**: Our data suggest that necroptosis may be involved on heme-induced pathogenesis/cell damage of VL, and reinforces the idea that L. chagasi parasite modulates host immune responses.

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ROLE OF HIGH-SENSITIVITY C-REACTIVE PROTEIN IN ISCHEMIC STROKE SUBTYPES

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Background: Stroke is a heterogeneous disease with servral risk factors. High sensitivity C reactive protein (hsCRP) is marker for cardiovascular and cerebrovascular diseases. Recent studies have shown that elevated hsCRP level is risk factor for ischemic stroke.

Aim: To investigate assocaiton of high hsCRP(>3mg/L) with acute ischemic stroke subtyeps in Indian patients

Metods: We recruited 210 consecutive patietns, admitted with acute ischemic stroke within 72 hours of onset at yasoda hospital Hyderabd. Study period was from April 2011 to March 2012. All patietns under tests as per standard protoclfor stroke. Seum hsCRP was assessed in all strokepatietns on the day of admission.

Results: In our study mean age was 612 years and men were 152 (72.3%).Out of 210 stroke patietns, 130(61.9%) had high hsCRP, 128(60.9%) had hypertension, 96(45.7%) diabetes, 89(42.3%) smoker 86(40.9%) alcholics and 82 (39%) had hyperlipidemia. Most common stroke subtype was large artery atherosclerosis in 86(40.9%) patients. High hsCRP was significanlty assocaited with hyperlipidemia (p=0.001), large artery atherosclerosis (p=0.01),cardioembolic stroke (0.001) and mortality (0.04). After adjustment using muliple logistic regression high hsCRP was assocated with cardioembolic stroke (odds ratio 3.4;95%CI:1.9-10.5) and large artery aterosclrosis (odds ratio 2.1;95%CI 1.5-3.8)

Conclusion: In our study, large artery atherosclerosis and cardioemboic stroke were significatnly assocated with high hsCRP,compated to other subtypes.

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ROLE OF MICROVESICLES (MVS) RELEASED DURING TRYPANOSOMA CRUZI- HOST CELL INTERACTION

ANDRES HERNAN MOJOLI; POLIANA DEOLINDO; IGOR DE ALMEIDA; MARCEL IVAN RAMIREZ.

OSWALDO CRUZ FOUNDATION, RIO DE JANEIRO - RJ - BRASIL.

Introduction

Recently the description of microvesicles (MVs) released from eukaryotic cells raised many questions about their function. MVs have been shown to be involved in intercellular communication, apoptosis, coagulant and immunological roles (Reviewed in J.Cell Biol. 2013 ;200(4):373-83). In this context, our group has been demonstrating the role of MVs in Trypanosoma cruzi, the causative agent of Chagas disease. The MVs production during the interaction T. cruzi metacyclic forms -host cells protect the parasite from complement lysis, enhance the parasite infectivity and promote a high infection in mice (J Immunol 2012 188(4):1942-52).

In this work we define as main aim how is the MVS release during the life cycle of T. cruzi and what is the role of these Mvs at the pathogenesis of Chagas disease?

Methods and results

We have prepared microvesicles from different forms and strains of T. cruzi using 1.10^6 parasites in contact with 1.10^5 THP1 cell (1 h at 37 C 1 mM Cacl2) Later, microvesicles were obtained after a cycle of centrifugations (1x 2000g x 5 min, 2 times 4000g x 30 min and 100000g x 1.5 hour).The microvesicles were quantified by flow cytometry and used in different essays. 1D-LC-MS/MS of all microvesicles-T. cruzi interaction were performed and analysed. Different strains of T. cruzi from cell derived trypomastigotes showed high number of MVs induction during contact with monocytes compared with other stages. Proteomic of MVs has shown that both cells, parasite and monocyte, contribute with membranes for the formation of these structures. However, trypomastigote membrane proteins were observed in higher number (25 %) than proteins from epimastigotes (4 %) and metacyclic (11 %).These vesicles presented phosphatidylserine (59%) and are important to enhance the parasite infectivity in vitro and in vivo. Experiments using NBD-PE fluorescent lipids showed that MVs can fuse and can be important in the cellular communication.

Conclusions

MVs carrying parasite antigens constitute a mechanism to modulate the communication between the parasite and the host cell. Cell derived trypomastigotes seem to participate at the Mvs genesis and these MVs could be involved in immunopathogenesis.

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ROLE OF MYCOBACTERIA CELL WALL COMPONENTS IN PHAGOSOME- LIPID BODY INTERACTION

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Introduction and Objectives: An efficient mechanism of mycobacterial survival inside the macrophages is the control of phagosome maturation arrest, such as the role of lipoarabinomannan (LAM) from M. Tuberculosis in the inhibition of phagosome-lysosome fusion. In addition, mycobacterial induction of lipid body biogenesis has also been implicated as a survival mechanism for this intracellular pathogen. Here we investigated the involvement of distinct components from mycobacterial cell wall in the relationship with LB and the correlation of this organelle with the trafficking of the Rab family proteins.**Methods and Results:** M. bovis bacillus Calmette-Guérin (BCG) and LAM, but not nonpathogenic mycobacteria M. smegmatis or non-coated latex beads, induced LB formation in macrophages in vitro **(**Mean ± SEM: from 2.3 ± 0.62 lipid bodies/cell in control; 2.32 ± 0.43 lipid bodies/cell in latex beads group; 2.13 ± 0.77 lipid bodies/cell in M. smegmatis group; 9.12 ± 0.99 lipid bodies/cell in LAM group; 16.68 ± 1.3 lipid bodies/cell in BCG group)**.** By immunogold staining, we showed ADRP-marked LB interacting with phagosomes during BCG infection in vivo. By fluorescent microscopy, it was observed that the interaction between LB and phagosomes is not dependent of bacterial viability. Also, we showed LB associated with phagosomes containing beads coated with LAM or PIM from M. tuberculosis or BCG, but not with non-coated beads. LAM coated beads interacts with LB 30% more than non-coated beads in bone morrow macrophages loaded with oleic acid **(**Mean ± SEM: from 48.5 ± 8.5 % in LAM coated beads-LB interaction group; 21.0 ± 11.0 % in non-coated beads-LB interaction group)**.** Moreover, Rab7 (late endosome marker) and its effector RILP but not Rab5 was co-localized in LB induced by BCG infection at 24h. By Transmission electronic microscopic, we observed that Rab7 was co-localized with LB in the site of interaction with an infected phagosome during the experimental BCG infection in vivo. Furthermore, the presence of LAM was observed co- localized with LB by fluorescence microscopy during BCG infection in vivo. **Conclusion:** These results suggest an involvement of mycobacterial cell wall components and Rab7 in the lipid body interaction with phagosomes. LB-phagosome interactions may have implications with the endocytic pathway proteins that could represent an exchange of their contents and an important escape mechanism of the host immune response.

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ROLE OF NEUTROPHIL RECRUITMENT TO LEGIONELLA LONGBEACHAE PATHOGENESIS

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Introduction: Legionnaires' disease is a severe and atypical pneumonia that is an important cause of mortality and morbidity worldwide. Bacteria from Legionella genus are the etiological agents of Legionnaires' disease. The most studied species of the genus is Legionella pneumophila, despite its higher importance in some countries, there is little information regarding the mechanisms by which host succumb to L. longbeachae infection. The main histological characteristic observed in lungs of infected individuals is an intense neutrophil infiltrate, therefore, in the present study, we aimed to analyze the role of neutrophil recruitment to L. longbeachae pathogenesis. To evaluate this, we used a murine model of infection in mice deficient for IL-17 and IL-1R, which are known to be important for neutrophil recruitment.

Methods and Results: To verify the importance of neutrophils during the L. longbeachae infection, we analyzed neutrophil recruitment to the lungs and survival of mice during L. longbeachae infection (10^5 L. longbeachae/mouse) in control mice, IL-17R$^{-/-}$ and IL-1R$^{-/-}$. As expected, differential cell count of bronchoalveolar lavage (BAL) revealed that IL-17R$^{-/-}$ and IL-1R$^{-/-}$ mice failed to recruit neutrophils ($6.4 \times 10^5 \pm 5.9 \times 10^5$ and $8.2 \times 10^5 \pm 5.2 \times 10^5$,respectively, vs $1.9 \times 10^6 \pm 1.1 \times 10^6$ to C57BL/6 control mice). In the survival test, these knockout mice were resistant to infection (IL-17R$^{-/-}$ showed a survival rate 40% greater than C57BL/6 control mice and survival rate of IL-1R$^{-/-}$ was 20% greater than survival rate of C57BL/6 mice).

Conclusion: These results indicate that neutrophils are important cells in the pathogenesis of L. longbeachae. The data suggest that a strong neutrophil recruitment and subsequent respiratory failure are responsible to death in experimental model of Legionnaires' disease caused by L. longbeachae.

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ROLE OF P2X7 RECEPTOR IN IMMUNE SYSTEM ACTIVATION IN RESPONSE TO PLASMODIUM CHABAUDI EXPERIMENTAL MALARIA

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Introduction: The erythrocyte rupture is a step of the life cycle of Plasmodium in which several molecules, including ATP, are released in the extracellular milieu. The P2X7 receptor is a purinergic receptor that recognizes extracellular ATP triggering many responses depending on the cell type involved. The aim of this study was to evaluate the role of P2X7 receptor signaling in experimental Plasmodium chabaudi malaria. **Methods and results:** P. chabaudi infection in $P2X7^{-/-}$ mice showed that these mice are more susceptible to the infection, with a lethality of 80% (n=5). Since phagocytes are very important to control parasitemia at acute phase of the disease, we decided to evaluate the sensitivity of these cells to ATP through permeabilization assays. In ex vivo assay, $CD11c^{+}$, $CD11b^{+}$ and $F4/80^{+}$ cell populations from spleen were sensitive to extracellular ATP in a P2X7-dependent way and the P. chabaudi infection acted as a component that increases this sensitivity. In vitro, bone marrow derived macrophages were also more sensitive to extracellular ATP in the presence of P. chabaudi-infected erythrocytes in a P2X7-dependent way. Phenotypic analysis of the spleen from infected $P2X7^{-/-}$ mice showed that, excepted for the $CD11b^{+}$ $Ly6G^{+}$ population, these mice have a lower number of phagocyte cells when compared to infected C57BL/6 mice (n=4). Moreover, phenotypic analysis of the liver from $P2X7^{-/-}$ mice showed that these mice also have a lower number of total cells when compared to C57BL/6 mice, and the same lower number of cells was seen to $CD4^{+}$, $CD8^{+}$, $CD11c^{+}$, $F480^{+}$ and $CD11b^{+}$ $Ly6C^{high}$ populations. However, as in the spleen, $CD11b^{+}$ $Ly6G^{+}$ population did not present more cells in C57BL/6 mice, with no difference to $P2X7^{-/-}$ mice. Furthermore, there was a slight deficiency in phagocyte activation, less IFN-γ production by splenic cells in the presence of parasitized erythrocytes and the number of cells that produce important cytokines as IL-10 and TNF-α was also lower in infected $P2X7^{-/-}$ mice in comparison with infected C57BL/6 mice (n=3). **Conclusion:** The increased susceptibility of $P2X7^{-/-}$ mice to the P. chabaudi infection is due to a deficient immune response, with a lower number of cells from immune system in the spleen and liver of these animals and, thus, lower production of important factors that help to control the infection.

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ROLE OF THE HIGH-SUGAR DIET INDUCED OBESITY ON IMUNOMODULATION OF THE EXPERIMENTAL INFECCTION OF THE MYCOBACTERIUM BOVIS BCG

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Introduction: Tuberculosis (Tb) is a public health problem with around 1.4 million deaths per year (WHO). In the lungs is observed an intense influx of cells to the site of infection where they can form structures called granulomas. It has been observed the differentiation of macrophages (MØ) in “foamy cells" in granulomas. The foam aspect of MØ is a reflex of intracellular lipid accumulation. Lipid body structural features, including lipid and protein composition may vary according to the cell type, activation state and inflammatory environment and thus may determine different cellular functions for lipid bodies. Obesity is another health problem worldwide, causing the deaths of almost 3 million of people. It is associated with chronic inflammatory response of white adipose tissue due to infiltration of MØ, responsible for overexpression of TNF-α and IL-6. Our objective was to evaluate the involvement of obesity in influx and activation of cells in experimental infection with M. bovis BCG in mice, aiming to clarify the physiopathology of Tb and the role of metabolic disorder in bacterium replication. **Methods and Results**: C57BL6 mice were divided in 2 groups fed with high-sugar diet or common chow. After 90 days, the mice were intrapleurally infected by BCG. The control received saline (Animal ethical approval #109/2012 CEUA/UFJF). The leukocyte influx and lipid body enumeration was performed at 24h after infection. It was observed an increase in the mass of fat in the high-sugar diet (Mean ± SEM: from: 0,112 ± 0,017 in control to 0,219 ± 0,002 in high-sugar group; n=10) and a significant reduction in influx of leukocytes into the pleural cavity (2,05 ± 0,366 in control to 20,100 ± 5,460 in infected on common chow group; from: 4,480 ± 0,615 in control to 8,080 ± 2,168 in infected on high-sugar diet group; n=5) as well as the neutrophils and eosinophils migration in obese mice. Also, there was less lipid body formation in obese compared with normal animals (from 1,200 ± 0,060 in control to 3,920 ± 0,738 in infected group on common chow; from1,713 ± 0,081 in control to 1,460 ± 0,102 in infected on high-sugar diet group). **Conclusion:** Our data suggest that the largest quantity of adipose tissue disadvantage the BCG. The fact the reduction in the lipid body formation and leukocyte migration in obese individuals indicated a correlation of obesity under the progression of experimental infection with M. bovis.

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SCHISTOSOMAL-DERIVED LYSOPHOSPHATIDYLCHOLINE TRIGGERS M2 POLARIZATION OF MACROPHAGES THROUGH TLR2 AND PPARΓ DEPENDENT MECHANISMS

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Introduction and Aim: Mansonic schistosomiasis is a disease caused by the trematode Schistosoma mansoni, endemic to tropical countries. S. mansoni infection in the host induces the formation of granulomas and potent polarization of Th2-type immune response. There is great interest in understanding the mechanisms used by this parasite that causes a modulation of the immune system in order to improve efficiency in treating this disease and to reproduce such modulation in the treatment of autoimmune diseases. Recent studies from our group demonstrated that lipids of S. mansoni, including lysophosphatidylcholine (LPC), have immunomodulatory activity. In the present study, our aim was to investigate the role of lipids derived from S. mansoni in the activation and polarization of macrophages and to characterize the mechanisms involved in this process.

Methods and Results: Peritoneal macrophages extracted from wild type or TLR2 deficient mice ($TLR2^{-/-}$) mice in a homogeneous C57BL/6 background were stimulated in vitro with lipids extracted from adult worms of S. mansoni. We demonstrated that total schistosomal-derived lipids as well as purified LPC induced a M2 profile of macrophage activation observed by increased expression of arginase-1, and production of IL-10 and PGE_2 after 24 h of stimulation. The involvement of the nuclear receptor PPARγ in macrophage response against LPC was investigated. Through western blot and immunofluorescence confocal microscopy we demonstrated that schistosomal-derived LPC induces increased expression of PPARγ in macrophages. Murine macrophages obtained from TLR2 deficient mice challenged with LPC in vitro showed lower expression of PPARγ in comparison to the wild, suggesting that this effect is modulated by TLR2. The LPC-induced increased lipid body formation and expression of arginase-1 were significantly inhibited by the PPAR-γ antagonist GW9662.

Conclusions: Together, these results demonstrate an immunomodulator role of schistosomal-derived LPC in activating macrophages to a profile of the type M2 through TLR2- and PPARγ-dependent mechanisms.

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SEARCHING FOR NEW ANTIGENS FROM PARACOCCIDIOIDES BRASILIENSIS THAT ASSOCIATED WITH ADJUVANT THERAPY INDUCE A PROTECTIVE RESPONSE IN EXPERIMENTAL PARACOCCIDIOIDOMYCOSIS.

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Introduction: Paracoccidioidomycosis (PCM) is an infectious disease caused by the dimorphic fungus Paracoccidioides brasiliensis. Among the deep mycoses, PCM is the most prevalent in Latin America and represents a major public health problem in countries where there is a higher incidence of the disease. In Brazil, PCM corresponds to the eighth leading cause of death among chronic or recurrent infections and parasitic diseases. Previous results from our group have shown that complete Freund's adjuvant (CFA) has a therapeutic effect in P. brasiliensis-infected mice. **Methods and Results:** In the current study, we propose to isolate P. brasiliensis antigens associated with the protective immune response induced by CFA in infected mice. Exoantigens (ExoAg) and somatic antigens (SoAg) were isolated from yeast cells of virulent Pb18 strain of P. brasiliensis, and electrophoresis of the preparations showed high complexity of proteins. Both antigenic preparations were able to induce delayed-type hypersensitive (DTH) reactions in BALB/c mice previously infected with the fungus, though more prominent and significant DTH reactions were elicited when the mice were treated with CFA. Moreover, these preparations induced a proliferation in CFSE-labeled $CD4^+$ spleen cells from mice infected with P. brasiliensis and treated with CFA significantly higher than those from only infected mice. **Conclusion:** These results suggest that ExoAg and SoAg contain antigens that are potential target to immunotherapeutic intervention in PCM. Others assays in vitro and in vivo are being conducted to isolate antigens from P. brasiliensis capable of inducing and/or enhancing the development of a protective cellular immune response.

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SELECTION OF IMMUNODOMINANT EPITOPES FROM THE RECOMBINANT PROTEIN PB40 OF PARACOCCIDIOIDES BRASILIENSIS

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Introduction: The Paracoccidioidomycosis (PCM) is the most prevalent systemic fungal disease in Latin America with high incidence in Brazil. The etiologic agent of PCM is the fungus Paracoccidioides brasiliensis. The treatment is very prolonged and expensive and in its absence can occur death. Therefore, vaccine strategies are important to improve treatment of the disease. Thus, the objective of this work is to select immunodominant peptides from the protein Pb40, identified as an antigen of the fungus, to use them as a possible vaccine against PCM.

Methods and results: Male BALB/c mice were immunized with the recombinant protein Pb40 (rPb40). The antisera were collected and were used to perform an Elisa test to monitor the effectiveness of the immunization. The peptide sequences were previously predicted, using bioinformatics tools, as linear B cell and T cell epitopes corresponding to rPb40 protein. The epitopes predicted were synthetized in a membrane using the spot method. To perform the selection, the spot membrane was incubated with the antisera from rPb40-immunized mice. Among the predicted epitopes, twelve were selected due to their high reactivity in comparison with the pre-immune antisera. The selected epitopes were synthetized and were conjugated with glutaraldehyde and used to vaccinate mice using CPG oligonucleotides as adjuvant. Immunized mice were infected with a virulent strain of P. brasiliensis (Pb18) and compared with the positive control showing reduction of viable fungal numbers (colony forming units). Additionally the antisera from mice vaccinated against conjugated peptides were collected and tested to select the immunodominat epitopes. From the reaction performed, among the twelve epitopes selected, three react positively with the spot membrane in comparison with the positive control.

Conclusion: We believe that the selected peptides are promising candidates to be tested as a possible vaccine to improve treatment of PCM, because peptides confer advantages over the use of recombinant proteins, such as greater purity, ease and less time of production and specificity of the immune response.

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SEROLOGICAL AND MOLECULAR DETECTION OF CYTOMEGALOVIRUS INFECTION IN RENAL TRANSPLANTED PATIENTS

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Introduction: Infection with human cytomegalovirus (CMV) occurs worldwide with the prevalence of anti-CMV being inversely related to population socioeconomic status. About 30% to 80% of patients undergoing solid organ transplantation acquire CMV infection. The aim of this study was to evaluate the prevalence of anti-CMV antibodies in patients undergoing renal transplantation at the University Hospital Onofre Lopes (HUOL), Natal, RN, as well as to perform the detection of CMV by polymerase chain reaction (PCR) in serum samples and peripheral blood mononuclear cells (PBMC). **Methods and Results:**This was a prospective study, which included patients who underwent renal transplantation at HUOL transplantation service, from August 2012 to April 2013. Serology for detection of IgG and IgM anti-CMV was performed before transplantation. Peripheral blood samples were collected from the recipient after transplantation and separation of PBMCs was carried out with Ficoll-Paque. DNA extraction was performed as QIAamp® DNA kit and PCR was performed in two steps (nested PCR) to amplify a conserved region of the CMV genome. Of the 30 patients included in the study, 50% were male and 50% female, median age 45 years, range 14-67 years old. Serological analysis before transplantation revealed that 87% (26/30) of patients were serum-positive for anti-CMV IgG and negative for IgM (IgG+/IgM-) and 13% (4/30) showed no IgG / IgM (CMV IgG-/IgM-). Regarding the molecular diagnosis of CMV in different clinical samples, 20% (6/30) of patients had viral DNA detected in PBMC while 10% (3/30) of patients were positive both in the serum and PBMC. Patients positive for CMV, by molecular detection in serum and/or PBMC, showed different symptoms, including fever, diarrhea and dysuria. Moreover, two patients positive for CMV presented graft dysfunction. **Conclusion:** In this study we observed a high prevalence of IgG antibodies specific for CMV. The molecular diagnosis has allowed the detection of active viremia in 10% of serum samples as well as the detection of latent cases, with the detection of cell-associated virus. As CMV infection is associated with increased predisposition to acute and chronic allograft rejection, molecular detection of infection is important for proper therapy administration, avoiding the progression of the disease and other complications.

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SEROLOGICAL EVIDENCE OF PARACOCCIDIOIDES SPP. INFECTION IN RATS INOCULATED WITH SOIL SAMPLES

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Introduction: Paracoccidioides spp. is a thermodimorphic fungus that causes paracoccidioidomycosis (PCM), the most prevalent systemic mycosis in Latin America, mainly in Brazil. PCM is a systemic granulomatous disease of chronic evolution that affects mainly male agricultural workers and the infection probably occurs by inhalation of fungal propagules. Although the disease has been described more than a century ago, the habitat of Paracoccidioides spp. is not defined until now. The identification of the fungus is important to clarify the form of infection. The few isolates of P. brasiliensis obtained from soil, suggests that it is the fungus habitat, as occurs with other pathogenic fungi. The objective of this study was to evaluate the use of immunological methods for detection of Paracoccidioides spp. infection in rats inoculated with soil samples from a Private Nature Reserve located in the Northern Region of Paraná state.

Methods and Results: A total of 103 soil samples were collected and after suspension in sterile saline containing chloramphenicol 50 µg/mL, the samples were intraperitoneally inoculated in Wistar rats. The serum of the animals was collected for six months and the immune response to exoantigen of P. brasiliensis was evaluated by indirect ELISA and Immunodiffusion. A positivity of 14.6% was observed by ELISA although no positivity was observed by immunodiffusion test. No isolate of Paracoccidioides spp. was obtained by culture of' spleen, lung, heart, kidney and liver fragments from seropositive animals in Sabouraud agar for 30 days at 36 °C. Epidemiological studies suggest that the niche of Paracoccidioides spp. is related to humid places, near lakes and rivers, with temperate or warm, rainy summers and dry winters and native areas. Most positive samples were collected at sites near creeks and in animal burrows.

Conclusion: The results suggested that the fungus is widely distributed in the study area. New collections will be held at these locations in order to obtain isolates of Paracoccidioides spp.

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SEROLOGICAL STUDY OF HANTAVIRUS IN RIO GRANDE DO NORTE, BRAZIL

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Introduction: Hantaviruses are classified as emerging viruses which cause two often fatal diseases: hemorrhagic fever with renal syndrome and the hantavirus cardiopulmonary syndrome. The natural reservoirs for hantaviruses in the Americas are rodents of the family Muridae, subfamily Sigmodontinae. The aim of this study was to evaluate the prevalence of antibodies to hantavirus in individuals of Rio Grande do Norte, Brazil. **Methods and Results**: The prospective study was conducted from February 2011 to July 2012, including individuals of rural population of Bento Fernandes and Espírito Santo, municipalities in Rio Grande do Norte. Samples of 3 mL of peripheral blood were collected from each participant and the detection of IgG antibodies to hantavirus was performed by enzyme immunoassay, using the recombinant N protein of the Araraquara hantavirus as antigen (ARAV rN). Of the 269 individuals (171 of Espírito Santo and 98 of Bento Fernandes) included in the study, 70% were female and 30% male. The mean age of participants was 26 years, range 02-85 years. Regarding to serology, no sample was positive for antibody IgG anti-hantavirus. **Conclusion**: From 1993 to 2012, 1635 cases of Hantavirus were confirmed in Brazil, with a lethality rate of 39.7%. Until the present time, two cases of the disease were reported in Rio Grande do Norte. Studies of hantavirus and its epidemiological behavior in different Brazilian regions, especially in areas epidemiologically considered as "silent", are important to the characterization of the real geographic extension of the risk for hantavirus infection in Brazil.

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SERUM LEVELS OF IL-10 AND TNF-ALPHA IN CHRONIC CHAGAS DISEASE PATIENTS

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Introduction: In Chagas disease, individuals chronically infected by the protozoan Trypanosoma cruzi may be asymptomatic or may present cardiac and/or digestive complications. It is well-know that the human immune response is related to the different clinical manifestations. Different patterns of cytokine serum levels have been previously described in patients with different clinical forms of chronic Chagas disease, but contradictory results were obtained. The aim of this study was to evaluate the serum levels of IL-10 and TNF-alpha in asymptomatic and chronic cardiac Chagas disease patients.

Methods and Results: Serum samples were collected from 80 patients with chronic Chagas disease. According to their clinical status, they were classified as asymptomatic or cardiac. The serum levels of IL-10 and TNF-alpha were measured by ELISA using commercial kits. The Shapiro-Wilk, Mann-Whitney and Kruskal-Wallis tests were applied to assess the differences between the groups of patients, and the significance level for all conclusions was set at 5%. The cytokines IL-10 and TNF-alpha were detected in all serum samples studied. However, the levels of these cytokines slightly varied between the groups of patients studied. The IL-10 serum levels were higher in patients with cardiomyopathy patients, mainly in those without heart enlargement. Although no significant difference was observed in TNF-alpha serum levels between the groups of patients studied, high levels of this cytokine were found in the chronic cardiac Chagas disease patients, mainly in those with heart dilatation.

Conclusion: The evaluation of IL-10 and TNF-alpha serum levels showed that these cytokines play an important role in the cardiomyopathy of chronic Chagas disease. Particularly, the IL-10 serum levels can be correlated with cardiac function, where lower levels were associated with heart enlargement. However the use of these cytokines as immunological markers that could aid treatment and improve management of chronic Chagas disease requires further investigations.

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SINGLE NUCLEOTIDE POLYMORPHISMS IN CANDIDATE GENES AND DENGUE SEVERITY IN CHILDREN: A CASE CONTROL, FUNCTIONAL AND META-ANALYSIS STUDY

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Introduction: Pathogenesis of dengue has been attributed to many factors over the years such as sequential infections by distinct serotypes, genetic and antigenic variations among viral strains and host genetic variations. Previous data have shown that host genetic polymorphisms play a role in disease susceptibility and severity. Our previous results showed, in a case control and functional study, that single nucleotide polymorphisms (SNPs) in genes like CLEC5A and DC-SIGN, were associated with outcome of dengue (severity or protection), but no association were found to TNF gene. Due to inconsistencies in the literature we performed a meta-analysis grouping our data with previous results to diminish the ambiguity for the association of SNPs in DC-SIGN and TNF genes. **Methods and Results**: genetic studies with sufficient genetic data, for SNPs rs4804803 (-336/DCSIGN) and rs1800629 (-308 TNF) in dengue severity were searched in databases and combined, eventually, with our data to generate -336 A/G DCSIGN and -308 A/GTNF meta-analysis. Consensus estimates of these SNPs indicated no association with dengue severity in the overall analysis (allele, genotype and carriers). But, an Asian subgroup analysis in the - 336 G>A DCSIGN, the G allele was associated with severe dengue (OR_{allele} =2.77; p=0.0001; OR carriers = 2.99; p=0.0001).**Conclusion**: Here, we propose that SNPs rs4804803 in DC-SIGN and rs1800629 in TNF genes are not associated with severe dengue in Brazilian population, although combined data of the literature suggest population-specific effect of the -336 DCSIGN SNP restricted to Asians.

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SMTL-13 REGULATES CELL DEATH AND PRO-INFLAMMATORY CYTOKINE PRODUCTION BY MACROPHAGES DURING INFECTION WITH MYCOBACTERIUM TUBERCULOSIS

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Introduction: Infection by Mycobacterium tuberculosis (Mtb) can lead to a latent state in which the host is able to control the pathogen growth. While effective cellular immune responses are critical to control Mtb growth inside macrophages, it has been demonstrated that mycobacteria-associated factors play an important role in the outcome of infection. We have previously described a novel secreted 13-kDa lectin in pathogenic Mtb (sMTL-13) and although a possible importance for this protein as a major mycobacterial antigen was shown in tuberculosis patients, fundamental questions on the biology of this lectin remain to be answered.

Methods and Results: Confocal microscopy analysis demonstrated that this lectin is detected inside macrophages upon infection with live Mtb as opposed to heat-killed Mtb. This result suggests that sMTL-13 is secreted at the intracellular milieu and could regulate bacterial-host interactions following infection. To further investigate the possible role of sMTL-13 during infection, a knock out mutant (ΔRv1419) was generated. Significant cell death was observed in ΔRv1419-infected macrophages as well as increased knockout intracellular growth compared to infection with wild type bacteria. Furthermore, it appears that the inflammasome platform is regulated by this lectin, since macrophages infected with ΔRv1419 display increased amounts of IL-1β in a caspase1/11-dependent manner, when compared with wild-type H37Rv-infected cell cultures. Moreover, lower levels of TNF were detected in ΔRv1419-infected macrophages 4 hours post-infection, suggesting that sMTL13 may be important for cell recognition.

Conclusion: We speculate that sMTL-13 present inside host cells regulates cell death and pro-inflammatory cytokine production as a survival strategy, allowing pathogen growth without leading host cells to a premature death.

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STUDY OF MELANIZED CRYPTOCOCCUS GATTII INFECTION IN MURINE MODEL

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Cryptococcus gattii is an encapsulated yeast that causes a fungal infection known as cryptococcosis. It is an emerging pathogen that affects immunocompetent individuals causing meningoencephalitis and pulmonary infection. Melanization is a process that influences the development of cryptococcosis by interfering on macrophage functions and contributing with the yeasty resistance to Reactive Oxygen Species. The aim of this study was to evaluate the influence of melanin on cryptococcosis development. We have infected SWISS / NIH mice with melanized and non melanized isolates of a C. gattii (L27/01) clinical sample. C. gattii was cultured in L-DOPA to induce melanization and to obtain melanized samples. The mice were infected through the intratraqueal method using $1x10^6$ colony forming units (CFU) per animal. These mice were clustered in two different groups: infected with melanized C. gattii and infected with non melanized C. gattii. The animals were monitored and euthanized after 10 days of infection. Brain and lungs were removed and analyzed. The organs were weighed and macerated in sterilized PBS. After 48 hours of incubation on Sabouraud-Dextrose Agar, the CFUs were counted and compared. Furthermore, bronchoalveolar lavage was performed with approximately 2.0 ml of total volume. This material was centrifuged to form the cell pellets used for total and differential cell counting. Considering the animals presenting pulmonary infection, no statistically significant difference in number of CFUs was observed between groups. However, a different result was obtained from the animals presenting brain infection. In this case, the yeasts were recovered only from the group infected with non melanized C. gattii. A statistically significant difference between groups submitted to infection with melanized and non melanized yeasts was observed. The group infected with melanized samples presented a lower cell counting. This suggests that melanization may reduce cell recruitment at infection site. The number of mononuclear cells was lower in the group infected with melanized yeasts, and neutrophil recruitment was identified only in this group. The results suggest that melanin synthesis is associated with tropism for the central nervous system (CNS) and may also influence the cell recruitment during immunological response. It is important to highlight the need of more studies for a better understanding of the relation between melanization process and host immune response.

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STUDY OF TGF-BETA 1 PATHWAY IN CLOSTRIDIUM DIFFICILE TOXIN A-INTOXICATED CELLS

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Introduction: Clostridium difficile is the most common anaerobic pathogen that causes diarrhea in hospitalized patients. Pathogenic strains of C. difficile produce two high molecular weight protein exotoxins, toxin A (TcdA) and toxin B (TcdB). Tcd A is recognized to induce fluid secretion, intestinal barrier dysfunction and diarrhea. TGF-beta participates of inflammatory processes, and it could affect cellular events like cell proliferation, differentiation and apoptosis. However, the involvement of this cytokine in C.difficileTcd A-intoxication is still unclear. The aim of this study is to investigate the role of TGF-beta on TcdA induced damage in intestinal epithelial cells.

Methods and Results: IEC-6 cells were seeded in cell culture plates and divide in different groups according to the treatment received: incubation only with medium (control), TGF-b (10ng/mL) or TcdA (10ng/mL) or both (TcdA + TGF-b). 24 hour later, we evaluated the apoptosis/necrosis using Annexin V assay by flow citometry. Migration was measured in 24 hours after a razor scrape of the cell monolayer. Cell proliferation was indirectly measured utilizing the Ki-67.The TGF-beta treatment significantly decreased the apoptosis/necrosis induced by TcdA, prevented decrease in cell proliferation (mean=16.27 Ki-67 positive cells/field) and cell migration (mean= 91.79 migrating cells/mm^2) induced by TcdA compare to the TcdA-cell intoxication (mean=3.59 Ki-67 positive cells/field and 72.74 migrating cells/mm^2, respectively). Conclusion: The TGF-beta reduced the TcdA-induced damage on viability, proliferation and cell migration events and this cytokine might play a protective role in C.difficile-induced disease.

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SUPPRESSOR OF CYTOKINE SIGNALING 2 (SOCS-2) PROTEIN DEFICIENCY RESULTS IN EXACERBATED LUNG INFLAMMATION AND SUSCEPTIBILITY INDUCED BY INFECTION WITH THE PATHOGENIC FUNGUS PARACOCCIDIOIDES BRASILIENSIS

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Introduction: Paracoccidioides brasiliensis and P. lutzii are the agents of paracoccidioidomycosis (PCM), the most prevalent deep mycosis in Latin America, endemic in South and Southeast regions of Brazil. The PCM presents a wide range of clinical manifestations and severity of the disease is related to the pattern of host immune response. The suppressor of cytokine signaling (SOCS) proteins are key controllers of cytokine responses, which can down-regulate specific cytokine signals and consequently modify the immune response. The study aimed to evaluate the participation of SOCS-2 protein in an experimental pulmonary infection induced by the dimorphic fungus P. brasiliensis in mice.

Methods and Results: Male C57BL/6 wild-type mice (WT) and SOCS-2-deficient mice (SOCS-$2^{-/-}$) were used in all experiments (CETEA-163/2012). After anesthesia, mice were infected with 10^6 yeasts of Pb18 strain, by intratracheal injection, while uninfected mice received buffered saline by the same route. Mice were evaluated for survival and after three or fifteen days of infection they were euthanized and their lungs removed for cytokine measurement by ELISA and evaluation of inflammatory infiltrates by myeloperoxidase (MPO), N-acetylglucosaminidase (NAG) and eoperoxidase (EPO) assays. Moreover, the fungal load was determined in bronchoalveolar lavage fluid (BAL) and lungs of infected mice. The results obtained showed that the absence of SOCS-2 resulted in 100% of death after 30 days of infection. This susceptibility to P. brasiliensis infection was associated with increased pulmonary fungal burden and important changes in the recruitment of inflammatory cells to both pulmonary parenchyma and the alveolar space. In addition, SOCS-$2^{-/-}$ mice showed a significant increase in the synthesis of proinflammatory mediators such as TNF-a, IL-1b, IL-6 and CXCL-1/KC as well as accumulation of neutrophils and eosinophils after the third day of infection and macrophages after 15 days in the lung tissue. This inflammatory response profile observed in SOCS-$2^{-/-}$ mice resulted in alteration of lung parenchyma, characterized by loss of alveolar architecture, which could result in loss of lung function. **Conclusion:** These results show that the signaling pathway involving SOCS-2 plays an important role in modulating the immune response during infection by the pathogenic fungus P. brasiliensis.

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SUSCEPTIBILITY OF MICE XID IN EXPERIMENTAL LEISHMANIA AMAZONENSIS INFECTION

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Introduction: Leishmaniasis belong to a group of diseases caused by protozoan parasites from Leishmania genus. The clinical forms depend both on the Leishmania species and the host response. L. amazonensis can lead to various clinical manifestations of leishmaniasis: localized cutaneous, diffuse cutaneous, and more rarely a severe form of visceral leishmaniasis. Experimental evidence suggests that B cells and/or antibodies are potentially involved in the development of infection caused by L. amazonensis. B-1 cells are a subtype of B lymphocytes whose role in the physiology of the immune system as well as in the pathogenesis of various diseases is still poorly understood. These cells have ability to engulf pathogens, to migrate to inflammatory focus and to modulate immune response in several experimental models, such as paracoccidioidomycosis and murine melanoma. In addition, B-1 cells produce large amounts of IL-10 cytokine, which plays a key role in immunosuppression in several diseases, such as leishmaniasis. Xid mice have naturally reduced B-1 cells have an increased resistance to experimental infection model by L. chagasi compared to Balb/c. However, the importance of these cells in leishmaniasis has not been clarified. The objective of this study was investigate the role of B-1 cells in experimental infection of L. amazonensis.

Methods and Results: Preliminary results demonstrated that Balb/c or Xid mice subcutaneously infected at the right hind-foot with $1x10^7$ promastigotes of L. amazonensis for 10 weeks Balb/c mice were more resistant than the XID mice with (41.0 ± 3.1%) minus the foot and Leishmania (21.8 ± 5.5%) spleen. These preliminary data suggest that B-1 may modulate the response to infection with L. amazonensis. Other studies were conducted to better understand the biological significance of these findings. The role of B-1 cells in the development of cutaneous leishmaniasis is also under investigation in our laboratory.

Conclusion: BALB/c mice had fewer parasites in the spleen and paw towards Xid mice after 10 weeks of infection.

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TH17 AND TREGS IMPAIR THE CONTROL OF MYCOBACTERIUM LEPRAE MULTIPLICATION: IN VIVO EVIDENCES FROM KNOCKOUT MICE STRAINS.

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Introduction: In the absence of a definitive experimental model for leprosy, the inoculation of Mycobacterium leprae in the mice footpads comprise a useful experimental tool in the study of leprosy. While a Th1/Th2 dichotomy is suggested to play a critical role in human leprosy outcome, the role of regulatory (Tregs) and Th17 subsets in disease pathogenesis remains unknown. In this study, we investigated the possible role of Tregs and Th17 cells in response to M. leprae comparing C57Bl/6(WT) and IL-17KO, IL-23KO, IL-6KO and CCR4KO mice strains. **Methods and Results:** Mice were infected with $1x10^4$ bacilli per footpad (according to classic Shepard´s technique) and after nine months were sacrificed. Samples (foodpads) were submitted to histopathological analysis and bacillary counting by cold Ziehl-Neelsen staining. As previously described, M. leprae footpad challenge did not resulted in the development of macroscopic leprosy-like lesions, but Ziehl-Neelsen staining demonstrated the presence of a significant bacillary load, associated with the presence of inflammatory cells (mainly epithelioid macrophages) in the surrounding connective tissue. As described to WT strain, no evidences of macroscopic lesions were observed in IL-17KO, IL-23KO, IL-6KO and CCR4KO mice strains. While no differences were observed between IL-17KO, IL-6KO and WT strains, our results showed a significant decrease in the numbers of bacilli in CCR4KO and IL-23KO strains when compared to WT mice. **Conclusions:** These results suggest a detrimental role for Th17 and Tregs subsets in the control of Mycobacterium leprae multiplication, since the absence of the Th-17-related cytokine IL-23 and the Treg-associated chemokine receptor CCR4 affect the multiplication of the M. leprae bacilli in vivo. Further studies are required to confirm such data and to clarify the mechanisms by which such molecules could be implicated in the control of M. leprae and possibly in the pathogenesis of leprosy.

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TH2 IMMUNE RESPONSE IN BRAZILIAN PATIENTS CHRONICALLY INFECTED WITH HEPATITIS C VIRUS (HCV)

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Introduction: Chronic hepatitis C has been associated with dysfunction of both T and B cells, which are represented by T $CD4^+$ cell exhaustion, autoimmunity and inefficient production of HCV neutralizing antibodies. Although the Th1 immune response has been well studied in this infection, the presence of a Th2 immune response is little known. In this work, we investigated the serum levels of IL-4, total IgE and Blomia tropicalis IgE antibodies in Brazilian patients chronically infected with HCV and evaluated their relationship with cryoglobulinemia, blood HCV load and liver histopathology. **Methods and Results:**Fifty untreated HCV patients without parasitic infection and 31 healthy controls participated of this study, approved by a local Ethics Committee. Forty-three patients were infected with HCV genotype 1, five with HCV genotype 3 and two with HCV genotype 2. All patients had HCV antibodies and blood HCV-RNA. Their HCV load varied from 19 x 10^3 to 57.5 x 10^6 IU/mL. Liver fibrosis and necroinflammatory activity were diagnosed using the METAVIR score, being verified that 22 out of 50 (44%) had advanced fibrosis (F3-F4) and 19 out of 50 (38.0%) moderate necroinflammatory activity (A2). Cryoglobulinemia was found in 24 out of 50 (48.0%) patients. The median of total IgE in HCV patients and controls was82 IU/mL and 62 IU/mL, respectively (P> 0.05), and high total IgE (> 100 IU/mL) was found in 24 out of 50 (48.0%) and in 11 out of 31(35.4%) controls (P> 0.05). High serum IgE level was not associated with moderate necroinflammatory activity or advanced fibrosis (P > 0.05). B.tropicalis IgE antibodies were detected in 4 out of 50 (8.0%) HCV patients whom had high total IgE level (median = 456 IU/mL), but their prevalence was lower than that previously reported for the local population. Measurable serum IL-4 level (> 2.0 pg/mL) was found in 10 out of 50 HCV patients (20.0%) with a median of 3.2 pg/mL (range 2 – 22 pg/mL). IL-4 level was not correlated with total IgE level or associated with cryoglobulinemia (P > 0.05), hepatic necroinflammatory activity or liver fibrosis. **Conclusions:**High IgE levels can be observed in important frequency in Brazilian patients with chronic HCV infection. However, they are not associated with IgE antibodies against B. tropicalis or increased serum IL-4 level. Moreover, this high IgE production is unrelated to cryoglobulinemia or liver injury caused by HCV infection.

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THE ATTEMPT OF A VACCINE DEVELOPMENT FOR ENTAMOEBA HISTOLYTICA INFECTION, USING A PARASITE SURFACE METALLOPROTEASE

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Introduction: Entamoeba histolytica is an enteric protozoan that causes amebiasis. This disease causes approximately 100,000 deaths per year and is considered by the WHO as one of the major health problems in developing countries. The life cycle of the parasite is relatively simple. However, the trigger that causes the parasite to switch to a pathogenic phenotype has not been fully understood. The invasive infection requires penetration of the intestinal wall in a protease-dependent process. Huston's group described a new metalloproteinase expressed on the E. histolytica surface, named EhMSP-1. Their results show that this protease is immunogenic and, in its absence, E. histolytica motility is reduced and its phagocytic ability is enhanced. The goal of this work is to investigate whether immunization of hamsters with a pool of fragments from the recombinant form of EhMSP-1 could protect the animals during subsequent challenge with the parasite. **Methods and results:** The extracellular portion of the EhMSP-1 encoding gene was cut in four 17kDa fragments and cloned in an Escherichia coli expression system. The fragments were produced from inclusion bodies by 8M Urea treatment and further purified. Simultaneously, the virulence of an HM1-IMSS Entamoeba histolytica culture was recovered by addition of 15-20 small pieces of normal hamster liver to an exponential growing tube. After 48 hours, the virulence was tested by checking the speed and number of ingested erythrocytes and the resistance to Complement activity. The virulence recovery protocol was successful. A group of 6 hamsters received $4x10^5$ trophozoites in the right liver lobe. The animals were sacrificed on the seventh day post-challenge. Marked development of amebic liver abscess (ALA) was observed. The livers were weighted and analyzed by histology. Large abscesses were observed around the parasites. A group of 10 hamsters is currently being immunized intraperitoneally with a pool of the four 17kDa fragments emulsified with Complete and Incomplete Freund Adjuvant. After 4 weeks, immunization success will be verified and the animals will be hepatically challenged with the trophozoites. **Conclusion:** Cloning and expression of fragments were successfully performed and the pool of protein is being currently utilized to immunize the animals. E. histolytica virulence was recovered and the trophozoites were able to induce ALA formation. The next assay will analyze the response profile of the immunized animals following hepatic challenge.

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THE CARDIOPROTECTIVE PHENOTYPE OF BRADYKININ B1 RECEPTOR DEFICIENT MICE CHRONICALLY INFECTED BY T. CRUZI UNVEALS A FUNCTIONAL LINK BETWEEN INFLAMMATORY EDEMA, HEART PARASITISM AND IMMUNOPATHOLOGY

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Introduction: Bradykinin and their metabolites exert their biological effects through the signaling of B2R (constitutively expressed by a wide range of cell types) and B1R (ubiquitously upregulated in injured/inflamed tissues). In a previous work, we showed that $B2R^{-/-}$ mice succumb to acute systemic challenge by Trypanosoma cruzi (Dm28 strain). Mechanistic studies have linked the susceptible phenotype of $B2R^{-/-}$ mice to impaired (i) maturation of splenic $B2R^{-/-}$ DCs and (ii) generation of type-1 $CD4^+$/$CD8^+$ T cells (Monteiro et al., 2007). Given the precedent that diabetic cardiomyopathy is attenuated in $B1R^{-/-}$ mice, here we asked whether activation of the kinin/B1R pathway in intracardiac inflammatory exudates may fuel T. cruzi parasitism and infection-associated immunopathology. **Methods and Results:** $B1R^{-/-}$ mice infected ip. with TCTs display (i) markedly reduced intracardiac parasitism as compared to WT mice (14 d.pi; 400% reduction) (ii) attenuated chronic myocarditis and heart fibrosis (85% and 65% reduction respectively). Since B1R engagement inhibited migration of autoreactive T cells to CNS in EAE models, we then checked if the reduced intracardiac parasitism of $B1R^{-/-}$ mice could result from enhanced infiltration of innate and/or adaptive effector cells into the chagasic heart. FACS analysis did not reveal significant differences in frequencies of $IFN\gamma^+$ $CD8^+$ and $CD4^+$ T cells both in the heart and spleen of WT and $B1R^{-/-}$ mice, nor in the expression of granzyme B in intracardiac $CD8^+$ T cells. Focusing on the lymphoid compartment, we did not find differences in the expression of activation marker CD44 in $CD8^+$ and $CD4^+$ T spleen cells. Interestingly, however, $B1R^{-/-}$ mice displayed reduced intracardiac frequencies of $Mac1^+Ly6C^{int}Gr1^{hi}$ neutrophils and $Mac1^+Gr1^{lo}$ LyC^{high} monocytes, a proinflammatory subset that aggravates heart pathology in classical models of myocardial infarction (27% and 38.2% reduction, respectively). Finally, in vitro assays using B1R and B2R antagonists showed that BK fuels T. cruzi infectivity in IFN-γ-primed macrophages (45% and 35% reduction by the respective antagonists).**Conclusion:** Collectively, our studies suggest that TCT may take advantage of prolonged inflammatory edema steered by kinins to persistently infect cardiovascular cells through the signaling of B1R, here defined as an inducible gateway for T. cruzi infection of cardiovascular cells (see accompanying abstract, Oliveira et al).

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THE CHEMOKINE RECEPTOR CXCR4 AND ITS LIGAND CXCL12 LIMIT FILARIAL INFECTION

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Introduction: Filariases are chronic diseases affecting 160 million people worldwide. Despite considerable effort to reduce disease burden, particularly through mass drug administration programs, filarial infections remain a major public health problem requiring new therapeutic approaches. In our study, we used Litomosoides sigmodontis as a well-established murine model of filarial infections. Previous studies have shown that the CXCL12 chemokine and its receptor CXCR4 participate to the mice resistance mechanism to the filarial infection, suggesting CXCR4 and CXCL12 as potential therapeutic targets. To decipher their role on the infection progression, we used a newly developed murine model of a rare combined human immunodeficiency disorder (WHIM: Warts, Hypogammaglobulinemia, recurrent Infections and Myelokathexis) caused by a gain of CXCR4 function and also characterized by a profound lympho-neutropenia. WHIM mice reproduce this leucopenia, which is associated with defective thymopoiesis and B-cell development, and lymph node disorganized architecture.

Methods and Results: Our results on filarial infection in those mice showed that filarial parasitic success was drastically decreased by 70% in WHIM mice compared to control wild-type mice. In addition, a significant neutrophilia became noticeable from 15 days post-infection, normalizing the circulating neutrophils to the control levels although the lymphopenia remained in the WHIM mice throughout the infection. Moreover, 6 hours post filarial infection, cell recruitment in the skin was more important in those WHIM mice, suggesting a stronger local immune response to L. sigmodontis.

Conclusion: Further analyses are currently conducted in order to elucidate the mechanisms behind this immune response in respect to the role of the CXCL12/CXCR4 axis in filarial infection.

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THE DEVELOPMENT OF HERPETIC HYPERALGESIA IS DEPENDENT OF IMMUNE RESPONSE IN DORSAL ROOT GANGLION

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Introduction: Herpes Zoster (HZ) is a disease caused by reactivation of latent herpesvirus Varicella Zoster (VZV) in the sensory ganglion, characterized by dermal rash and pain. VZV infects only humans, and there are no animal models available to study the disease. However, a murine model of Herpes Simplex 1(HSV-1) infection on the hind paw skin has been used to study HZ, since mice develop HZ-like lesions and pain-related responses. There are no data available about the immune response in dorsal root ganglion (DRG) of these mice, neither the relationship between immune response and the development of hyperalgesia. Thus, the aim of this study was to evaluate immune cells and inflammatory mediators present in DRGs and its relationship with herpetic hyperalgesia. **Methods and Results:** Briefly, mice were depilated and 2 x 10^5 plaque forming unities (PFU) of HSV-1 were inoculated in the skin of the right hind paw. Mice were observed daily and behavioral tests were performed at different periods. The DRGs L1-L6 were collected for evaluation of cellular infiltration (flow cytometry), western blot analysis (GFAP and COX-2 expression) and PCR (TNF-a and COX-2 mRNA expression).Viral load was measured by quantitative Real-Time PCR. In some groups mice were treated with anti-TNF-a (1ug/i.t/day). Mice developed hyperalgesia from 3 to 21 dpi only in the ipsilateral (ips) paws. Approximately 50% of mice showed persistent hyperalgesic behavior until 45 dpi. A higher viral load was detected in DRGs L4, L5 and L6 of infected mice at 7 dpi. We also observed an intense activation of satellite glial cells in ips DRGs (GFAP expression). In infected mice, a higher mRNA expression of COX-2 and TNF-a was detected in ips DRGs. Moreover, blockage of TNF-a reduced the development of herpetic hyperalgesia. We also observed an intense inflammatory infiltrate composed by neutrophils and macrophages in ips DRGs at at 7dpi. T CD4+ lymphocytes infiltration was detected at 7 and 15 in ips DRGs, while T CD8+ cells were found only from 15 dpi. **Conclusions:** Our results show the presence of an intense inflammatory infiltrate in DRGs of infected mice, and the early expression of inflammatory mediators that contribute for the induction of herpetic hyperalgesia.

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THE EFFECT OF AEDES AEGYPTI SALIVA ON IMMUNE RESPONSE INDUCED BY VIRAL PARTICLES IN MODEL IN VITRO.

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Introduction: Dengue, transmitted horizontally by arthropods such as Aedes aegypti, can be facilitated by the action of the mosquito's saliva. Participation of vector saliva in disease transmission has long been recognized. Saliva inoculated during blood feeding modulates the immune response allowing the infection to become established. On the other hand, anti-vector saliva immunity may protect the host against some vector-borne diseases Studies with other hematophagous reveal that these diseases may be favored by the actions anti-hemostatic and immunomodulatory properties of saliva, by regulating the host immune response potentiating infectious events. Thus, this study aimed to evaluate the immunomodulatory effect of the saliva of Aedes aegypti in dengue virus infection in vitro. **Methods:** The viral antigen DENV 2 and the Aedes aegypti saliva, were provided, gently, by Instituto Evandro Chagas and Universidade Federal de Ciências da Saúde de Porto Alegre. In all tests were used spleen cells from BALB / c mice at a density of $2x10^5$ cells / ml. For cell proliferation essay, spleen cells were incubated with Con A (5µg/mL) or DENV 2 (5µg/mL) in presence of Aedes aegypti saliva (1,2 salivary gland) in 5% CO_2 at 37°C for 24 hours. Ten micro liters of methylthiazoletetrazolium was added 4 hours before the determination of the proliferative index, each group was performed with three replications. The production of nitric oxide (NO) was measured as nitrite (NO2-) by Griess reaction. Values were expressed in proliferative index and percentage of dead cells. Statistical Analysis was used one-way ANOVA,* $p \leq 0.05$. **Results and Discussion:** In proliferation assay, our results showed that after 24 hours, all stimulus (ConA and DENV) induced significant proliferation of spleen cells, whereas the saliva was able potentiate the proliferation induced by these stimuli (CON A= 1.45 ± 0.173; DENV = 1.65 ± 0.25, CON A+SV= 2.49 ± 0.37; DENV +SV= 3.43 ± 0.27). In the NO measure at 24 and 48 hours, cells when stimulated with DENV or ConA in the presence of Aedes aegypti salivary glands produce high levels of NO in vitro when compared to the control without stimulus and saliva. At time of 48 hours, the levels of NO in the stimulated groups the presence of saliva are still high compared with the groups without saliva, however, the production of this mediator lower when compared to the time of 24 hours especially with the stimulus of DENV 2.

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THE IMMUNE RESPONSE OF TWO DISTINCT MICE STRAINS TO LEPTOSPIRA INTERROGANS INFECTION

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Introduction

Leptospirosis is an infectious disease caused by pathogenic Leptospira that is worldwide spread. Rodents, such as rats and mice are asymptomatic carries of leptospires. In this work, we studied the immune response of the mice strains C57Bl/6 and A/J (C5 deficient) against the infection by L. interrogans sorovar Kennewicki strain Fromm.

Methods and Results

The animals were infected with 1.5 x 10^8 leptospires and sacrificed in the third and sixth day after the infection. We observed that, when compared to C57Bl/6, the A/J strain gained less weight and required three more days to develop splenomegaly. Besides, A/J mice had a reduced number of blood leukocytes in the third day, demonstrating a greater difficulty to control the infection. In the third day, all animals of A/J strain presented live bacteria in the kidneys and liver, against 80% of C57Bl/6. Hepatic enzymes activity were higher in A/J mice than in C57Bl/6 sera, which could reflect a possible liver injury. Serum from A/J mice had also an increase in the levels of TNF-a, IFN-g, MCP-1 and IL-6. The increased concentration of TNF-a could reflect the reduced number of blood leukocytes and the limited gain of weight presented by A/J, since this protein may inhibit the hematopoiesis and cause cachexia when present in high concentrations. In the kidney of both strains, there was an increase of TNF, IL-6, IL-12(p40) and IL-10 at sixth day, which could have contributed to the bacteria elimination from the organ.

Conclusion

In conclusion, our results suggest that A/J strain is more sensitive to the infection by Leptospira than C57Bl/6. The role of the component C5 to this in vivo model of Leptospira infection remains to be further investigated.

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THE LEWIS Y ANTIGEN AS A MEDIATOR OF THE INFLAMMATORY RESPONSE IN LEPROSY

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The Lewis Y antigen is a difucosylated tetrasaccharide inducible pro-inflammatory cytokines such as interleukin 1ß and TNF-a, which may regulate its expression in endothelial cells. Under these circumstances, the expression of Lewis Y is necessary in the rolling and adhesion of immature dendritic cells (DCs) over endothelial cells.And so, the migration transendotelial dendritic cells is effected by the interaction between the endogenous ligands, which express the antigen Lewis Y as ICAM-2 on endothelial cells and ICAM-3 in lymphocytes, with the CD209ou CD-SIGN (dendritic cell specific ICAM 3 grabbing nonintegrin) receptor present in dendritic cells. The present study evaluated the interrelationship of genetic susceptibility to leprosy in relation to the indirect immunohistochemistry detection of the Lewis Y antigen expression in the epidermis of individuals with different clinical forms of leprosy. Lewis Y antigen was detected in 59% (32/54) of individuals with leprosy, as defined by the reactivity with the anti-Ley mAb. Although no expression of these antigens was observed in the same histological structur of epiderme of healthy individuals.This antigen was located in different areas of the epidermis, the vascular endothelium, histiocytes, lymphocytes, epitelioides cells , sweat glands, whereas expression in neutrophils and nerve was detected at a low frequency. The homogeneous reactivity pattern was often associated with the severity of inflammatory lesions. The expression of these antigens showed no significant dependence with the various clinical spectrum of the disease. The expression of Lewis Y antigens concentrated preferentially in endothelial cells, which seems to confirm the evidence of the angiogenic effects of these antigens, which plays an active role in inflammatory processes, functioning as adhesion molecules, inducing or mediating cell adhesion to vascular endothelium. These results implicate a role of the Lewis Y determinant as mediator of angiogenic responses, when DC-SIGN are recruited from blood into tissues to patrol the M. leprae antigens and finally they initiate immune response. This activation-associated with up-regulated in Lewis Y expression seem to represent the mechanism for bacterium destruction and the inflammation process in peripheric nerves, which is the main cause of morbidity of leprosy individuals. In fact, this observation suggests that Lewis Y blood group antigen could be served as new targets for therapy of the disease.

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THE LTB4 PARTICIPATES IN THE CONTROL OF LEISHMANIA INFANTUM INFECTION TROUGH TH17-DEPENDENT MECHANISM

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Introduction Visceral leishmaniasis (VL) is a chronic and potentially fatal disease caused by Leishmania donovani and Leishmania infantum, being the last one endemic in Brazil. Although the protection is associated with Th1 pattern, some works report that leukotrienes (LTs) are also important in protective host responses to infection. However, there is no information whether LTs participate in the host response to VL. Thus, our aim was to determine the role of LTB4 in the parasite control.

Methods and results: Bone marrow-derived dendritic cells (BMDC) (10^6) were infected with L. infantum promastigotes form (10^7) and LTB_4 production was evaluated 24 hours after infection by Elisa assay. To investigate the role of LTB_4 in the parasite restriction, 5-LO knockout (KO) (129-Alox5) and wild-type (WT- sv/129) mice were infected with L. infantum promastigotes forms ($1x10^7$ parasites / mice - i.v. route). At 6th week post infection, the spleens and livers were harvested to evaluate parasites burden (limiting dilution assay). Our results show that, in vitro, BMDC produced high amounts of LTB_4 when infected with parasite. Furthermore, KO mice were more susceptible to infection, showing higher parasites numbers into the spleen and liver when compared to the control group (WT). The susceptibility to infection it was directly related to reduction of pro-inflammatory cytokines released in to the organs such as TNF and IL-17, but not IFN-γ by ELISA. Phenotyping inflammatory cells by flow citometry, neither neutrophils nor dendritic cells were altered in the absence of lipid mediator. Interestingly, the genetic ablation of 5-LO reduced significantly $CD4^+$T cells-producing IL-17, but not interferes in Th1 or regulatory T cells. **Conclusion:** These data suggest that participation of LTB_4 in the control of VL may be correlated to induction of Th17 pattern of immune response. However, the mechanism by which LTB4 elicited Th17 maintains to be elucidated. Understanding of this mechanism will help development of new control strategies for the disease.

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THE P2X7 RECEPTOR CONTRIBUTES TO PROTECTION AND TO AMELIORATE THE CLINICAL MANIFESTATIONS OF BLOOD-STAGE PLASMODIUM CHABAUDI MALARIA

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Introduction: Malaria, a disease caused by the protozoan parasite Plasmodium, remains a serious healthcare problem in developing countries. The blood stage of infection is responsible for all symptoms associated with malaria, which are mostly related to excessive activation of the immune system. Recently, it has been shown that innate immune receptors are able to detect signals released by damaged cells as ATP. P2X7 receptor (P2X7R) detects extracellular ATP and therefore could contribute to activate the immune response to Plasmodium. **Methods and results:** Six-to-eight-week-old C57BL/6 (B6) and P2X7R$^{-/-}$ (B6 background) female mice were infected by intraperitoneal injection with 10^6 parasitized erythrocytes. Serum samples of B6 mice were collected before and after the erythrocyte rupture and the ATP was quantified using ATP Bioluminescence Assay Kit. ATP levels were significantly higher after the erythrocyte rupture. Erythrocytes were removed from blood cell preparations by Percoll gradient separation (70%), to assess whether the ATP released from infected and non-infected erythrocytes is able to permeabilize spleen cells. Supernatants from lysed infected erythrocytes induced an increase in permeabilization of CD4$^+$ T cells and CD11c$^+$ cells. We observed that infection by P. chabaudi is capable of up-modulating the ATP-induced permeabilization of CD4$^+$ T cells and CD11c$^+$cells. P2X7R$^{-/-}$ mice showed a deficient expansion of splenic T and B cell populations and consequent production of IFN-γ and antibodies on day 7 p.i.. Therefore, we then analyzed several clinical manifestations of the acute phase of malaria. P2X7R$^{-/-}$ mice had difficulty in controlling parasitemia and failed to reestablish body temperature and weight up to the seventh day of infection. **Conclusion**: Our results suggest that the recognition of extracellular ATP by P2X7R contributes to parasite control and to ameliorate the clinical manifestations of blood-stage Plasmodium chabaudi malaria.

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THE ROLE OF CRYPTOCOCCUS NEOFORMANS CAPSULE AND PHOSPHOLIPASE B1 IN MODULATION OF EOSINOPHILS ACTIVATION

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Introduction: Cryptococcus neoformans is a human pathogenic yeast, which causes pneumonia and granuloma formation, with high levels of eosinophils in systemic circulation and tissues, in immunocompromised individuals. Its capsule is a well known virulence factor responsible for evade immune system. C. neoformans is also capable of secreting phospholipase B1 which is essential for its dissemination to central nervous system. Furthermore, C. neoformans phagocytosis by eosinophils followed by cytokine release has been reported in infected mice. However, the modulation of eosinophils lipid metabolism by C. neoformans and the role of its capsule and phospholipase B1 in this process remain unclear. Here we investigate the role of C. neoformans capsule and phospholipase B1 in the activation of eosinophils, investigating the lipid body biogenesis and the role of DP1, DP2 and PGD_2 synthase in this process.

Methods and Results: Human eosinophils were incubated for 2 h at 37°C with C. neoformans strain B3501 and its acapsular mutant Cap67, and strain H99 and its deleted mutant and reconstituted yeasts for phospholipase B1. In addition, eosinophils were stimulated with the capsule components GXM and GalXM, and yeast-derived lipid extract. In order to characterize the signaling pathway involved in eosinophil activation, cells were pre-treated with inhibitors of PGD synthase (HQL-79), DP1 (BWA868C) and DP2 (Cay10471). Biogenesis of lipid bodies was determined by flow cytometer and confocal microscopy analysis. The experiments were done with 5 donors. The acapsular mutant triggered, while the wild type inhibited lipid body formation in human eosinophil. Phospholipase B1 deleted H99 also decreased lipid body formation compared to its wild type. Pre-treatments of eosinophils prior to infection showed that PGD synthase does not participate in lipid body biogenesis. Cap67 and H99 deleted strain triggered DP2 dependent lipid body formation. Capsule components failed to induce, whereas B3501-derived lipid extract participates of the inhibition of lipid bodies.

Conclusions: Our results showed that C. neoformans are capable of direct activation of human eosinophils, triggering DP2 dependent and PGD synthase independent lipid body biogenesis. Taken together, our findings suggest that C. neoformans capsule and its major components, along with its phospholipase B1 and total lipid extract may play an important role in immune response evasion by this fungus.

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THE ROLE OF LIPID ANTIGEN PRESENTATION THROUGH CD1 MOLECULE IN FUNGAL INFECTION

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Introduction: Fungi represent important pathogens in immunocompromised patients. Fungal diseases can arise from either a lack of recognition by the immune system or overinflammatory response activation. Therefore, antigen molecules recognition is important to trigger the immune response. The remarkable discovery of CD1 antigen presentation pathways provides a new perspective on microbial immunity by showing how T cells can recognize lipid antigens from pathogens as well as self-lipids. Here we evaluated the modulation of CD1 expression in fungal infections caused by Paracoccidioides brasiliensis (Pb) and Cryptococcus neoformans (Cn).

Methods and Results: In order to verify if the fungi can induce the expression of CD1 and modulate the infection profile, we infected human monocytes to analyzed the Group1 I CD1 molecules (CD1a, CD1b and CD1c) and murine peritoneal macrophage to investigate Group II CD1d molecule expression with isolated a virulent strain of PB (PB18), a non virulent strain of PB (PB265), the capsulated strain of Cn (B3501), and its mutant acapsular Cn (CAP67), and a other serotype of Cn (H99). We analyzed: (I)The expression of CD1a, Cd1b, Cd1c and Cd1d by Flow Cytometry and (II) Confocal microscopy to view the expression of CD1a and CD1d, and (III) infected mice C57BL/6 with different fungal isolated and to analyze CD1d expression in lung by histopathology. Our data suggested that P. brasiliensis and C. neoformans virulent strains could reduce the expression of CD1 molecules. The capsulated strain of Cn triggered a down regulation of all CD1 isoforms compared to acapsular strain which induced significant higher levels of CD1a expression. In addition, the CD1 expression during infection is also correlated to the pattern of virulence of fungi isolates.

Conclusion: Taken together our data demonstrated that the fungi P. brasiliensis and C. neoformans developed a mechanism to evade immune response by reducing the expression of CD1 and consequently the TCD8+ activation, which can play an important role in pathogen survival.

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THE ROLE OF LIPID DROPLETS BIOGENESIS IN PARACOCCIDIOIDES BRASILIENSIS INFECTION

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Introduction: The dimorphic fungus Paracoccidioides brasiliensis is the etiological agent of Paracoccidioidomycosis, a human systemic granulomatous disease that affects mainly the rural population of South America. The initial inflammatory and innate immune response caused by this fungus is still poorly understood. The present work aimed to investigate whether the inflammatory activation marker lipid droplets are involved in the differential immune response triggered by virulent and low virulent fungi P. brasiliensis. In addition, we analyzed the immunomodulatory properties of lipids from P. brasiliensis cell wall. **Methods and Results**: In order to verify whether two different strains of P. brasiliensis, a virulent (PB18) and low virulent (PB265) are capable of inducing different lipid droplets formation in vivo, C57BL/6 mice were intratracheally infected with P. brasiliensis yeast cells. After 30 days (chronic infection) the presence of lipid droplet biogenesis was analyzed in pleural and peritoneal lavage by flow cytometry, as well as in the lung tissue by immunohistochemistry. Our results demonstrated that the high virulent fungus strain Pb18 induced significantly higher number of lipid droplets in vivo compared to low virulent fungus strain Pb265 in all conditions analyzed. We next investigated the cellular and molecular mechanisms involved in lipid droplets formation induced by P. brasiliensis in vitro. Peritoneal macrophages were previously treated or not with different pharmacological inhibitors: GW9662 ($PPAR_{\gamma}$ antagonist), JSH-23 (Inhibitor of NFκB), C75 (inhibitor of fatty acid synthase), Metil-b-ciclodextrin (disruptor of lipid-rafts), Piceatanol (tyrosine kinase Syk inhibitor), and lipid droplet biogenesis was analyzed by flow cytometry, In addition, CFU assay was also performed in order to verify the role of the molecules involved in lipid droplet formation in the yeasts killing. Lipid droplet biogenesis was dependent of the integrity of lipid rafts, fatty acid synthase, tyrosine kinase Syk, and $PPAR_{\gamma}$. It was independent of the transcription factor NFkB. Furthermore, P. brasiliensis killing was dependent of a functional fatty acid synthase.

Conclusion: Taken together our data that P. brasiliensis triggered lipid droplets formation in a specific pathway, that can be involved in fungus killing, which may be therefore a potential target of pharmacological intervention against infection by this pathogenic fungus.

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THE ROLE OF OXIDATIVE STRESS IN THE BIOLOGY OF TRYPANOSOMA CRUZI

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Introduction: In vitro, T. cruzi is readily uptaken by macrophages and triggers respiratory burst. However, little is known about the role of ROS in T. cruzi infection. The main consequence of oxidative stress is the formation of DNA lesions, which can result in genomic instability and lead to cell death. Guanine is the base that is most susceptible to oxidation and 8-oxoguanine (8-oxoG) is the most common and deleterious lesion. The so-called GO-system is a three-component 8-oxoG repair pathway. In bacteria, MutT, MutY and MutM constitute this system. **Objective:** The aim of this study was to investigate the importance of 8-oxoG during parasite infection of mammalian cells and to investigate the parasite burden in macrophages from C57BL6 wild-type mice (C57BL/6) and from mice deficient in NADPH phagocyte oxidase (phox KO). **Methods and results:** Parasites overexpressing the TcMTH enzyme (homologous to MutT of bacteria) and heterologously expressing Escherichia coli MutT were used to infect macrophages. Macrophages isolated from murine peritoneal cavity of C57BL/6 and phox KO were infected with culture trypomastigotes of wild-type and recombinant parasites for different times and the parasite burden was analyzed by optical microscopy. Our results demonstrate that both macrophages uptook parasites similarly. Both wild-type and recombinant parasites had the same capacity of infecting macrophages. The modified parasites (MutT and TcMTH) presented enhanced replication inside murine inflammatory macrophages from C57BL6 mice when compared with control parasites. Interestingly, when phoxKO macrophages were infected with these parasites, we observed a decreased number of all parasites when compared with macrophages from C57BL/6. In phox KO macrophages we did not observe exacerbation of the infection. **Conclusions:** Our results indicate a paradoxical role for ROS. In large quantities, ROS can cause damage to parasites and our results highlight the importance of the 8-oxoG repair system for cell viability, since recombinant parasites were more successful in C57BL6 macrophages. On the other hand, ROS also can function as an important signal to induce the proliferation of parasites, since the multiplication of the parasites becomes reduced in macrophages devoid of ROS produced by phox. This study indicates that ROS contributes to T. cruzi growth inside macrophages and increases overall parasitism.

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THE TNFRP55 MODULATE THE INFLAMMATORY RESPONSE IN LEISHMANIA AMAZONENSIS INFECTION

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Introduction: The cytokine tumor necrosis factor (TNF) is required for resistance to several pathogens, such as Listeria monocytogenes, Candida albicans, Trypanosoma cruzi and Leishmania major. One protective function of this cytokine is the ability to synergize with IFN-y to induce the expression of iNOS by macrophages, leading to NO production and the killing of parasites. Two cognate receptors for TNF have been described: the TNFR1 (TNFRp55) and the TNFR2 (TNFRp75). TNFR1 promotes cell survival and inflammation or, alternatively, can induce apoptosis. Although many studies had demonstrated that TNF plays a central role in the outcome of many infection models, the role of this cytokine in L. amazonensis infection remains to be completely understood. The objective of this study was to evaluate the role of TNFRp55 in infection by L. amazonensis.

Methods and Results: Our data did not show differences in parasite load, lesion size and production of TNF-α, IFN-γ and IL-10 by lymph node cells stimulated in vitro with the parasite antigen, between C57BL/6 wild-type and TNFRp55$^{-/-}$ mice, 8 weeks post infection. After 16 weeks, an increase in lesion size was seen in the TNFRp55$^{-/-}$ mice, but the parasite load was not different between the groups. At this time of infection, the production of TNF-α, IFN-γ and IL-10 were the same for both groups, but knockout mice showed a higher arginase activity in the footpad, which can reflect a higher inflammatory infiltration. Interestingly, at the beginnig of infection, larger lesions were seen in wild type mice.

Conclusion: These data suggest that TNFRp55 plays an immunomodulatory effect in this infection model that may be is important for the resolution of the inflammatory process, be by mediating may apoptosis, but TNFRp55 was not essential for the control of the parasite replication.

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THE USE OF LPG MUTANTS OF LEISHMANIA MAJOR TO EVALUATE THE ROLE OF THESE MOLECULES IN INNATE IMMUNE RECOGNITION OF LEISHMANIA

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Introduction: Leishmaniasis is the second largest parasitic disease in the world and is caused by parasites of the Leishmania genus. Promastigotes forms of the parasite express several glycoconjugates, which are either secreted or anchored to the parasite surface. Lipophosphoglycan (LPG) is the most abundant and plays important roles in parasite colonization of the intestinal tract of the sand fly and in infectivity and pathogenesis in the mammalian host. Furthermore, LPG is important for modulation the host immune responses to favor the establishment of mammalian infection. Up to date is no information about the recognition of Leishmania sp. LPG by innate immune receptors. Here we optimized macrophage infection with LPG mutatns of L. major to further evaluate the role of LPG for parasite and innate immune recognition.

Methods and Results: To evaluate the role of LPG in parasite phagocytosis and survival inside macrophages, we infected bone marrow-derived macrophages (BMDMs) with a L. major strain deficient either on LPG1 or LPG2 genes. We determined the internalization kinetics by counting the intracellular parasites within macrophages. This was measuring using either stationary phase promastigotes and metaciclic promastigotes. To verify if LPG is important to parasite survival inside BMDMs, we determined the percentage of infected cells and amastigotes/cell. Our results showed that the percentage of BMDMs infected with LPG2 stains after 1 or 3 hours of infection was similar to that observed in WT parasites, indicating that LPG is not essential for parasite internalization. By other hand, we found that in the absence of LPG, the percentage of infected cells was decreased, a feature that can be complemented by expression of LPG molecules in trans in the parasite mutants for LPG.

Conclusion: These data support previous observation indicating that LPG is important to parasite survival. LPG is required for parasite multiplication within host cells. In the absence of LPG, macrophages control by L. major infection, suggesting a role of this glycoprotein as immunomodulator. Subsequent, studies will be conducted on the interaction of LPG and activation of the innate immune system to better understand the complex host-parasite interaction in Leishmaniasis.

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TLR-2 AND TLR-4 EXPRESSION IN PATIENTS WITH CARDIAC FORM OF CHAGAS DISEASES - PRELIMINARY RESULTS

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Introduction: Chagas disease is a neglected disease and a serious public health problem. In Brazil, there are about 3 million people infected with higher prevalence of chronic cases. In order to have the adaptive immune response, cellular receptors, such as TLR-2 and TLR-4 are essential for the recognition and performance of effectors mechanisms against T. cruzi. Our objective was to evaluate the expression of the receptors TLR-2 and TLR-4 in patients with cardiac form of Chagas disease. **Methods and results:** The expression of receptors TLR-2 and TLR-4 in CD3 and CD14 was analyzed by flow cytometry. In CD3, the percentage of TLR4 expression was significantly higher in patients (32.05 ± 9.25) compared to the control group (0.42 ± 0.22). No differences were found in expression of TLR2 and TLR2/TLR4 coexpression in both groups. Regarding CD14, patients showed a significantly lower percentage of expression of TLR2 (48.38 ± 13.25) and TLR4 (0.17 ± 0.2) compared to the control group (98.99 ± 0.99 and 60.25 ± 30.44) respectively. There was no significant difference in coexpression of the receptors in this cell type. **Conclusion:** Our results suggest that chronic chagasic patients with the cardiac form of the disease have a higher expression of TLR-4 in lymphocytes and lower expression of TLR2 and TLR4 in monocytes, suggesting a possible involvement of these receptors in the development of the immune response.

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TLR-5, NF-KB AND IL-8 RESPONSES AND VIABILITY OF INTESTINAL EPITHELIAL CELLS CHALLENGED WITH PATHOGENIC AND COMMENSAL ESCHERICHIA COLI'S

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Introduction: Enteroaggregative Escherichia coli (EAEC) is one of the major pathogens responsible for diarrheal diseases in developing countries. Such pathogen was prevalent in studies that investigated children from low-income communities of Fortaleza, Ceara-Brazil. This study evaluated intestinal damage induced by EAEC on cell viability and innate immune response of intestinal epithelial cells. **Methods and Results:** Rat intestinal epithelial cells (IEC-6) were cultured and infection was performed with one of the following bacterial strains: EAEC 042 strain, EAEC wild type strain (isolated from a malnourished child), and Escherichia coli HS (commensal strain). IEC-6 were seeded in 96-well plates at $2,5x10^4$ cells/well and cultured for 24 hours. Cells were then infected and incubated for 3 hours, washed and gentamicin-treated. After 12, 24 and 48 hours, cell proliferation was evaluated by adding WST-1 reagent, following measurement at spectrophotometer. Transcription levels of TLR-5, NF-kB and IL-8 genes were quantified right after infection and at 6 and 12 hours later. Statistical analysis was conducted with ANOVA and Bonferroni adjustment. A significant reduction on cell viability of infected groups with EAEC 042 and wild type strains at 12, 24 and 48h was detected ($p<0.05$), while E. coli HS strain did alter viability only at 48h ($p<0.05$). For IEC-6 immune responses, IL-8 transcription was the most increased after bacterial contact (400 fold increase). NF-kB transcription was increased after contact with both EAEC 042 and wild type strains right after removal of bacteria, but not for E. coli HS, while all infected groups showed increased expression at 12 hours time point. IL-8 transcription was increased for all infected groups and TLR-5 transcription was increased after EAEC 042 and wild type strains at time zero ($p<0.05$). Interestingly, TLR-5 transcription levels were decreased after 6 hours ($p<0.05$). **Conclusion:** Acute infection with EAEC 042 and wild type strains led to worse impact than with E. coli HS strain. Reduction on transcription of TLR-5 and IL-8 genes was consistent with the removal of bacterial contact. E. coli HS did not cause increase on TLR-5 transcription levels.

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TNF-ALPHA IS REQUIRED FOR PRIMING EFFECTOR IMMUNE RESPONSES AGAINST NEOSPORA CANINUM

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Introduction: The protozoan parasite Neospora caninum has been associated to abortions in cattle since the early 1990's, and the infection leads to major economic impact to the segment. TNF-a is rapidly elicited during the acute phase of the infections. Produced mainly by activated macrophages, its actions are required for the induction of systemic inflammation. Given the importance of this cytokine during acute infectious processes, its role becomes important target for understanding the pathologies arising from clinical neosporosis. **Methods and Results:** C57BL/6 wild type (WT) and genetically deficient mice in TNF-α receptor I (p55, TNFRI-/-), and double knockout for receptors I and II (p55 and p65, TNFRI/II-/-), were infected intraperitoneally with a sublethal ($1x10^6$) or lethal dose ($3x10^7$) of N. caninum tachyzoites, by the intraperitoneal route. The animals were monitored every two days for their body weights and survival, and bled every week for serological analysis, during four weeks. We found that TNFRI-/- and TNFRI/II-/- animals showed increased survival and reduced weight loss during the acute phase of infection, if compared to WT mice. We also found a role for TNF-a in the production of immunoglobulins during chronic infection by protozoa. We observed that, regardless of the presence of TNF-α signaling, mice showed production of specific IgM to soluble antigens of the parasite. However, the recognition of antigenic targets of the parasite by specific IgG, as well as its subclasses, were severely compromised TNF receptor deficient mice. **Conclusion:** These results demonstrate the role of TNF-α signaling in the induction of acute systemic inflammatory responses and B cell class switch during infection by the protozoan N. caninum.

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TOXOPLASMA GONDII HEAT SHOCK PROTEIN 70KDA (TGHSP70) DETECTION IN THE BRAIN IS INDICATIVE OF POOR PROGNOSIS IN TOXOPLASMIC ENCEPHALITIS

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Introduction: Under stress conditions, Toxoplasma gondii tachyzoites converts to bradyzoites, and vice versa, and express TgHSP70, which is known to represent a danger signal during T. gondii infection. The aim of this work was to determine whether TgHSP70 mRNA and/or protein expression in the brain is associated with susceptibility of mice.

Methods and Results: BALB/c and C57BL/6 mice (n=5 per group) were intraperitoneal infected with 10 ME49 T. gondii cysts and sacrificed 7, 32 and 56 days post infection (d.p.i.). Another group was treated with dexamethasone (DXM, 10mg/mL in drinking water) from 32 to 56d.p.i. and then sacrificed. TgHSP70 mRNA in the brain was evaluated by qPCR, whereas tissue parasitism and TgHSP70 expression in the brain were detected by immunohistochemistry and quantified using Image J® software. Acute infected (7d.p.i.) C57BL/6 and BALB/c mice did not show any difference in relative TgHSP70 mRNA expression or in histological scores ($P>0.05$) or in protein intensity inside cysts. Nevertheless, chronically infected C57BL/6 mice presented about 20 to 40 times more TgHSP70 mRNA expression in the brain in 32 d.p.i. (9084.0 ± 4351.0 against 239.4 ± 66.4, $P<0.05$) and 56 d.p.i. (2440.0 ± 728.5 against 107.3 ± 37.8, $P<0.05$) than BALB/c mice. In accordance, chronically infected C57BL/6 mice presented higher TgHSP70 protein expression and an increased number of cysts compared to BALB/c mice. Additionally, brain cysts from C57BL/6 were strongly marked with specific anti-TgHSP70 antibody compared with those from BALB/c. Moreover, chronically infected C57BL/6 mice also presented higher histological alterations in the brain, in comparison with BALB/c mice. Treatment with DXM was able to reduce histological changes in the brain of both mouse lineages. Nonetheless, DXM-treated C57BL/6 mice presented 7 times more parasite burden (515.3 ± 245.8 against 72.8 ± 8.5, $P<0.05$) and also three times more TgHSP70 mRNA in the brain (7849.0 ± 2315 against 2440.0 ± 728.5, $P<0.05$) than untreated mice on 56 d.p.i.. This profile was not observed in BALB/c DXM-treated mice (201.7 ± 95.7 against 107.3 ± 37.8, $P>0.05$).

Conclusion: These results suggest that TgHSP70 expression and/or protein detection in the brain is an indicative of highly active infection and is related to a poor prognosis.

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TOXOPLASMA GONDII-INDUCED TH1 MEMORY CELLS AGGRAVATE EXPERIMENTAL SUBLETHAL SEPSIS

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Introduction: Several studies of parasite-host interaction have focused on a single pathogen interaction. However, in natural environment, the host is commonly exposed to multiple pathogens sequentially or simultaneously. In this context, we hypothesized that chronic infection by Toxoplasma gondii can modulate the host response against sepsis induced by Cecal Ligation and Puncture (CLP). M**ethods and Results:** To test this hypothesis, C57BL/6 mice were orally infected with 5 cysts of T. gondii and after 40 days post-infection, these mice were subjected to sub-lethal CLP (SL-CLP). We found that mice chronically infected by T. gondii were more susceptible to SL-CLP. Despite of showing improvement on bacterial killing and increased recruitment of cells to the site of infection, these mice displayed intestinal tissue damage and increased IFN-γ-producing $CD4^+$ T cells in the peritoneal cavity. These mice had also increased pro-inflammatory cytokines (IFN-γ, TNF-α, IL-6 and IL-1β) which induced increased nitric oxide (NO) production observed within 24 hours after SL-CLP. Because we observed this early Th1 activation during SL-CLP, we investigated whether these cells were primed during the T. gondii infection. To explore this point, we found that these mice had increased T. gondii-induced $CD4^+$ and $CD8^+$ memory T cells that were responsible to produce IFN-γ and TNF-α during SL-CLP. When these mice were treated with anti-IFN-γ or anti-TNF-α antibodies, we found an increased host survival. The increased pro-inflammatory cytokines are able to promote the hypotension observed during SL-CLP of mice previously infected by T. gondii. **Conclusion:** We demonstrate that chronic infection with T. gondii aggravates SL-CLP by promoting Th1 memory cells, which induce increased pro-inflammatory cytokines, leading to hypotension and predisposes to septic shock. **Financial support:** CAPES, CNPQ, FAPESP, SES-MT.

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TRYPANOSOMA CRUZI AND THEIR MOLECULES DO NOT REDUCED CELL VIABILITY OF NEUTROPHILS DURING NET LIBERATION

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Introduction: Neutrophils release extracellular traps (NETs) which are composed of DNA and granular proteins. NETs are produced by a mechanism called NETose that shows different classical signals from apoptosis and necrosis processes. These extracellular structures capture and kill several pathogens. Recent results from our group presented that parasite Trypanosoma cruzi and their soluble molecules are able to induce formation and liberation of NETs from human neutrophils. At the moment, there are no studies demonstrating the presence or absence of classical signals of cell death during NETose process induced by parasite T.cruzi. In this study, our aim was to evaluate the cell viability and the DNA degradation in human neutrophils incubated with parasite T.cruzi and their soluble molecules. **Methods and Results:** Neutrophils ($2X10^5$) were isolated from human peripheral blood and incubated with different number of parasite and different concentrations their soluble molecules by 1-4 hours at 37^oC. After the incubations, cell viability have been evaluated using MTT assay and Neutral Red. Degradation of DNA has been assayed by agarose gel electrophoresis. Through the period of generation of NETs (1-4 hours) none of concentrations of parasites or their soluble molecules were able to reduce cell viability. These results have been reproduced by MTT salt and Neutral Red assays. We also observed an increase of viable cells in positive control (neutrophils incubated with PMA) compared to negative control (neutrophils incubated with Hank´s). Analyses of agarose gel electrophoresis showed that neutrophils incubated with T.cruzi and their soluble molecules do not present degraded DNA. The same results were obtained when neutrophils were incubated with Hank´alone. In these samples, electrophoretic profile of DNA was a simple band with high molecular weight. On the other hand, apoptotic neutrophils showed several bands with different molecular weight, a classical DNA ladder. **Conclusion:**Together, our results indicate that neutrophils remain viable and do not show DNA degradation in the presence of the parasite T.cruzi or their soluble molecules, suggesting absence of death cell signals during NETosis process induced by that parasite.

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UPREGULATION OF T LYMPHOCYTE APOPTOTIC MARKERS IS ASSOCIATED TO CELL ACTIVATION DURING THE ACUTE PHASE OF DENGUE

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Dengue fever, a public health problem in Brazil, may present severe clinical manifestations as result of an increased vascular permeability and coagulation disorders. T cell activation is a critical event for an effective immune response against infection, including the production of a range of cytokines. We aim to reveal processes that modulate the virus-cell interaction, with an emphasis on cell death mechanisms. PBMCs from healthy individuals and dengue infected patients clinically classified were obtained from heparinized venous blood. Extra and intracellular staining were made by FACS. The analysis of apoptotic proteins profile expression (Human Apoptosis Kit Array) was done using PBMCs lysates obtained from patients and controls. As results, we observed up regulation of CD29 expression (CD4 Controls 33.3 ± 5.6%; DF with WS 48.9 ± 19.7% and severe dengue 51.1 ± 22.2% - CD8 Controls 28.7 ± 5.3%; DF with WS 40.8 ± 17.5% and severe dengue 52 ± 25%) and CD107a expression (CD4 controls 2.5 ± 2.1%; DF with WS 33.3 ± 15.9%; severe dengue 18.6 ± 11%; and CD8 controls 2.5 ± 2.1%; DF with WS 35.5 ± 16.2%; severe dengue 18.6 ± 11%) indicated that in patients the majority of T cells express the activation and cytotoxic phenotype. Higher frequency of CD95 was presented in T lymphocytes especially in those T cells with cytotoxic activation profile (CD4 controls 28.9 ± 8.8%; DF with WS 71.9 ± 16.4%; severe dengue 74.7. ± 15.6%; and CD8 controls 19 ± 13.4%; DF with WS 62.5 ± 29%; severe dengue 71.2 ± 20.1%). We demonstrated that both T cells subsets expressed low levels of anti-apoptotic Bcl-2 (CD4 controls 14.8 ± 8.5%; DF with WS 44.2 ± 27.2%; severe dengue 42.8. ± 21.8%; and CD8 controls 14.6± 14.6%; DF with WS 46.5 ± 32.6%; severe dengue 54.3. ± 25.2%). Corroborating these data, DNA fragmentation was observed in PBMCs from patients during dengue infection, confirms apoptosis of T lymphocytes. Analysis of the apoptosis-related proteins expression profile shown that some apoptotic molecules are over expressed in PBMCs from dengue infected patients at acute phase. Our data support virus modulation of apoptotic factors in PBMCs from patients at acute phase of disease. The immune scenario generated as a result of infection and or the virus itself may be interfering in activation and cell death. These mechanisms are relevant to understand and clarify the immunopathogenesis and disease severity. **Financial support:** FIOCRUZ - IOC/Biologia Parasitária, CNPq and FAPERJ.

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UREASE OF HELICOBACTER PYLORI AND ITS INTERACTION WITH PLATELET MEMBRANE RECEPTORS

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Introduction Helicobacter pylori is a gram-negative bacterium that colonizes the human stomach, and is considered a risk factor associated to gastric and duodenal ulcers through mechanisms not yet fully understood. Recent studies show a positive correlation between the infection with H. pylori and cardiovascular diseases. Urease produced by H. pylori (HPU) is considered a virulence factor since its ureolytic activity enables the bacterium to survive in the acidic medium of the stomach. Our group has shown that HPU induces platelet aggregation independent of its ureolytic activity, requiring ADP secretion through the 12-lipoxygenase pathway, a signaling cascade also triggered by collagen. Here our aim is to investigate the interaction of Helicobacter pylori urease with known platelet membrane receptors in rabbit platelets.

Methods and Results A recombinant HPU produced in Escherichia coli and purified by ion exchange and gel filtration chromatographies was used for the experiments. His-tagged recombinant UreA e UreB chains produced in E. coli were purified by Ni affinity chromatography. rHPU and its isolated chains were tested in rabbit platelet aggregation assay in a Lummi-aggregometer and in a SpectraMax in 96 well plates. Our results show that: rUreA alone has no effect on platelets but it inhibited collagen-induced platelet aggregation in a dose-dependent manner. rUreB caused partial inhibition of collagen-induced aggregation and also interferes with ADP-induced platelet aggregation. This peptide may be the fragment responsible for platelet aggregation induced by HPU, even though some differences in the kinetics of platelet aggregation could be seen. Platelet aggregation induce by HPU is inhibited by antibodies against glycoprotein VI (GPVI), a collagen receptor in platelets.

Conclusion Our data show that activation of platelets by HPU shares at least partially the signaling cascade triggered by collagen, a platelet physiological agonist. This newly described pharmacological property of HPU reinforces the hypothesis that this protein could play an important role in the pathogenesis of the cardiovascular diseases indirectly caused by H. pylori.

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UREASE OF HELICOBACTER PYLORI: ROLE IN INFLAMMATION

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Introduction: Ureases (EC 3.5.1.5), nickel-dependent enzymes that hydrolyze urea into NH_4 and CO_2, are present in plants, fungi and bacteria. The spirochete Helicobacter pylori is the etiological agent of gastric ulcers and is possibly involved in the development of gastric cancer. Urease produced by H. pylori (HPU) is considered a virulence factor since its ureolytic activity enables the bacterium to survive in the acidic medium of the stomach. Previous data of our group showed that HPU induces paw edema in a dose- and time-dependent manner. Here, purified HPU was evaluated for biological effects not related to its enzymatic activity. We investigated: 1) the induction of lipoxygenase expression by HPU-activated neutrophils; 2) the role of HPU on neutrophil apoptosis; 3) the chemotatic potential of HPU on human neutrophil migration; 4). the production of reactive oxygen species by HPU-stimulated human neutrophils.

Methods and Results: Recombinant urease produced in Escherichia coli was purified by ion exchange and gel-filtration chromatrographies and used to evaluate biological effects independent of its enzyme activity. Treatment of human neutrophils with HPU (100 nM) leads to a 2.4-fold increase in lipoxygenase levels, determined by immunoblotting, and a decrease (40.5% compared to control) in apoptosis. HPU is able to induce the expression of Bcl-X_L, an anti-apoptotic enzyme, and the degradation of Bad, a pro-apoptotic protein. HPU-induced neutrophil chemotaxis was 88% of that observed for fMLP (100 nM), a strong chemoattractant used as positive control. The anti-apoptotic and chemoattractant activities of HPU are abolished by AA861, a 5-LO inhibitor. HPU is also able to induce the production of reactive oxygen species by HPU-activated human neutrophils (approximately 2-fold as control).

Conclusion: These newly described pharmacological properties indicate that HPU could play an important role in the pathogenesis of the gastrointestinal disease caused by H. pylori.

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USE OF INFECTIOUS CLONE TECHNOLOGY TO STUDY THE ROLE OF A HIGHLY CONSERVED ENVELOPE PROTEIN PEPTIDE FOR DENGUE VIRUS INFECTIVITY

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Introduction: In the last years the role of highly conserved regions of structural proteins of the most divergent virus in nature to the generation of broadly neutralizing antibodies has been showed. For Influenza, some of these antibodies are against cryptic peptides of hemagglutinin, which are exposed at some time of viral cycle. Moreover, it was demonstrated that mutations in those conserved regions can impair in viral infectivity of different forms. In this context, we began to look in Dengue viruses for proteins that are functionally correlated to those found in Influenza. The Dengue virus Envelope (E) protein was chosen because it is the most abundant protein at the virion's envelope surface and also it is the major antigenic determinant.

Methods and Results: Using structural bioinformatics analysis we have found a highly conserved peptide in E protein domain II that is exposed only in the trimeric form. In order to find functions and hypothesizing that mutations in this peptide might interfere with viral infectivity, we designed mutations in its sequence that would either disrupt its secondary structure or affect possible protein/protein interactions. Moreover, a series of mutants were designed to evaluate the same region of the E protein of other members of the Flaviviridae, in the context of the DENV protein, recreating regions of West Nile Virus and Tick-borne encephalitis virus. All DNA fragments containing mutations were synthetized and inserted into the infectious clone of the DENV 1. The mutant viruses resulting from the new infectious clones will be tested in standard growth curves and whether any of these show advantages or disadvantages to infect cells, we will investigate if they interfere with viral adsorption, assembly, replication or other moments of the viral cycle.

Conclusion: Since structurally conserved regions of viruses are major targets to the development of immunotherapies and vaccines, with this study we hope to increase the knowledge regarding the function of highly conserved E protein peptides.

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