Epidemiologia - Epidemiology

EP01 - EVALUATION OF TRIPANOCIDAL AND LEISHMANICIDAL PROPERTIES OF 2,3,5 TRIMETOXIACETOFENONA CHALCONES.

Pacheco, L. K. *(Universidade Federal de Santa Catarina)* Eger, I. *(Universidade Federal de Santa Catarina e Universidade do Vale do Itajaí)*; Duarte, A. M.C. *(Universidade Federal de Santa Catarina)*; Nunes, R. K. *(Universidade Federal de Santa Catarina)*; Fermino, J. S. *(Universidade Federal de Santa Catarina)*; Yunes, R. A. *(Universidade Federal de Santa Catarina)*; Grisard, E. C. *(Universidade Federal de Santa Catarina)*; Steindel, M. *(Universidade Federal de Santa Catarina)*

Leishmaniasis and Chagas disease affect several million people in tropical and subtropical regions. The chemotherapy available for the treatment of these infections is restricted to a few drugs with limited efficacy and serious side-effects. Chalcones are compounds of the flavonoid family with a broad spectrum of biological activities. In this work we have assessed the *in vitro* activity of 28 synthetic chalcones derived from 2,3,5 trymethoacetophenone against culture forms of *Trypanosoma cruzi* Y strain and *Leishmania braziliensis* 2904 strain. Epimastigotes and promastigotes forms were grown in LIT medium (10% FBS) and Schneider′s medium (5% FBS), respectively, and had their concentration adjusted to 5x106 parasites/ml. Bioassays were carried out at 27^{o}C for 72h incubating 180μL of the parasites suspension/well in the presence of the 20μL of each compound (1 to 500μM), solubilized in DMSO. As controls, parasites were incubated with 1% DMSO or 100μM of benznidazole (*T. cruzi*) or 10μM amphotericine B (*L. braziliensis*). Activity of the compounds was assessed by counting the number of surviving parasites in Neubauer chambers. Toxicity assays were carried out by the MTT method using Vero cells. The obtained data were compared by ANOVA and CC_{50} and IC_{50} values were calculated by linear regression analysis. Two chalcones (J10 and J25) presented trypanocidal activity (IC_{50} of 33.4 and 88.8μM) and five (J06, J10, J12, J14 and J25) presented leishmanicidal activity IC_{50} range between 12.8 and 147.8μM. All active compounds revealed a reduced cytotoxicity to Vero cells ($CC_{50}>$ 278.6μM). The presented data suggests that this class of chalcones may be promising molecules for the development of new drugs for Chagas disease and leishmaniasis chemotheraphy. Antiparasitic assays of these chalcones against *T. cruzi* and *Leishmania braziliensis* intracellular amastigote forms are under investigation.
Supported by CNPq and UFSC.

EP02 - COMMUNITY ASSAY OF A VACCINE ANTI-AMERICAN CUTANEOUS LEISHMANIASIS (ACL) IN CARATINGA, MINAS GERAIS.

Mendonça Mendes, A.M.M. *(Universidade Federal de Ouro Preto - NUPEB)*; Vidal Siqueira, L.M.V.S. *(Universidade Federal de Ouro Preto - Escola de Farmácia)*; Marrocos de Rezende, S.M.R. *(Secretária Estadual de Saúde-MG)*; Cecílio de Paula, J.C.P. *(Secretária Municipal de Saúde de Caratinga)*; Santos Dias, E.S.D. *(CPqRR/FIOCRUZ/MG)*; Mayrink, W.M. *(Universidade Federal de Minas Gerais - ICB)*; Lins Machado-Coelho, G.L.L.M.C. *(Universidade Federal de Ouro Preto - Escola Famárcia)*

ACL is a parasitic disease responsible for considerable morbidity in affected individuals. In Rio Doce valley (Minas Gerais) the ethiological agent is *Leishmania (Viannia) braziliensis*. The transmission of that disease occurs mainly in peridomestic and wild environmental, impairing the vector control. For this reason, the vaccine is the best prevention means. The objective of this study was to determine the effectiveness of ACL vaccine using a community assay. The assay was carried out in localities of seven census sectors with constant ACL transmission, selected in random way to receive vaccine or placebo. The population data were obtained by census and growth rate, whereas ACL morbidity data were obtained from the registrations of the Reference Center of Leishmaniasis, in Caratinga. The analysis was accomplished in SPSS, Map Info and SatScan softwares, using UTM coordinates obtained by GPS. Results: The assay population was composed of 11,773 individuals, 4.8% of which were excluded due to LTA in the past, 6.6% for naturally positive Montenegro's test (MT) and 27.9% for refusal. Among the 6,834 individuals eligible 50,2% were male and 49.8% female, 8.9% were 1 to 4 years-old, 13.1% (5-9), 25.9% (10-20), 44%(21-59) and 8% (60-80). Differences in proportion of vaccine/placebo by sex and age groups were not observed. The conversion rate of MT was 70%, in accordance to other studies. The forecast for the end of this assay is the next epidemic loop, foreseen for 2007 March. Supported by: FAPEMIG; SES/MG; SVS/MS

EP03 - Leishmania parasites can be isolate from biopsies of LTA patients of the Center-West region of Brazil by in vitro or in vivo process.

Pires, ASP *(Universidade Federal de Goiás)*; Oliveira, JGO *(Universidade Federal de Goiás)*; Dorta, MLD *(Universidade Federal de Goiás)*; Oliveira, MAPO *(Universidade Federal de Goiás)*; Lima, GMCAL *(Universidade Federal de Goiás)*

Leishmaniasis is a vector-born protozoan disease. Occurring in several forms, the disease is generally recognized for its cutaneous form which causes non-fatal, disfiguring lesions, although the potentially fatal visceral form cause thousands of deaths. Our objective was to isolate leishmanias from biopsies of patients of the Center-West region of Brazil in culture or in experimental animals (C57Bl/6 IFN-g KO) for

further investigations. The parasites were obtained from patients with LTA attended in the HDT Hospital of Goiânia. All five isolates of Leishmania obtained were identified as L. (V.) braziliensis. The biological characterization consisted on the growth curves evaluation in vitro (5x105 parasites/ml cultivated during 15 days) and in vivo (kinetic of infection in mice C57Bl/6 IFN-g KO and BALB/c; 5x106 parasites in the paw). The curves of growth in vitro showed that the stationary phase was achieved in the 4th day for the isolates UAF5, PLR6 and WSS5, in the 5th day for the EFSF6 and in the 6th day for the HPV6. The duration of the stationary phase was similar to all isolates, finishing in the 11th day. The kinetic of infection of IFN-g deficient mice was interrupted when the footpad lesions were measureless: the mice inoculated with the isolate PLR6 or UAF5 were sacrificed in the 5th week of infection, those with HPV6 or EFSF6 in the 6th week; and with WSS5 in the 10th week. The lesions in BALB/c mice were less severe, growing up to 5th week for the majority of the isolates, except for the PLR6 that was up to 3rd week, regressing after that period. We conclude that is possible to isolate and cultivate L. (V.) braziliensis by processing biopsies in vitro as in vivo. This specie is the most common Leishmania between the patients with LTA of the Center-West region of Brazil.

EP04 - CHARACTERIZATION OF LEISHMANIA SPECIES FROM CENTRAL REGION OF BRAZIL.

BOTELHO, T.A.B. *(Lab. de Imunobiologia das Leishmanioses do IPTSP/UFG)*; BASTOS, R.P.B. *(Imunobiologia das Leishmanioses do IPTSP/UFG)*; GOSCH, C.S.G. *(Imunobiologia das Leishmanioses do IPTSP/UFG)*; PEREIRA, L.I.A.P. *(Departamento de Doenças Infecciosas e Parasitárias do IPTSP/UFG)*; PEREIRA, A.J.C.S.P. *(Hospital de Doenças Tropicais do Estado de Goiás)*; DIAS, F.R.D. *(Imunobiologia das Leishmanioses do IPTSP/UFG)*; DORTA, M.L.D. *(Imunobiologia das Leishmanioses do IPTSP/UFG)*

American Cutaneous leishmaniasis (ACL) is an endemic disease caused by at least six Leishmania species from the subgenus Viannia and Leishmania. In the Central region of Brazil autochthonous cases have been increasing, indicating the need for new control strategies. The aim of the present work was to characterize Leishmania species from patients with ACL from Central-West region. The present report describes seventy cases of ACL which were attended in the outpatient department for leishmaniasis of Anuar Auad Hospital in Goiânia, Goiás. The patients were from Goiás (75.7%), from Mato Grosso (18.6%) and Tocantins state (5.71%). Diagnostic procedures included clinical symptoms evaluation, exposure history, direct microscopy visualization of lesion biopsies, hystopathological analysis of lesion sections, immunologic methods such Montenegro skin test, detection of antibodies by ELISA and IFI and detection of Leishmania DNA by PCR. In 70 samples examined, Leishmania species characterized from 61 patients (87.5%) were identified as Leishmania Viannia, whereas in 7 (9.72%) were identified as Leishmania (Leishmania) amazonensis and in 2 patients (2.77%) PCR was negative. Direct visualization of parasites by HE staining was possible for 62 (78.68%) of samples. When immunohistochemical test was used, parasites were detected in 19 (29.23%) of 65 samples. The Montenegro skin test was positive for 54 patient (98.18%) and negative for 1 patient (1.81%). Anti-Leishmania antibodies were detected by IFI and ELISA assay in 62 (91.17%) and 65 (94.2%) serum samples, respectively. The sensibility of PCR for detection of Leishmania species was 97.1%. Species identification is crucial for the diagnosis, for clarifying the epidemiology of the disease, for the correct prognosis, for leading treatment regimes and for control measures. Financed by CNPq/PADCT and CAPES.

EP05 - MOLECULAR DIAGNOSIS OF CANINE VISCERAL LEISHMANIASIS AND THE Leishmania SPECIES IDENTIFICATION BY PCR-RFLP

QUARESMA, P. F. *(CPqRR)*; MURTA, S. M. F. *(CPqRR)*; FERREIRA, E. C. *(CPqRR)*; LIMA, A.C.V.M.R. *(CPqRR)*; GONTIJO, C. M. F. *(CPqRR)*

Considering the importance of the domestic dog in *Leishmania* transmission cycle, in this study we investigated the efficacy of PCR for canine visceral Leishmaniasis (CVL) diagnosis. We analyzed samples from skin, blood and bone marrow from 217 dogs living in Belo Horizonte/MG/Brazil, an endemic area for CVL. These dogs were clinically classified as: asymptomatic (73) and symptomatic (144). The presence of the parasite in clinical samples was investigated by amplifying a 120 bp fragment of *Leishmania* conserved region of kDNA, which is present in all species of this genus. Our results showed that the PCR was specific and sensitive being able to detect DNA fragments up to 0.1fg. PCR results were compared with those from the conventional parasitological and serological examinations, previously carried out in our laboratory. Out of the 73 asymptomatic dogs, 94% showed to be positive through PCR analysis in at least one of the clinical samples. Of the seronegative asymptomatic dogs, 93% showed to be PCR-positive in some of the tissues and, finally, out of the animals with negative parasitological examination, 88% were PCR-positive. Among symptomatic dogs, 98% of the dogs showed to be PCR-positive, out of which 97% of the seronegative dogs were PCR-positive, and 96% with negative parasitological tests showed to be PCR-positive in at least one of the clinical samples. PCR-RFLP KDNA was employed with aim toward species identification. All one hundred twenty three positive PCR amplifications submitted to RFLP, showed to be positive for *L. chagasi*. Our results suggest that the prevalence of *Leishmania*infection might be underestimated when only serological examinations are performed in CVL surveys in endemic areas. Such findings point to the need for a more sensitive diagnosis method to be used in endemic areas in which there are a large number of asymptomatic animals.

EP06 - Identification of *Leishmania sp.* from autochthonous human cases of American Cutaneous Leishmaniasis (ACL) in southern Brazil by PCR-RFLP.

Scholl, D. *(Universidade Federal de Santa Catarina)*; Eger, I. *(Universidade do Vale do Itajaí)*; Rossetto, A. L. *(Universidade do Vale do Itajaí)*; Specht, C. M. *(Universidade do Vale do Itajaí)*; Mattos, M. S. *(Instituto de Pesquisa Clínica Evandro Chagas)*; Grisard, E. C. *(Universidade Federal de Santa Catarina)*; Steindel, M. *(Universidade Federal de Santa Catarina)*

The first cases of autochthonous ACL were described in 1990 in the western region of the Santa Catarina State (SC). A few years later, a foci of ACL was detected in the northeastern region of the State at the Piçarras municipality. Characterization of the parasites isolated from these areas revealed the presence of both *L. (L.) amazonensis* and *L. (V.) braziliensis*. Since then, an increasing number as well as an expansion of the geographical distribution of ACL cases has been recorded in SC, especially in the Itajaí River Valley, where more than 70 autochthonous ACL cases were detected since 2005. The aim of this study was to identify the *Leishmania* species in skin biopsies from 48 autochthonous ACL cases detected in 2005 by PCR-RFLP. Patients from distinct SC Municipalities (Aurora - 02, Balneário Camboriú - 11, Blumenau - 08, Brusque - 01, Chapecó - 01, Itajaí - 01, Itapema - 16, Lontras - 01, Luis Alves - 02, Maravilha - 01, Nova Trento - 01 and Piçarras - 03) were submitted clinical examinations and biopsies at reference centers and the samples collected in 70% ethanol and submitted to DNA extraction by standard phenol-chloroform. PCR was performed using primers 150 e 152 directed to the conserved region of kDNA minicircle and 5μl of the amplification products were submitted to restriction with 1U of HaeIII or AvaI for 3h at 37^{o}C as previously described. Restriction fragments were then separated in 10% poliacrylamide gels and stained by ethidium bromide. Parasites from all samples were charaterized as subgenus *Viannia* probably *L. (V). braziliensis*, suggesting that this is the predominant species in the Santa Catarina State, southern Brazil. Further biochemical and molecular characterization of isolated strains are in progress along with clinical and epidemiological studies in the area.
Supported by CNPq/UFSC.

EP07 - Evidence for a South American-Amazonian variant gene repertoire in *Plasmodium falciparum*

Albrecht, L. *(Universidade de São Paulo)*; Ferreira, RGM *(Universidade de São Paulo)*; Castineiras, C *(Universidade São Paulo)*; Fischer, W *(Universidade de São Paulo)*; Wunderlich, G *(Universidade de São Paulo)*

The *Plasmodium falciparum var* multigene family encodes the highly polymorphic *P. falciparum* erythrocyte membrane proteins 1 (PfEMP1) which are associated with the pathology of this organism. PfEMP1s have an important role in cytoadherence and immune evasion of infected erythrocytes. In this study, we analyzed the sequences of over two thousand partial *var* genes in isolates from different regions throughout the world, including sequences from almost 60 Brazilian isolates amplified and sequenced in our laboratory. 785 different Brazilian sequences, 927 from Kenya, 124 from Gabon, 106 from other African sites, 353 from Asia and 51 from Venezuela were aligned using ClustalX1.83, and the results from the Identity Matrix were employed for further analysis. Sequences with over 95% identity were considered identical. We then calculated the sequence overlap index (SOI) which contemplates the number of shared sequences between regions and the total number of sequences per region. We found the highest SOI between Venezuelan and Brazilian isolates, while the comparison of Brazilian and Kenyan isolates and Asian and Brazilian isolates showed 30 times lower SOIs when compared to Brazilian and Venezuelan isolates. These results suggest that the *var* genes from Brazilian isolates are significantly shared with those from Venezuelan isolates, while only conserved or ancestral sequences seemed to be shared between Brazilian and Asian or African isolates. We also analyzed the presence of specific conserved motifs in sequences from different regions and the result will be presented. Supported by FAPESP

EP08 - Analysis of single-nucleotide polymorphisms in the *crt-o* and *mdr1* genes of *Plasmodium vivax* among chloroquine resistant isolates from the Brazilian Amazon region

Orjuela, P. *(Depto. de Parasitologia, Instituto de Ciências Biomédicas, USP)*; Simões, F. *(Fundação de Medicina Tropical do Estado do Amazonas)*; Chechuan, Y. *(Fundação de Medicina Tropical do Estado do Amazonas)*; Costa, M. *(Fundação de Medicina Tropical do Estado do Amazonas)*; Yamamoto, M. M. *(Depto. de Parasitologia, Instituto de Ciências Biomédicas, USP)*; Koyama, C. F. *(Depto. de Parasitologia, Instituto de Ciências Biomédicas, USP)*; Alecrim, G. *(Fundação de Medicina Tropical do Estado do Amazonas)*; del Portillo, A. H. *(Depto. de Parasitologia, Instituto de Ciências Biomédicas, USP)*

Chloroquine resistance (CQR) is threating to use this cheap and non-toxic drug as the first-line treatment against *Plasmodium vivax*. CQR in *P.falciparum*, the most deadly species, have been previously associated with mutations in the *pfmdr1* and *pfcrt* genes. Orthologues of these genes, *pvmdr1* and *pvcrt-o*, have been described in *P.vivax*, but to date few studies associating mutations in these genes with the CQR phenotype have been conducted. In this work we report a single-nucleotide polymorphisms (SNPs) analysis of *pvmdr1* and *pvcg10* among CQR isolates from the Brazilian Amazon region. Our analysis included both translated and 5′ and 3′ untranslated regions (UTRs) of these genes in order to test the possibility that, besides SNPs, changes in the expression levels of these genes due to mutations in

regulatory regions, may be associated to CQR. CQ sensitive and resistant isolates were obtained from a control study conducted at FMT - AM during September 2004-February 2005, in Manaus, Brazil. At the day of parasitological recurrence of the cases, the criteria to define CQR among isolates from this study considered the dosage of CQ plasma levels exceeding the minimal effective concentration for sensitive parasite strains (MEC-$\geq$10ng/ml), according to the WHO 28-day Protocol of Efficacy of Chloroquine for the Treatment of *P.vivax* Malaria. Thus, these parasites are unarguably resistant to CQ. Three samples of CQ sensitive and CQR parasites were used for genomic DNA extraction. *pvmdr1* and *pvcrt-o* open reading frames, 5′ and 3′ UTRs were amplified by PCR, cloned and sequenced. Chromatograms were evaluated and assembled into contigs which were screened for SNPs. No association was found between CQR phenotype and SNPs in *pvmdr1* coding regions. SNP analysis of *pvcrt-o* and 5′ and 3′ UTRs of both genes is currently being conducted in order to determine whether there are associations among mutations and CQR parasites.

EP09 - COCCIMORPH: a real-time diagnostic tool based on automatic image recognition of protozoan parasites of the genus *Eimeria*

Castañón, C.A.B. *(Universidade de São Paulo)*; Fernandez, S. *(Laboratório Biovet S.A.)*; Fraga, J.S. *(Universidade de São Paulo)*; Costa, L.F. *(Universidade de São Paulo)*; Gruber, A. *(Universidade de São Paulo)*

Coccidiosis of the domestic fowl is an enteric disease caused by seven species of the genus *Eimeria*. Species discrimination is classically performed using morphological features or PCR-based assays. Here we describe an approach of automatic feature extraction for shape characterization of the distinct species. We used digital images of oocysts, a round-shaped stage presenting inter-specific variability. Three groups of features were used: curvature characterization, size/symmetry, and internal structure quantification. Species discrimination was performed with a Bayesian classifier using Gaussian distribution. A database comprising 3891 micrographs was constructed and samples of each species were employed for the training process. The classifier presented an overall correct classification rate of 85.75%. A standalone program was implemented in C^{++} language and installed on a web server. Thus, a remote user can access a web page, upload an image, and obtain a real-time electronic diagnosis. We foresee that such a system would allow for a reliable diagnosis, with no need of biological sample transportation between the farms and a reference laboratory. This represents an important achievement, since live sample traffic may potentially represent a threat of disease dissemination. Compared to other diagnostic approaches, our system does not require personnel with skills on parasite identification or molecular biology techniques. The incorporation of other parasites to the system may increase the scope of applicability of this electronic diagnostic tool. Coccidian protozoa and helminth eggs, by presenting morphology similar to *Eimeria* oocysts, are the obvious candidates. Considering the decreasing prices of high resolution digital cameras, our system is relatively cheap. In fact, a microscope with a camera and an adapter tube represents the minimum required apparatus. Concluding, we believe that COCCIMORPH demonstrates the feasibility of using computer-assisted systems to provide an interesting alternative for the rapid diagnosis of parasites.

Support: CAPES and CNPq **E-mail:** argruber@usp.br

EP10 - Microsatellite markers of *Eimeria acervulina*, *E. tenella* and *E. maxima* reveal a high genetic homogeneity across strains of different geographic sources

Viotti, A.P. *(Universidade de São Paulo)*; Fernandez, S. *(Universidade de São Paulo)*; Sobreira, T.J.P. *(Universidade de São Paulo)*; Solis, J. *(Laboratório Biovet S.A.)*; Gruber, A. *(Universidade de São Paulo)*

Chicken coccidiosis is caused by seven species of the genus *Eimeria*. Molecular assays are available for species discrimination, but strain differentiation is rather complex due to the lack of reliable discriminative features. This work aimed at developing microsatellite markers for the intra-specific discrimination of the three most relevant *Eimeria* species: *E. acervulina*, *E. tenella* and *E. maxima*. Potential candidate *loci* were selected from the *E. tenella* Genome Project and from *E. acervulina* and *E. maxima* ORESTES sequences generated in our laboratory. The sequences were analyzed with the program Tandem Repeats Finder, and the best candidates selected using TRAP. From a total of 57 markers tested for *E. acervulina*, 14 presented both species-specificity and intra-specific polymorphism, with an average of 3.3 alleles per *locus*. For *E. tenella*, 99 markers were screened, yielding 21 polymorphic *loci*, with 2.4 alleles per *locus*. In case of *E. maxima*, work is still underway and we have obtained so far a total of 11 polymorphic markers. These microsatellite markers allow for the theoretical differentiation of more than 53-million haplotypes for *E. acervulina*, 4.5-million for *E. tenella* and 18,000 for *E. maxima*, respectively. No correlation was observed between the geographical origin and the genetic distance of the strains. The allelic polymorphism and heterozygosity found were low, resulting in an overall low genetic diversity. This highly homogeneous genetic structure of isolates from different continents is noteworthy and is probably due to multiple factors. The intensive use of a limited set of anticoccidial drugs and chicken lineages along decades, together with the use of highly standardized conditions for nutrition and bird rearing, may have contributed to select genetically similar parasites. Microsatellite genotyping of *Eimeria* isolates from wild birds, not submitted to drugs or intensive poultry production conditions, may clarify this point.

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S/A

EP11 - Chagas disease epidemiological study at 'El Maco', Sucre State, Venezuela.

Figuera, L. *(Instituto de Investigaciones en Biomedicina y Ciencias Aplicadas-UDO)*; Herrera, L. *(Instituto de Zoología Tropical-UCV)*; Carrasco, H. *(Instituto de Medicina Tropical-UCV)*; Martínez, C. *(Instituto de Medicina Tropical-UCV)*; Zambrano, E.A. *(Instituto de Biomedicina-UCV)*; Espinoza, R. *(IVSS, Hospital Miguel Pérez Carreño)*; Marchan, E. *(Instituto de Investigaciones en Biomedicina y Ciencias Aplicadas-UDO)*

The Official control program of Chagas disease in Venezuela was abandoned resulting in an actual increase of transmission (Anez et al., 2003, Bol. Malariol. San. Amb. Vol. 43). San Pedro is a rural village of Sucre (northeastern Venezuela) with human seropositivity for *T. cruzi* (25%) and *Panstrongylus geniculatus* infestation of human dwellings with 68% (15/22) of TcI bugs infection (Figuera *et al.*, 2004, 2005, XXXI and XXXII Annual Meeting on Basic Research in Chagas Disease). In June 2006 we carried out a re-visitation of El Maco community (San Pedro-riverside area at 324 slm) where 67% of the people are less than 20 years old. Peridomestic triatomines and reservoirs were captured with community participation in two nights (50 Tomahawk mammals traps overnight-capture effort of 1/5 for night) 20-30 km away dwellings including xenodiagnosis over mammals. Six *P. geniculatus* attracted by light were collected, 3 of them positives for *T. cruzi*. 2 *Didelphis marsupialis*, 1 *Proechymys* sp and 1 *Rattus norvegicus* were captured. Mean parasitemia in NMRI mice (1.4 x 10^4 trypomastigotes/ml blood) was obtained from IP inoculation of 1.5 x 10^5 flagelates/ml of intestinal contents of *P. geniculatus* and *Rhodnius prolixus* of positive xenodiagnosis from 1/2 trapped *D. marsupialis*. These findings revealed the occasional appearance of *P. geniculatus* without domestic colonization. Peridomiciliar enzootia by *T. cruzi* maintained by *P. geniculatus* and *D. marsupialis* could be confirmed by parasitological analysis in the murine model. These results revealed risk factors for active transmission of Chagas disease at El Maco. Molecular characterization of *T. cruzi* subpopulations from vectors and reservoirs and human serological evaluation are in progress (Grant: FONACIT No. G-2005000827).

EP12 - Serology as tool to identify invasive amoebiasis in endemic areas

Fernandes, HC *(Universidade Federal de Minas Gerais)*; Pires, EM *(Universidade Federal de Minas Gerais)*; Busatti, H *(Universidade Federal de Minas Gerais)*; Costa, JO *(Universidade Federal de Minas Gerais)*; Povoa, MM *(Instituto Eveandro Chagas, Belém)*; Silva, MCM *(Instituto Evandro Chagas, Belém)*; Gomes, MA *(Universidade Federal de Minas Gerais)*

Entamoeba histolytica infect the human gut. Generally it lives in the lumen without producing symptoms. In 10% of infections, amoebae invade the tissue producing necrosis responsible for colitis behind another dyspeptic symptoms. There, through the blood, the amoebae disseminate to other organs, and the most common is the liver. The symptoms are similar to other liver infections. The difference between invasive (symptomatic) and non-invasive (asymptomatic) disease is important to treatment schedule definition. Microscopy is the main diagnostic to confirm amoebiasis suspicion. However, it is not conclusive. *E. dispar*, a non pathogenic amoeba, similar to *E. histolytica* is frequently found infecting the man. These amoebae are only distinguished from each other by molecular tools, which have been in standardization process yet. Identification of stool antigens constitutes a secure method to distinguish both amoebae. In spite of this, it does not distinguish invasive from non-invasive infection. In this context, the serology could be a good tool to clarify the invasive infection etiology. Nevertheless, the power of discrimination of serology in endemic areas can be reduced, resulting to false positives. In this study, the serology was evaluated to identify invasive infections in patients from Belém, a well-known endemic area of amoebiasis. It was used 84 sera from patients with or without intestinal symptoms and 3 with suspect of hepatic amoebiasis. These sera were processed to ELISA. The plates were coated with 0.8 ug of antigen pool obtained from 5 *E. histolytica* strains. Among a total of 87 sera, 13 presented positive serology, all of these were symptomatic ones. Ten patients presented intestinal amoebiasis and 3 hepatic one. No asymptomatic case was positive to serology. These results suggest that serology is a good tool to identify *E. histolytica* invasive infections even, in endemic areas, which contributes to the success of the treatment.

Imunologia - Immunology

IM01 - Comparative study of the IgG isotype-specific immune response against *Leishmania mexicana* in Venezuelan patients with localized and diffuse cutaneous leishmaniasis

Paniz-Mondolfi, A. E. *(Instituto de Biomedicina)* Pérez-Alvarez, A. M. *(Instituto de Biomedicina)*; Rodríguez, V. *(Instituto de Biomedicina)*; Ulrich, M. *(Instituto de Biomedicina)*; Convit, J. *(Instituto de Biomedicina)*

Leishmaniasis comprises a wide spectrum of diseases caused by protozoan parasites belonging to the Leishmania genus. Clinical and immunological features of this affectation present diverse characteristics, being localized cutaneous Leishmaniasis (LCL) the benign pole of the disease while diffuse cutaneous leishmaniasis (DCL), often of difficult clinical manipulation, represents the malign pole of this parasitosis. By Western Blot analysis, we evaluated sera from patients with LCL as well as with DCL, with the aim of assessing the specific pattern of IgG-isotypes recognition against a *Leishmania mexicana* homogenate. Vast majority of antigenic elements ranged between 50 to 220 kDa, when total IgG was evaluated. Conversely, both groups of patients showed elevated reactivity when IgG1 isotype was evaluated, although recognition to low molecular weight (MW) antigens ($< 50kDa$) was very poor in patients with LCL. Concerning IgG4, we found that patients with LCL had undetectable levels of this isotype in contrast with patients with DCL which presented high recognition to diverse MW antigens. When these sera were evaluated by ELISA, using formaldehyde-fixed parasites as antigen, results were very dissimilar regarding the IgG1 isotype when compared with Western Blot. We found that, as reported previously, IgG1 isotype from patients with DCL were unable to recognize formaldehyde-fixed parasite antigens in comparison with patients with LCL, which presented elevated recognition to them. The IgG4 levels in patients with LCL were very low when compared with patients with DCL, in agreement with Western Blot analysis. Although the role of antibodies in Leishmania infection is not clear, IgG4 elevated levels in patients with DCL found in our study must be investigated. Conversely, our results suggest that other than superficial antigens may be responsible of the recognition by IgG1 in patients with DCL. In addition, linear antigenic determinants may also be involved in this differential response.

IM02 - Advances in Epitope Mapping of the *Leishmania (Leishmania) amazonensis* CPB COOH-Terminal Region

Pereira, B.A.S. *(Fundação Oswaldo Cruz)*; Alves, C.R. *(Fundação Oswaldo Cruz)*

Leishmania species are important pathogens with worldwide distribution and have emerged as excellent models for studies concerning the cell-mediated immune response, having the parasite's cysteine-proteinases (CPs) been described as important components with immunomodulatory properties. Three types of CPs have been more studied: CPA, CPB and CPC. CPB has two distinctive characteristics: it presents a COOH-terminal extension of about 100 aminoacids (aa) and it is present, in the parasite genome, as multiple genes organized in a tandem array. Previous studies have shown that CPB is able to affect the balance between Th1 and Th2 immune responses in a murine model. Our current project is focused on studying the effects of CPB COOH-terminal portion from *L.(L.) amazonensis* (CysPep) in the murine infection model. We are have used publicly avaiable on-line softwares to select peptides derived from the CysPep that may interact with MHC molecules, having elected two criteria for our selection: the peptide should be able to survive proteossomal cleavage and the peptide should be able to bind to murine MHC class I (H-2) molecules. We have used the programs PAProC, NetChop and MAPP to check for capacity to survive proteossomal cleavage and the programs SYFPEITHI and RankPep to check for capacity to bind H-2 molecules. Twenty-one peptides (ranging from 8 to 11aa) were selected and are being chemically synthesized by FMOC strategy for use in biological assays in vivo. Also, computational molecular docking assays are being conducted to confirm the capacity of these peptides to bind correctly into the active site of H-2 molecules (specifically, H-2k^d, H-2k^k, H-2K^b and H-2D^b) and still be able to interact with T cell receptors. The results from this study may provide a reliable and relatively fast procedure to select molecules with immunomodulatory properties from parasites.Financial support:PAPES IV(Fiocruz/CNPq-400148/2006-4) and CAPES.Laboratório de Biol.Molecular e Doenças Endêmicas-IOC,Fiocruz-Avenida Brasil,4365-CEP-21045-900-RJ

IM03 - THE C3H/HePas MICE STRAIN AS A MODEL TO STUDY THE MECHANISM OF RESISTANCE TO *Leishmania* (*L.*) *amazonensis* INFECTION.

Carmo, E. V. S. *(Universidade Federal de São Paulo)*; Haapalainen, E. F. *(Universidade Federal de São Paulo)*; Katz, S. *(Universidade Federal de São Paulo)*; Fedeli, C. E. C. *(Universidade Federal de São Paulo)*; Santos, N. M. *(Universidade Federal de São Paulo)*; Barbiéri, C. L. *(Universidade Federal de São paulo)*

Leishmania (*L.*) *amazonensis* infection was studied in two mouse strains, BALB/c and C3H/HePas. Following disease evolution in these two strains for 10 weeks it was observed that C3H/HePas mice exhibited foot lesions significantly smaller compared to those presented by BALB/c mice. Thin sections of foot lesions from C3H/HePas mice showed a high inflammatory reaction characteristic of a granulomatous process with presence of epithelioid and plasma cells,

frequent neutrophils, and very few infected macrophages. In contrast, in BALB/c mice foot lesions there was a predominance of macrophages harboring a large number of amastigotes and very few lymphocytes and neutrophils. These results suggested the participation of innate immunity mediated by neutrophils in the resistance of C3H/HePas strain to *L.* (*L.*) *amazonensis* infection. Depletion of neutrophils was performed by administration of Rb6-8C5 antibody in C3H/HePas mice. Although peripheral blood of depleted animals presented a neutrophil reduction of 90% as evaluated by FACS, neutrophil depletion did not alter significantly the course of infection in C3H/HePas mice. These results suggest that other mechanisms may be involved in control of *L.* (*L.*) *amazonensis* infection in this murine model. CD8+ lymphocytes have been implicated in resolution of *L.* (*L.*) *amazonensis* foot lesions in vaccinated BALB/c mice (Colmenares et al., 2003, Infect. Immun. 71: 3172-3182). The possible involvement of these cells in the resistance of C3H/HePas is now considered and CD8+ depletion experiments are currently in progress. Supported by FAPESP.

IM04 - Histopathological and parasitological analysis of ear tissue biopsies from dogs naturally infected with *Leishmania (Leishmania) chagasi*, treated with liposome-encapsulated meglumine antimoniate

ARRUDA, F.C.S *(UFMG)*; MOURA, E.P. *(UFMG)*; LIMA, W.G. *(UFMG)*; SAMPAIO, W.M. *(UFMG)*; MELO, F.A. *(UFMG)*; MUDADO, M.A. *(UFMG)*; FONTES, C.A. *(UFMG)*; RIBEIRO, R.R *(UFMG)*; MICHALICK, M.S.M. *(UFMG)*; TAFURI, Wg.L *(UFMG)*

Canine visceral leishmaniasis is a zoonose caused by *Leishmania (Leishmania) chagasi* and transmitted to man and animals by infected blood sucking sand flies of the genus *Lutzomyia*. Dogs are considered the mainly domestic reservoir of the disease because they present an intense cutaneous parasitism. The aim of this study was to evaluate the impact of the treatment with liposomal formulation of meglumine antimoniate (LMA) in the intensity of inflammatory process and in the tissue parasitism. Thirty six dogs naturally infected with *Leishmania chagasi* received four i.v. doses of LMA (6,5 mg Sb/kg, n=12), empty liposomes (n=12) or untreated (n=12) at 4 days intervals. Fragments of the ear were collected before the treatment and 60 days after the treatment. The fragments were fixed in formalin (10%) and they were paraffined for histological (H&E staining) and immunohistochemical analysis (streptoavidin-peroxidase method to detect amastigotes forms of *Leishmania*). Our previous results have showed a significantly increase in the number of inflammatory cells in groups treated with LMA (p=0,0388) and empty liposome (p=0,0279), while there was no difference in control group. However, the treatment appeared not interfere in the tissue parasitism load in all groups analyzed. These results have suggested that treatment stimulates the migration of inflammatory cells for the site of inflammation, but it might not interfere in the parasitism. Financial Support: CNPq (grant 472287/01-0-NV), FAPEMIG (grant EDT-2124/03), UFMG

IM05 - Evaluation of L. chagasi, L. braziliensis or L. amazonensis freeze-thawed vaccine associated with a pCI-neo-p36(LACK) vaccine in BALB/c challenged with L. chagasi

Grenfell, RFP *(Universidade Federal de Ouro Preto)*; Marques-da-Silva, EA *(Universidade Federal de Ouro Preto)*; Souza, MC *(Universidade Federal de Ouro Preto)*; Serafim, TD *(Universidade Federal de Ouro Preto)*; Coelho, EF *(Universidade Federal de Minas Gerais)*; Fernandes, AP *(Universidade de Minas Gerais)*; Rezende, SA *(Universidade Federal de Ouro Preto)*

American visceral leishmaniasis is a chronic disease that can reach 98% of human mortality when not treated. Due to its severity and high incidence, vaccination has become an important approach to protection. Several vaccines have been tested but none of then had obtained effective protection. Amongst then, freeze-thawed antigen and DNA vaccines. It has been demonstrated that both lead to a partial protection in liver and spleen and the first one induces a protective response with IL-4 and IFN-γ while the second directs to a strong Th1 response with IFN-γ release, being able to induce a long-term cellular immunity. In our study, BALB/c mice were vaccinated with an association of two vaccines, one subcutaneous containing 50μg of *L. chagasi*, *L. braziliensis* or *L. amazonensis* freeze-thawed antigen and an intramuscular one with 100μg of pCI-neo-p36(LACK). Then the mice were challenged intravenously with 1×10^7 *L. chagasi* promastigotes at 2 or 12 weeks after booster. The DNA vaccine was constituted of LACK (*Leishmania* homologue of receptors for activated C kinase) expression gene. LACK is a 36kDa protein highly conserved in all species and life cycle stages of *Leishmania*. Initially, the freeze-thawed vaccines protection capabilities in liver and spleen was tested by quantitative limiting dilution culture 5 weeks after challenge and then was measured the production of IL-4 and IFN-γ by ELISA. Our results showed a significant protection in mice that received *L. chagasi* freeze-thawed antigen. In addition, the same protocol was applied to the association of the 2 vaccines and the results demonstrated a higher protection when freeze-thawed *L. chagasi* and pCI-neo-p36(LACK) was administered with the detection of IFN-γ and IL-4, being more significant in those sacrificed in 2 weeks after challenge and in the association of the vaccines in the comparison with the freeze-thawed alone.

Financial support: FAPEMIG

IM06 - Soluble Serine Proteases From *L. amazonensis* as Disease-promoting Components of the Intramuscular LaAg Vaccine.

Guedes, H. L. M. *(Universidade Federal do Rio de Janeiro)*; Rezende-Neto, J. M. *(Universidade Federal do Rio de Janeiro)*; Pinheiro, R. O. *(Universidade Federal do Rio de Janeiro)*; de Simone, S. G. *(FIOCRUZ)*; Rossi-Bergmann, B. *(Universidade Federal do Rio de Janeiro)*

We showed previously that intramuscular pre-vaccination with a crude promastigote lysate of *L. amazonensis* (LaAg) leads to exacerbated disease in BALB/c mice. Serine proteases (SP) play crucial roles in host-parasite interaction. In this work, we investigated the role of SP in enhanced mouse suscetibility after vaccination with LaAg. We treated LaAg with irreversible SP inhibitors (PMSF, TLCK, TPCK). We compared *in vivo* the effect of pre-immunization with LaAg and LaAg treated with SP inhibitors (LaAg-iSP). BALB/c mice were injected with two doses of 25μg of LaAg or LaAg-iSP prior to infection with fluorescent *L. amazonensis*. After infection, cutaneous hypersensitivity response and the course of infection were both monitored by the footpad thickness. At the end of experiment, the parasite loads were assessed by the fluorescent intensity of the infected footpad lysates and the production of cytokines was measured in the lesion-draining lymph node cells. Besides, we purified serine proteases from aqueous and detergent-soluble extracts of promastigotes of *L. amazonensis* using a single step with aprotinin-agarose chromatography. These were named soluble SP (S-SP) and detergent-soluble SP (DS-SP) respectively. These fractions were first evaluated *in vitro* as to their capacity to modulate cytokine production of fresh lesion-draining lymph node cells. S-SP was evaluated *in vivo* by an immunization as described above. We found that pre-immunization with LaAg-iSP depress the capacity of mice to mount a disease-associated Jones-Mote hypersensitivity response; promote the control of lesion growth, decrease TGF-β and IL-10 production opposite to observed with LaAg. In vitro, we observed that S-SP induced TGF-β in the fresh lesion-draining murine lymph node cells. *In vivo*, S-SP promoted increased Jones-Mote type hypersensitivity response and susceptibility to subsequent infection, similar to found previously with LaAg. Treatment of S-SP with SP inhibitors reduced Jones-Mote hypersenitivity response. In conclusion, serine proteases are disease-promoting component of intramuscular LaAg vaccine.

IM07 - Opsonic requirements for uptake of *Leishmania amazonensis* amastigotes by human dendritic cells.

Bosetto, M. C. *(Instituto de Biologia - Universidade Estadual de Campinas)*; Giorgio, S. *(Instituto de Biologia - Universidade Estadual de Campinas)*

Leishmania amazonensis, a species transmitted mainly in the Amazon region in Brazil, is associated with localized cutaneous lesions. In their mammalian hosts,*Leishmania* are obligate intracellular parasites that mainly reside in macrophages and dendritic cells (DCs). The parasites require cell surface molecules to ensure their recognition and uptake by the host cells, for example, the entry of *Leishmania* promastigotes is mainly mediated by complement receptors, Fc receptors and mannose-fucose receptors on macrophages surface. Less is known about the mechanism of amastigotes into human dendritic cells. In the present study, we investigated the mechanisms of entry of *L. amazonensis* amastigotes isolated from nude mice lesions (Igs are not detectable at the surface of these parasites) into human DCs, verifying in vitro what receptors or biomolecules are used for parasite binding and internalization. DCs were generated from adherent mononuclear cells in human blood and cultured for 7 days in ISCOVEs medium supplemented with rhGM-CSF and rhIL-4. On day 7, DCs were infected with amastigotes previously opsonized with healthy human serum and serum from patients with leishmaniasis. These sera were also decomplemented and depleted of antibodies. In additional experiments, amastigotes were pretreated with soluble heparin and cell cultures were preincubated with soluble mannan before infection. Opsonization of amastigotes with the sera increased in 50% the DC infection rate when compared to unopsonized amastigotes, and the depletion of serum components (antibodies and complement) decreased about 40% the entrance of the parasites into DCs. We were unable to implicate mannose-fucose receptors in uptake of *L. amazonensis* amastigotes by human DCs. These results demonstrated that uptake of *L. amazonensis* amastigotes appears to occur primarily through the Fc receptors and complement receptors. Our study help understand the *Leishmania*-human DC interaction and the immunoregulation of cutaneous leishmaniasis. Supported by CNPq and FAPESP

IM08 - CHITOSAN MICROPARTICLES AS POTENTIAL CARRIERS OF AN ANTILEISHMANIAL LACK DNA INTRANASAL VACCINE.

Pinto, E.F. *(Universidade Federal do Rio de Janeiro)*; Costa, B.L.S. *(Universidade Federal do Rio de Janeiro)*; Delmório, P. *(Instituto de Pesquisas Tecnológicas do Estado de São Paulo)*; Ré, M.I. *(Instituto de Pesquisas Tecnológicas do Estado de São Paulo)*; Rossi-Bergmann, B. *(Universidade Federal do Rio de Janeiro)*

We have previously shown the effectiveness of intranasal vaccination against cutaneous leishmaniasis in mice using LACK DNA (Pinto et al, 2004). Chitosan has attracted considerable attention as a new non-toxic polymer having favourable characteristics such as biocompatibility and mucoadhesiveness. Additionally, chitosan has a great potential for complexation with negatively charged DNA plasmids, due to its

cationic characteristics. Also, chitosan can partially protect DNA from nuclease degradation, what is potentially suitable for mucosal delivery of the antileishmanial DNA vaccines. In this work, chitosan microparticles were prepared and crosslinked with the biocompatible reactant d,l-glyceraldehyde. These microparticles were tested for LACK DNA superficial absorption and LACK DNA release kinetic in simulated biological medium. For LACK DNA absorption, 50mg chitosan microparticles and 1mg LACK-DNA were incubated by stirring (400 rpm) for 2h / 37^{o}C in pH 5.5. After formation, the microparticle suspension was centrifuged for 15 min at 14,000 rpm and the non-entrapped DNA in the supernatant was measured using a spectrometer. The amount of encapsulated DNA in the microparticles was calculated by measuring the difference between the amount of DNA added to the microparticle preparation solution and the non-entrapped DNA remaining in the aqueous phase after microparticle formation. Yields around 86% demonstrated the high complexation of the crosslinked chitosan microspheres with LACK DNA. For release kinetics, LACK DNA-loaded chitosan microparticles were incubated at 37^{o}C, pH 5.5 under stirring (400 rpm) and supernatant aliquots were collected at various times. Release kinetics showed that 10% of total added LACK DNA was released after 20min, with 90% of total DNA being released after 4hs. The high yield of complexation and the release kinetics profiles obtained in this work suggested that cross-linked chitosan microparticles are suitable mucoadhesive biodegradable carriers to improve the efficacy of the LACK DNA intranasal vaccine to be tested against cutaneous and visceral leishmaniasis.

IM09 - Dynamics of CD4$^+$ and CD8$^+$ lymphocyte subsets in dogs with visceral leishmaniasis submitted to multiple dose treatment with liposome-encapsulated meglumine antimoniate

Moura, E.P. *(Universidade Federal de Minas Gerais)*; Ribeiro, R.R. *(Universidade Federal de Minas Gerais)*; Alves, C.F. *(Universidade Ferderal de Minas Gerais)*; Lima, W.G. *(Universidade Federal de Minas Gerais)*; Sampaio, W. M. *(Universidade Federal de Minas Gerais)*; Melo, F. A. *(Universidade Federal de Minas Gerais)*; Arruda, F.C.S. *(Universidade Federal de Minas Gerais)*; Michalick, M.S.M. *(Universidade Federal de Minas Gerais)*; Tafuri, Wg. L *(Universidade Federal de Minas Gerais)*

The development of the classical picture of visceral leishmaniasis (VL) has not been completely identified, but *Leishmania* -specific cellular immune response seems to play fundamental role in the control of infection. The CD4$^+$ and CD8$^+$ T-cell response appears to be critical for the host resistance. We evaluated the impact of the treatment with liposomal formulation of meglumine antimoniate (LMA) in dynamics of CD4$^+$ and CD8$^+$ lymphocyte subsets. Mongrel dogs naturally infected with *L. chagasi* received 4 iv doses of LMA (6,5 mg Sb/kg, n=12), empty liposomes (n=12 animals) and untreated (n=12) at 4 days intervals. Peripheral blood was collected 30 and 60 days after treatment. Cells were separated per density gradient and quantified by flow cytometric analysis. The treatment with LMA had no influence in CD4$^+$T cells levels. However, the group treated with empty liposome showed lower frequency of T CD4$^+$lymphocytes than the control group, 30 days after treatment. There was no difference in frequency of T CD8$^+$ lymphocytes in the group treated with LMA. In the other analyzed groups we observed a tendency of low on the levels of T CD8$^+$ lymphocytes (60 days after treatment) although this difference has not been significant. When the CD4$^+$/CD8$^+$ relation obtained from each dog was evaluated, it was shown to be significantly higher in the control group, 60 days after treatment, compared with the group treated with LMA. We also observed that in the group treated with empty liposome, the CD4$^+$/CD8$^+$ relation increased comparing both moments of analyses. These results showed that treatment with LMA maintains high CD8$^+$ lymphocytes levels, during an enlarged period, in peripheral blood, suggesting the importance of this lymphocyte class in protective immunological response. Interesting, the treatment with empty liposome modified CD4$^+$ levels, suggesting this formulation is capable of module the immune response.

Financial support: FAPEMIG

IM10 - Immunization of dogs with a recombinant cysteine proteinase from *Leishmania* (*Leishmania*) *chagasi* (rLdccys1) in a endemic area of visceral leishmaniasis (Teresina, Piauí-Brazil)

Pinheiro, P.H.C. *(Faculdade NOVAFAPI)*; Dias, S.S. *(Universidade Federal de São Paulo/Escola Paulista de Medicina)*; Ferreira, J.H.L. *(Faculdade NOVAFAPI)*; Katz, S. *(Universidade Federal de São Paulo/Escola Paulista de Medicina)*; Barbiéri, C.L. *(Universidade Federal de São Paulo/Escola Paulista de Medicina)*

The development of a protective vaccine against canine visceral leishmaniasis represents a major goal in achieving efficient control of this widespread disease. A cysteine proteinase of 30 kDa (p30) was identified in *L.* (*L.*) *chagasi* amastigotes and shown to induce lymphoproliferative responses mediated by $CD_4{}^+$ Th1 lymphocytes and partial protection against challenge with *L.* (*L.*) *chagasi* in BALB/c mice (Pinto et al., 2000 Int. J. Parasitol. 30, 599-607). Expression of the gene encoding the p30 antigen from *L.* (*L.*) *chagasi* , *Ldccys1*, in the pHis vector resulted in a recombinant protein of 47 kDa, rLdccys1. In the present study the lymphoproliferative responses induced by rLdccys1 were evaluated in immunized dogs from Teresina, Piauí, Brazil. A group of 10 non-infected dogs was used in this study. Group 1 received 50 μL of PBS. Group 2 was immunized with 800 μg of *Propionibacterium acnes* by subcutaneous route. Group 3 was immunized with 80 μg of rLdccys1 plus 800 μg of *P. acnes*. Group 4 was immunized with 80 μg of rLdccys1. After immunization, peripheral blood mononuclear cells (PBMC)

from immunized dogs were used for *in vitro* proliferation assays. Interferon-γ (IFN-γ), interleukin 4 (IL-4) and interleukin 10 (IL-10) were measured in the supernatants from PBMC by ELISA assay. Higher stimulation indexes (SI $\simeq$ 10) were induced by rLdccys1 in PBMC from group 3 dogs compared to those from groups 1, 2 and 4. Significant levels of IFN-γ (3.5 ng/mL) and absence of IL-4 and IL-10 were observed in the PBMC supernatants from group 3 dogs. These results indicate that responses to rLdccys1 are driven toward a Th1 profile that may lead to protective immunity and encourage us to extend immunization studies with rLdccys. Supported by FAPESP and NOVAFAPI.

IM11 - SPLENECTOMY DOES NOT ALTER THE IMMUNE RESPONSE AGAINST LEISHMANIA MAJOR IN BALB/c AND C57BL/6 MICE

Maioli, T.U. *(Universidade Federal de Minas Gerais)*; Venâncio, L.F.S. *(Universidade Federal de Minas Gerais)*; Assis, F.A. *(Universidade Federal de Minas Gerais)*; Carneiro, C.M. *(Universidade Federal de Ouro Preto)*; Faria, A.M.C. *(Universidade Federal de Minas Gerais)*

The spleen is an important lymphoid organ that plays a critical role in the protection against various infectious agents. It is located in the abdomen and is the largest filter of the blood. All circulating antigens reach the spleen where a large collection of B and T cells are activated (Pabst, 1988). Splenectomy is still a common practice and several reports have shown that this surgery increases susceptibility to infection by microorganisms and decreases Th1 type responses (Karunaga, 2005; Panitsas, 2004).The aim of this study was to evaluate the immune response of splenectomized mice against Leishmania major. To test the effects of splenectomy, BALB/c and C57BL/6 mice had their spleens removed by surgery after general anesthesia and the experiments were performed one month afterwards. Mice were injected with 1x106 L.major in the footpad and lesion development was followed weekly. Differential leukocyte counts were performed and levels of serum immunoglobulins and cytokines from tissue culture supernatants were measured by sandwich ELISA. Hystological sections of tissues from different organs were also analyzed after hematoxilin/eosin staining. We observed no difference in lesion development, parasite levels (PL) nor in histological analyzes of infected footpads of splenectomized (S) as compared to control C57BL/6 (C) mice. A similar result was obtained in BALB/c C and S mice for these parameters. Blood leukocyte counts were also comparable between groups as well as serum immunoglobulin and cytokine production in cell supernatants (IL-4 and IFN-gamma) in both strains of mice. Moreover, the classical Th1 response of C57BL/6 against L. major was maintained after splenectomy, as well the Th2 response developed by BALB/c mice. Although the spleen plays an important role in protective immunity against microorganisms, but it seems to be not necessary for immune responses against L. major. Financial support: FAPEMIG and CNPq.

IM12 - Atorvastatin interferes with resistance against Leishmania major infection in C57BL/6 mice

Ferraz, F.O. *(Universidade Federal de Ouro Preto)*; Tafuri, W.L. *(Universidade Federal de Minas Gerais)*; Santos, L.A.V.D.R. *(Universidade Federal de Minas Gerais)*; Santiago, H.C. *(Universidade Federal de Minas Gerias)*; Teixeira, M.M. *(Universidade Federal de Minas Gerais)*; Afonso, L.C.C. *(Universidade Federal de Ouro Preto)*; Vieira, L.Q. *(Universidade Federal de Minas Gerais)*

Statins, 3-hydroxy-3-methyl-glutaryl coenzyme A (HMG coA) reductase inhibitors, are cholesterol-lowering drugs used in human beings and their utilization as large-scale prophylactic agents has been widely suggested. Several reports also have established that these compounds act as immunomodulatory and anti-inflammatory agents. Some statins, particularly atorvastatin, promote the inhibition of type 1 immune responses, impairing the production of IFN-γ, and the augmentation of type 2 responses, increasing the production of IL-4 and especially IL-10. The indiscriminate employment of statins may alter a patient/s immune response profile against infectious diseases, such as leishmaniasis. Resistance to infection with Leishmania major, a causative agent of cutaneous leishmaniasis in the Old World, depends on the production of high levels of IFN-γ, being characterized as a type 1 immune response.Thus, the effect of atorvastatin treatment on the immune response in a L. major-resistant strain (C57BL/6) was assessed, to assert if the drug is capable of altering the immune response profile in these mice. C57BL/6 mice were infected with $1x10^6$ stationary forms of L. major in the hind footpads and divided in two groups. After 15 days of infection, the atorvastatin treatment (10mg/Kg per o.s.) was started. The control group received phosphate-buffered saline (PBS). Lesion progression in the treated group was more expressive, presenting significantly larger lesions than its control counterpart. Accordingly, parasitism in the infection site at 4, 8 and 10 weeks post-infection was higher in the treated group. The histopathological data were compatible with the parasite titer in the lesions.Surprisingly, the atorvastatin-treated mice presented a higher production of IFN-γ in the draining lymph node. However, higher levels of IL-10 were also detected. These findings suggest that the extended treatment with atorvastatin interferes with resistance against L.major infection in mice, augmenting the production of IL-10, therefore impairing the adequate action of IFN-γ in the parasite control.
Support:FAPEMIG/ CAPES/CNPq

IM13 - COMPARATIVE EVALUATION OF PROTECTION LEVELS INDUCED BY NUCLEOSIDE HYDROLASE (NH) AND A2-DNA IMMUNIZATION IN BALB/c MICE AGAINST *Leishmania chagasi* INFECTION

Zanin, F.H.C. *(Departamento de Analises Clinicas-Faculdade de Farmacia/UFMG)*; Coelho, E.A.F *(Setor de patologia Clinica-Colégio Técnico-UFMG)*; Marques-Da-Silva, E.A. *(Laboratorio de Imunopatologia-UFOP)*; Rezende, S.A. *(Departamento de Analises Clinicas-Escola de Farmacia-UFOP)*; Tavares, C.A. *(Departamento de Bioquimica e Imunologia-ICB/UFMG)*; Gazzinelli, R.T. *(Departamento de Bioquimica e Imunologia ICB/UFMG)*; Fernandes, A.P. *(Departamento de Analises Clinicas-Faculdade de Farmacia-UFMG)*

The A2 and NH antigens are shared by various *Leishmania* species, including *L. chagasi*, *L. donovani* and *L. amazonensis* and they were shown to induce partial protection in BALB/c mice against *L. donovani* and *L. amazonensis* (A2) or against *L. donovani* and *L. mexicana*(NH). We investigated the protective efficacy of NH DNA and A2 DNA vaccines, administered isolated or in association, in BALB/c mice against *L. chagasi* challenge infection. Animals were immunized *i.m.* with 100 μg of plasmids (NH-DNA, A2-DNA or NH-DNA/A2-DNA), 3-weeks interval, and challenged endovenously, after 4 weeks the last dose, with 1x10 ^{7} promastigotes of *L. chagasi*. Control mice received saline or empty vector. IFN-γ, IL-10 and IL-4 levels were evaluated in splenocytes cultured before and 35 days after challenge infection. Parasite burdens at spleen and liver were also evaluated. A2-DNA immunized group was protected against *L. chagasi*, as indicated by a significant reduction on parasite burden at both liver and spleen. Significantly higher IFN-γ and lower IL-4 and IL-10 levels were detected in these mice. NH-DNA immunized mice produced, before challenge, significant elevated of IFN-γ and IL-4 levels. After infection, however, these mice showed a reduction in IFN-γ production and persistent IL-4 and IL-10 levels. NH-DNA/A2-DNA group was partially protected against *L. chagasi*, in spite of these animals produced, after challenge infection, high levels of IFN-γ, IL-4 and IL-10 cytokines. Our results suggest that high IFN-γ and low IL-4 and IL-10 levels are important requirements for inducing protection against American Visceral Leishmaniasis caused by *L. chagasi*. Supported by FAPEMIG, CNPq.

IM14 - ROLE OF ECOTIN-LIKE SERINE PEPTIDASE INHIBITORS OF *Leishmania major* IN PARASITE INTERNALIZATION AND SURVIVAL IN MURINE MACROPHAGES.

Faria, M. S. *(Instituto de Biofísica Carlos Chagas Filho)*; Eschenlauer, S. *(Wellcome Centre for Molecular Parasitology - University of Glasgow,)*; Santos, C. C. *(Instituto de Biofísica Carlos Chagas Filho)*; Coombs, G. H. *(Wellcome Centre for Molecular Parasitology - University of Glasgow)*; Mottram, J. C. *(Wellcome Centre for Molecular Parasitology - University of Glasgow)*; Lima, A. P. C. A. *(Instituto de Biofísica Carlos Chagas Filho)*

Bacterial ecotins are potent competitive inhibitors of Clan PA, family S1 serine peptidases such as trypsin, cathepsin G and neutrophil elastase (NE), and also inhibit enzymes that participate in the coagulation cascade. Three ecotin-like genes, *ISP*1, *ISP*2 and *ISP*3, were identified in the genome of *Leishmania major*. However, no Clan PA serine peptidases are encoded in the *Leishmania* genome, which raises the possibility that the inhibitors modulate the activity of host enzymes. *L. major ISP*2 and *ISP*3 double null mutants ($\Delta isp2/3$) were generated by homologous recombination and these parasites were used to analyse different parameters of the host-parasite interaction. $\Delta isp2/3$ promastigotes were internalized by peritoneal macrophages of either BALB/c or C57B6 mice more efficiently than wild type parasites after 3 h, but intracellular parasites failed to survive at high numbers after 72 h. The adhesion of $\Delta isp2/3$ promastigotes to macrophages at low temperature was likewise increased as compared to wild type. Furthermore, the incubation of macrophages with $\Delta isp2/3$ parasites, but not with wild type, led to an increase in the uptake of FITC-coupled latex beads. Addition of the serine peptidase inhibitor aprotinin, of recombinant ISP2 or of a selective inhibitor to cathepsin G significantly reduced phagocytosis of null mutant parasites, while internalization of wild type parasites was unaffected. Taken together, our data suggest that ISPs play important roles in modulating the activity of host serine peptidases during the interaction between *Leishmania* and macrophages.

IM15 - PROTECTIVE IMMUNE RESPONSES TO MURINE VISCERAL LEISHMANIASIS INDUCED BY A RECOMBINANT CYSTEINE PROTEINASE FROM *Leishmania (L.) chagasi*, RLDCCYS1, AND THE *Ldccys1* GENE.

Ferreira, J. H. L. *(Universidade Federal de São Paulo)*; Gentil, L. G. G. *(Universidade Federal de São Paulo)*; Fedeli, C. E. C. *(Universidade Federal de São Paulo)*; Dias, S. S. *(Universidade Federal de São Paulo)*; Katz, S. *(Universidade Federal de São Paulo)*; Barbiéri, C. L. *(Universidade Federal de São Paulo)*

The implication of a 30 kDa cysteine proteinase from *Leishmania (L.) chagasi*(p30) in partially protective cellular immune responses against homologous infection mediated by $CD4^{+}$ Th1 cells was previously demonstrated in BALB/c mice (Pinto et al., 2000, Int. J. Parasitol. 30, 599-607). Expression of the gene encoding the p30 antigen from *L.*

(L.) chagasi, *Ldccys1*, in pHis vector resulted in a recombinant protein of 47 kDa, rLdccys1. In the present study, two schedules of immunization were tested in BALB/c mice, rLdccys1 in combination with either *Propionibacterium acnes* or Bacille Calmette Guerin (BCG) as adjuvants, and a plasmid carrying the *Ldccys1* gene (pcDNA3+*Ldccys1*) followed by a booster with rLdccys1. In genetic immunization experiments animals received three intramuscular doses of 100 μg pcDNA3+*Ldccys1* plus CpG ODN with a two-week interval among them, followed by a prime-boost with 25μg rLddcys1 plus CpG ODN. Controls received PBS or empty pcDNA3. Immunization with rLdccys1 included three subcutaneous doses of 25 μg rLdccys1 plus either 200μg *P. acnes* or $1x10^6$ BCG with a week interval. Controls received either PBS or adjuvants alone. Two weeks after immunization animals were challenged with $1x10^7$ amastigotes of *Leishmania (L.) chagasi* by intravenous route. Both immunization protocols induced a significant protection against *L. (L.) chagasi* infection as evaluated by a very low parasite load in the spleen of immunized mice compared to controls. However, our results indicated that DNA immunization induced a more effective protection compared to that observed in animals immunized with the recombinant protein. Evaluation of immune responses elicited in animals immunized with either rLdccys1 or *Ldccys1* gene showed a significant secretion of IgG2a antibodies, IFN-γ, and nitric oxide, indicating a predominance of Th1 responses. Supported by FAPESP.

IM16 - Efficacy of Parenteral Vaccination with the Aqueous Extract of Leishmania. Amazonensis Promastigotes in the Prevention of Cutaneous Leishmaniosis

Rezende Neto, J.M.R.N *(Universidade Federal do Rio de Janeiro)*; Guedes, H.L.M.G *(Universidade Federal do Rio de Janeiro)*; Bergmann, B.R.B. *(Universidade Federal do Rio de Janeiro)*

Leishmaniasis is a group of diseases caused by protozoa of the Leishmania genus that affects millions of people worldwide. Different clinical manifestations are observed in infection with distinct parasite species. While L. amazonensis is the main agent of the anergic diffuse cutaneous leishmaniasis, L. braziliensis is the agent of hyperergic mucocutaneous leishmaniasis. Previously, we showed that intramuscular pre-vaccination of BALB/c mice with crude promastigote lysate of L. amazonensis (LaAg) but not of L. braziliensis (LbAg) in the absence of adjuvants leads to exacerbated disease. In the present work, we prepared aqueous extract of LaAg and LbAg by ultracentrifugation (100.000ug) and evaluated in vitro their T cell anergic activity by the MTT assay and the modulation of NO production by macrophages using the Griess method. In vivo, we evaluate the preimmunization with aqueous extract of LaAg and LbAg prior to L. amazonensis infection. We observed that the aqueous extract of LaAg significantly inhibited lymphoproliferation, at levels similar to achieved with whole LaAg. Interestingly, whole LaAg inhibited NO production by peritoneal cells, but not in the aqueous extract. In vivo, two intramuscular injections with 25fÝg of aqueous extract of LaAg increased the resistance of BALB/c mice to subsequent infection with L. amazonensis-GFP, but not of LbAg. Using 100fÝg of saponin as adjuvant, LaAg and the aqueous extracts of both LaAg and LbAg induced significant protection. Protection was accompained by increased IFN-$f^{\times}$ production in the lesion-draining lymph node cells. In conclusion, the aqueous extract of LaAg displays capacity to protect against L. amazonensis infection in the highly suscestible mice.

IM17 - Densitometry analysis using a histomorphometrical method to determine he intensity of expression of CR3 (CD11b/CD18) in spleens of dogs naturally infected with *Leishmania chagasi*.

Lima, W.G. *(Universidade Federal de Minas Gerais)*; Caliari, M.V. *(Universidade Federal de Minas Gerais)*; Michalick, M.S.M. *(Universidade Federal de Minas Gerais)*; Tafuri, W.L. *(Universidade Federal de Minas Gerais)*; Tafuri, Wg. L. *(Universidade Federal de Minas Gerais)*

Leishmania chagasi are parasites transmitted to vertebrate host by the bit of sand flies and infect cells of the mononuclear phagocytes. In Visceral leishmaniasis dogs represent the domestic reservoir of the disease. Complement receptors 3 (CR3) appears to make a quantitatively greater contribution to this interaction contributing to survive of parasite inside mononuclear vertebrate host cells. The aim this work was to evaluate a quantitative standardization method using histomorphometrical analysis in order to determine the intensity of expression of CR3 in spleen of dogs naturally infected with *Leishmania chagasi*. Asymptomatic and symptomatic mongrel dogs obtained from Sabará/MG were sacrificed with lethal dose (1ml/kg) of sodic Thiopental (2,5%) and T-61 (0,3ml/kg). Spleen fragments were collected and frozen in Tissue Medium Freezing. The streptoavidin-peroxidase immunohistochemical was carried out for determinate the CD11b/CD18. Immunolabeled cells by CD11b/CD18 were quantified by histomorphometrical analysis using KS300 software. To determinate the optical densities of CR3 expression were captured random black and white images in microcamera JVC-TK1270 with deactivated RGB signal. All images were captured by polarization in GG495 interference filter. The optical expression was determinate by the Software analyzing 200 positive cells on images using a standardized macro following basic procedures for development of a morphometric methodology. The optical density was obtained from the ratio of transmitted light to incident light at the 500 nanometros proposed by Beer's law (OD = -logI/I). Our results have showed no differences on optical density expression to CR3 proteins on spleen in different clinical groups of animals. Analyzing the quantity of expression has demonstrated a higher presence of positive CD11b/CD18 cells in symptomatic animals comparing to asymptomatic ones. Also, the spleen parasitism load was higher in symptomatic animals.

Taken together these results we could consider a parallel between the number of parasites and CD11b/CD18 positive cells.
Financial support: FAPEMIG

IM18 - CHITOSAN MICROPARTICLES AS A DELIVERY SYSTEM FOR MUCOSAL VACCINES AGAINST LEISHMANIASIS: PRÉ-CLINICAL STUDIES OF ACUTE TOXICITY AND ALLERGENICITY.

Costa, B.L.S. *(Instituto de Biofísica Carlos Chagas Filho - UFRJ)*; Pinto, E.F. *(Instituto de Biofísica Carlos Chagas Filho - UFRJ)*; Delmório, P. *(Instituto de Pesquisas Tecnológicas do Estado de São Paulo)*; Ré, M.I. *(Instituto de Pesquisas Tecnológicas do Estado de São Paulo)*; Rossi-Bergmann, B. *(Instituto de Biofísica Carlos Chagas Filho - UFRJ)*

We have previously shown the effectiveness of nasal vaccination against cutaneous leishmaniasis in mice using either free particulate leishmania antigen (LaAg) or LACK DNA. Chitosan is a mucoadhesive polymer that is able to open tight junctions and allow the paracellular transport of molecules across mucosal epithelium. It is therefore potentially suitable for mucosal delivery of the antileishmanial vaccines. In this work we prepared and tested the safety of gliceraldehide crosslinked chitosan microspheres to be used as delivery systems for both the antileishmanial vaccines. For studies of allergy the animals received 2 x 2 mg doses of microspheres either by the s.c. or nasal routes with a 7 day interval. Upon challenge with the microspheres in the footpad, the local swelling was measured. Serum IgE levels, eosinophilia and neutrophilia were measured after 48h. For studies of acute toxicity the animals received 3 x 8 mg doses of microspheres by the i.p. route on days 0, 1 and 4. On day 7, the local cell influx and nitric oxide production was measured in the peritoneal cavity. Systemic levels of TGO and TGP transaminases as well as creatinine were measured in the serum. Administration of a total of 4 mg of microspheres either by the s.c or nasal routes did not induce detectable systemic allergy, as observed by unaltered IgE serum levels and absence of eosinophilia or neutrophilia. Also, no local allergy (edema) was seen. Intraperitoneal administration of a total of 24 mg of microspheres induced only a low increase in nitric oxide production in the peritoneal cavity, but no cell influx was observed. The high load of chitosan microspheres did not affect the TGP, TGO and creatinine serum levels. These results demonstrate that microparticles of chitosan are a safe and promising delivery systems for LaAg and LACK DNA nasal vaccines against leishmaniasis.

IM19 - INFLUENCES OF ATP METABOLISM IN *Leishmania* INFECTION

Marques da Silva, E. A. *(Departamento de Ciências Biológicas / NUPEB / Universidade Federal de Ouro Preto)*; Lima Jr, D. S. *(Departamento de Ciências Biológicas / NUPEB / Universidade Federal de Ouro Preto)*; Oliveira, J. C. *(Departamento de Ciências Biológicas / NUPEB / Universidade Federal de Ouro Preto)*; Fietto, J. L. R. *(Departamento de Bioquímica e Biologia Molecular / Universidade Federal de Viçosa)*; Afonso, L. C. C. *(Departamento de Ciências Biológicas / NUPEB / Universidade Federal de Ouro Preto)*

Leishmania parasites are not capable to realize "de novo"synthesis of purine nucleus. They need to obtain these substances from ATP that is present in the extracelular medium. In this study we provide new evidences of the correlation between the *Leishmania* capability to hydrolize ATP and the establishment or maintaining of infection. First, we looked at the presence of apyrases - diphosphohydrolases well described and characterized to other trypanosomatids - in the genetic and protein expression levels in *Leishmania* species with different grades of virulence. In order to verify this, we performed RT-PCR for GDPase (ecto-enzyme) and NTPDase (soluble apyrase) that revealed the presence of mRNA to both enzymes in *Leishmania (L.) amazonensis* (PH8 strain) and *Leishmania (L.) major* (Friedlin strain) but not in *Leishmania (V.) braziliensis* (M2903 strain). In parallel, Western Blotting analysis using sera against recombinant *Trypanosoma cruzi* apyrase revealed higher expression of apyrases in *L. amazonensis*, the most virulent specie, if compared with the enzyme expression of the other two species. Since *Leishmania* species may also hydrolyze AMP and produce adenosine, an immunomodulatory substance, we tested the administration of 50 μM adenosine at the moment of *L. braziliensis* inoculation in C57BL/6 mice footpad in order to verify the effects of this substance in lesion development. This treatment slightly delayed the lesion healing, and was followed by higher production of IFN-γ by lymph node cells eight weeks after inoculum as determined by ELISA test. A higher parasite load in the foot tissue three weeks after inoculum as determined by quantitative limiting dilution culture and histology procedures was also observed in adenosine-treated mice. Together, these results corroborate the idea that ATP hydrolysis and adenosine production may favor *Leishmania* infection.
Financial Support: FAPEMIG, CNPq, CAPES

IM20 - INFLAMMATORY RESPONSE IN THE SKIN OF DOGS NATURALLY INFECTED WITH *L. (L.) chagasi.*

Ibarra, M.C.F. *(Faculdade de Medicina da USP)*; Laranjeira, D. *(Faculdade de Medicina da USP)*; Rizzardi, R.L. *(Faculdade de Medicina da USP)*; Tomokane, T.Y. *(Faculdade de Medicina da USP)*; Feitosa, M. *(Universidade Estadual de São Paulo)*; Corbett, C.E.P. *(Faculdade de Medicina da USP)*; Laurenti, M.D. *(Faculdade de Medicina da USP)*

Aiming the evaluation of dogs naturally infected with *L.(L.)chagasi* as a source of infection on the transmissibility

of the parasite to the vector, we studied the histopathological changes in paraffin skin section stained by HE; and the parasitism ,the expression of macrophage and CD3 by immunohistochemistry. Ninety-eight dogs sacrificed by the Zoonosis Control Center of Araçatuba city, São Paulo State were used. For the diagnosis, clinical features, direct parasite search in lymph nodes and spleen smears, as well as antibodies detection by ELISA were performed. According to the clinical signs, the dogs were divided in four groups: symptomatic 28,6% (28/98), oligosymptomatic 18,4% (18/98) and asymptomatic 20,4% (20/98), with positive parasite search and/or antibody detection; and negative 32,6% (32/98), with negative parasite search and antibody detection. The skin lesions were characterized by dermal chronic inflammatory infiltrate formed by macrophages, lymphocytes and plasma cells, which were present in 58% of the animals. It varied between discrete to intense, as well as focal or diffuse. The epithelioid granulomas were evident in the skin of same animals. The parasite detection in the skin by immunohistochemistry was positive in 57,1% (16/28) in symptomatic, 38,8% (7/18) in oligosymptomatic and 35,0% (7/20) in asymptomatic group. Macrophages were positive in all clinical groups with different intensities. CD3+ cells were present in 46,4% (13/28) in symptomatic, 55,6% (10/18) in oligosymptomatic and 45,0% (9/20) in asymptomatic group. From 32 animals, which present CD3+ cells in the skin, 6 showed intense parasitism, 4 moderates, 6 discrete and 16 negative. The number of CD3+ cells was inversely proportional to the number of parasites in the skin. Our data suggest that the presence of the immune competent cells in the skin could collaborate for the clearance of parasites impairing the transmissibility to the vector . Supported by FAPESP, UNESP and LIM50 HC-FMUSP.

IM21 - TOLL-LIKE RECEPTOR (TLR)-MEDIATED TRIGGERING OF MICROBICIDAL RESPONSE AGAINST *Leishmania*.

Oliveira, A. C. *(Universidade Federal do Rio de Janeiro)*; Secca, C. *(Universidade Federal do Rio de Janeiro)*; Noronha, R. *(Universidade Federal do Rio de Janeiro)*; Santiago, M. F. *(Universidade Federal do Rio de Janeiro)*; Saraiva, E. *(Universidade Federal do Rio de Janeiro)* Bellio, M. *(Universidade Federal do Rio de Janeiro)*

TLRs are key sensors of the innate immune response and different members of the TLR family recognize distinct pathogen-associated molecules. Recent data demonstrated that lipophosphoglycan (LPG), a prominent promastigote surface glycoconjugate, activates both macrophages and NK cells through TLR2. It has also been demonstrated a role for TLR3 in leishmanicidal activity *in vitro* and for TLR4 in the control of mouse infection, though the current knowledge of the function and significance of different TLRs in Leishmania infections is still very limited. In the present work, we first developed a novel methodology using flow citometry to study the interaction between *L. amazonensis* promastigotes and murine peritoneal macrophages. Using this methodology, we compared the leishmanicidal activity *in vitro* of elicited peritoneal macrophages from TLR2-null mice with their respective wt controls, C57BL/6-derived macrophages. The infection rate after 1 h of interaction between *L. amazonensis* and macrophages, at 5:1 ratio, attained the same level in TLR2-null and in wt cells. However, we found that after 24 or 48 h of culture, the percentage of infected cells was significantly lower in wt compared to TLR2 KO macrophages. The generation of reactive oxygen species (ROS) in response to infection was also evaluated and ROS levels were lower in TLR2 KO macrophages. Moreover, we demonstrated, by confocal microscopy, that the infection with *L. amazonensis* promastigotes of HEK cells, stable transfected with the TLR4/YFP and MD-2 proteins, triggers the MyD88-dependent activation pathway. Together, our results demonstrated that both TLR2 and TLR4 are involved in leishmanicidal activity against *L. amazonensis* promastigotes. Financial support: CNPq, PRONEX/FAPERJ, CAPES, FAPERJ and FUJB.

IM22 - Evaluation of chemokine production during the infection of IL-12 p40 deficient mice by *Leishmania braziliensis*

Lima Junior, D.S. *(DECBI / NUPEB / Universidade Federal de Ouro Preto)*; Marques-da-Silva, E.A. *(NUPEB/ Universidade Federal de Ouro Preto)*; Vieira, L.Q. *(Departamento de Bioqímica e Imunologia ICB / UFMG)*; Afonso, L.C.C. *(DECBI / NUPEB / Universidade Federal de Ouro Preto)*

Amongst the species causing of cutaneous leishmaniasis in Brazil, *L. braziliensis* is distinguished for being the main responsible species for this disease in the country, as well as its envolvement in the majority of the cases of mucous injuries. In this work, deficient mice in the subunidade p40 of IL-12 (IL-12p40 -/-) and C57BL/6 were infected with parasites of phase stationary and sacrificed 6 and 12 weeks of infection. At these time points we evaluate the course of infection through weekly measurement of the development of the lesion and the expression of message for chemokines in the spleen and in the lesion by Reverse Transcriptase-PCR. Lesion development was stabilized from 5 or 6 weeks of infection onwards. However, dissemination of the parasite to the spleen and the appearance of metastatic lesions in the non-injected footpads were observed by 12 weeks of infection. In relation to the production of chemokines and their receptors, a significant increase in the footpads (and to a smaller degree in the spleen) in the expression of CCL5 (RANTES), CCL10(IP-10), CXCL9 (MIG), CCR1, CCR2, CCR5 was observed in IL-12-p40-/- animals at 6 weeks of infection. On the other hand, in C57BL/6 mice we observed an increase in CCL-10, CXCL-9 and CCR5 message in the footpad, which was smaller than the presented by IL-12p40 -/- mice. An increase in CCL2 in the spleen of C57BL/6 mice at this time point was also observed. The increased expression of CCL10 and CXCL9 in the primary lesions in IL-12p40-/- mice was

maintained at 12 weeks of infection and a small increase of these plus CCL2 and CCL5 was observed in the spleen, suggesting a role for NK cells in the control of chronic infection in these animals.
Financial Support: CNPq, FAPEMIG, PIBIC-CNPq

IM23 - HYPOXIA REDUCES *Leishmania amazonensis* INFECTION IN MACROPHAGES LACKING INDUCIBLE NITRIC OXIDE SYNTHASE (iNOS$^{-/-}$)

Degrossoli, A *(Universidade Estadual de Campinas)*; Colhone, MC *(Universidade Estadual de Campinas)*; Arrais-Silva, WW *(Universidade Estadual de Campinas)*; Bosetto, MC *(Universidade Estadual de Campinas)*; Gadelha, FR *(Universidade Estadual de Campinas)*; Giorgio, S *(Universidade Estadual de Campinas)*

Hypoxia, a microenvironmental factor present in diseased tissues such as tumor and leishmaniasis, has been recognized as a specific metabolic stimulus. Experimental hypoxia has been reported to induce macrophage resistance to *Leishmania amazonensis* infection. Nitric oxide (NO) is one of the major effectors molecules for macrophage killing of *Leishmania* and previous experiments suggest no correlation between NO production and control of infection in macrophages under hypoxic conditions. In this study, we exposed peritoneal macrophages from inducible nitric oxide synthase knockout (iNOS$^{-/-}$) mice (C57Bl/6) to hypoxia (6% O2) and evaluated macrophage susceptibility to *L. amazonensis* infection, nitrite and reactive oxygen species (ROS) production, and tumor necrosis factor-α (TNF-α) release. When *L. amazonensis* infection rate was examined, iNOS$^{-/-}$ macrophages in hypoxia showed a decreased of 32% in the percentage of infected cells and a decreased of 29% in the number of amastigotes per cell as compared to cell culture under normoxic condition. Infected iNOS$^{-/-}$ macrophages in hypoxia produced only a background concentration of nitrite (2$\pm$0,16 μM) not significantly different from that produced by infected cells under normoxia (1,3$\pm$0,8 μM). Although iNOS$^{-/-}$ macrophages produced ROS when stimulated with phorbol 12-myristate 13-acetate (PMA; 2,7-fold higher level than unstimulated cells), *L. amazonensis* infected macrophages showed low ROS production under normoxic and hypoxic conditions as compared to unstimulated cells. The TNF-α release was assessed by a bioassay and results indicated that infected iNOS$^{-/-}$ macrophages produced similar quantities of TNF-α in both normoxic (0,66$\pm$0,1 U/mL) and hypoxic (0,54$\pm$0,2 U/mL) conditions. This study indicates that hypoxia induces iNOS$^{-/-}$ macrophages resistance to *L. amazonensis* infection and other leishmanicidal factors, besides NO, ROS and TNF-α, are involved in the macrophage resistance to parasite under hypoxic condition. Supported by FAPESP, CAPES and CNPq.

IM24 - Histopathological and immunocytochemical analysis of 1366 canine ear skin biopsies of animals naturally infected with *Leishmania (Leishmania) chagasi* throughout May 2003 to May 2006.

Figueiredo, M. M. *(Universidade Federal de Minas Gerais)*; Lima, W. G. *(Universidade Federal de Minas Gerais)*; Amaral, F. N. *(Universidade Federal de Minas Gerais)*; Amaral, M. E. *(Universidade Federal de Minas Gerais)*; Moura, E. P. *(Universidade Federal de Minas Gerais)*; Sampaio, W. M. *(Universidade Federal de Minas Gerais)*; Ribeiro, V. M. *(Universidade Federal de Minas Gerais)*; Michalick, M. S. M. *(Universidade Federal de Minas Gerais)*; Tafuri, W. L. *(Universidade Federal de Minas Gerais)*; Tafuri, W. L. *(Universidade Federal de Minas Gerais)*

Canine Visceral Leishmaniasis (CVL) is a zoonosis and a chronic systemic disease caused by *Leishmania chagasi* in the New World. We describe a histological picture and immunocytochemical retrospective study of canine skin ears biopsies. We analyzed the histology (inflammation) and parasite tissue load. Ears skin biopsies of a 1366 animals were provided from Clinical Veterinaries. These samples were received and processed for histological studies in the Department General Pathology (ICB/UFMG). Thus, histological and immunocytochemical analysis were carried out on paraffined skin sections. The immunocytochemistry method was the streptoavidin peroxidase for *Leishmania* detection. Our results have demonstrated 220 positive animals (16.13%). All animals showed a general chronic inflammatory reaction picture whereas the mononuclear exudate was diffuse in the upper dermis and localized mainly in the deep dermis. The exudate was mainly composed by plasma cells, macrophages and lymphocytes. In studies carried by Sollano-Gallego et al., (2004) the histopathological scenario in naturally infected dogs with L. infantum was consisted of a diffuse granulomatous inflammatory reaction. However, in our study, the presence of a granuloma formation in the dermis was not observed. On the other hand, there is a straight relation between the intensity of the inflammatory reaction and the parasitism. In fact, the statistical analysis (Spearmen correlation test) confirmed that a higher parasitism in parallel to a chronic inflammation. However, this relation was not strong based on the media coefficient relation (r^2=0,328). It means that the parasite load determine an inflammatory reaction, but not necessarily an intense inflammatory process. Also, we have concluded that is rare to find negative animals with an intense chronic inflammation or *vice and versa*.

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IM25 - Cytokines Profile in the Draining Lymphnodes of Mice Infected with *L.(L.)amazonensis* and *Lu. longipalpis* Saliva

Pernichelli, T. *(Faculdade Medicina USP)*; Secundino, N. F. C. *(Laboratório de Entomologia Médica)*; Pimenta, P.F.P. *(Lab de Entomologia Médica)*; Corbett, C.E.P. *(Faculdade de medicina USP)*; Laurenti, M.D. *(Faculdade de medicina USP)*; Tomokane, T.Y. *(Faculdade Medicina USP)*

We have shown that saliva of *Lu.longipalpis* exacerbates the*L.(L.)amazonensis* infection and that this effect is more prominent in the presence of saliva from colonized than field captured vectors. Flow cytometry analysis suggested that mice infected in the presence of saliva develop an inflammatory reaction in the early phase of infection that could facilitate parasite survival and the establishment of the infection characterized by higher number of CD14+ cells and lower of CD3+, CD4+, CD8+ cells. In order to characterize the Th1/Th2 cellular immune response by cytokine profile, C57BL/6 mice were inoculated subcutaneouslly into the hind footpads with 106 promastigotes of *L. (L.) amazonensis* in the absence or presence of half pair of salivary gland of colonized and field captured *Lu.longipalpis*. Popliteal lymph nodes were collected at 24 hours, 7, 30 and 60 days post-infection (PI); and their cellular suspensions were cultured under specific antigen stimulation. The supernatant of the cellular cultures were processed for IL-2, IL-12, IL-10, IL-4 and INF-γ Caputre ELISA. In the acute phase of infection, no significant levels of IL-2 and INF-γ were observed. At 24 hours, lower levels of Th1 and Th2 cytokines (IL-4, IL-10 and IL-12) were observed in saliva group compared to animals infected only with parasites; but at 7 days PI, IL-12 level increased in the field captured saliva group and it was similar to the level of only parasite infected group. In the chronic phase of infection no difference was observed in Th1 cytokines between the groups, but Th2 cytokines were present in higher concentration in both saliva groups compared to only parasite group. Our data showed that saliva supressed both Th1 and Th2 cytokines production in the acute phase of infection which could be responsable for the predominance of Th2 response in the chronic phase. Supported: FAPESP, LIM50/HC-FMUSP.

IM26 - An attempt to the establishment of an intradermal infection model for studies of murine visceral leishmaniasis.

Serafim, T.D. *(DECBI / NUPEB / Universidade Federal de Ouro Preto)*; Marques-da-Silva, E.A. *(NUPEB / Universidade Federal de Ouro Preto)*; Grenfell, R.F.P. *(NUPEB / Universidade Federal de Ouro Preto)*; Reis, A.B. *(DEACL / NUPEB / Universidade Federal de Ouro Preto)*; Rezende, S.A. *(DEACL / NUPEB / Universidade Federal de Ouro Preto)*

Leishmania can cause a broad spectrum of disease manifestations collectively known as leishmaniasis. In the New World, the agent responsible for visceral leishmaniasis is *Leishmania chagasi*, which can be fatal if patients are not treated. Most studies in leishmaniasis use the murine model of infection, however this model is not able to demonstrate the real mechanisms of interaction between parasite and host, because it uses the inoculation of promastigotes forms by endovenous route and this does not mimic the natural course of infection. In order to separate live organisms from cellular debris present in late log phase of culture, parasites were separated on a Percoll^{TM} gradient. These parasites were more motile and presented a more homogeneous aspect. To study the progression of infection, parasites were inoculated in the ear pinnae of BALB/c mice, animals were sacrificed on second, fourth and sixth week after infection and parasite load was estimated in liver, spleen and lymph nodes. Furthermore, their spleen and lymph node cells were stimulated with *L. chagasi* antigen to study the cytokines produced in response to the parasite. We observed that parasites were detected in the lymph nodes in all the experiments. Furthermore parasites were detected in the spleen and liver in all weeks studied but a great heterogeneity was observed among animals in each group. Thus, our results show the difficulty to reproduce the natural model of infection and improvements are being done, such as separation of metacyclics forms by a Ficoll^{TM} gradient.

Financial Support:FAPEMIG / CNPq - PIBIC

IM27 - Acylated and deacylated saponins of *Quillaja saponaria* mixture as adjuvants for the FML-vaccine against visceral leishmaniasis.

Oliveira-Freitas, E *(Instituto de Microbiologia Prof Paulo de Góes, CCS, UFRJ)*; Casas, CP *(Instituto de Microbiologia Prof Paulo de Góes, CCS, UFRJ)*; Borja-Cabrera, GP *(Instituto de Microbiologia Prof Paulo de Góes, CCS, UFRJ)*; Santos, FN *(Instituto de Microbiologia Prof Paulo de Góes, CCS, UFRJ)* Nico, D *(Instituto de Microbiologia Prof Paulo de Góes, CCS, UFRJ)*; Souza, LOP *(Nucleo de pesquisas de Produtos Naturais)*; Tinoco, LW *(Nucleo de pesquisas de Produtos Naturais, CCS, UFRJ)*; Palatnik, M *(Hospital Universitário Clementino Fraga Filho, CCS, UFRJ)*; Parente, JP *(Nucleo de pesquisas de Produtos Naturais, CCS, UFRJ)*; Palatnik-de-Souza, CB *(Instituto de Microbiologia Prof Paulo de Góes, CCS, UFRJ)*

The adjuvant of the FML-vaccine against murine and canine visceral leishmaniasis, the Riedel de Haen saponin mixture, was fractionated by ion exchange chromatography on DEAE-cellulose to afford one TLC homogeneous *Quillaja saponaria* Molina QS21 saponin fraction (18.0%), a mixture of two deacylsaponins (19.4%), sucrose (39.9%), sucrose and

glucose (19.7%), rutin (0.8%) and quercetin (2.2%), that were identified by comparison of ^{1}H and ^{13}C NMR spectroscopy. The QS21 shows the typical aldehyde group in C-23 (65% equatorial) and a normonoterpene moiety acylated in C-28. The deacylsaponins show the aldehyde group but do not have the normonoterpene moiety. Balb/c mice were vaccinated with 150μg of FML antigen of *Leishmania Donovani*and 100μg of each obtained fraction and further challenged by infection with 10^8 amastigotes of *Leishmania chagasi*. The safety analysis and the effect on humoral and cellular immune responses and in clinical signs showed that the QS21 saponin and the deacylsaponins are the most active adjuvant compounds of the Riedel the Haen saponin mixture. Both induced the highest and non-significantly different increases in DTH, CD4+ T lymphocytes in spleen, IFNy *in vitro*, body weight gain and the most pronounced reduction of parasite burden in liver (95% for QS21 and 86% for deacylsaponins; p>0.05). While the QS21 showed mild toxicity, significant adjuvant effect on the anti-FML humoral response before and after infection, and decrease in liver relative weight, the deacylsaponins showed no toxicity, less haemolysis and antibody and DTH responses increased mainly after infection, still inducing a stronger *Leishmania*-specific *in vitro* splenocyte proliferation. Our results confirm in the Riedel de Haen saponin extract the presence of deacylsaponins normonoterpene-deprivated which are non-toxic and capable of inducing a specific and strong immunoprotective response in vaccination against murine visceral leishmaniasis.

Support: PRONEX, CNPQ, FINEP, FUJB-CEPG-UFRJ, FAPERJ, CAPES.

IM28 - *Leishmania amazonensis*: Liposomes carrying membrane proteins of amastigotes are able to induce partial protective immunity to cutaneous leishmaniasis in BALB/c mice

Santos, F.R. *(Universidade de São Paulo)*; Ferraz, D.B. *(Universidade de São Paulo)*; Ciancaglini, P. *(Universidade de São Paulo)*; Ramalho-Pinto, F.J. *(Universidade de São Paulo)*

The general objective of the present study was to investigate if membrane proteins of *L. amazonensis* amastigotes could induce protective immunity to infection in BALB/c mice. To prepare crude amastigote extracts, parasites were sonicated and mixed with detergent (0.5 mg/ml of protein: 0.1% SDS). As for proteoliposomes, we have used a protocol in which lipids DPPC:DPPS:cholesterol (5:1:4 w/w) were incubated with 0.5 mg of solubilized amastigote proteins (Santos et al, 2006). To assay protective immunity, BALB/c mice received an intraperitoneal injection of 10, 20 or 40 μg of proteoliposome and challenged in the footpad with 10^6 *L. amazonensis* promastigotes. The protection was evaluated for up to 12 weeks by measuring the footpad lesions with a caliper, weekly. At 12 wk post infection, Balb/c mice immunized with 10 or 20 μg of proteoliposomes showed a maximum protection of 25 and 35%, respectively, as compared to mice that received only buffer. On the other hand, mice immunized with 40 μg of proteoliposomes displayed a maximum protection of 70% at 6 wk, decreasing afterwards to 50% at 8 wk and 40% at 12 wk. These results indicate that BALB/c mice immunized with these proteoliposomes are able to interfere with *L. amazonensis* infection in a dose-dependent manner. The fact that higher doses are more effective in inducing anti-leishmania immunity indicates that, with this protocol, by increasing the amount of proteoliposomes, we may eventually attain levels of sterile immunity. Supported by: CNPq, CAPES and FAPESP.

IM29 - Histopathological aspects of the inflammatory reaction induced by paraffin tablets and *Leishmania major* in mice AKR/J deficient in the fifth complement serum factor: a previous work.

Ferreira, V.M *(Universidade Federal de Minas Gerais)*; Melo, A.L. *(Universidade Federal de Minas Gerais)*; Tafuri, W.L. *(Universidade Federal de Minas Gerais)*; Tafuri, Wg.L. *(Universidade Federal de Minas Gerais)*

Previously work using the subcutaneous implantation of paraffin-tablets associated to *Leishmania major* experimental in mice has shown that monocytes-macrophages seems to act more as host cell than being effector cells (Tafuri et al. 2000). In this work, we experimentally induced an inflammatory model that using implanting paraffin tablets under the skin of C57BL/6 mice and C5 deficient (AKR/J mice) mice serum. The animals were separated in three groups. The first group had paraffin tablets subcutaneously implanted in the dorsal region, the second group was experimentally infected with *L. major* promastigotes and the third group received paraffin tablet (220,0mg x 1,0 cm x 0,5 cm) implantation and was infected with promastigotes of *L. major* (1 x 10 ^{7} promastigotas/0,1mL) immediately thereafter. After 15, 21 and 30 days of the implantation and experimental infection by *L. major*, the animals were sacrificed and skin and inflammatory capsule were collected for histopathology. After 21^{th} - 30^{th} day, the microscopical analysis of skin of all groups of animals in which the paraffin tablets were implanted showed a delicate granulation tissue (fibroblasts, small blood vessels and leukocytes). All animals only inoculated with *L. major* showed moderate chronic inflammatory reaction with macrophages parasitized with *L. major*. However, C5-/- mice has showing the discrete histopathological picture, with less cellular exudation. These findings might indicate that the fifth component (C5) of complement system plays in mediating the local accumulation of inflammatory cells, that the important role for the resolution of leishmaniasis cutaneous. Future studies will be done to confirm these results.

IM30 - Immunotherapy against visceral leishmaniasis with the nucleoside hydrolase-DNA vaccine of *Leishmania donovani*.

Gamboa-León, R *(Instituto de Microbiologia, Prof. Paulo de Góes", CCS, UFRJ)*; Paraguai de Souza, E *(Instituto de Microbiologia Prof Paulo de Góes, CCS, UFRJ)*; Borja-Cabrera, GP *(Instituto de Microbiologia Prof Paulo de Góes, CCS, UFRJ)*; Santos, FN *(Instituto de Microbiologia Prof Paulo de Góes, CCS, UFRJ)*; Grechi, Juliana *(Instituto de Microbiologia Prof Paulo de Góes, CCS, UFRJ)*; Myashiro, LM *(Instituto de Microbiologia Prof Paulo de Góes, CCS, UFRJ)*; Pinheiro, RO *(Instituto de Biofísica "Prof. Carlos Chagas Fo")*; Dumonteil, E *(Lab de Parasitologia, Centro de Investigaciones Regionales Dr. Hideyo Noguchi")* Palatnik-de Sousa, CB *(Instituto de Microbiologia Prof Paulo de Góes, CCS, UFRJ)*

The nucleoside hydrolase (NH36) of *Leishmania (L.) donovani* is a vital enzyme which releases purines or pyrimidines of foreign DNA to be used in the synthesis of parasite DNA. As a bivalent DNA vaccine, the VR1012-NH36 was immunoprotective against visceral and cutaneous murine leishmaniasis. In this work we tested the immunotherapy against *L.(L.) chagasi* infection, using two doses of 100μg or 20μg VR1012-NH36 vaccine (*im* route), and, as a possible immunomodulator, aqueous garlic extract (8mg/kg/day by the *ip* route), which was effective in immunotherapy of cutaneous murine leishmaniasis. Liver parasitic load was significantly reduced following treatment with 100μg (91%) and 20μg (77%) of the DNA vaccine, and by 20μg DNA vaccine and garlic extract (76%) (p=0.023). Survival was 33% for saline controls, 100% for the 100μg vaccine, and 83 and 67%, for the 20μg vaccine with and without garlic extract addition, respectively. Garlic treatment alone did not reduce parasite load (p>0.05), but increased survival (100%). The NH36-DNA vaccine was highly effective as a new tool for the therapy and control of visceral leishmaniasis, while the mild protective effect of garlic might be related to an unspecific enhancement of IFN-y secretion. **Support**: PRONEX, CNPQ, FINEP, FUJB-CEPG-UFRJ, FAPERJ, CAPES.

IM31 - The FML-vaccine Leishmune ® against canine visceral leishmaniasis: a transmission blocking vaccine.

Saraiva, EM *(Instituto de Microbiologia Prof Paulo de Góes, CCS, UFRJ)*; Figueiredo Barbosa, A *(Instituto Oswaldo Cruz)*; Santos, FN *(Instituto de Microbilogia Prof Paulo de Góes, CCS, UFRJ)*; Borja-Cabrera, GP *(Instituto de Microbilogia Prof Paulo de Góes, CCS, UFRJ)*; Nico, D *(Instituto de Microbilogia Prof Paulo de Góes, CCS, UFRJ, RJ)*; Souza, LOP *(Instituto de Microbilogia Prof Paulo de Góes, CCS, UFRJ, RJ)*; Mendes-Aguiar, CO *(Instituto de Microbilogia Prof Paulo de Góes, CCS, UFRJ, RJ)*; Paraguai de Souza, E *(Instituto de Microbilogia Prof Paulo de Góes, CCS, UFRJ, RJ)*; Fampa, P *(Instituto de Microbilogia Prof Paulo de Góes, CCS, UFRJ, RJ)*; Palatnik-de-Sousa, CB *(Instituto de Microbilogia Prof Paulo de Góes, CCS, UFRJ, RJ)*

Transmission blocking vaccines are one of the control strategies for vector transmitted protozoan diseases. Antibodies raised in the vaccinated host prevent the development of the parasite in the insect vector, interrupting the epidemiological cycle. The FML antigen of Leishmania donovani in combination with saponin (FML-vaccine and Leishmune ®) induced 92-97% of protections against zoonotic visceral leishmaniasis. We assayed the ability of FML to inhibit Leishmania donovani and Leishmania chagasi procyclic promastigote-binding to dissected *Lutzomya longipalpis midgets*. We found a dose-dependent inhibition, more pronounced on *L. donovani* (80%) than on *L. chagasi* promastigotes (p<0.001). On the other hand, the Fab-IgG serum fraction of Leishmune® vaccinated dogs (IgG2 predominant), also inhibited parasite binding in a dose-response (p<0.0001) with an equally potent effect against *L. donovani* or *L. chagasi* (p=0.061). The transmission blocking properties of the Leishmune® vaccine was also assessed by an *in vivo* membrane assay, with sand flies fed with 1.5 x 10^7 amastigotes, human blood and, vaccinated or normal control dog sera. Significantly higher values were found in rate of infection (p<0.025) and intensity of infection (number of parasites/insect) (p<0.05) of control sand flies, making a very reduced infection index (20.7%) in the vaccine group. Our results disclosed that the Leishmune® vaccine is a TBV, and that the dog antibodies present in sera, even 12 months after vaccination, lead to a significant effective protection of 79.3 %. Support: PRONEX, CNPQ, FINEP, FUJB-CEPG-UFRJ, FAPERJ,CAPES.

IM32 - Early induction of $CD8^+CD25^+CD28^-$ Regulatory T cells during *Leishmania major* and *Leishmania amazonensis* co-infection.

González Lombana, C.Z. *(Universidade Federal de Minas Gerais)*; Seixas-Rego, V.A. *(Universidade Federal de Minas Gerais)*; Macedo, J.P *(Universidade Federal de Minas Gerais)*; Afonso, L.C.C *(Universidade Federal de Ouro Preto)*; Vieira, L.Q. *(Universidade Federal de Minas Gerais)*

Infection of mice with Leishmania spp. that cause cutaneous leishmaniasis has led to an understanding of many immunological events that are required for a successful host response towards these protozoan parasites. Experiments developed in our laboratory have demonstrated that C57BL/6 mice previously infected with *L. major*, restrain pathogenic responses induced by *L. amazonensis* infection. To understand how Th cell activities are regulated in this model, we examined the frequency and phenotypes of T cells in the draining lymph

node at various time points. C57BL/6 mice, which had been previously inoculated into the left hind footpad with 10^4 metacyclic promastigotes of *L. major*, were infected with 10^4 metacyclic promastigotes of *L. amazonensis* in the contralateral footpad and the course of infection and the phenotype of T cells in DLN were assessed ex vivo using flow cytometry for up to 10 weeks. *L. major* pre-infected mice showed a frequency of CD4 and CD8 T cells comparable to mice infected just with *L. amazonensis* (control) at three weeks of infection. However, at six weeks of infection the frequency of CD4+ and CD8+ T cells in control mice was lower than in pre-infected mice. The response to polyclonal stimuli was similar in both groups. The frequency of CD4 and CD8 T cells producing IFNγ in mice previously infected with *L. major* was higher compared with those of control mice at 3 and 6 weeks. Interestingly, we found that at 3 weeks, *L. major*-pre-infected mice had higher frequency of CD8+CD25+CD28- T cells than mice infected only with *L. amazonensis*, 53 and 43 percent respectively. These data indicate that the control of lesion progression caused by *L. amazonensis* in C57BL/6 mice pre-infected with *L. major* is related with the early induction of T cells bearing the regulatory phenotype. Support: CNPq, FAPEMIG and CAPES.

IM33 - IMMUNOLOGICAL MARKERS CORRELATE WITH CLINICAL ASPECTS IN LESION CELLS FROM PATIENTS WITH CUTANEOUS LEISHMANIASIS

Faria, D. R. *(Universidade Federal de Minas Gerais)*; Antonelli, L. R. *(Universidade Federal de Minas Gerais)*; Machado, P. *(Universidade Federal da Bahia)*; Schriefer, A. *(Universidade Federal da Bahia)*; Carvalho, L. P. *(Universidade Federal da Bahia)*; Carvalho, E. M. *(Universidade Federal da Bahia)*; Gollob, K. J. *(Universidade de Minas Gerais)*; Dutra, W. O. *(Universidade Federal de Minas Gerais)*

Human cutaneous leishmaniasis (CL) is a parasitic disease caused by the protozoa Leishmania, which are intracellular parasites that can lead to the establishment of chronic lesions and cause a major public health problem in Brazil. In an attempt to understand the clinical relevance of immunological data, several immunological phenotypes determined using confocal microscopy in lesions of patients with CL were correlated with clinical measurements of the disease (Montenegro skin test (MST), lesion area and time of infection). Our results showed a negative correlation between the intensity of MST and numbers of IFN-γ, TNF-α and IL-10 producing cells. In contrast, we observed a positive correlation between the number of CD8+IFN-γ and the number of CD68+TNF-α cells and MST intensity. Moreover, a positive correlation was observed between the lesion area and the number of IFN-γ+, IL-10receptor+ and iNOS+ cells. This correlation was also seen for the absolute number of CD4+ and CD8+ cells. The analysis also demonstrated a positive correlation between the time of infection and the number of IL-10receptor+ and iNOS+ cells. These results suggest that events of cell activation in leishmaniasis and lymphocyte/macrophage migration are correlated with clinical indicators of human leishmaniasis. Financial support: CNPq/PADCT, CAPES, PRONEX, WHO and TMRC/NIH.
Financial support:CNPq-PADCT / CAPES / PRONEX - FAPEMIG / WHO / TMRC-NIH

IM34 - THE IMPORTANCE OF USING DIFFERENT SEROLOGICAL APPROACHES IN CLINICAL STUDIES BEFORE AND AFTER TREATAMENT OF CUTANEOUS LEISHMANIASIS

Bastos, RP *(Imunologia - IPTSP/UFG/GO)*; Sobrinho, JOC *(Imunologia - IPTSP/UFG/GO)*; Porto, LB *(Imunologia - IPTSP/UFG/GO)*; Guimarães, AB *(Imunologia - IPTSP/UFG/GO)*; Costa, FO *(Imunologia - IPTSP/UFG/GO)*; Pereira, LIA *(Imunologia - IPTSP/UFG/GO)*; Pereira, AJCS *(Hospital de Doenças Tropicais de Goiânia)*; Dias, FR *(Imunologia - IPTSP/UFG/GO)*; Dorta, ML *(Imunologia - IPTSP/UFG/GO)*

L.(V.)braziliensis is the most important specie responsible for the American cutaneous leishmaniasis (ACL) in Brazil. Despite the scientific and technological advances, the diagnosis of ACL still presents important gaps and usually requires the combination of immunological and parasitological tests. The purpose of this study was to evaluate the performance of different serological approaches to identify the profile of sera IgG antibody and early markers for leishmaniasis infection. The serological response to leishmanial antigens was evaluated using Western blot analysis and enzyme-linked assay (ELISA) by monitoring changes in titers to specific proteins before and after therapy. Sera from ACL patients were screened for IgG and IgG subclass-specific reactivity against a soluble antigen of promastigotes forms of L.(V.)braziliensis (LbAg). Western blot analysis indicates prominent reaction of IgG1 and IgG3 with polypeptides of 16, 20, 26, 30, 32, 35, 38, 45, 50, 65, 80, 91, 105, 110, 113 and 120 kDa, followed by IgG2 and IgG4 with 16, 20, 30, 32, 35, 38, 45, 50, 65, 80, 91, 105 and 120 kDa. There was fall in numbers of bands detected by IgG subclass following treatment. Seven bands, 16, 20, 50, 65, 80, 105 and ~120 kDa had significantly higher intensities than the others bands and were defined as being immunodominant. The seroprevalence for IgG was 85% and for IgG subclass, IgG1, IgG2, IgG3 and IgG4, was 90%, 25%, 40% and 30%, respectively. The IgG1 was prevalent and detected in high levels in patients with LTA before and after treatment and we found a significant decrease of antibody levels of the subclasses IgG3, IgG2 and IgG4 after treatment. The sensibility and specificity of Western blot for IgG subclass detection were higher than IgG detection by ELISA, seems to strengthen the safe use of this test for the diagnosis of Human ACL.

IM35 - INTRANASAL IMMUNIZATION WITH A PARTICULATED LEISHMANIA ANTIGEN PROTECTS BALB/C MICE AGAINST VISCERAL LEISHMANIASIS

Gomes, DCO *(Instituto de Biofísica Carlos Chagas Filho-UFRJ)*; Buscacio, LFA *(Instituto de Biofísica Carlos Chagas Filho-UFRJ)*; Rossi-Bergmann, B *(Instituto de Biofísica Carlos Chagas Filho-UFRJ)*

Intranasal vaccination is an effective way to promote local and systemic specific responses against not only respiratory but also systemic infectious diseases. The easy administration, lower antigen dose and lower degradation of the antigen, are the main positive factors of its use in relation to the oral route. Recently, we demonstrated the capacity of the intranasal immunization with whole *L. amazonensis* promastigote antigens (LaAg) to promote protective immune responses against murine cutaneous leishmaniasis caused by *L. amazonensis*. In the present work, we investigated the effectiveness of the intramuscular (i.m.) and intranasal (i.n.) administration of LaAg and whole *L. chagasi* promastigotes antigens (LcAg) in protecting mice against visceral leishmaniasis. BALB/c mice were immunized i.n. (by nasal instillation) or i.m. (by intramuscular injection into the hind leg thigh) with 10 ug of LaAg or LcAg and a booster dose 7 days later. Vaccinated and control mice were challenged i.v. with 10^7 *L. chagasi* promastigotes 7 days after the second dose. LaAg and LcAg i.n. vaccinated mice displayed significantly lower parasite loads (determined by limiting diluition assay) in the spleen 1 month after infection, as compared with non-vaccinated or LcAg i.m.-vaccinated. Splenocytes of LaAg or LcAg i.n. vaccinated showed a significantly increase in nitric oxide (NO) production (determined by Griess assay) in relation to control or LcAg i.m vaccinated mice. In addition, LaAg- or LcAg- i.n. vaccinated groups produced higher amounts of IFN-γ during antigen recall by spleen cells as compared with i.m. LcAg or control group. Our results suggests that intranasal immunization with whole leishmanial antigens can be an important strategy to induce a protective response not only against cutaneous but also against visceral leishmaniasis. Financial support: CNPq

IM36 - Myelofibrosis in Canine Visceral Leishmaniasis: a preliminary study

Mudado, M.A. *(Universidade Federal de Minas Gerais)*; Martins, G.C. *(Universidade Federal de Minas Gerais)*; Lima, W.G. *(Universidade Federal de Minas Gerais)*; Amaral, M.E. *(Universidade Federal de Minas Gerais)*; Moura, E.P. *(Universidade Federal de Minas Gerais)*; Sampaio, W.M. *(Universidade Federal de Minas Gerais)*; Tafuri, Wg. L *(Universidade Federal de Minas Gerais)*

The myelofibrosis can be idiopathic or seen in lymphoproliferative or myeloproliferative deseases, myelodisplasias, systemic mastocytosis, metastasis of epithelial malignant neoplasias, and in non neoplastic diseases. In the literature, the association of myelofibrosis with human kala-zar has been reported. So far, it has not showed in dogs with visceral leishmaniasis disease. Dogs are the principal domestic reservoir to the human visceral leishmanasis. Liver, spleen, lymph nodes and bone marrows are the organs most affected by parasite. The aim of this work is evaluate the parasite tissue load in bone marrows and its correlations with clinical and histopathological aspects of the disease in dogs. Mongrel dogs, naturally infected with *L. chagasi* were obtained from the City Hall of Santa Luzia/MG. Animals were classified following a different clinical status as asymptomatic and symptomatic dogs. They were all sacrificed with lethal dose of Sodic Thiopental (1ml/Kg) and T61 (0,3ml/kg). After the necropsy, fragments of bone marrow were collected and fixed in buffer formalin at 10% and processed for histopathological analyses (hematoxilin-eosin and Silver Stainings). The immunohistochemistry technique (streptoavidin-peroxidase method) was carried out to determine the amastigotes forms of *Leishmania* in paraffined tissue sections. A preliminary result has showed a same histopathological picture for asymptomatic and symptomatic animals with hyperplasic bone marrow and mononuclear cells predominance on all region of the tissue. Differences on parasite tissue load for the clinical groups have not been found (p=0.8421; Mann-Whitney test). Until now, dates obtained showed no myelofibrosis in any case and no correlation between the parasitism load and collagen deposition on tissue (r^2=0.0041). Sponsors: Fapemig and CNPq

IM37 - IMMUNE RESPONSES INDUCED IN DOGS (BEAGLES) BY VACCINATION WITH THE ANTIGEN A2 AGAINST VISCERAL LEISHMANIASIS

Costa, M. M. S. *(Universidade Federal de Minas Gerais)*; Coelho, E. A. F. *(Universidade Federal de Minas Gerais)*; de Freitas, E. *(Universidade Federal de Minas Gerais)*; Michalick, M. S. M. *(Universidade Federal de Minas Gerais)*; Gazzinelli, R. T. *(Universidade Federal de Minas Gerais)*; Fernandes, A. P. *(Universidade Federal de Minas Gerais)*

The development of a vaccine against canine visceral leishmaniasis (VL) is an alternative approach for interrupting the VL domestic cycle and has been recommended by WHO. In addition, it is recommended that this vaccine allows the serological differentiation of vaccinated and infected dogs, by means of conventional serological tests. In this work, we aimed to investigate the immune responses induced by vaccination with A2 antigen, as recombinant protein. A2 is an amastigote stage-specific antigen that is protective against *L. (L.) donovani* and *L. (L.) chagasi* infections in mice. Beagles were immunized on days 0, 21 and 42. One month after the third dose, animals were infected with 1 x 10^7 promastigotes. Levels of IFN-γ in culture supernatants of peripheral blood mononuclear cells (PBMC) were assessed by sandwich

ELISA. Before infection, stimulation with rA2 resulted in significantly increased ($p < 0.05$; Kruskal-Wallis) IFN-γ levels in PBMC of vaccinated animals, as compared to the control group. Accordingly, significantly increased levels of total IgG and IgG2 anti-A2 antibodies were detected in sera of vaccinated animals. No significant increase in IgG1 anti-A2 antibodies were observed in vaccinated animals. Moreover, there was no significant increase in total IgG, IgG2 and IgG1 to total parasite antigens (LcPA) in all groups. In the Infected (I) group, there were significant differences in levels of anti-rA2 IgG1 and IgG2 antibodies, as compared to the levels before infection. In addition, in these animals significant differences were observed in levels of IgG1 anti-LcPA antibodies as compared to the levels of Vaccinated and Infected group. Immunization with rA2 antigen induces Th1 response, characterized by increased levels of IFN-γ and anti-A2 specific IgG2 antibodies and low levels of IgG1 antibodies. In addition, it allows the serological differentiation between vaccinated and infected animals, an important requirement for a canine VL vaccine in Brazil.

IM38 - Natural resistance induces TH1 bias and increases the possibility of chronic states in a model of cutaneous leishmaniasis.

De Almeida, M C *(Universidade De Brasília-IB)*; Moreira, H *(Universiade De Brasília -MAT)*

In a previous model of leishmaniasis (De Almeida, M. C. & H. N. Moreira. 2003. Leishmaniasis: A Dynamical System Approach. Rev Inst Med Trop S Paulo. 45: (Suppl 13)123-124) we obtained the main described TH (T helper) patterns, stability solutions, of leishmaniasis. In a refinement of the model, we divided our parameters in sensible and resistant hypothetical mice. Noteworthy, we manipulated independently natural and adaptive immunity. Some new obtained results were: 1. High initial number of reactive clones are not associated with a silent phase in low dose models 2. Antigen dose irrespective to genetic background determines the T helper response 3. Low antigen doses are associated with prolonged initial silent phase (lag) of infection 4. Low antigen doses are associated with longer parasite curves 5. In a sensible model increasing natural resistance favours TH1 dominance 6. The chronic states - stable TH1 infection, stableTH1/TH2 infection, stable TH2 infection are associated to high natural resistance 7. In chronic states, except stable TH2 infection, the number of parasites is very low in the stability points 8. Both high natural resistance and increased activation induced cell death are important in TH1 stable infection 9. Numerical simulation indicates very strong natural resistance in TH1/TH2 infection. One interpretation is that we need in this case a non permissive cell for the parasite. Because of multigenic nature of resistance in leishmaniasis, is possible to admit a model in which natural resistance is high, due to expression of genes involved primarily in impairing nutrition or macrophage environment for leishmania. They could not be linked to expression of antigen presenting molecules or cytokine receptors in the cell harbouring the parasite, but even in this case, by reason of dynamics, the natural resistance drives the response to TH1 side. Acknowledgements This work is sponsored by FAPDF.

IM39 - EVOLUTION OF THE CUTANEOUS IMMUNE RESPONSE TO EXPERIMENTAL *Leishmania (Viannia) braziliensis* INFECTION IN THE RHESUS MONKEY(*Macaca mulatta*) MODEL

Souza-Lemos, C. *(Instituto Oswaldo Cruz)*; de-Campos, S. N. *(Instituto Oswaldo Cruz)*; Porrozzi, R. *(Instituto Oswaldo Cruz)*; Grimaldi, G. *(Instituto Oswaldo Cruz)*

The outcome of leishmanial infection of macrophages depends on factors particular to each host-parasite combination, involving not only intrinsic properties of the parasite, but also genetically determined characteristics of the host cell or of its interactions with immunocompetente cells. LTA caused by *L. (V.) braziliensis* has a diversity of clinical forms and the histopathology of the disease is complex. Differential expression of cytokines parallels the variation observed on the nature of cellular inflammatory infiltrate, but large variation in the densities of different cell types is usually found among patients, which probably reflects different stages of the disease. Several immunological mechanisms may account for the tissue-damaging state observed in these patients. We have shown that *M. mulatta* appears to have significant advantages over conventional laboratory animals in terms of modeling the human disease, in particular for studying the interactions between parasite and host determinants for infection, disease (virulence) and cure in *L. (V.) braziliensis* LTA. The goal of present study was to characterize the histological events with time in *L. (V.) braziliensis*-infected macaques developing self-healing cutaneous lesions. The pathological findings included a typical cell-mediated immunity-induced granulomatous reaction (consisting of an aggregation of epitheloid cells and multiple Langhans-type giant cells, surrounded by lymphocytes and plasma cells) that had an effect on the control of parasite replication. Computer-assisted automatic counting of expressed leukocyte immunophenotypes (such as CD20, CD3, CD4, CD8, CD68 and HLA-DR) revealed consistent variation regarding proportions of these cell types. Of note, however, the frequencies of CD4+ were higher than CD8+ T during the evolution of skin lesions. Taken together with our previous work (Parasitology 2003, 127:437-447), these studies led us to propose the macaque model for further assessing (under more controlled conditions than are possible in clinical studies) the correlates of local and systemic immune responses and the disease evolution.

IM40 - PARTIAL PROTECTION AGAINST *Leishmania (Viannia) braziliensis* CUTANEOUS LEISHMANIASIS (CL) IN THE PRIMATE *Macaca mulatta* FOLLOWING VACCINATION WITH DNA ENCODING THE LEISHMANIAL TRYPAREDOXIN PEROXIDASE (TryP)

de Oliveira, A.P. *(Instituto Oswaldo Cruz)*; Porrozzi, R. *(Instituto Oswaldo Cruz)*; Blackwell, J. M. *(Cambridge Institute for Medical Research)*; Grimaldi Jr., G. *(Instituto Oswaldo Cruz)*

Leishmaniasis affects 12 million people worldwide. The artificial induction of protective immunity using second-generation vaccines against leishmanial parasites would be essential for ultimate control of the human disease. Nevertheless, there is still much to be done in assessing the effectiveness of vaccination in the absence of a natural challenge. Although several new approach used in modern vaccinology are being investigated in mouse models of CL, the relevance of vaccine trials in mice to the human system remains speculative. The homology between the *M. mulatta* immune system and that of humans has led to the belief that the efficacy of those antigens selected as being candidates for an anti-*Leishmania* vaccine in this model may represent suitable vaccine candidates against this parasite in humans. Although used less than the mouse, this model can be protected effectively by *Leishmania* vaccination (Infect Immun 2001, 69:4103). Here we investigated in the macaque model of *L. (V.) braziliensis* CL the protective efficacy of the TryP protein delivered as either an homologous DNA/DNA or heterologous DNA/MVA prime-boost strategy. Vaccinated monkeys with either the TryP_DNA or TryP_MVA construct showed no adverse systemic or local effects. The antigen-specific serum IgG and IgG1 antibody responses were increased after the last boost in monkeys receiving both vaccine formulations. Prior to the challenge, none of the control and vaccinated animals exhibited either parasite-specific lympho-proliferative responses or positive DTH responses, as measured by the leishmanin skin test. However, multiplex analysis of cytokines in the blood of vaccinated macaques exhibited augmented in vitroTryP-induced multiple cytokine production [IL-1β, TNF-α, MIP-1α, IL-12 and IFN-γ]. Of note, vaccinated monkeys showed a reduced development of lesion size, compared with controls. The findings point to the feasibility of using vectored DNA as an appropriate antigen delivery system for inducing inflammatory responses, which is another component of an effective vaccine.

IM41 - EVALUATION OF ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA) USING LEISHMANIAL CRUDE OR SPECIFIC ANTIGENS FOR SERODIAGNOSIS OF SYMPTOMATIC AND ASYMPTOMATIC *Leishmania infantum* VISCERAL INFECTION (VL) IN DOGS

Costa, MVS *(FIOCRUZ)*; Porrozzi, R *(Fiocruz)*; Teva, A *(Fiocruz)*; Ferreira, AL *(Universidade Federal do Espírito Santo)*; Santos, C *(Universidade Federal do Espírito Santo)*; Falqueto, A *(Fiocruz)*; Fernandez, AP *(Universidade Federal de Minas Gerais)*; Grazzinelli, RT *(ufmg)*; Campos-Neto, A *(The Forsyth Institute)*; Grimaldi, G *(Fiocruz)*

Diagnostic research in VL has been hampered by the lack of a gold standard. When a reference test with sub-optimal sensitivity for case ascertainment is used, true VL cases are missed and therefore included in the group of controls. A flawed reference test can substantially affect validity estimates of new tests under scrutiny, thus the sensitivity and specificity estimates of serologic tests might be biased to some extent, depending on the controls and reference test used. Therefore, the requirements of a serological test for canine VL may very. For confirmatory purposes of clinically suspected cases, test specificity is most important, whereas a high sensitivity is most essential in surveillance programs to detected asymptomatic carriers. Such animals are a potential source of infection for sandfly vectors. Here we have evaluated the effectiveness of ELISA for the detection of anti-*Leishmania* antibodies in serum samples from symptomatic and asymptomatic *L. infantum*-infected dogs from an endemic rural area of VL in Southeast Brazil. The ELISA was carried out using microtiter plate wells coated with either soluble promastigote-derived antigens or the recombinant K26, K39 and A2 proteins. We have also enrolled control samples to achieve adequate precision for the sensitivity estimates of the ELISA, thus supporting the suitability of using this diagnostic test for *Leishmania* mass-screening surveys and intervention campaigns. The different antigens assayed by ELISA rarely agreed on the proportion of positives in a sample, thus proving to be independent in their antibody reactivity. The serologic test performance was dependent on stage of the disease. While the levels of anti-leishmanial antibodies were highest in sick dogs, the sensitivity of antibody detection was generally lower in early or asymptomatic infections. The levels of IgG, IgG1 and IgG2 specific for *L. infantum* were also variable among groups, so one must be careful in extrapolating results.

IM42 - Histopathological and Immunohistochemical Investigations of the Hepatic Compartment Associated with Parasitism and Biochemical changes in Canine Visceral Leishmaniasis

Giunchetti, R. C. *(Universidade Federal de Ouro Preto)*; Abreu, R. T. *(Universidade Federal de Ouro Preto)*; Roatt, B. M. *(Universidade Federal de Ouro Preto)*; Mayrink, W. *(Universidade Federal de Minas Gerais)*; Carneiro, C. M. *(Universidade Federal de Ouro Preto)*; Corrêa-Oliveira, R. *(Fundação Oswaldo Cruz)*; Martins-Filho, O. *(Fundação Oswaldo Cruz)*; Marques, M. J. *(Universidade Federal de Alfenas)*; Tafuri, W. L. *(Universidade Federal de Ouro Preto)*; Reis, A. B. *(Universidade Federal de Ouro Preto)*

The histopathological evaluation of the liver is essential for the understanding of inflammatory processes in canine visceral leishmaniasis (CVL). Three clinical groups of dogs naturally infected with *Leishmania chagasi* [i.e. asymptomatic AD, n = 12), oligosymptomatic (OD, n = 12) and symptomatic (SD, n = 17)] were assessed and compared with a group of non-infected dogs (NID, n = 11). Liver fragments were stained with haematoxylin-eosin and evaluated histologically for parasite density [Leishman-Donovan unit (LDU) index and anti-*Leishmania* immunohistochemical assay]. Correlation analyses were carried out in order to determine possible associations between histological and parasitological parameters, and between these and measured biochemical parameters including albumin, plasma globulin and total protein levels, and albumin/globulin ratio. Intense reaction of the Kupffer cells, capsule and portal inflammation, and the presence of intralobular granulomas, were observed in the different clinical groups. These findings appear to relate to the pathogenesis of hepatomegaly, representing the histological basis of hypertrophy and hyperplasia of liver parenchyma in CVL. Dogs in the SD group presented a higher frequency of parasitism compared with the AD group, illustrating the direct relationship between clinical symptoms and frequency of hepatic parasitism. Inflammatory alterations were more intense in the SD group and were associated with parasitism, suggesting that the presence of the parasite in the hepatic parenchyma triggers the inflammatory reaction in the portal tract and in the parenchyma. The results also indicated an association between histopathological liver changes and the progression of biochemical alterations.
Financial Support:FAPEMIG / CNPq / CPQRR-Fiocruz / UFOP / UFMG

IM43 - The spleen in canine visceral leishmaniasis

Giunchetti, R. C. *(Universidade Federal de Ouro Preto)*; Vitoriano-Souza, J. *(Universidade Federal de Ouro Preto)*; Mayrink, W. *(Universidade Federal de Minas Gerais)*; Tafuri, W. L. *(Universidade Federal de Ouro Preto)*; Carneiro, C. M. *(Universidade Federal de Ouro Preto)*; Corrêa-Oliveira, R. *(Fundação Oswaldo Cruz)*; Martins-Filho, O. A. *(Fundação Oswaldo Cruz)*; Marques, M. J. *(Universidade Federal de Alfenas)*; Reis, A. B. *(Universidade Federal de Ouro Preto)*

Canine visceral leishmaniasis (CVL) is a disease caused by the protozoan *Leishmania chagasi* and transmitted by the bite of phlebotomine sand fly vectors. The main clinical findings are skin lesions, hepatosplenomegaly, lymphadenopathy, glomerulopathy and anemia. Studies on histophatology in CVL present only report microscopic lesions. In this context, the aim of the present study was to make immunopathological study in spleen biopsies from asymptomatic (AD, n=12), oligosymptomatic (OD, n=12) and symptomatic (SD, n=16) CVL-bearing dogs as well as 11 healthy controls (NID). These results of microscopical analysis of the spleen were make association statistical analysis with different histopathological results and parasitism level by immunohistochemical. In addition, we evaluated the correlation between histopathological picture of spleen lesions/parasitism and leukocytes in peripheral blood and splenocytes (CD4+, CD8+, CD5+, Thy-1+, CD14+, CD21+) accessing by flow cytometry. Our data demonstrated that frequency in all infected dogs presented histopathological pattern consistent with white pulp depletion with macrophage infiltrated, red pulp hypertrophy/hyperplasia and congestion, in comparison to NID. Statistical analysis suggested that cell reactivity in different compartment in spleen may be stimulated by parasite load. The correlation was presented among peripheral blood and histological parameters by AD in CD4+, CD14+ and CD21+. Taken together, the correlation was presented between splenocytes and histological parameters by AD in CD4+, CD21+ and OD in CD21+. These findings suggest participative reaction in leukocytes emigration from peripheral blood to distinct compartment in spleen reporting of the mononuclear cells regarding immune pathological events associated with CVL outcome.
Financial Support:FAPEMIG / CNPq / CPQRR-Fiocruz / UFOP / UFMG

IM44 - Evaluation of the Humoral Immune Response Against Total Antigens and LPG from Leishmania braziliensis and L. amazonensis in Patients with Cutaneous Leishmaniasis Before and After Treatment

Passamani, R. *(Universidade Federal de Ouro Preto)*; Stransk, B. *(Universidade Federal de Ouro Preto)*; Toledo, V.P.C.P. *(Universiade Federal de Minas Gerais)*; Mayrink, W. *(Universidade Federal de Minas Gerais)*; Afonso, L.C.C. *(Universidade Federal de Ouro Preto)*

In this work, we evaluated the humoral response of patients with Cutaneous Leishmaniasis, against total antigens and lipophosphoglycan (LPG) from Leishmania before and after treatment with imunochemoterapy (IC) or conventional

chemotherapy(CC). Analysis of the IgG reactivity profile, by Western-blotting against L. braziliensis antigen, demonstrated that although the recognition of some epitopes was common amongst different patients, a high degree of variability was observed. Comparison of the profiles before and after treatment demonstrated that, in general, patients that received the IC protocol presented an increased intensity of recognition while those treated by CC presented the opposite result. However, in both groups, we did not observe qualitative variations in the profile. Evaluation of the IgM response by ELISA against total L. braziliensis antigen showed no differences before and after treatment in the CC group. Curiously, IgM levels were slightly increased after treatment in patients treated by IC. Total IgG levels decreased in the CC group after treatment but were not altered in the IC group. This IgG response was characterized by the presence of IgG1 and IgG3 and no IgG2 or IgG4. When purified LPG from L. braziliensis was used as antigen in the ELISA test a high IgM response and low IgG (total and isotypes) was detected in both groups. Curiously, even though LPG expression in amastigotes is decreased, we did observe any differences in reactivity before and after treatment regardless of the protocol. Interestingly, the response to LPG purified from L. amazonensis was consistently smaller than that against LPG from L. braziliensis. Given the fact that the patients come from an area where L. braziliensis is the predominant causative agent, this result demonstrate a certain degree of specificity in the response, suggesting that there may be significant structural differences between the LPG from the two Leishmania species.

Support:FAPEMIG/Rede Mineira de Biomoléculas/CNPq

IM45 - Relationship between Canine Visceral Leishmaniosis and the *Leishmania (Leishmania) chagasi* Burden in Dermal Inflammatory Foci

Dores-Moreira, N. *(Universidade Federal de Ouro Preto)*; Ker, H. G. *(Universidade Federal de Ouro Preto)*; Aguiar-Soares, R. D. O. *(Universidade Federal de Ouro Preto)*; Mayrink, W. *(Universidade Federal de Minas Gerais)*; Carneiro, C. M. *(Universidade Federal de Ouro Preto)*; Corrêa-Oliveira, R. *(Centro de Pesquisas René Rachou)*; Martins-Filho, O. A. *(Centro de Pesquisas René Rachou)*; Tafuri, W. L. *(Universidade Federal de Ouro Preto)*; Reis, A. B. *(Universidade Federal de Ouro Preto)*; Giunchetti, R. C. *(Universidade Federal de Ouro Preto)*

The skin is the first point of contact with organisms of the genus *Leishmania* from sand fly vectors, and apparently normal skin of sick dogs harbors amastigote forms of *Leishmania chagasi*. In relation to visceral Leishmaniosis (VL), the ear skin was examined in 10 uninfected dogs (UD) and in 31 dogs dogs naturally infected with *L. chagasi*. The infected animals consisted of 10 asymptomatic dogs (AD), 12 oligosymptomatic dogs (OD) and nine symptomatic dogs (SD). A higher parasite burden was demonstrated in SD than in AD by anti-*Leishmania* immunohistochemistry ($P < 0.01$), and by Leishman Donivan Unit (LDU) indices (P= 0.0024) obtained from Giemsa-stained impression smears. Sections stained with haematoxylin and eosin demonstrated a higher intensity of inflammatory changes in SD than AD ($P < 0.05$), and in the latter group flow cytometry demonstrated a correlation (P=0.05/r=0.7454)between the percentage of CD14+ monocytes in peripheral blood and chronic dermal inflammation. Extracellular matrix assessment for reticular fibers by staining of sections with Masson trichrome and Gomori ammoniacal silver demonstrated a decrease in collagen type I and an increase in collagen type III as the clinical signs increase. The data on correlation between cellular phenotypes and histological changes seemed to reflect cellular activation and migration from peripheral blood to the skin, mediated by antigenic stimulation. The results suggested that chronic dermal inflammation and cutaneous parasitism were directly related to the severity of clinical disease.

Financial Support:FAPEMIG / CNPq / CPQRR-Fiocruz / UFOP / UFMG

IM46 - Control of lesions in TNFRp55-/- mice infected with Leishmania major may be partially achieved with stem cells or thalidomide.

Seixas, V.A.R. *(Universidade Federal de Minas Gerais)*; Macedo, J.P. *(Universidade Federal de Minas Gerais)*; Afonso, L.C.C. *(Universidade Federal de Ouro Preto)*; Vieira, L.Q. *(Universidade Federal de Minas Gerais)*

Tumor necrosis factor (TNF) has an essential role in the activation of infected macrophages to kill *Leishamania major* after activation with IFN-γ. Mice in which the TNF receptor 1 was deleted by homologous recombination (TNFRp55-/-) resolve parasitism in the footpad when infected with *L. major*, but more slowly than C57Bl/6 wild-type. More interestingly, even after the levels of parasites at the site of infection were undetectable, TNFRp55-/- did not resolve lesions, and an intense inflammatory infiltrate was present after 25 weeks of infection. The level of apoptosis in TNFRp55-/- mice is lower than C57BL/6. The deficiency of apoptosis allows cells to maintain chemokine production, attracting cells to the inflammatory site. In this work we looked for ways to resolve lesions in TNFRp55-/- mice. Eleven-week infected mice were treated with $5x10^6$ cells bone marrow cells from femur and tibia from healthy TNFRp55-/-. A significant difference in lesion sizes was found from week 17 of infection trhough week 36, but the parasite burden did differ significantly between treated and non-treated groups. e then treated mice with bone marrow cells at three different time points: 6, 11 and 19 weeks post-infection. Treated mice presented lower lesions 2 weeks after treatment till 22 weeks of infection. Another approach was to treat mice with thalidomide, a TNF inhibitor. Thalidomide also has other effects, including the upregulation of IFN-γ production. TNFRp55-/- mice infected with *L. major* were treated with thalidomide orally at 6 weeks of infection (30 mg/kg/day) for 30 days. Smaller lesions were found from 7 weeks of infection. Our data suggest that the

lesions due to infection with L. major in TNFRp55-/- can be controlled. The mechanisms for this control may be the downregulation of TNF production or the repair of tissues by bone marrow stem cells. Supported by CNPq, CAPES and FAPEMIG.

IM47 - Pre-infection of C57BL/6 Mice with *Leishmania major* Leads to control of Lesion Progression Caused by *Leishmania amazonensis.*

SEIXAS, V.A.R. *(Universidade Federal de Minas Gerais)*; MACEDO, J.P. *(Universidade Federal de Minas Gerais)*; GONZALEZ LOMBANA, C.Z. *(Universidade Federal de Minas Gerais)*; AFONSO, L.C.C. *(Universidade Federal de Ouro Preto)*; VIEIRA, L.Q *(Universidade Federal de Minas Gerais)*

Infection of mice with *Leishmania spp.* that cause cutaneous leishmaniasis has led to an understanding of many immunological events that are required for a successful host response against these protozoan parasites. Many studies have demonstrated that the host response is determined by the species or strains of *Leishmania* and also that this response may vary in the same mouse strain. Since C57BL/6 mice infected with L. major develop a Th1 response that leads to spontaneous resolution of lesions and knowing that the susceptibility of these mice to *L. amazonensis* results from an inability to mount this response, we assessed the hypothesis that the previous induction of a Th1 response elicited by *L. major* would modify the outcome of infection with *L. amazonensis.* For that purpose, we infected the left ear (group 1) with $1x10^4$ metacyclic forms of *L. major* or the right hind footpad (group 2) and, after three weeks, we infected the left ear with $1x10^4$ metacyclic forms of *L. amazonensis.* The lesion development was followed for six weeks after the infection with *L. amazonensis.* Both lesion sizes were significantly smaller when compared to their control counterparts (group 3 - mice infected with $1x10^4$ metacyclic forms of *L. amazonensis*in the left ear). The parasite load in the footpads of group 2 animals was nil, and in the ears was significantly smaller when compared to group 1 and 3. Group 1 mice presented a higher parasitism in the right ear (*L.major*) as well as the left ear (*L. amazonensis*) when compared to control ones. These data suggest the occurrence of *Leishmania* species interchange between ears.These data indicate that in C57BL/6 mice, pre-infection with L. major allowed the control of lesion progression caused by *L. amazonensis.* Support: CNPq, FAPEMIG and CAPES.

IM48 - Inflammatory mononuclear phagocyte: phenotypes and adhesion molecule expression in leishmaniasis

HERMIDA, M. D. *(FUNDAÇÃO OSWALDO CRUZ)*; MACEDO, M. P. *(FUNDAÇÃO OSWALDO CRUZ)*; MENGEL, J. O. *(FUNDAÇÃO OSWALDO CRUZ)*; DOS SANTOS, W. L. C. *(FUNDAÇÃO OSWALDO CRUZ)*

Leishmania infection modulates integrin function in inflammatory phagocytes. Such change in integrin function may interfere with cell migration and with antigen presentation to the immune system. The aim of this study was to identify the inflammatory phagocytes populations susceptible to *Leishmania* infection and the potential changes in their migratory capabilities. In this part of the study we examined the profile of mononuclear leukocyte populations present after 4 days of peritonitis induced in Balb/c mice with thiogicolate, their susceptibility to *Leishmania* infection *in vitro* and changes in expression of adhesion molecules after infection. The inflammatory mononuclear phagocytes present in the peritoneal exudates expressed CD11b (78±6), CD11c (28±15) and F4/80 (65±9). A higher proportion of infected cells was observed among CD11b+ than CD11b- cell populations: CD11b+/CD11c+ (77%), CD11b+/CD11c- (68%) in comparison with CD11b-/CD11c- (30%) and CD11b-/CD11c+ (25%), ANOVA P=0.0023. Among the CD11c+ infected cells 28% also expressed MHC-II, and this expression was high in 6% of the cells. Although no change in the intensity of adhesion molecule expression was observed in infected cells, there was a reduction in the number of cells expressing CD11b (10% reduction), F4/80 (21% reduction) and CD11c (15% reduction). Now we are examining the profile of adhesion molecule expression on specific cell populations and their capability to transport *Leishmania* to the draining (parathymic) lymph node, using injection and tracking experiments combined with immunohistochemistry. support: FAPESB, CNPQ, CAPES

IM49 - NKT Cells Are Required for Vaccine-Induced Immunity Against *Leishmania amazonensis* in IL-12P40$^{-/-}$*C57BL/6Mice*

HERNANDEZ, MX -S *(Universidade Federal de Minas Gerais)*; GOLGHER, D. B. *(Universidade Federal de MInas Gerais)*; SILVA, I. C. C. *(Universidade Federal de Minas Gerais)*; BATISTA, L. A. C. B. *(Universidade Federal de Minas Gerais)*; AFONSO, L.C.C *(Instituto de Ciências Biologicas)* VIEIRA, L.Q. *(Instituto de Ciência Biológicas)*

Adoptive or vaccine-induced protection against leishmaniasis is largely dependent on cell mediated type 1 immune response and IL-12-driven IFN-γ production. Surprisingly, previous data from our laboratory described the efficiency of vaccination to *L. amazonensis* and, in addition, IFN-γ production was found up regulated in vaccinated IL-12p40$^{-/-}$ mice. Also it was verified that CD8$^+$ T cells are not required for vaccine-induced immunity against *L. amazonensis* infection in these mice. The aim of this study was to evaluate the role of other cells types, such as NK and NKT cells, in protection against *L. amazonensis* in vaccinated mice. IL-12p40$^{-/-}$mice were immunized with two inoculations, seven days apart, of Leishvacin (Biobrás, Montes Claros, MG, Brazil) plus Corynebacterium parvum as adjuvant. Twenty-eight days later, animals received a booster and were infected

with *L. amazonensis* seven days later. NK and NKT cells were depleted by treatment of vaccinated mice with anti-NK1.1 mAb PK136 or control antibody on day - 6, - 3, + 4, + 7 and once weekly after *L. amazonensis* infection. Non vaccinated infected mice were used as a control group. Infection was followed for 8 weeks. NK1.1 depleted/vaccinated group developed larger lesions when compared to vaccinated group and similar lesion compared with control group. The parasite burdens in the NK1.1-depletedvaccinated group were similar to control group. In addition, the control group showed reduction of NKT cells in spleen and lymph node compared with the vaccinated group, suggesting a role for NKT cells in control of lesions. Interestingly, the vaccinated group had higher frequency of dendritic cells in the lesion. Such results suggest that NKT cells play an important role in *L. amazonensis* vaccination in IL12p40$^{-/-}$animals contributing to lesion control. Support: CAPES,FAPEMIG and CNPq

IM50 - THE PROTECTIVE RESPONSE AGAINST LEISHMANIA BRAZILIENSIS IS MEDIATED BY TCD8+ CELLS RECRUITMENT INDUCED BY LUTZOMYIA LONGIPALPIS SALIVA

Carregaro, V. *(Faculdade de Medicina de Ribeirão Preto- Universidade de São Paulo)*; Costa, D.L. *(Faculdade de Medicina de Ribeirão Preto- Univbersidade de São paulo)*; Brodyskin, C. *(3Centro de Pesquisa Gonçalo Muniz, Fundação Oswaldo Cruz (FIOCRUZ),)*; Barral, A.M. *(3Centro de Pesquisa Gonçalo Muniz, Fundação Oswaldo Cruz (FIOCRUZ),)*; Barral-Netto, M. *(3Centro de Pesquisa Gonçalo Muniz, Fundação Oswaldo Cruz (FIOCRUZ))*; Cunha, FQ *(Faculdade de Medicina de Ribeirão Preto- Universidade de São Paulo)*; Silva, JS *(Faculdade de Medicina de Ribeirão Preto-Universidade de São Paulo)*

Introduction and Objective: Studies suggested that saliva of the vectors Lutzomyia sp. and Phlebotomus sp. is important in the establishment of Leishmania infection. Conversely, prior exposure of mice to bites of uninfected sand flies or presensitization with salivary gland sonicated (SGS) of Lutzomyia longipalpis and Phlebotomus papatasi resulted in a protection against the parasite. However, no work had demonstrated which cells are involved on the salivary immunoprotective effect. In the present study, we investigated the inflammatory response induced by saliva of Lutzomyia longipalpis and its influence in the course of Leishmania braziliensis infection. Methods and Results: BALB/c mice were inoculated intradermally once or three times into ear with SGS or PBS, with or without of 2 x 103 L. braziliensis promastigotes. We observed that mice injected three times with SGS presented reduced numbers of inflammatory cells in the dermal compartment, except for CD3+CD8+ cells that were increased, when compared with mice inoculated once. These mice showed an increase percentage of the CD3+IFN-gama+ cells as well CD11b+ IL-12+. Moreover, the presensitization with saliva enhanced the expression of IP-10, MIP-1alpha; and MIP-1beta and a decreased expression of chemokine receptors mRNA expression. Injection of SGS three times induced a protective effect against infection with L. braziliensis, resulting in a reduced lesion size and lower levels of parasites in the dermal compartment. The pattern of resistance induced by SGS was correlated with the initial recruitment of CD3+CD8+ cells and expression of IFN-gama that was detected during the entire course of infection. Conclusions: The results suggest that the protection against Leishmania braziliensis infection induced by Lutzomyia longipalpis injection is due to production of chemotactic factors, which lead to recruitment and activation of CD8+ T cells at the inoculation site, that in their turn produce cytokines pattern that mediates the protection.

IM51 - A combination of recombinant canine IL-2, IL-12 and Leishmania chagasi crude antigens induce greater lymphoproliferative response than antigen alone in dogs living in a visceral leishmaniasis endemic area

Pereira, A.M. *(Fundação Oswaldo Cruz)*; dos Santos, L.R. *(Universidade Federal da Bahia)*; dos-Santos, W.L.C. *(Fundação Oswaldo Cruz)*; Melo, C.G. *(Universidade Federal da Bahia)*; Santana, C.C. *(Fundação Oswaldo Cruz)*; Pontes-de-Carvalho, L. *(Fundação Oswaldo Cruz)*; Oliveira, G.G.S. *(Fundação Oswaldo Cruz)*

Zoonotic visceral leishmaniasis (VL) is an infectious disease caused by *Leishmania chagasi* that affects dogs and humans. Susceptibility to disease development is associated to low in vitro lymphoproliferative and Th1 antigen-specific immune responses. As an initial assessment of the possible therapeutical benefits of using citokines in canine VL, the effect of recombinant canine IL-2 and IL-12 upon in vitro lymphoproliferative response of 26 stray dogs living in a VL endemic area (Jequié, Bahia) was evaluated. Out of these 26 animals, 19 exhibited clinical manifestations compatible with VL, whereas 21 had positive results in at least one immunological test performed (ELISA for anti-*L. chagasi* IgG antibodies, delayed type hypersensitivity skin test and in vitro antigen-specific lymphoproliferation) or in spleen-aspirate culture for *Leishmania*. Peripheral blood mononuclear cells (PBMC), stimulated with parasite crude antigens alone or in combination with either recombinant canine IL-2, recombinant canine IL-12, or both, showed stimulation indexes of 17.5±4.2 , 28.2±8.4, 28.6±6.3 and 43.0±10.7, respectively. Statistical analysis carried out with Friedman's test indicated that only the combination of IL-2 and IL-12 promoted significantly greater proliferative effect than that induced by antigen alone ($P = 0.0016$). These results demonstrate that a combination of IL-2 and IL-12 would be beneficial in canine visceral leishmaniasis treatment. Further investigation in respect to IFN-gamma and IL-10 secretion in such conditions is being undertaken.

IM52 - ANALYSIS OF TH1-TYPE IMMUNE RESPONSE INDUCTION IN MICE IMMUNIZED WITH A Leishmania chagasi AMASTIGOTE RECOMBINANT ANTIGEN ADMINISTERED IN COMBINATION WITH DIFERENT ADJUVANTS OR AS PLASMID DNA

dos Santos, L.R. *(Universidade Federal da Bahia)*; Fraga, R.E. *(Universidade Federal da Bahia)*; Pereira, A.M. *(Fundação Oswaldo Cruz)*; Pontes-de-Carvalho, L. *(Fundação Oswaldo Cruz)*; Oliveira, G.G.S. *(Fundação Oswaldo Cruz)*

Visceral leishmaniasis (VL), caused by *Leishmania chagasi*, is an infectious disease where dogs are considered the major reservoir of the causal agent. An effective canine vaccine might contribute to human and dog VL control. We are evaluating a panel of *L. chagasi* amastigote recombinant proteins aiming to identify antigens capable of inducing Th1 immune response, considered protective in dogs. Herein, the immunogenicity of a recombinant antigen, rLc9, administered in combination with different adjuvants or as plasmid DNA, was evaluated. Groups of BALB/c mice were injected 3 times sc., at 3-week intervals, with (i) saline, (ii) 100 micrograms of rLc9 alone or associated to (iii) Freund's adjuvant, (iv) peanut oil, (v) saponin, (vi) alum or (vii) 50 micrograms of pcDNA3.1-mIL-12 or with (viii) 50 micrograms of Lc9 encoding plasmid (pBKCMV-Lc9) or (ix) negative control plasmid. DNA was injected through intramuscular injections followed by electroporation. After the last dose, anti-total promastigotes antigens IgG and anti-rLc9 IgG, IgG1 and IgG2a antibody production was accessed by ELISA on day 10 and groups (i), (viii) and (ix) were infected with *L. chagasi* promastigotes on day 30. Murine splenocytes stimulated with rLc9 in vitro lymphoproliferative response and cytokines production were assessed after immunization and 2 months after the infection. Splenic parasite burden was determined by limiting dilution assay 2 months after the infection. Lc9 alone or in combination with adjuvants, as well as pBKCMV-Lc9 promoted specific IgG production. However, immunization protocol with rLc9-saponin and pBKCMV-Lc9 were the only ones to show a predominant IgG2a-IgG1 specific antibody production and IFN-gamma expression after in vitro stimulation with rLc9. In rLc9-saponin immunized animals, lymphoproliferative response to rLc9 and expression of IL-5 was also observed. No protection against infection was observed in the tested group, perhaps because the intensity of the TH1 immune response was not strong enough.

IM53 - Definition of immunoinflammatory markers for susceptibility in canine visceral leishamniasis (CVL)

Santana, C.C.S. *(Fundaçao Oswaldo Cruz-Bahia)*; Pinheiro Jr, N.F.P *(Fundação Oswaldo Cruz -Ba)*; Freitas, L.A.R.F. *(Fundaçào Oswaldo Cruz-Ba)*; Freitas, L.A.R.F. *(Fundaçào Oswaldo Cruz-Ba)*; Vassallo, J.V. *(Universidade Estadual de Campinas)*; dos-Santos, WLCS *(Fundação Oswaldo Cruz-BA)*

The aim of this study wasto identify immuno-inflammatory patterns in the spleen associated with canine visceral leishmaniasis. Spleen samples were obtained from stray dogs captured in an endemic area of visceral leishmaniasis. The animals were grouped into four categories as follows: a) potentially resistant to visceral leishmaniasis: with positive anti-L chagasi DTH and negative spleen culture for Leishmania sp.; b) potentially susceptible to visceral leishmaniais: with negative anti-L chagasi DTH and positive spleen culture for Leishmania sp.; c) undefined susceptibility to visceral leishmaniasis: with positive DTH and positive spleen culture for Leishmania sp.; d) non-infected: negative anti-L. chagasi DTH and serology (ELISA) and negative spleen culture for Leishmania sp. Analysis was performed using conventional histology and morphometry. The significance of the differences between groups was examined using one way ANOVA or Fisher's exact test when recommended. Animals in the potentially susceptible group presented higher frequency of perisplenitis ($15/28$, $P = 0,0001$), granuloma ($8/28$, $P = 0,0008$), structural disorganization ($14/28$, $P = 0,0001$), and atrophy of the lymphoid follicles ($11/29$, $P = 0,0167$), and marginal zone ($12/29$, $P = 0,084$). The data presented herein shows important changes in the white pulp of the spleen associated with visceral leishmaniasis. Such alterations may interfere with the role of the spleen in the surveilance of blood-born antigens. We are now examining the changes in leukocyte populations in these spleens and the profile of cytokine expression using real time RT-PCR. Support: FAPESB, CNPq

IM54 - IFN-γ-dependent and NOS2-independent mechanisms of resistance to *Leishmania amazonensis*

Macêdo, J. P. *(Universidade Federal de MInas Gerais)*; Seixas, V. A. R. *(Universidade Federal de MInas Gerais)*; Santos, L. A. R. *(Universidade Federal de MInas Gerais)*; Vieira, L. Q. *(Universidade Federal de MInas Gerais)*

The main mechanism involved in control of Leishmania in mice is the production of NO by activated machrophages. However, mice that do not express the nitric oxide synthase (iNOS-) present some resistance to infection when compared to mice that do not express interferon-gamma (IFN-γ-), the main macrophage-activating cytokine. Hence, we raised the hypothesis that reactive oxygen species (ROS) might be responsible for the partial resistance to infection in iNOS- mice. To test this hypothesis, elicited peritoneal machrophages from iNOS-, wild type (wt) and mice lacking the gp91 chain of the phagocyte NADPH oxigenase (phox) were used for in vitro infections. Our data reveal that machrophages from iNOS- mice are more susceptible to infection with *L. amazonensis*. Curiously, when macrophages from iNOS- were

treated with IFN-γ, there was a slight but significant inhibition of parasite growth. This datum points towards a IFN-γ dependent, iNOS independent pathway of inhibiting parasite growth in machrophages. It is possible that the lack of NO exacerbates the production of ROS, which would partially control the growth of parasites. On the other hand, phox-/- mice don't show large difference when compared with WT. This study sheds light on other mechanisms involved in the control of growth of *L. amazonensis* in machrophages. Support: CNPq, CAPES and FAPEMIG.

IM55 - *Lutzomyia longipalpis* salivary gland homogenates induce vasodilation and vascular permeability increase in the hamster cheek pouch.

Svensjo, E. *(Universidade Federal do Rio de Janeiro)*; Lemelle, N. S. *(Universidade Federal do Rio de Janeiro)*; Scharfstein, J *(Universidade Federal do Rio de Janeiro)*; Saraiva, E.M. *(Universidade Federal do Rio de Janeiro)*

Saliva of sand flies contains potent pharmacological components that facilitate *Leishmania* transmission. Apart from assisting vectors needs in obtaining their blood meals, the sand fly saliva induces host responses that modulate inflammation and anti-parasite immunity. In the present study we explored the potential contribution of *L. longipalpis* saliva to the invasion and infection of *Leishmania* parasites. Salivary glands were dissected from non-fed, non-infected sand flies, sonicated and stored at -80°C until used in intravital microscopy experiments. Salivary gland homogenate (SGH), equivalent to half a salivary gland was applied topically to cheek pouches of anesthetized hamsters ($n = 5$) after i.v injection of FITC-dextran. Arteriolar diameters and vascular permeability changes were measured at 5 min intervals. SGH caused an immediate arteriolar dilation ($P < 0.05$) at 2 min following its application and reached a maximal diameter of $52 \pm 20\%$ ($mean \pm SD$) above pre-SGH diameter at 15 min. Vascular permeability responses increased ($P < 0,05$) to maximum of $44 \pm 8\%$ above pre-application control level at 15 min. A secondary challenge with SGH at 60 min after the first application resulted in a significant dilation (52 ± 27 %, $P < 0.05$) but there was no increase in vascular permeability. Secondary challenge only evoked discrete responses in vascular permeability indicating that the vascular bed was desensitized, as observed after ischemia/reperfusion injury or topical application of different *Leishmania* parasites. Hamsters ($n = 4$) treated with bradykinin-2-receptor antagonist HOE-140 plus SGH showed similar dilation effects but a smaller increase in vascular permeability than SGH alone. Salivary gland homogenate (SGH) induced reproducible effects and can be used for further studies on the role of salivary gland components for *Leishmania* transmission. Supported by: CNPq, FAPERJ.

IM56 - SERUM RESPONSE IN C57BL/6J, BALB/C AND SWISS MICE IMMUNIZED WITH IRRADIATED TACHYZOITES OF *Toxoplasma gondii* RH STRAIN AND ORAL CHALLENGE WITH ME-49 STRAIN

Galisteo Jr., A.J. *(Instituto de Medicina Tropical de São Paulo)*; Zorgi, N.E. *(Instituto de Medicina Tropical de São Paulo)*; Baptista, J.A. *(Instituto de Pesquisas Energéticas e Nucleares)*; Hiramoto, R.M. *(Instituto Adolfo Lutz)*; do Nascimento, N. *(Instituto de Pesquisas Energéticas e Nucleares)*; de Andrade Jr., H.F. *(Instituto de Medicina Tropical de São Paulo)*

Toxoplasmosis, a prevalent widespread infection in man and animals, is mainly transmitted by oral route, through ingestion of oocysts from water and food contaminated with cat feces or infected animal tissue cysts in undercooked meat. Vaccine development implies in effective intestinal immunity, the first site of parasite entry. Radiation (255Gy/60-Co) sterilized *T. gondii* RH strain tachyzoites (RST) induced significant protection when parentally administered, similar to chronically infected and acute disease protected animal. We study the humoral immune response in C57Bl/6j, Balb/c and Swiss mice immunized with 10^7 RST, by oral or parenteral 3 biweekly administration. *T.gondii* antigens specific ELISA for IgG, IgA, IgG1, IgG2a and IgG2b detection was performed in weekly blood samples during immunization. After 2 weeks, immunized and control animals were challenged with 10 cysts of ME49 strain p.o. Protection was determined at the 30th day for *T. gondii* cysts counting in brain. All immunized mice groups presented significant protection when challenged with ME-49 cysts; however, Balb/c mice showed better protection levels, with only one positive animal on brain microscopic analysis. IgG production in the serum of the animals was higher in groups immunized by i.p route; however, IgA and IgG1 levels were higher in Balb/c mice immunized by oral route. This higher protection found in Balb/c group could probably also be related to the Th2 response, demonstrated by higher IgG1 levels. All these data provide insights in oral immunization schedules for toxoplasmosis prevention, suggesting that oral immunization could be an alternative in the prevention of toxoplasmosis and the block of chain transmission. Galisteo Jr., A.J. is a fellow of CNPq (141404/2004-3). This work was supported by CNPq and LIMHCFMUSP-49

IM57 - Strain specific antibodies using synthetic peptide ELISA in the diagnosis in *T. gondii* infections

Obeid, M.A. *(Instituto de Medicina Tropical de São Paulo)*; Tsutsui, V.S. *(Instituto de Medicina Tropical de São Paulo)*; Marciano, M.A.M. *(Instituto de Medicina Tropical de São Paulo)*; Galisteo Jr., A.J. *(Instituto de Medicina Tropical de São Paulo)*; de Andrade Jr., H.F. *(Instituto de Medicina Tropical de São Paulo)*

Toxoplasma gondii is a highly prevalent heteroxenous apicomplexan, affecting warm-blooded vertebrates. Despite

asymptomatic chronic infections in most people, toxoplasmosis could cause acute infection with lymphadenopathy, chorioretinitis, and fatal encephalitis in immunosupressed patients or fetus. *T. gondii* is one unique species but there are at least 3 phenotypic and genotypic defined strains, with diverse virulence and geographical distribution. In Brazil, *T. gondii* types I and III strains were found, without isolation of type II strain. The diagnosis of each strain type relies on isolation and genotype analysis, a time-consuming and difficult task. Recently, there are reports of strain specific epitope markers, which allow strain identification by specific antibody production. We tested a set of human serum samples, previously screened for anti-*T. gondii* antibodies, to evaluate its parasite strain-specificity using synthetic peptides. ELISA were performed using *T. gondii* total lysate and synthetic peptides from dense granule antigens, GRA6-I/III (LPRERVNVFDY) and a derived sequence from GRA6-II (LHPGSVNEFD). The coupling efficiency to the carrier protein bovine serum albumin via glutaraldehyde was over 35%, by using ^{3}H-proline and ^{3}H-leucine. We found that most sera reacted more intensely with I/III peptide, but a few sera presented higher reaction with type II peptide. These findings corroborate the high prevalence of types I and III strains in Brazil. Further studies with synthetic peptides using association of specific peptides could improve the strain identification in *T. gondii* infection, allowing screening of specific strain related diseases. This work is supported by LIMHCFMUSP, CNPq and Fundap.

IM58 - Detection of Anti-*Toxoplasma gondii* Antibodies in Dogs from northwest region of São Paulo state, Brazil

Aureliano, D. P. *(Insttituo Adolfo Lutz)*; Motoie, G. *(Instututo Adolfo Lutz)*; Tolezano, J.E. *(Instituto Adolfo Lutz)*; Taniguchi, H.H. *(Instituto Adolfo Lutz)*; Hiramoto, R.M. *(Instituto Adolfo Lutz)*

Toxoplasma gondii is an obligate parasite widespread throughout the world. It affects warm-blooded animals, including man, birds and domestic animals. The infection occurs by ingestion of oocysts excreted in faeces of infected felines or by ingestion of raw and undercooked meat containing cysts. Infected individuals with *T. gondii* are generally asymptomatic but ocular damage can occur. The microorganism can cause serious diseases in immunocompromised patients and fetus of primary infection of pregnant woman. *Toxoplasma* infection in man and dogs are very common as demonstred by various serological surveys. Considering this fact, it is possible to use dogs as an important tool environmental indicator of *Toxoplasma gondii* contamination. We have evaluated serum samples of domestic animals from Araçatuba, Guararapes, Mirandópolis and Andradina, São Paulo state, Brazil. A total of 200 blood samples were analyzed by enzyme-linked immunosorbent assay (ELISA). The sera-prevalence of *T. gondii* infection was determined by a specific IgG ELISA with respectively 55,1%, 57,1%, 40% and 52% in these cities. The positive percentage of samples in all cities was 51%. These results were obtained from domestic dogs and show the risk those animals have been exposed. Probably the population has a similar exposure risk. To sum up, both dogs and humans can be exposed to common sources of infection what is an indication that the parasite is disseminated in the urban areas.

IM59 - EXPRESSION, ANTIGENICITY AND CIRCULAR DICHROISM STUDIES OF THE RECOMBINANT MEROZOITE SURFACE PROTEIN-3 ALPHA AND BETA OF *PLASMODIUM VIVAX*.

Jimenez, M.C.S. *(Departamento de Análises Clínicas e Toxicológicas)*; Ricci, R. *(Departamento de Análises Clínicas e Toxicológicas)*; Ramos, C.H. *(Centro de Biologia Molecular Estrutural)*; Chitnis, C.E. *(International Centre for Genetic Engineering and Biotechnology)*; Barnwell, J.W. *(Division of Parasitic Diseases)*; Galinski, M.R. *(Division of Parasitic Diseases)*; Rodrigues, M.M. *(Universidade Federal de São Paulo)*; Soares, I.S. *(Departamento de Análises Clínicas e Toxicológicas)*

In previous immuno-epidemiological studies of the naturally acquired antibody responses to Merozoite Surface Protein 1 (MSP-1) of *Plasmodium vivax*, we had evidence that the responses to distinct blood stages antigens could be differentially regulated. The present study was designed to evaluate the antigenicity of the recombinant proteins representing the Merozoite Surface Protein-3 alpha and 3 beta of *P. vivax* in 220 individuals from the State of Pará, in the north of Brazil. Recombinant proteins representing the C-terminal region of MSP-3 alpha and different regions of MSP-3 beta (N and C-terminal and full-length protein) were expressed in *Escherichia coli* from vectors histidine-tagged. Circular Dichroism (CD) spectroscopy was used to probe the secondary structure of each one recombinant protein representing MSP-3 alpha and MSP-3 beta. These recombinant proteins were compared in their ability to bind to IgG antibodies of serum samples collected from malaria exposed individuals. Recombinant proteins representing of the region II of Duffy Binding Protein (PvDBP-RII) and C-terminal region of MSP-1 ($MSP1_{19}$) were also tested for comparison. During patent infection with *P. vivax*, the frequency of individuals with IgG antibodies to MSP-3 alpha and at least one recombinant protein representing MSP-3 beta were 68.2% and 79.1%, respectively. The frequency of individuals with IgG antibodies to $MSP1_{19}$ was higher (95.0%) and of the greater magnitude. In contrast, the frequency of individuals with IgG antibodies to PvDBP-RII was lower (44.5%), however the frequency of responders increased after the second episode of the disease. Individually, the antibody levels to MSPs significantly declined nine months after treatment, except to full-length MSP-3 beta. Our results provide the first observations on human antibody responses to MSP-3 alpha and MSP-3 beta and confirm a complex regulation of the immune response to distinct blood stage antigens that can contribute to the high risk of re-infection in these individu-

als.
Support:CAPES.

IM60 - CD34$^+$ cells in the spleen during experimental rodent malaria.

Hermida, F. P. H. *(Instituto de Ciências Biomédicas)*; Fernandes, E. R. F. *(Faculdade de Medicina de São Paulo)*; Vieira, D. P. V. *(Intituto de Pesquisas Energéticas e Nucleares)*; de Andrade, H. F. A. Jr. *(Instituto de Medicina Tropical de São Paulo)*

The spleen is composed of 3 distinct but functionally associated structures forming the white pulp and the red pulp. An essential organ for malaria control, it is responsible for parasite clearance through cell filtration network in the red pulp. The increased load of rapid growing parasites implies in the need of huge and quick amplification of this network in order to control parasitemia and host survival. The amplification involves the participation of several cell types, including myeloid precursors from the endothelial and myelocytic lineages, usually presenting the CD34 in their surface. We study the distribution of CD34$^+$ cells, by immunohistochemistry, in the spleen of experimental rodent malaria models. Groups of C57Bl/6j mice infected with two strains of Plasmodium chabaudi, the CR self-controlled non-lethal strain, and the AJ strain, usually lethal, were killed daily after infection with 106 parasites, until 10th day, when all AJ infected mice died. CD34$^+$ cells were rarely detected in the red pulp of normal spleen, but after malaria, they started to appear in large numbers in the red pulp, at early times, without evident significant parasite control, with similar spleen distribution both in non-lethal and lethal models. As the red pulp increases, some CD34$^+$ positive cells were seen inside lymphoid follicles in the white pulp. Marginal sinus and the red pulp achieved an activated state when parasitemia control starts in the non-lethal model, which was not found in the lethal model. Some foci of erythroid metaplasia were seen in the red pulp. These data suggests that the CD34$^+$ cells participate on the early stages of spleen amplification in rodent malaria, but the exact participation of those cells, if as active immune cell promoter or a supporting cell for filtration network amplification remains to be established by further studies.

IM61 - : IgG Avidity and serology using T.gondii rROP2 in the detection of acute infection in IgM screened pregnant women.

Macre, M.S. *(INSTITUTO DE MEDICINA TROPICAL/INSTITUTO DE CIÊNCIAS BIOMÉDICAS USP)*; Angel, S.O.A. *(Laboratorio de Parasitología Molecular)*; Andrade Jr, H.F. *(Faculdade de Medicina da Universidade de São Paulo)*

Toxoplasmosis is a worldwide protozoan infection who affects $\tilde{2}$0% of the human population. Usually non-symptomatic, this infection cause severe disease to the fetus during acute maternal infection. Usually performed by IgG and IgM assay, the diagnosis of acute infection became difficult, due to the extremely sensitivity of new IgM commercial assays. IgG avidity was an alternative assays, but with difficult standardization, and the use of recombinant antigens could results in a better efficient test. We standardized an IgG avidity assay using rROP2 recombinant protein in the solid phase, testing their efficiency in the detection of acute infection in 160 serum from IgM + pregnant women. Conventional high binding plates were recovered with 10ug/ml of rROP2 dissolved in 8M Urea PBST and used in conventional ELISA assays, with one washing with two different chaotropes, Urea or Ammonium Thiocyanide. Avidity was calculated using restricted O.D. ratio (Avr). Most samples presented rROP2 specific antibodies, with good correlation (r2=0.31) but some *T.gondii* positive samples give no results in rROP2 assay and false negatives also appear, with a greet but perfect agreement (kappa index 0,72). *T.gondii* IgA titers also correlated (r=0.16) with rROP2 IgG. Avidity using rROP2 was similar to *T.gondii* IgG avidity, but it was related more to the type of chaotrope used. This recombinant assay could be an alternative to the diagnosis of acute infection in patients, but until more samples were tested, its result must be associated to others tests.

IM62 - Virulence and organ dissemination of two strains of *Toxoplasma gondii* with two different genetic profile in a bird experimental model

Carmo, CV *(Instituto de Medicina Tropical de São Paulo)*; Meireles, LR *(Instituto de Medicina Tropical de São Paulo)*; Tsutsui, VS *(Instituto de Medicina Tropical de São Paulo)*; Marciano, MAM *(Instituto de Medicina Tropical de São Paulo)*; Andrade Jr., HF *(Instituto de Medicina Tropical de São Paulo)*

Toxoplasma gondii is a parasitic protozoan with high prevalence and an exceptional capacity of infecting birds and mammals, being transmitted in a felid-prey system by carnivorism and oocysts production. In spite of being highly clonal, *T. gondii* isolates present characteristic isoenzymes profile and mouse virulence, classified in three main strains (I, II and III). Those biological characteristics could be selected according to the prey-predator association, as large felid-ruminants strains less virulent than cat-birds or small animals associated strains, switch necessarily must be more efficient and reproductive. We study the virulence of two strains of *T. gondii* with two different genetic profiles (RH type I and ME-49 type II) in an experimental model of birds. Groups of one-day old chicks were inoculated with 1000 tachyzoites of RH or ME-49 strains or brain cysts of ME-49 previously infected mice, by subcutaneous injection. No sign or symptom of acute disease was found in any bird, without any death. After 30 days, the animals were killed and the organs (brain, heart, lung and liver) were analyzed by histology and PCR for *T.gondii* B1 gene. *T.gondii* nucleic acids were found in all organs analyzed, independent of strains or parasite form

used for infection, with adequate internal controls showing that all animals presented disseminated infection. By histology of RH infected birds, the heart was the more affected, with clear inflammatory foci and agents. SNC and liver also presented discrete inflammatory foci and the lung was less affected. ME-49 infected birds presented a less evident organ involvement, main in the lung and brains, especially in those who were infected with tissue cysts. The strain RH induced more lesions and probably more dissemination of the infection, but ME-49 strain also induced disseminated infection, as detected by PCR. Virulence of *T.gondii* strain could be related to prey-host successful association.

IM63 - EFFICIENCY OF SEROLOGICAL DIAGNOSIS OF TOXOPLASMOSIS AS RELATED TO CENTRAL OR DISTANT LABORATORIES

Mioranza, S.L. *(Universidade de São Paulo)*; Andrade Jr, H.F. *(Universidade de São Paulo)*; Meireles, L.R. *(Instituto de Medicina Tropical de São Paulo)*; Alvim, R.P. *(Instituto de Medicina Tropical de São Paulo)*

Toxoplasmosis importance is directly related to low prevalent severe diseases, as congenital toxoplasmosis, and SNC involvement in immune-deficient patients. Patients at risk are diagnosed by specific antibody tests, usually in pre-natal care or preexisting diseases definition in immune-deficient or HIV patients, for specific or fetal therapy. Several commercial kits are used, as IFA, hemmaglutination, ELISA and others in large centers. Those tests are performed according to instructions without external standards in recently developed urban areas in Brazil, as Paraná state. We compare the serological diagnosis in samples from patients previously diagnosed at the Central Laboratory in the city of Cascavel-PR, using commercial assay, retested with controlled assays in a metropolitan reference center, in the Protozoology Laboratory/IMTSP. Samples from 342 patients which toxoplasmosis serology established at the Central Laboratory of Cascavel, PR , were re-assayed by IFA and in house IgG ELISA. Comparison between frequency and titers in each assay and laboratory were analyzed by Kappa agreement, sensitivity and specificity indexes and concordance. Using IFA as golden standard, our sample was composed by 189 (55,26%) positive and 153 (44,74%) negatives. When IFA was compared to In House ELISA, there are a greater agreement (0.98225) with high sensitivity (99.5% - 95% IC 96.6-100.0) and specificity (98.7% - 95%IC 94.9-99.8). The original results, as tested in Cascavel, showed lower sensitivity (97.9% - 95% IC 94.3-99.3), and agreement (0.9705). The numbers of false positive was similar in ELISA assays, but false negatives were higher (2%) in commercial assays. Careful search of anti-*Toxoplasma gondii* IgG in high prevalence recently developed regions, as Paraná, must be adequately performed, but commercial assays performed by local testers resulted in false negatives that could affect the adequate treatment. Reference reports and adequate training and checking must be established for improvement in the toxoplasmosis diagnosis in those rapid developing areas.

IM64 - Inhibition properties of the antibody response to *Plasmodium vivax* Duffy binding protein in an area with unstable malaria transmission.

Cerávolo, I. P. *(Centro de Pesquisas René Rachou, FIOCRUZ)*; Souza-Silva, F. A. *(Centro de Pesquisas René Rachou, FIOCRUZ)*; Fontes, C. J. F. *(Universidade Federal de Mato Grosso)*; Braga, E. M. *(Universidade Federal de Minas Gerais)*; Krettli, A. U. *(Centro de Pesquisas René Rachou, - FIOCRUZ)*; Souza, J. M. *(Instituto Evandro Chagas)*; Adams, J. N. *(University of Notre Dame)*; Brito, C. F. A. *(Centro de Pesquisas René Rachou)*; Carvalho, L. H. *(Centro de Pesquisas René Rachou, FIOCRUZ)*

Plasmodium vivax Duffy binding protein (DBP) function in the erythrocyte invasion process is critical for parasite growth and pathogenesis in human infections. This makes DBP a logical target for vaccine-mediated immunity, but little is known about anti-DBP antibodies induced by infection and their ability to inhibit DBP-erythrocyte interactions (Michon et al.,*Infect. Immun.* 68:3.164-3.171, 2000). In the present study, we examined the ability of sera from different populations of the Brazilian Amazon - an area of markedly unstable malaria transmission - to inhibit the erythrocyte-binding function of the ligand domain of the DBP (region II, DBPII), expressed on the surface of cultivated mammalian cells. We find that long-term exposure to malaria in the Amazon area elicits an inhibitory antibody response against different DBPII variants. The inhibitory efficacy of the immune inhibition did not have a strong correlation with the antibody responses quantified by ELISA. Of potential importance for vaccine development, the three different DBPII variants tested were equally recognized by sera from asymptomatic and symptomatic *P. vivax* infections. Supported by UNICEF/UNDP/Word Bank/WHO/TDR, CNPq FIOCRUZ (PAPES IV).

IM65 - *PLASMODIUM VIVAX* APICAL MEMBRANE ANTIGEN 1: COMPARATIVE RECOGNITION BY HUMAN ANTIBODIES OF RECOMBINANT PROTEINS REPRESENTING DIFFERENT PORTIONS OF THE ECTODOMAIN.

Múfalo, B. C. *(Universidade de São Paulo)*; Soares, I. S. *(Universidade de São Paulo)*

The Apical Membrane Antigen 1 (AMA-1) of *Plasmodium sp* is one of the leading candidate antigens being studied as a vaccine against malaria. This merozoite protein has an ectodomain that can be divided into three domains (DI, DII, and DIII). We have previously shown the ectodomain of the *Plasmodium vivax* AMA-1 is highly immunogenic during nat-

ural human infections. To investigate the antibody responses directed against different portions of AMA-1, we expressed as bacterial recombinant proteins each of the domains separately or in combination (DI-II and DII-III). Their recognition by IgG (and subclasses) and IgM antibodies were evaluated using 100 *P. vivax*-infected sera collected from Brazilian endemic region. We found that the frequencies of sera which recognize the recombinant proteins containing the domain II was higher (65% for DII, 59% for DI-II, and 58% for DII-III) and of greater magnitude. In contrast, the frequencies of individuals with IgG antibodies to individual DI and DIII were lower (13% and 12%, respectively). Overall, IgG1, IgG3 and IgG4 antibodies were prevalent to all proteins. On the other hand, the frequency of individuals that presented IgM antibodies to each domain was low ranging from 4% (DIII) to 36% (DII-III). Our results provide the first observations on human antibody responses to individual *P. vivax* AMA-1 domains and suggest that the DII is particularly immunogenic during natural infections. Recombinant proteins containing this domain can be explored in future studies for the induction of protective immunity against malaria *vivax*. Supported by FAPESP and CNPq (Instituto do Milênio-IMTEV).

IM66 - Retina autoantibodies in sera from patients with toxoplasmosis.

Costa, T.B. *(Instituto de Medicina Tropical de São Paulo)*; Yamamoto, J.H. *(Departamento de Oftalmologia e Otorrinolaringologia)*; Cursino, S.R.T. *(Instituto de Medicina Tropical de São Paulo)*; Meireles, L.R. *(Instituto de Medicina Tropical de São Paulo)*; Mioranza, S.L. *(Instituto de Medicina Tropical de São Paulo)*; Marciano, M.A.M. *(Instituto de Medicina Tropical de São Paulo)*; Cardoso, R.P.A *(Instituto de Medicina Tropical de São Paulo)*; Andrade Jr, H.F. *(Instituto de Medicina Tropical de São Paulo)*

Toxoplasmosis is a disease that affects millions of people worldwide, with most infected patients without symptoms. In some groups, the infection causes diseases, as a lymphonodular febrile acute syndrome, or a chronic retinochoroiditis. In immune-compromised patients and the fetus, acute or reactivated disease severely affects CNS, amongst other complications. The diagnosis of the disease is established with serological tests involving IgM for the acute phase, and IgG for the chronic phase. In ocular toxoplasmosis the diagnosis is established with ophthalmologic examination and the lesions are often necrotic with destruction of the neuronal retina and choroids. Scars from those lesions are found in up to 20% of specific IgG positive patients. There are several reports dealing with autoimmune processes in the eye, as ophthalmia sympatica, and most patients with active *T.gondii* retinochoroiditis also present lymphoproliferative response to retinal antigens. In order to study humoral autoimmunity in the eye involvement of toxoplasmosis, we developed a crude retinal human antigen ELISA, using retina extracted from discarded eyes from cornea transplantation, using a urea-SDS-protease inhibitors solution. Sera from 489 patients, previously tested for toxoplasmosis, were used this assay and the results sorted using an arbitrary threshold, as low responders and high responders. There is a higher frequency of high responders in patients who had evidence of positive serology against *T.gondii* (153/285, 53.7%), when compared to negative patients (83/183, 45.4%), which was significant but at border levels (Fisher exacts 0.03 and Odds ratio = 1.4). There are some negative patients who presented higher levels of anti-retina antibodies and we cannot exclude that the *T. gondii* infection is only partially responsible for those autoantibodies. This data is highly suggestive and corroborate the participation of an autoimmune response in *T.gondii* retinochoroiditis. This work is supported by LIMHCFMUSP, CNPq and Fundap.

IM67 - *Plasmodium berghei* sporozoites develop in mammalian skin

, T. A. V. *(Dpt of Medical Parasitology, New York University School of Medicine)*; , P. S. *(Dpt of Medical Parasitology, New York School of Medicine)*

Plasmodium sporozoites, the infectious stage of the malaria parasite, are injected into the dermis by infected Anopheline mosquitoes. From here they enter the blood circulation and go to the liver where they invade hepatocytes and develop into exoerythrocytic stages (EEFs). Until recently, it was thought that sporozoites spent little time in the skin and had to go to the liver to complete their development. We now have data demonstrating that some sporozoites remain at the injection site for long periods of time. Using confocal microscopy and GFP-expressing sporozoites, we studied the fate of those parasites that stay in the skin. Our results showed in the 72 hours sporozoites develop into to what appear to be mature EEFs judging from the size and the shape of the parasites. Follow-up over several days revealed that these putative EEFs could persist until at least 15 days. These skin EEFs were observed for both *Plasmodium berghei* and *Plasmodium yoelii*, two different rodent malaria parasites and in different rodent hosts, suggesting that *Plasmodium* development in the skin of the mammalian host may be a common property of *Plasmodium* species. Immunization of mice with irradiated sporozoites significantly decreased their number. Skin EEFs may be a useful tool to study the biology of these stages and to learn more about the host immune response to preerythrocytic stages of *Plasmodium*.

IM68 - ROLE OF MIF IN EXPERIMENTAL TOXOPLASMA GONDII INFECTION IN GENETICALLY RESITANT AND SUSCEPTIBLE HOSTS.

Mesquita, J.S. *(Inst.Microbilogia Prof. Paulo de Góes/UFRJ)*; Queiroz, LF *(Hosp. Univ. Clementino Fraga Filho/UFRJ)*; Madi, K. *(Hosp. Univ. Clementino Fraga Filho/UFRJ)*; Bozza, MT *(Inst. Microbiologia Prof. Paulo de Góes/UFRJ)* Cavalcanti, MG *(Hosp.Univ.Clementino Fraga Filho/UFRJ)*

Introduction: Host genetic background determines susceptibility (C57BL/6) or resistance (Balb/c) to Toxoplasmosis *T. gondii* infection is a result of host ability to control parasite burden and regulate immunopathology. Macrophage-migration inhibitory factor (MIF) is a pro-inflammatory cytokine which contribute to both protective or pathogenic responses in parasitic infections. Herein, we investigate the role of MIF in pro-inflammatory-induced pathology and parasite control in genetically distinct mice infected with *T.gondii*. **Material and Methods**: Wild type (B6, Balb/c) and MIF knock out (MIFKO, C57BL/6xSv129, Balb/c) mice were infected with 100 cysts of ME-49 strain of *T. gondii* perorally and with 20 cysts, via ip. For histopathologycal analysis, tissue samples were stained with HE. Parasite burden was determined by counting the number of cysts in brain homogenates. **Results**: Mortality was higher in B6wt mice when compared to MIF KO(n=5-7,wt; n=5-7, MIF; p<0,01). But, Balb/c and Balb/cMIFKO survival rates were not different. Only B6wt mice have increased weight loss when compared to MIFKO (15,2g±1,6 versus 22.3g±2,8; respectively; p<0,05) but not Balb/c and Balb/cMIFKO. Histological analysis showed that inflammatory infiltration were increased in the liver and brains (CNS) of wt when compared to MIFKO mice in both genetic backgrounds though only B6wt presented ileitis. Total cell numbers from mesenteric lymph nodes(MLN) were augmented in B6wt (80,3± 0,58 x 10^6 cells) when compared to MIFKO (31,75± 5,53 x 10^6 cells, p<0,01). And the same difference was observed in Balb/c versus Balb/cMIFKO. At CNS, parasite burden was decreased in MIFKO when compared to wt mice(B6 x MIFKO, 1550± 50 x 333±202; Balb/c x MIFKO, 61±12 x 4± 3, respectively, p<0.05) . **Conclusion**: Our data suggest that MIF decreases resistance to Toxoplasmosis in genetically susceptible but not in resistant hosts. Nonetheless, MIF regulates both pro-inflammatory response and parasite burden independently of mice strain.

IM69 - In situ expression of anti-*(E. histolytica)* and anti-*(E.dispar)* antibodies in liver: effects against trophozoites and on the extension of hepatic lesions

Costa, C. A. X. *(Universidade Federal de Minas Gerais)*; Nunes, A. *(Universidade Federal de Minas Gerais)*; Gomes, M. A. *(Universidade Federal de Minas Gerais)*; Caliari, M. V. *(Universidade Federal de Minas Gerais)*

Entamoeba histolytica is a protozoan parasite which causes amebiasis in man. The mechanisms involved in generating lesions in the liver are not still completely understood, as well as the role of immune responses raised against trophozoites. Trying to reach these issues, the aims of our study was to verify the in situ expression of anti-trophozoite antibodies and their effects on the amount of amoebas and lesion extension in parasitized tissues. Twenty-five hamsters were inoculated intra-hepatically with 100,000 trophozoites of *E. histolytica* and *E. dispar* strains. Groups of five animals were necropsied in days 1, 2, 3, 6 and 8 after inoculation. Liver fragments were stained with streptoavidin-peroxidase immunohistochemistry for detection of trophozoites and anti-trophozoites antibodies. The optical density measures were obtained in the Carl Zeiss image analyzer. The necrotic lesions were significantly bigger in liver infected with E. histolytica than *E. dispar* . Most of the trophozoites of both amoebas presented positive reaction for anti-trophozoite antibodies with stronger optical densities in *E. histolytica* ones, indicating lower amount of antibody bound on their surfaces $Mann - Whitneytest, p = 0.0306$. These findings may be evidences of the enhanced *E. histolytica* ability to evade humoral immune response compared to *E. dispar* which may be related to the amount and types of cysteine proteinases produced by *E. histolytica* trophozoites . Supported by FAPEMIG

IM70 - Vesicles shed by *Trypanosoma cruzi* modulate pro-inflammatory cytokines and mice infection by the parasite

Torrecilhas, A.C.T *(Universidade de São Paulo)*; Tonelli, R.R *(Universidade de São Paulo)*; Pavanelli, W.R. *(Universidade de São Paulo-Faculdade de Medicina de Rib)*; Silva, J.S *(Universidade de São Paulo-Faculdade de Medicina de Ribeirão Preto)*; Colli, W *(Universidade de São Paulo)*; Alves, M.J.M *(Universidade de São Paulo)*

Infective trypomastigote forms of *T.cruzi* release vesicles into the culture medium that are rich in Tc85 and alpha-galactosyl-containing molecules. These vesicles induce potent inflammatory host immune response and enhance *in vitro* host cell invasion by parasite (Torrecilhas et al., submitted). The role of the vesicles on cytokine production and infection *in vivo* are being investigated. The results shown that pretreatment of isolated peritoneal macrophages with

vesicles for 24 hs induces a hyporesponsive state to a second vesicle stimulation, as measured by a significant decreased in TNF-alpha and IL-12 production. Spleen cells isolated from BALB/c mice previously treated with vesicles and infected with 500 blood forms of parasite (Y) produce high levels of IL-10, IL-4 and NO when stimulated in vitro with Con-A or T-Ag (antigen) (15 dpi) and lower levels of TNF-alpha and IL-12 when compared with the controls (PBS). Our preliminary data suggest that pretreatment of BALB/c mice with total shed or one isolated fraction (P2 fraction) increases the susceptibility to *T. cruzi* infection. Accordingly, the mortality of total shed- or P2- treated mice reached 80% by day 15-16 and 100% by day 20-22, whereas all animals of the control group died only after 30 days pos infection. The results herein described show the modulation of pro-inflammatory cytokines and the higher susceptibility to *T.cruzi* infection by the total fraction of vesicles shed by the parasite or by the P2 fraction and may be considered as a parasite virulence factor. Supported by: FAPESP and CNPq

IM71 - SIZE OF INFECTIOUS DOSE CONTROLS THE TEMPO OF *Trypanosoma cruzi* MULTIPLICATION AND THE KINETICS OF SPECIFIC CD8+ T CELL EXPANSION.

Tzelepis, F. *(UNIFESP-EPM, São Paulo, SP, Brazil)* Rodrigues, M.M. *(UNIFESP-EPM, São Paulo, SP, Brazil)*

CD8+ T lymphocytes play a critical role in the naturally acquired immunity during experimental infection with *T. cruzi*. Recently, we described that the kinetics of expansion of *T. cruzi* specific CD8+ T cells during experimental infection is distinct from other pathogens such as virus, bacteria or malaria parasites. To gain further knowledge on the parameters that control this unusual kinetics, we investigated the tempo of parasite multiplication and the kinetics of expansion of CD8+ T cells specific for the Amastigote Surface Protein-2 (ASP-2) in mice infected with different doses of *T. cruzi*. For that purpose, C57BL/6 mice were infected i.p. with 10E2, 10E3, 10E4 or 10E5 trypomastigotes of the Y strain. The increasing doses of parasites administered significantly accelerated the peak parasitemia from day 12-13 (10E2) to day 5 (10E5) post-challenge. The CD8+ T-cell response to ASP-2 was monitored by the in vivo cytotoxic activity to the epitope IYNVGQVSI. We observed that increasing doses of parasites significantly accelerated the in vivo cytotoxic activity to this epitope. For example, by day 10 post infection, the in vivo cytoxicity of mice infected with 10E5 parasites was close to 100% while the other groups varied from 0 to 40%. By day 20 post-infection, the in vivo cytoxicity of all mouse groups was close to 100%. The acceleration of CD8+ T cell activation was confirmed by estimating the number of Interferon-gamma producing cells by the ELISPOT method. To our knowledge, our study provides the first evidence suggesting that the tempo of parasite multiplication can be a key factor influencing the expansion of specific CD8+ cytotoxic T cells. Supported by FAPESP and CNPq (Instituto do Milênio-IMTEV).

IM72 - TLR-9 activator CpG ODN 1826 is as a potent adjuvant generating long lived protective CD8+ T cells against *Trypanosma cruzi* infection.

Alencar, B.C.G. *(UNIFESP-EPM)*; Araújo, A.F.S. *(UNIFESP-EPM)*; Tzelepis, F. *(UNIFESP-EPM)*; Penido, M.L.O. *(UFMG-MG)*; Gazzinelli, R.T. *(CPRR and UFMG)* Rodrigues, M.M. *(UNIFESP-EPM)*

Immunizations with recombinant proteins in the presence of conventional adjuvants rarely elicit CD8+ T cell-mediated immune responses. Vaccine formulations containing recombinant proteins capable of cross-priming specific CD8+ T cells could be of great interest. We have recently described that vaccination with recombinant proteins of the Amastigote Surface Protein-2 (ASP-2) of *T. cruzi* in the presence of alum and the TLR-9 activator CpG ODN 1826 induced specific CD8+ T cells and remarkable protection against lethal *T. cruzi* infection (Infect. Immun, 73:6017-25). Here, we explored some of the mechanisms underlying the activation and expansion of these protective CD8+ T cells. From our studies we concluded that the presence of CpG ODN 1826 significantly improved the number of H-2Kk-restricted CD8+ T cells specific for the epitope TEWETGQI and the protective immunity against a lethal challenge with T. cruzi. Complete protection was long lived, being detected as far as 5 months after the last immunizing dose. We also observed that the induction of specific CD8+ T cells was independent of CD4+ T cells and negatively regulated by the presence of CD25+ cells. Finally, we established that after challenge of immunized mice, specific CD8+ cells displayed a swift and robust anamnestic response. From our results, we concluded that a TLR-9 activator may be a reliable adjuvant to elicit long-lived protective CD8+ T cells against parasitic infections. Supported by: CNPq (Instituto do Milênio-IMTEV), FAPESP and FAPEMIG

IM73 - In vitro trypanocidal activity of *Erythrina speciosa* (Andrews) is not related with control of infection in Swiss mice

Graça-de-Souza, VK *(Departamento de Microbiologia, Universidade Estadual de Londrina)*; Malvezi, A.D. *(Hematologia, HUNP- Universidade Estadual de Londrina)*; Menolli, R.A *(Universidade Estadual do Oeste do Paraná)*; Marguti, I. *(Departamento de Imunologia, Universidade São Paulo)*; Rizzo, L.V. *(Departamento de Imunologia, Universidade São Paulo)*; Yamada-Ogatta, S.F. *(Departamento de Microbiologia, Universidade Estadual de Londrina)*; Pinge-Filho, P. *(Departamento de Ciências Patológicas, Universidade Estadual de Londrina)*; Faria, T.J *(Departamento de Química, Universidade Estadual de Londrina)*

We evaluated the anti-trypanosomal activity of an ethanolic extract of *Erythrina speciosa* Andr. (Leguminosae-Papilionoideae) (Et-Es) on epimastigote forms of *Trypanosoma cruzi*. Et-Es, at 250 to 1000 μg/mL, inhibited *T. cruzi* growth by 50 to 75% after 72 h incubation. Daily administration of Et-Es (50mg/kg body weight) in Swiss mice, beginning 4 h after inoculation with 5 x 10^3 trypomastigote forms of *T. cruzi*, provoked a significant increase in parasitemia when compared to non-treated infected mice. Infected mice survived for 16 days after inoculation. Infected mice treated with Et-Es showed 100% mortality but survived longer (day 22 post infection). In addition, we determined the effect of Et-Es on hematological parameters and nitric oxide production (NO). Infected mice showed a slight anemia and increase in NO production by macrophages from the peritoneal cavity, which were not modified by treatment with Et-Es. These studies indicate that in vitro trypanocidal activity of Et-Es is not relevant to the control of infection in mice.

IM74 - Oral immunization with *Phytomonas serpens*, a tomato parasite, prevent thrombocytopenia and leukopenia during acute phase of experimental infection with *Trypanosoma cruzi* (Y strain)

Specian, A.F.L *(Curso de Farmácia, Universidade Norte do Paraná)*; Malvezi, A.D. *(Setor de Hematologia, Hospital Universitário Norte do Paraná)*; Graça-de Souza, V.K. *(Departamento de Microbiologia, Universidade Estadual de Londrina)*; Rizzo, L.V. *(Departamento de Imunologia, Universidade São Paulo)*; Yamada-Ogatta, S.F. *(Departamento de Microbiologia, Universidade Estadual de Londrina)* Pinge-Filho, P. *(Departamento de Ciências Patológicas, Universidade Estadual de Londrina)*

Strong anemia, thrombocytopenia and leukopenia are observed during acute infection with *Trypanosoma cruzi*, the parasitic protozoan agent of American trypanosomiasis or Chagas*ŝprime* disease. The parasite sheds trans-sialidase, an enzyme able to mobilize the sialyl residues on cell surfaces, which is distributed in blood and is a virulence factor. Recently we showed that oral immunization with *Phytomonas serpens*, a tomato parasite, with lack activity trans-sialidases, modulates parasitemia and prevents mortality of C57BL/6 mice infected with lethal dose of *T. cruzi* (Y strain). Since the sialic acid content on the platelet surface is crucial for determining the half-life of platelets in blood, we examined the effect of protective immunity induced with *P. serpens* on the development of thrombocytopenia and leukopenia in acute phase of *T. cruzi* infection. We found that C57BL/6 mice inoculated with 5000 sanguineous trypomastigotes forms (sublethal dose) by the intraperitoneal route reduced the platelet and leukocytes count by 50% on day 14 pos-infection. More importantly, these alterations were prevented by oral immunization with *P. serpens* (2 x 10^8 promastigotes forms). Normal mice and immunized showed none altered hematological parameters. Results reported here strongly support the hypothesis that the use of the lower trypanomatids lacking trans-sialidase activity as a safer source of immunogenic agents for Chagas*ŝprime* disease can contribute to enhance parasite clearance and reduce parasite load and thus inhibit the progression of chronic disease. Financial support: PROPG-UEL, FAEP, CAPES, CNPq, FAPESP

IM75 - Myocardial expression of inflammatory mediators during experimental *T. cruzi* infection.

Cutrullis, R. A *(Hospital de Niños Dr. Ricardo Gutiérrez)*; Petray, P. B *(Hospital de Niños Dr. Ricardo Gutiérrez)*; Postan, M *(Instituto Nacional de Parasitología Dr. Mario Fatala Chabén)*; Corral, R. S *(Hospital de Niños Dr. Ricardo Gutiérrez)*

Chemokines (CHK) have been shown to play a major role in the influx of immune cells in numerous disease models where they participate in control of pathogens and/or contribution to chronic inflammation. In the present study, we have determined by semi-quantitative RT-PCR the kinetics of RNAm expression of a panel of inflammatory mediators, including CHK (CCL5/RANTES, and its receptor CCR5; CXCL9/MIG), cytokines (IFN-γ, TNF-α) and the inducible form of nitric oxide synthase (iNOS), within the heart along the course of experimental *T. cruzi* (RA strain, highly virulent) infection. Acutely (7-28 dpi) infected mice displayed high parasitemia level and developed acute myocarditis, characterized by amastigote nests, infiltration of mononuclear cells and increased expression of IFN-γ, TNF-α, CCL5, CCR5, CXCL9 and iNOS in cardiac tissue. CHK were expressed since the first days of infection when IFN-γ was absent or poorly detected, suggesting that other proinflammatory stimuli and/or parasite antigens could be triggering CHK up-regulation in murine chagasic cardiomyopathy. At the early chronic stage (57 dpi), inflammatory infiltrates in the heart were still present but significantly re-

duced, in correlation with low tissue parasitism and subpatent parasitemia. Accordingly, enhanced expression of the inflammation markers studied in the infected myocardium was restricted to CXCL9 and IFN-γ. In view of the above results, we conclude that RNAm expression of proinflammatory cytokines, iNOS, CHK and their receptors in the heart of *T. cruzi*-infected mice varies during the acute and chronic phases of infection. This expression pattern is linked to the host's immune response raised by this protozoan parasite, whose inflammatory mechanisms are involved in the erradication of *T. cruzi* as well as the induction or amplification of tissue damage.

IM76 - SECRETION OF CXC CHEMOKINES BY MACROPHAGES LINKS TLR2-DEPENDENT SENSING OF *Trypanosoma cruzi* TO MECHANISMS THAT STEER INNATE IMMUNITY VIA BRADYKININ RECEPTORS.

Schmitz, V. *(Biofísica)*; Andrade, D.S. *(Biofísica)*; Almeida, I.C. *(University of Texas)*; Diaz, B.L. *(Biofísica)*; Scharfstein, J. *(Biofísica)*

Plasma extravasation is a common endothelium response to tissue injury provoked by pathogens. Recently, we developed a subcutaneous model of T. cruzi (Dm28c) infection to study the role of the kinin system in immunity. First, we showed that tissue culture trypomastigotes (TCT) evoke edematogenic inflammation by activating the kinin system through mechanisms that involve cooperative signaling of TLR2 and the B_2-bradykinin receptor (B_2R). Analysis of the early stages of inflammation revealed that TCT activate neutrophils/endothelium in a TLR2 dependent manner. Owing to disruption of endothelial barrier function, the levels of blood-borne kininogens (i.e. kinin precursor proteins) are rapidly raised in infection sites. Once associated to extracellular matrix, kininogens are processed by parasite proteases (i.e., cruzipain), generating high-levels of bradykinin in peripheral tissues. Acting as maturation signals for dendritic cells, the short-lived kinins steer adaptive immunity via the B2R/Il-12 dependent pathway. In the present study, we studied how the presence of this pathogen is sensed by immune sentinel cells. Analysis of TCT interaction with bone marrow derived mast cells (BMMCs) showed that mast cells do not degranulate (β-hexosaminidase secretion). In contrast, measurements of secretion of CXC chemokines revealed that mouse macrophages (WT, TLR4def, $B_2R^{-/-}$, resident or TG-elicited) vigorously responded to TCT. Notably, $TLR2^{-/-}$ macrophages did not secrete appreciable amounts of KC or MIP-2, neither responded to tGPI-mucin (TLR2 ligand) stimuli. Consistent with these results, epimastigotes and metacyclic trypomastigotes, both of which fail to evoke inflammatory responses in vivo, did not induce CXC chemokine secretion in macrophages. Addition of ACE inhibitors mildly up regulated secretion of KC/MIP-2 and the effect was blocked by the B_2R antagonist. Collectively, our studies suggest that secretion of CXC chemokines secretion by macrophages, a TLR2-dependent response, sets in motion an inflammatory cascade that steers innate immunity through DCs via the bradykinin signaling pathway. Support: FAPERJ,CNPq

IM77 - Activation of the innate kinin pathway is critical for development of host resistance to acute intraperitoneal infection with *Trypanosoma cruzi*

Monteiro, A.C.S. *(Biofísica)* Schmitz, V. *(Biofísica)*; Morrot, A. *(Intracellular Parasite Biology Section, Laboratory of Parasitic Diseases)*; Gazzinelli, R.T. *(Laboratório de Imunopatologia)*; Pesquero, J. *(Departamento de Biofisica)*; Tanowitz, H. *(Department of Medical and Molecular Parasitology)*; Scharfstein, J. *(Biofísica)*

We have previously reported that exogenous bradykinin (BK) activates immature DC via the constitutive BK receptor (B_2R), thus stimulating Th1 adaptive immunity (Aliberti et al., 2003). Extending these findings to the context of experimental infection, recent studies demonstrated that endogenous liberation ok kinins by T. cruzi upregulates type-1 immunity in a subcutaneous model of infection (Monteiro et al., submitted). To investigate the importance of the kinin signalling route in resistance to acute infection, C57BL/6 WT ($B_2R^{+/+}$) and $B_2R^{-/-}$ mice were systemically (ip.) infected with two alternative T. cruzi strains, DM28c and Brazil. Our results showed that $B_2R^{-/-}$ mice displayed a higher blood parasitemia (15 d/ 30 d pi.) and succumbed (100%) to the acute infection. Analysis of Ag-specific recall responses by splenic T cells showed that IFN-γ production was impaired in $B_2R^{-/-}$ mice. Considering that trypomastigotes drive dendritic cell (DC) maturation in vitro through B_2R, we then asked if this mechanism is also relevant in the in vivo settings. This was addressed by injecting trypomastigotes intravenously in Balb/c mice, followed by assessment of IL-12 production. FACs and cytokine data showed that IL-12 production by splenic $CD11c^+$ DCs was upregulated in infected mice. Importantly, mice pre-treatment with the specific B_2R antagonist (HOE-140) showed diminished IL-12 production. Collectively, these results suggested that parasite induced activation of the B_2R kinin pathway upregulates type-1 responses, thus contributing to resistance against acute infection. Supported by: CNPq, CAPES and FAPERJ.

IM78 - Reactivation of *Trypanosoma cruzi* infection after ciclophosphamide treatment can be correlated with parasite genetic diversity

Santos, D.M. *(Universidade Federal de Ouro Preto)*; Silva, R.R. *(Universidade Federal de Ouro Preto)*; Martins, T.A.F. *(Universidade Federal de Ouro Preto)*; Queiroz, F.B. *(Universidade Federal de Ouro Preto)*; Caldas, I.S. *(Universidade Federal de Ouro Preto)*; Veloso, V.M. *(Universidade Federal de Ouro Preto)*; Carneiro, C.M. *(Universidade Federal de Ouro Preto)*; Lana, M. *(Universidade Federal de Ouro Preto)*; Bahia, M.T. *(Universidade Federal de Ouro Preto)*

The factors involved in the reactivation of chagasic infection are not clear enough and may be related to selective host immune depletion or parasite genetic diversity. To evaluate the role of the parasites genetic in *T.cruzi* infection reactivation induced by ciclophosphamide immunosuppression, groups of 32 Swiss mice were inoculated with *T.cruzi* clonal stocks classified as *T.cruzi* I (Cuicacl1, P209cl1, Gambacl1, SP104cl1) and *T.cruzi* II (Bug2148cl1, MNcl2, IVVcl4, MVBcl8) were used. Infected animals were treated with ciclophosphamide when was with subpatent parasitemia still during the acute phase (AP), and chronic phase (CP). Animals infected with *T.cruzi* I stocks showed 82,6% and 47,5% of parasitemia reactivation during the AP and CP, respectively, being observed 0%, 100%, 100% and 100% of parasitemia reactivation in animals inoculated with clones SP104cl1, Gambacl1, Cuicacl1 and P209cl1, respectively in the AP, and 80%, 40%, 50% and 20% in CP. On the other hand, animals infected with *T.cruzi* II showed only 4,1% of parasitemia reactivation when immunosuppressed during the AP and 0% in CP. However the heart and skeletal muscle lesions of animals infected by *T.cruzi* I were similar to those observed in controls infected and not immunosuppressed group (CINI). By the way an increasing of encephalic lesions in immunosuppressed animals during the CP in relation to CINI was observed. Although parasitemia reactivation was not observed in animals infected with *T.cruzi* II clones, an increase of inflammatory process in the heart and skeletal muscle, but not in the brain was observed among animals infected with Bug2148cl1. Our results showed that the genetic diversity of *T.cruzi* influences the reactivation of the infection after immunosuppression and corroborates the working hypothesis subjacent to the model clonal theory in *T.cruzi* .

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IM79 - DNA vaccination against *Trypanosoma cruzi* infection with different genes expressed during the amastigote stage

Silveira, E. L. V. *(Universidade Federal de São Paulo)*; Claser, C. *(Universidade Federal de São Paulo)*; Rodrigues, M. M. *(Universidade Federal de São Paulo)*

In previous studies, we have shown that protective immunity against lethal de *T. cruzi* infection can be elicited in highly susceptible A/Sn mice following vaccination with a gene expressed during amastigote stage. Based on these results, our objective in the present study is to use the genetic vaccination of A/Sn mice to search for new protective genes/antigens expressed in amastigotes of *T. cruzi*. For that purpose, we cloned several genes previously described as being expressed during this stage. They included the HSP70 (heat shock protein), MTP70 (mitochondrial heat shock protein), RibpS4 and RpL7a (ribosomal proteins), EF2 (elongation factor), TcG4, TcG8, ASP 5340 and ASP 7015 (surface proteins), H2b (histone), PAR2 (paraflagellar rod protein) and Tc24 and FlCBP (flagellar calcium binding proteins). Cloned genes were inserted in our vector pIgSP which contains the pcDNA3 backbone and the sequence encoding the mouse Igk chain signal peptide. So far we were able to immunize A/Sn mice with plasmids containing the genes RibpS4, RpL7a, TcG4 e ASP 5340 and H2b. Sera from these mice recognized amastigotes by immunofluorescence. We are currently evaluating the protective immune response following a challenge with trypomastigotes of the Y strain.

IM80 - Assessment of the monoterpene, glycidic and triterpene-moieties/ contributions to the adjuvant function of the CP05-saponin of *Calliandra pulcherrima* Benth

Nico, D *(Instituto de Microbiologia Prof Paulo de Góes, CCS, UFRJ)*; Santos, FN *(Instituto de Microbiologia Prof Paulo de Góes, CCS, UFRJ)*; Borja-Cabrera, GP *(Instituto de Microbiologia Prof Paulo de Góes, CCS, UFRJ)*; Grechi, J *(Instituto de Microbiologia Prof Paulo de Góes, CCS, UFRJ)*; Myashiro, LM *(Instituto de Microbiologia Prof Paulo de Góes, CCS, UFRJ)*; Palatnik, M *(Hospital Universitário Clementino Fraga Filho, CCS, UFRJ)*; Palatnik-de-Sousa, CB *(Instituto de Microbiologia Prof Paulo de Góes, CCS, UFRJ)*

The CP05 saponin from *Calliandra pulcherrima* Benth, shows remarkable similarities to the QS21 saponin of *Quillaja saponaria* Molina. Both shared a monoterpene hydrophobic moiety, a glycidic chain attached to the triterpene C-28, and three sugars attached to C-3. Different from QS21, the CP05 does not show the aldehyde group in triterpene C-4 involved in TH1 response. Balb/c mice were immunized either intact saponin (CP05), the monoterpene-deprived (BS), the C28 carbohydrate-deprived (HS) or the sapogenin fraction, in formulation with the FML antigen of Leishmania donovani and challenged with $2x10^8$ amastigotes of *L. chagasi*. While the CP05 induced 90% survival and 92.1% parasite reduction, a 100% survival and 94.1% protection were detected after the BS-vaccine treatment, indicating that the monoterpene acylated moiety, absent in the BS vaccine, is not necessary for the induction of a protective global TH1 response. Only the DTH response of BS vaccines was mildly lower than that of CP05 vaccinees. Maximal anti-FML antibody, CD4+ and CD8+ *Leishmania* specific lymphocytes, IFN y splenocyte

secretion, reduction in parasite load and survival was also detected for the BS vaccine. The HSFML vaccine showed diminished responses in all tested variables, except for IFNy secretion, indicating that the integrity of the carbohydrate moiety attached to C-28 is mandatory for the these functions. No protection was induced by the sapogenin-FML indicating that the CP05 triterpene which lacks the C4 aldehyde group, is not a immunostimulating compound. **Support:** PRONEX, CNPQ, FINEP, FUJB-CEPG-UFRJ, FAPERJ, CAPES.

IM81 - HIGH IL-10 EXPRESSION IS ASSOCIATED WITH BETTER CLINICAL PROGNOSIS AND MAY BE GENETICALLY DETERMINED IN INDIVIDUALS WITH CHAGAS/ DISEASE

Costa, G. C. *(Universidade Federal de Minas Gerais)*; Moreira, P. R. *(Universidade Federal de Minas Gerais)*; Rocha, M. O. C. *(Universidade Federal de Minas Gerais)*; Menezes, C. A. *(Universidade Federal de Minas Gerais)*; Gollob, K. J. *(Universidade Federal de Minas Gerais)*; Dutra, W. O. *(Universidade Federal de Minas Gerais)*

Chronic Chagasic Cardiomyopathy (CChC) is the main cause of disability and death in human Chagas disease, in which arrhythmia and heart failure are the most clinically relevant syndromes. Our group, as well as others, have previously shown that peripheral blood mononuclear cells (PBMC) from indeterminate patients (I) display an immunomodulatory profile, characterized by high expression levels of IL-10, as compared with PBMC from non-chagasic individuals. Whether this fact is a consequence of disease, with multiple factors influencing this response, or a result of genetic polymorphisms is unclear. Functional studies demonstrated a high production of IL-10 by individuals carrying the GG genotype for the -1082 G/A polymorphism, located in the promoter region of the IL10 gene. In this study we demonstrate, using RFLP polymorphism analysis of 197 individuals, that the IL-10 gene polymorphism is associated with the establishment of the indeterminate clinical form of Chagas disease. We also measured IL-10 expression levels (flow cytometry analysis) by PBMC from chagasic individuals and confirmed that indeterminate patients display a higher intensity of IL-10 production. Moreover, we observed that high levels of IL-10 were positively correlated with left ventricle ejection fraction parameters (LVEF), analyzed as a measure of cardiovascular function. These results show that the occurrence of the IL-10 gene polymorphism is associated with the immunomodulatory profile seen in indeterminate patients, suggesting a protective role for the GG genotype. Hence, high levels of IL-10 are associated with better clinical prognosis. Thus, susceptibility to cardiac disease could be genetically determined in chagasic individuals. Financed by: WHO/TDR, NIH, CNPq and CAPES.

IM82 - Evaluation of memory T cell responses specific for *Trypanosoma cruzi*during treatment with benznidazole in chronic Chagas disease patients.

Bertocchi, G *(Hospital Eva Perón, Buenos Aires, Argentina)*; Pérez, D *(Instituto Nacional de Parasitología Dr. M. Fatala Chabén, Buenos Aires Argentina)*; Alvarez, MG *(Hospital Eva Perón, Buenos Aires, Argentina)*; Armenti, AH *(Hospital Eva Perón, Buenos Aires, Argentina)*; Viotti, RJ *(Hospital Eva Perón Buenos Aires, Argentina)*; Albareda, MC *(Instituto Nacional de Parasitología Dr. M. Fatala Chabén, Buenos Aires Argentina)*; Lococo, BE *(Hospital Eva Perón Buenos Aires, Argentina)*; Postan, M *(Instituto Nacional de Parasitología Dr. M. Fatala Chabén, Buenos Aires Argentina)*; Tarleton, RL *(Center for Tropical and Emerging Global Diseases, UGA, Athens, United States.)* Laucella, SA *(Instituto Nacional de Parasitología Dr. M. Fatala Chabén, Buenos Aires Argentina)*

The aim of this study was to determine the effect of the etiological treatment with benznidazole on memory T cell responses specific for *Trypanosoma cruzi.*Thirty three chronic Chagas disease patients with no or mild disease aged 30-50 years of age were recruited for this study based on seropositivity for *Trypanosoma cruzi* infection and clinical evaluation. Chemotherapeutic treatment consisted of 5mg benznidazole/kg/day for 30 days. All the participants were submitted to serological, clinical and immunogical evaluation before treatment (time 0) and 2, 6, 12 months post-treatment. IFN-gamma ELISPOT responses to a *Trypanosoma cruzi*-lysate preparation were evaluated with peripheral blood mononuclear cells in order to determine the frequency of memory T cells specific for *Trypanosoma cruzi.* The frequency of IFN-gamma producing T cells specific for *Trypanosoma cruzi* significantly decreased in the treated patient group (n=24) compared to non-treated (n=9) (Mann-Whitney U test on post-treatment/pre-treatment differences treated vs non-treated P= 0,031), 12 months after follow-up. ELISPOT responses became negative in 8 out of the 24 (33%) patients receiving benznidazole whereas T cell responses remained unaltered in all non-treated patients (Fisher exact test P=0,035). In 4 out of the 24 treated patients, the decrease in ELISPOT responses was associated to a significant decrease in the levels of antibodies specific for *Trypanosoma cruzi.* None of the patients showed clinical alterations during follow-up. We showed for the first time that memory T cell responses specific for *Trypanosoma cruzi* decreased after treatment with benznidazole during the chronic phase of *Trypanosoma cruzi* infection. Conversely, the levels of *Trypanosoma cruzi* antibodies remained unaltered in most patients. These findings suggest a higher sensibility of the ELISPOT technique for the early detection of parasite clearance during chemotherapy and might be useful to assess the success of therapy.

IM83 - Trypanosoma cruzi modulates the profile of memory CD8+ T cells in chronic Chagas disease patients.

Albareda, MCA *(Instituto Nacional de Parasitología Dr mario Fatala Chaben)*; Laucella, SL *(Instituto Nacional de Parasitologia Dr Mario Fatala Chaben)*; Olivera, CO *(Instituto Nacional de Parasitologia Dr Mario Fatala Chaben)*; Alvarez, MGA *(Hospital Interzonal General de Agudos Eva Perón)*; Armenti, AA *(Hospital Interzonal General de Agudos Eva Perón)*; Bertochi, GB *(Hospital Interzonal General de Agudos Eva Perón)*; Tarleton, RLT *(Center for Tropical and Emerging Global Diseases)*; Postan, MP *(Instituto Nacional de Parasitología Dr. Mario Fatala Chabén)*

Previous studies from our laboratories have shown a high frequency of IFN-g ELISPOT responders to a parasite lysate among patients with mild clinical disease and a low frequency of responders among those with severe form of the disease, indicating an inverse correlation between T. cruzi-specific T cell responses and disease severity. In order to ascertain whether the lower responses found in chagasic subjects with severe clinical forms of the disease is related to a process of clonal exhaustion in the T cell population, we analyzed phenotypic markers indicative of maturation status of T cells and the expression of Annexin-V, a marker for apoptosis. The results showed that the T. cruzi-specific CD8+ T cell population in chronically infected subjects is enriched in early differentiated (CD27+CD28+) T cells, regardless the disease status of the patients. In contrast, the frequency of CD27+CD28+CD8+ T cells in the total memory CD8+ T cell population decreases as disease becomes more severe, while the proportion of fully differentiated memory (CD27-CD28-) CD8+ T cells increases. Accordingly, the levels of spontaneous apoptosis of CD8+ T cells in subjects with heart dysfunction were higher (Mean±SD 35.49±9.84) as compared to asymptomatic subjects (Mean±SD= 15.73±11.87; $p<0.05$) and controls (Mean±SD= 20.21±9.26; $p<0.05$). In addition, the percentage of CD8+ T cell undergoing apoptosis was inversely correlated with the frequency of IFN-g-producing memory T cells (r=-0.53; p=0.03) and the percentage of IL-7 receptor-expressing CD8+T cells was significantly decreased in chronic chagasic subjects (Mean±SD= 61.4±12.9) as compared to the controls (Mean±SD 76.6±9.7; $p<0.05$). Altogether these results are consistent with the hypothesis of a gradual clonal exhaustion in the CD8+ T cell population in chronic T. cruzi infection. We hypothesize that persistent exposure to the parasite drives the antigen-experienced CD8+ population to a late differentiated status, largely incapable of effector function and with signs of senescence.

IM84 - THE ROLE OF HEME OXYGENASE IN THE *Trypanosoma cruzi*-*Trypanosoma cruzi* INDUCED MYOCARDITS IN MICE INFECTION.

Pavanelli, W.R. *(Universidade de São Paulo)*; Gutiérrez, F.R.S. *(Universidade de São Paulo)*; Mariano, F.S *(Universidade de São Paulo)*; Milanezi, C.M *(Universidade de São Paulo)*; Cunha, F.Q. *(Universidade de São Paulo)*; Silva, J. S. *(Universidade de São Paulo)*

Introduction: The over expression of Heme Oxygenase (HO) protect organs/tissues from immune-mediated injury, either through prevention of oxidative damage or via a local immunomodulatory influence on infiltrating inflammatory cells. Here, we aim to investigate the immunomodulatory functions of HO in experimental infection with *T. cruzi*. Methods: Females of BALB/c mice were infected intraperitoneally with 1000 trypomastigote forms of T. cruzi (Y strain) and daily treated with the chemical inhibitor (ZnDPBG) of inducible and constitutive HO for 10 days and the parasitemia and survival evaluated. At days 10, 15 and 20 after infection, heart, liver and muscle skeletal were collected and histopathology analysis were performed by HE staining. ELISA assays were also performed for cytokine detections in these organs. The presence of CD4 and CD8 T cells and IFN-γ in the heart was also determined by immunohistochemistry (IH) assays. In addition, we assessed the levels of nitric oxide (NO) in the sera of infected mice and the phenotype of regulatory T cells [Treg] of spleen. Results: We found that ZnDPBG-treated mice are more susceptible to infection, since they exhibited higher parasitemia and mortality compared with the control group. Furthermore, we observed an increased influx of inflammatory cells into the cardiac tissue of treated mice, mainly of increased CD4 and CD8 T cells. Moreover, an increased production of IFN-γ was detected IH and ELISA in the heart tissue of ZnDPBG-treated and infected mice. In addition, the amount CD4+CD25+ T cells expressing Foxp3, GITR, and CTLA-4 was decreased in the spleens of ZnDPBG-treated mice on day 10 p.i compared with the controls. These data suggest that HO plays a role in the regulation of immune response against the parasite and modulates the influx of inflammatory cells to the heart tissue of *T. cruzi*-infected mice. \ Financial support: CNPq and FAPESP.

IM85 - NADPH phagocyte oxydase knockout mice succumb to infection with *Trypanosoma cruzi*

Santiago, Helton C. *(Universidade Federal de Minas Gerais)* González Lombana, C.Z. *(Universidade Federal de MInas Gerias)*; Seixas-Rego, V.A. *(Universidade Federal de Minas Gerias)*; Alves de Oliveira, R. *(Centro de Pesquisas Rene Rachou-Fiocruz)*; Tafuri, W. *(Universidade Federal de Minas Gerais)*; Romanha, A.J. *(Centro de Pesquisas Rene Rachou-Fiocruz)*; Teixeira, M.M. *(Universidade Federal de Minas Gerias)*; Radi, R. *(Universidade de la Republica-Montevideo)*; Vieira, L.Q. *(Universidade Federal do MInas Gerais)*

The microbicidal properties of reactive oxygen species (ROS) are well recognized, but little importance has been attributed to them during infection with *Leishmania* or *Trypanosoma cruzi* . However, we have found that mice deficient in nitric oxide synthase 2 ($NOS2^{-/-}$) are less susceptible to infection with *L. major* than mice deficient in IFN-γ (GKO), suggesting that there is an IFN-γ dependent but NOS2 independent mechanism of resistance to this parasite. In order to investigate this issue, we infected NADPH phagocyte oxydase deficient mice (gp91phox $^{-/-}$) with *L. major* or *T. cruzi* and followed the course of infection. We found that the course of infection in gp91phox$^{-/-}$ mice did not differ significantly from the course of infection in wild-type mice. In contrast, when infected gp91phox$^{-/-}$ mice with T. cruzi all mice succumbed to infection between day 15 and 21, while wild-type mice did not. Interestingly, gp91phox$^{-/-}$ mice have similar or reduced parasitemia and similar levels of IFN-γ and TNF in serum and spleen cell culture supernatant when compared to wild-type controls. Further investigation demonstrated increased serum levels of NO_x at day 15 of infection. The association between this free RNS and mortality is under investigation, but we speculate that the high levels of NO in sera of p91phox$^{-/-}$ mice induce tissue damage and shock. This issue is currently under investigation. Supported by CAPES and CNPq.

IM86 - Secretory repertoire of mast cells in *Trypanosoma cruzi* infection

Meuser-Batista, M. *(Instituto Oswaldo Cruz - FIOCRUZ)*; Corrêa, J.R. *(Instituto Oswaldo Cruz - FIOCRUZ)*; Meuser, M.B. *(Instituto Oswaldo Cruz - FIOCRUZ)*; Henriques-Pons, A. *(Instituto Oswaldo Cruz - FIOCRUZ)*

Bone marrow-derived mast cells circulate in the blood and lymphatic vessels and migrate to tissues and cavities, where they assume mature morphologic and functional characteristics under influence of local micro-environmental factors. They react to multiple physical, biological and chemical non-specific and specific stimuli, secreting diverse chemical mediators and cytokines pre-storaged in granules that are important for cell recruitment, endothelial activation, antigenic presentation, immunomodulation, exerting a central role in innate and acquired immunity. Regarding to the heart, mast cells have been implicated in cardiovascular diseases, such as myocarditis, ischaemic heart disease, experimental myocardial infarction, heart failure, transplant-related fibrosis and hypertensive heart disease. However, their role in myocarditis induced by *Trypanosoma cruzi* infection is poorly explored. In this work we used male CBA mice infected with CL strain of *T. cruzi* and employed an enzymatic protocol to dissociate cardiac mast cells in the acute phase. We purified cardiac and peritoneal mast cells with two different percoll-based protocols for mRNA isolation and qualitative RT-PCR method. We observed a peculiar pattern of cytokines (IL-1β, IL-2, IL-4, IL-6, IL-10, IL-12, TGF-β, IFN-γ and TNF-α), chemokines (MIP-1α, JE/MCP1 and RANTES), apoptosis inducing molecules (Fas and $P2X_7$), histamine receptors (H1, H2, H3 and H4) and iNOS expression that might be involved in the progression of the myocarditis. Supported by Fundação Oswaldo Cruz and CNPq.

IM87 - Kinin receptors promote *Trypanosoma cruzi* uptake by human circulating monocytes and induce modulatory changes in expression of cytokines and co-stimulatory molecules

Coelho, J. S. *(Universidade Federal de Minas Gerais)*; Scharfstein, J. *(Universidade Federal do Rio de Janeiro)*; Amaral, F. N. *(Universidade Federal de Minas Gerais)*; Galvão, L. M. C. *(Universidade Federal de Minas Gerais)*; Gollob, K. J. *(Universidade Federal de Minas Gerais)*; Dutra, W. O. *(Universidade Federal de Minas Gerais)*

Previous studies of the interaction of tissue-culture derived trypomastigotes (TCT) with endothelial cells and cardiomyocytes appointed the G-protein coupled bradykinin receptors (B2 or B1 subtype) as transducers of Ca2+-dependent responses leading to cellular invasion. Generation of the kinin agonist, presumably occurring in host-parasite synapses, is thought to depend on cruzipain-mediated processing of cell-bound kininogens. Once released, kinins either bind to their cognate GPCRs, or are swiftly degraded by metallopeptidases, such as the angiotensin converting enzyme (ACE). Here we checked if addition of ACE inhibitor (captopril) and/or specific antagonists for the B1 and B2 receptors could influence the outcome of TCT (Y strain) interaction with human monocytes. As read outs, we determined the number of parasites internalized after 3, 48 and 96 hours of interaction. In parallel, we checked if activation of the kinin signaling pathway was coupled to changes in the expression of surface molecules (CD80, CD86 and HLA-DR) and immunoregulatory cytokines. Our results showed that treatment with the ACE inhibitor did not enhance monocyte infectivity. However, monocyte infection was partially reduced in cultures supplemented with either B2 or B1 antagonists. Interestingly, we found that blockade of the B1 kinin receptor (inducible), but not B2 kinin receptors, increased CD80

expression by non-infected cell, suggesting that it is normally down-regulated by the low level of B1 agonists spontaneously generated in such cultures. Intriguingly, cultures exposed to TCT no longer showed this effect, suggesting that the B1 receptor responsiveness is modulated. Furthermore, IL-10 was upregulated in infected cells pre-treated with captopril and specific B1 antagonist (but not with B2 antagonist). In conclusion, parasite uptake by monocytes is partially dependent on kinin receptor signaling. Further work is required to characterize the influence of kinin regulation in monocyte function. Financing agency: CAPES, CNPq

IM88 - Increase of the relative weight and lymphatic alterations of mice experimentally infected with *Trypanosoma cruzi* treated with iron chelator

Andrade, V.L. *(Núcleo de Pesquisa em Ciências Biológicas)*; Francisco, A.F. *(Núcleo de Pesquisa em Ciências Biológicas)*; Vieira, P.M.A. *(Núcleo de Pesquisa em Ciências Biológicas)*; Martins, H.R. *(Núcleo de Pesquisa em Ciências Biológicas)*; Lana, M. *(Núcleo de Pesquisa em Ciências Biológicas)*; Bahia, M.T. *(Núcleo de Pesquisa em Ciências Biológicas)*; Tafuri, W.L. *(Núcleo de Pesquisa em Ciências Biológicas)*; Pedrosa, M.L. *(Núcleo de Pesquisa em Ciências Biológicas)*; Carneiro, C.M. *(Núcleo de Pesquisa em Ciências Biológicas)*; Arantes, J.M. *(Núcleo de Pesquisa em Ciências Biológicas)*

Male Swiss mice (n=80) thirty days old were divided in four experimental groups: (IT) infected with *T. cruzi* and treated; (INT) infected with *T. cruzi* and not-treated; (NIT) control treated (not-infected with *T. cruzi* and treated) and control (NINT - not-infected with *T. cruzi* and not-treated). Animals treated received daily doses (5mg/animal/day) of desferrioxamine (DFA) during 14 days before the infection with 500 blood forms of Y *T. cruzi* strain by intraperitoneal route. After infection, treated groups received DF during 21 days. We have analyzed the weight of the animals, the relative weight and the histopathological alterations, as well as tissue parasitism in the heart, liver, spleen and lymphnodes when sacrificed in 14th and 21th DAI. DFA treatment decreased significantly the body weight of animals infected or not. Between the 14th and 21th days after infection, an increase of the relative weight of the heart, spleen, liver and lymphnodes were observed only in the INT group. The infected treated (IT) or not treated (INT) animals presented similar histological alterations in the heart. The liver of the IT group, presented lower intensity of alterations and the evaluation of the lymphatic organs demonstrated early immune response in IT group. Differences in relation to tissue parasitism in the studied organs of the IT or INT animals was not observed. DFA treatment decreases the weight of the animals independent of the infection. The stability of the heart weight of the infected and treated animals possibly is related to the development of an early immune response of the host, since it was observed an increase of the lymphatic organs relative weight and lesions in the 14th DAI. This work was supported by CNPq, FAPEMIG and UFOP.

IM89 - Impact of the inoculum source and immunossupression on cardiac lesions during acute *Trypanosoma cruzi* infection in dogs

Souza, S.M. *(Laboratório de Imunopatologia)*; Santos, V.R.L. *(Laboratório de Imunopatologia)*; Paiva, N. C. N. *(Laboratório de Imunopatologia)*; Veloso, V.M. *(Laboratório de Parasitologia)*; Araújo, F.M.G. *(Laboratório de Parasitologia)*; Reis, A.B. *(Laboratório de Imunopatologia)*; Bahia, M.T. *(Laboratório de Parasitologia)*; Lana, M. *(Laboratório de Parasitologia)*; Tafuri, W.L. *(Laboratório de Imunopatologia)*; Carneiro, C.M. *(Laboratório de Imunopatologia)*

Experimental canine model of *Trypanosoma cruzi* infection has been shown to mimetize the various aspects of Chagas disease. Our group has been demonstrated that the source of the inoculum (blood-BT or metacyclic trypomastigotes-MT) influences the evolution of the immunological and parasitological aspects of the acute phase of *T. cruzi* infection in dogs. We observed at the peripheric blood a predominance of CD8+T cells in MT group. Considering the histological picture, a major characteristic of Chagas disease is a myocarditis constituted primarily of mononuclear cells, during both, acute and chronic phases of the disease. So, the aim of this study was to evaluate the histopathological aspects of the heart during acute phase in BT and MT *T. cruzi* infection associated or not to immunosuppressive therapy (azathioprine-2mg/kg/daily during 42 days) and the macrophage contribution to the inflammatory infiltrate. Parasitemia was significantly higher in animals infected with MT than BT. The immunosuppressive therapies lead to higher levels of parasitemia however, no differences were observed among MT and BT. Dogs were necropsied during acute phase (42 days of infection) and the heart was collected. The heart of dogs infected with BT showed more intense acute myocarditis characterized by a focal and diffuse exudation of mononuclear cells and collagen neoformation than MT group. Dogs infected and immunossupressed showed acute myocarditis more destructive than not-immunossupressed, characterized by diffuse exudation of mononuclear cells. We have performed, by immunohistochemistry, a quantification of macrophages and parasites. Parasites and macrophages were observed more frequently in immunossupressed group. Our results suggest that the early activation of CD8+T-cells, observed in peripheric blood, seams to play a pivotal role controlling the immunopathology observed during acute Chagas disease. Supported by CNPq, FAPEMIG, CPqRR-FIOCRUZ, UFOP

IM90 - Development of cardiomyopathy in canine Chagas disease correlates with in situ high IFN-γ and low IL-10 production

Guedes, P.M.M. *(Universidade Federal de Minas Gerais)*; Veloso, V.M. *(Universidade Federal de Ouro Preto)*; Souza, S.M. *(Universidade Federal de Ouro Preto)*; Lana, M. *(Universidade Federal de Ouro Preto)*; Afonso, L.C.C. *(Universidade Federal de Ouro Preto)*; Caliari, M.V. *(UNiversidade Federal de Minas Gerais)*; Carneiro, C.M. *(Universidade Federal de Ouro Preto)*; Chiari, E. *(Universidade Federal de Minas Gerais)*; Galvão, L.M.C. *(Universidade Federal de Minas Gerais)*; Bahia, M.T. *(Universidade Federal de Ouro Preto)*

The aim of this study is to evaluate IL-10 and IFN-γ production in the heart, presence of cardiomegaly and their correlation with cardiac inflammation and fibrosis in *Beagle* dogs infected with *Trypanosoma cruzi*. Twenty-four *Beagle* dogs were inoculated with 4.0 x 10^3 trypomastigotes/kg of the Y, Berenice-78 or ABC *T. cruzi* strains. The animals were euthanasied 30 days and two years after inoculation, and eight uninfected dogs were used as control. The heart weight was determined and related with animal weight and macroscopic alteration. For histopathological analysis tissue fragments were collected from the right atrium, processed by Hematoxylin-Eosin and Masson Tricromic, and through a computerized morphometrical analysis (KS300/Carl Zeiss) for quantifying the areas occupied by cardiac muscle, inflammation and fibrosis. *In situ* production of IFN-γ and IL-10 was determined in right atrium by RT-PCR. The animals infected with Berenice-78 strain (peak: 5000 tryp/0.1 ml) showed lower parasitemia than Y strain (10.000 tryp/0.1 ml) and ABC strain (21.800 tryp/0.1 ml). The histopathological analysis showed severe myocarditis in all infected dogs during the acute phase. Discreet cardiomegaly and fibrosis were observed in 50%, 25% and 50% of the animals infected with Y, Berenice-78 and ABC, respectively. In the chronic phase cardiomegaly, inflammation and fibrosis in 100%, 50% and 100% of the animals infected with Y, Berenice-78 and ABC were, respectively, observed. The cytokine production *in situ* during acute phase was similar in the animals infected with the different strains of the *T. cruzi*. However, during chronic phase only dogs infected with Berenice-78 strain produced IL-10. Our results indicate that animals infected with Berenice-78 strain, which produced IL-10 in situ during chronic phase, had less cardiac alterations (myocarditis, fibrosis and cardiomegaly) than infected them with Y and ABC strains, which produced high IFN-γ levels and did not produce IL-10. Supported by:PRONEX,CNPQ,FAPEMIG,UFOP and UFMG

IM91 - The role of 5-Lipoxigenase-derived lipid mediators during the experimental *Trypanosoma cruzi* infection in mice.

Canavaci, A. M. C. *(FCFRP - USP)*; Sorgi, C. A. *(FCFRP - USP)*; Morais, F. R. *(FCFRP - USP)*; Martins, V. P. *(FCFRP - USP)*; Cunha, F.Q *(FMRP - USP)*; Rossi, M.A. *(FMRP - USP)*; Faccioli, L. H. *(FCFRP - USP)*; Nomizo, A. *(FCFRP - USP)*

The arachidonic acid derived metabolites are potent lipid mediators with a key role in immune and inflammatory responses. Accumulating studies have indicated that 5-lipoxigenase (5-LO) converted lipid mediators as leukotrienes and lipoxins acts modulating the host immune response against infectious agents. The precise role of leukotrienes and lipoxins during the protozoan infection is unknown. In this work we evaluate the *T. cruzi* (Colombian strain) infection in 129 mice and 5-lypoxigenase deficient mice (5-LOko). Our results show LTB_4 and LTC_4 are produced during the *Trypanosoma cruzi* infection by 129 mice and that 5-LOko infected mice are more resistant to parasite infection than control mice as judge by the lower number of blood circulating parasites, decreased number of parasite nest and inflammatory cells in the heart and skeletal muscle and low rate of mortality. The resistance of 5-LOko mice to *T. cruzi* infection is associated with the increased capacity of spleen cells to produce cytokines as IL-6 and IL-12, the reduced capacity to produce TNF-α and Nitric Oxide (NO); presence of increased number of spleen cells expressing Gr-1^+, Gr-1^+CD11c$^+$, F4/80$^+$ and CD19$^+$; reduced numbers o spleen cells expressing CD4$^+$CD69$^+$, CD4$^+$CD44$^+$, CD8$^+$CD69$^+$ and CD11b$^+$. Importantly, our results indicate that differently of the infected control mice that showed an expansion of CD4$^+$CD25$^+$ during the infection, the 5-LOko infected mice do not present expansion of CD4$^+$CD25$^+$ cells. Altogether our results indicate that 5-lipoxigenase (5-LO) converted lipid mediators contribute negatively to generation of the effective immune response to controlling parasite in both the blood and tissues by modulating the production of innate response cytokines, NO and the regulating the number of some subpopulation of cells during the infection. Financial support: FAPESP, CNPq and FCFRP - USP

IM92 - *Trypanosoma cruzi*: different immunoglobulins profiles in experimental infections in dogs

Vital, WC *(Universidade Federal de Ouro Preto)*; Pinto, LCL *(Universidade Federal de Ouro Preto)* Ker, HG *(Universidade Federal de Ouro Preto)*; Martins, HR *(Universidade Federal de Ouro Preto)*; Bahia, MT *(Universidade Federal de Ouro Preto)*; Lana, M *(Universidade Federal de Ouro Preto)*; Tafuri, WL *(Universidade Federal de Ouro Preto)*; Veloso, VM *(Universidade Federal de Ouro Preto)*; Carneiro, CM *(Universidade Federal de Ouro Preto)*; Reis, AB *(Universidade Federal de Ouro Preto)*

T. cruzi develops a complex biological cycle that requires an invertebrate vector and mammalian hosts. Previous studies in our laboratory have demonstrated that dogs experimentally infected with Berenice-78 *T. cruzi*-strain presented a longer patent-parasitemia, and myocarditis of variable degree. Despite these parasitological and pathological findings, little is known about the humoral immune response during acute phase of the infection in dogs. Herein we have performed a detailed follow-up investigation of serological profile by ELISA using specific monoclonal anti-canine isotypes (IgG, IgG1, IgG2 and IgM) employing a soluble epimastigotes *T. cruzi* antigen. Eight dogs were intraperitoneally inoculated either with 2,000 metacyclic trypomastigotes (MT) or blood trypomastigotes (BT) of BE-78 *T. cruzi* strain, per Kg/body/weight. Four uninfected dogs were included as a control group. Our results showed higher levels of IgG and IgG2 isotype at 21th days after infection in the MT group. On the other hand, higher levels of IgM were observed in BT during acute phase. BT group showed significant increased levels of IgG and IgM until 35 days after infection. Furthermore, no alterations were detected with IgG1 in all groups in comparison to control. Taken together, these findings emphasize the importance of the inoculum source, suggesting that vectorial or transfusional routes of *T. cruzi* infection may trigger distinct parasite-host interaction during acute Chagas disease. Ours results still suggest also that the early production of IgG and IgG2 seems to play a pivotal role control of the immunopathology observed in acute canine Chagas disease. In addiction, these data suggested that the route of infection can be an important element driving the immune response in infected dogs. Therefore the different profile of class and subclass of immunoglobulins could be contribute for the development of immunophatogenesis of canine experimental infections.

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IM93 - IL-4 Induces a Wide-Spectrum Signaling in CD8+ T cells

Pinheiro, A. A. S. *(Johns Hopkins University)*; Morrot, A. *(Johns Hopkins University)*; Chakravarty, S. *(Johns Hopkins University)*; Bream, J. *(Johns Hopkins University)*; Irusta, P. *(Johns Hopkins University)*; Zavala, F. *(Johns Hopkins University)*

IL-4 plays a central role in regulating the immune response. It controls the specificity of Ig class switching in B cells and the differentiation of TCR-stimulated naïve CD4+ T cells to the TH2 phenotype. In CD4+ T cells, the IL-4 effect depends upon signaling through a receptor complex consisting of the IL-4Ralpha chain and the common gamma chain, resulting in a series of phosphorylation events mediated by Jak1 and Jak3. Two major IL-4 signaling pathways are mediated by PTB domain proteins and Stat6, which are involved in the control of cell growth and gene activation. It was described that CD4+T cells are essential to the development of CD8+ T cell responses against malaria parasite liver stage and that the cross-talk between these cells is mediated by IL-4. More recently, in a comparative study using normal and IL-4R knock out TCR transgenic CD8+ T cells specific for *P. yoelii* epitope, it was shown that IL-4 acts directly on CD8+ T cells through IL-4R and this interaction is important to sustain a long living memory cell population. In this study we characterize the activation of the Jak/Stat and IRS-2/PI-3K/PKB pathways induced by IL-4 in CD8+ T cells. Our results strongly suggest that both mechanisms are involved in the anti-apoptotic effect of IL-4. We found that IL-4 induces an intense signaling activity on CD8+ T cells, including phosphorylation of different Stats and PKB. Importantly, we found that CD8+ T cells, compared to CD4+T cells, display greatly diminished transcriptional levels of SOCS genes, which negatively control cytokine pathways of cell differentiation. Supported by: CNPq/NIH

IM94 - Kinetics of murine mononuclear phagocytes migration from the inflammatory site to the draining lymph node

Malta, R.O. *(Fundação Oswaldo Cruz)*; Pereira, D.C.B. *(Fundação Oswasdo Cruz)*; Júnior, N.P. *(Fundação Oswasdo Cruz)*; dos Santos, W.L.C.S. *(Fundação Oswasdo Cruz)*

Leishmania infection modulates integrin function in inflammatory phagocytes, impairing their adhesion to connective tissue. Such change in integrin function may influence the migratory pattern of the phagocytes capable of transporting the parasite. The aim of this study is to define the effect of *Leishmania* infection on inflammatory mononuclear phagocyte migration from an inflammatory site to the dining lymph node. In this part of the study we examined the kinetics of normal mononuclear phagocyte migration from thioglicolate-induced peritonitis to the draining lymph node. Peritonitis was induced in adult Balb/c mice. The cell populations present in the peritoneal exudates and the histopathological changes in the draining (para-thymic) lymph node were examined 1, 2, 4, 6, 10, 20 and 40 days after injection. Mononuclear phagocytes were the predominant cell in

the peritoneal exudate from day 1 (76,9 %) to day 20 (84,33 %). Polymorphs were expressively present on day 1 (17,47 %) and 2 (9,52 %) decreasing in the following days. Lymphocytes, present in small amounts in the early days after injection, became the predominant cell on the day 40 (62,93 %). Thioglicolate-containing mononuclear phagocytes were observed in the sub-capsular area of the lymph nodes in the first day after injection and in the interior of the lymphoid tissue after the second day. Sub-capsular sinus dilatation and remodeling of lymph node architecture following massive phagocyte infiltration was observed from the second day after the stimuli. Now we are examining early stages of the process and performing experiments with *Leishmania*-infected cells.

IM95 - THE ROLE OF THE MANNAN-BINDING LECTIN ACCEPTORS IN THE LECTIN COMPLEMENT PATHWAY ACTIVATION BY *Trypanosoma cruzi*

Cestari, I.S. *(Instituto Oswaldo Cruz)*; Mogami, S. *(Instituto Oswaldo Cruz)*; Ramirez, M.I. *(Instituto Oswaldo Cruz)*

Trypanosoma cruzi evades the complement system to establish the infection. The complement is activated by the classical (CP), lectin (LP) , or alternative pathway (AP) culminating with parasite lysis. Previous studies showed that trypanosomatids lysis is mediated by AP according to the absence of antibodies with trypanolytic activities in Normal human Serum (NHS, Rimoldi, MT et al., 1989). Although reports suggested that AP is responsible for *T cruzi* lysis, our experiments with NHS 25% and EGTA(CP-LP inhibitor)-treated NHS 25% showed that 96% of Y strain epimastigote forms are lysed with the three activated pathways after 10 minutes while AP lysed 50% in 30 minutes at 37^{o}C. These results suggest a significant involvement of CP and LP at the beginning of complement activation. Parasites were preincubated at 4^{o}C with NHS (MBL bind to parasite surface in a Ca^{++}-dependent process) followed by EGTA-treated NHS 25% (inhibiting the classical pathway) allowing to compare the synergistic effect of LP-AP to AP. In 10 minutes, 5% of parasites survived LP-AP treatment while 20% survived AP reinforcing the rapid activation of LP. Furthermore, the role of AP in the complement activation by *T. cruzi* was remarkable in a competition assay incubating NHS 25% with increasing mannose concentrations (0,1-40 mM) and *T. cruzi* lysis was inhibited in a dose dependent manner (26 to 72%). To analyse the role of mannan-binding lectin acceptors on the surface of epimastigote forms in complement activation, kinetics with NHS 25% and tunicamycin (n-glycosylation inhibitor) treated parasites (2μg/ml) at 5-10 minutes showed survival of 53,6% and 30% while non-treated parasites survived to 38,6% and 16,3%, respectively. Taken together our results suggest that the lectin pathway is the main complement pathway activated by *T. cruzi* at the first contact with NHS.

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