Bioquimica - Biochemistry

BQ01 - Acyl-CoA-binding protein expression profile in *Rhodnius prolixus*

Alves, M. *(Instituto de Bioquímica Médica, Universidade Federal do Rio de Janeiro)*; Majerowicz, D. *(Instituto de Bioquímica Médica, Universidade Federal do Rio de Janeiro)*; Grillo, L. A. M. *(Instituto de Bioquímica Médica, Universidade Federal do Rio de Janeiro)*; Almeida, C. B. *(Instituto de Bioquímica Médica, Universidade Federal do Rio de Janeiro)*; Paiva-Silva, G. O. *(Instituto de Bioquímica Médica, Universidade Federal do Rio de Janeiro)*; Gondim, K. C. *(Instituto de Bioquímica Médica, Universidade Federal do Rio de Janeiro)*

Acyl-CoA esters have many functions in cell metabolism, such as energy production and cell signaling. Acyl-CoA-binding protein (ACBP), that is a highly conserved 10 kDa intracellular protein, binds long straight-chain acyl-CoA esters with very high affinity and protects acyl-CoA esters from hydrolysis. Prediction of three-dimensional structure of ACBP from *Rhodnius prolixus* using CPHmodels 2.0 showed that it has four helices in up-down-down-up conformation, as it was described for other ACBPs. Using RT-PCR, ACBP gene expression was detected in anterior and posterior midgut, fat body, ovary, flight muscle and salivary glands, and it was highest ($\tilde{5}$ fold) in posterior midgut. Expression analysis of ACBP gene in the midgut by Real-Time PCR showed a great increase after blood meal, and it was very high ($\tilde{7}$-fold increase) on the first day after feeding and decreased after it. In the fat body, expression of ACBP gene was highest on the eleventh day after feeding and, in the other days, expression was relatively constant. In order to investigate the mechanisms involved in the control of ACBP gene expression, the possible role of ecdysone was analyzed. Injection of 2 ng of 20-hydroxyecdysone into unfed females inhibited the expression of ACBP gene in approximately 30%. When females were fed immediately after the injection of 20-hydroxyecdysone, the ACBP gene expression was decreased by 25%. These results suggest that this hormone may be involved in the control of ACBP expression, and this is under investigation. Supported by CNPq, PIBIC/UFRJ, Faperj and CAPES.

BQ02 - Effect of Ketoconazole on growth, morphology, infectivity and in the pattern expression of flagellar glycoproteins in *Leishmania (Viannia) braziliensis*

Yoneyama, K. A. G. *(Universidade Federal de São Paulo)*; Takahashi, H. K. *(Universidade Federal de São Paulo)*; Straus, A. H. *(Universidade Federal de São Paulo)*

Cholesterol plays an important role in the properties of cell membranes in mammalian cells. *Leishmania* and *Trypanosoma* synthesize ergosterol using a similar biosynthetic pathway that pathogenic fungi. Previous work carried out in our lab showed that ergosterol from *Leishmania (Viannia) braziliensis* presents an essential role on lipid rafts maintenance on plasma membrane. In order to better characterize the role of ergosterol on *Leishmania*, the effect of different concentrations of ketoconazole (an inhibitor of ergosterol synthesis) on *L. (V.) braziliensis* was analyzed. Low ketoconazole concentration as 18 nM caused significant decrease of parasite growth rate (about 90%) and macrophage infectivity (84%). Also drastic changes on parasite morphology were observed such as rounded body, presence of one or more truncated flagella and two or more nucleus, suggesting that ketoconazole might inhibit of cytokinesis process. By indirect immunofluorescence with monoclonal antibody (mAb) SST-3, which recognizes specifically a triplet glycoprotein (200 to 160 kDa), expressed exclusively in parasite flagellum, it was observed that ketoconazole treatment apparently did not alter the distribution of this flagellar glycoprotein on truncated flagellum. On the other hand, by Western blotting it was observed a drastic alteration in band profile of flagellar glycoproteins recognized by mAb SST-3. Other biochemical studies are under investigation in order to better understand the role of ergosterol in flagella organization. The present data suggest that ketoconazole is a promising drug against *L. (V.) braziliensis*. Supported by FAPESP and CNPq.

BQ03 - Phosphoproteome during nutritional stress in *Trypanosoma cruzi*

Chung, J. *(UNIVERSIDADE FEDERAL DE SÃO PAULO)*; Ferreira, L.R.P. *(UNIVERSIDADE FEDERAL DE SÃO PAULO)*; Ramos, T. *(UNIVERSIDADE FEDERAL DE SÃO PAULO)*; Schenkman, S. *(UNIVERSIDADE FEDERAL DE SÃO PAULO)*

Phosphorylation is one of the most common post-translational modifications of proteins and is a key event in intracellular signaling. Therefore, phosphorylation combined with proteomic analysis has been used as important tool to elucidate many biological processes. In trypanosomatids a large number of protein kinases were predicted by genome sequence, suggesting that phosphorylation must be particularly important in these organisms. For example, transformation of *Trypanosoma cruzi* epimastigotes into metacyclic trypomastigotes is induced by factors like, nutritional stress, differences in temperature and pH variation. Very little is known about the signals that induce the process of metacyclogenesis. In the present work compared the phosphorylation profiles between epimastigotes that suffered nutritional stress (incubation at different time points in the chemically defined TAU medium) and control parasites (stationary phase epimastigotes). 2D gels from were visualized by specific stains for phosphorylated proteins and total proteins (Pro-Q Diamond and Sypro Ruby respectively (Molecular Probes). Phosphorylated proteins were also isolated from *T. cruzi* total protein extracts after enrichment

by affinity chromatography and identified by mass spectrometry analysis. Among the identified phosphorylated proteins, some have their function correlated with cellular nutritional stress response, growth and metabolism. Listing two of them are Rad23 (UV excision repair RAD23-like protein) and cystathionine beta-synthase. Supported by FAPESP and FINEP

BQ04 - PROTEOMIC ANALYSIS OF BENZNIDAZOLE SUSCEPTIBLE AND RESISTENT *TRYPANOSOMA CRUZI* TRYPOMASTIGOTES

Lopes dos Santos, S. *(Instituto René Rachou)*; Silva-Pereira, R.A. *(Instituto René Rachou)*; Romanha, A.J. *(Instituto René Rachou)*

INTRODUCTION: Chagas' disease is a major public health problem in Central and South America, where 16-20 million people are infected with *Trypanosoma cruzi*. Nitroheterocyclic drugs, benznidazole (Bz) and nifurtimox (Nf), are currently used to treat Chagas' disease. Both drugs have frequent side-effects, very low anti-parasitic activity in long-term chronic forms of the disease and variable efficacy according to the geographical area. The existence of strains naturally resistant and susceptible to Bz and Nf drugs was previously described. Efforts are necessary to provide a better understanding of *T. cruzi* drug resistance mechanisms, which may lead to the identification of new drug targets and new chemotherapeutic tools. These efforts must be directed to *T. cruzi* human host forms, trypomastigotes and amastigotes. Furthermore, proteomic analysis is particularly important because the regulation of gene expression in *T. cruzi* occurs by post-transcriptional mechanisms. OBJECTIVE: The aim of this work is to identify trypomastigote proteins potentially involved in the Bz resistance mechanisms. METODOLOGY: Trypomastigote forms from two populations selected in vivo, Bz resistant (BZR) and Bz susceptible (BZS) (Murta e Romanha, 1998), were maintained in Vero cell monolayers and collected. The resistance/susceptibility phenotype was determined by an *in vitro* biological assay, in which trypomastigotes released from cells under the Bz activity were counted. RESULTS: Preliminary results have shown that BZR population is 1.75-fold more resistant to Bz than BZS population. Comparative proteomic analysis between BZR and BZS trypomastigote forms is being carried out by bi-dimensional electrophoresis and the identification of differentially expressed proteins will be performed by mass spectrometry and similarity searching against the *T. cruzi* protein databases. Financial support: PDTIS/FIOCRUZ - IRR - FAPEMIG

BQ05 - Proteases activities in recently field-isolated *Trypanosoma cruzi* strains: identification, expression characterization among I and II groups strains.

Fampa, P. *(Fundação Oswaldo Cruz/ RJ)*; Santos, A.L.S. *(Universidade Federal do Rio de Janeiro)*; Ramirez, M.I. *(Fundação Oswaldo Cruz/ RJ)*

Trypanosoma cruzi presents a heteroxenous life cycle including invertebrate and vertebrate hosts. The taxon is known as a heterogeneous species consisting of several sub-populations of the parasite. *T. cruzi* I group is associated to sylvatic transmission cycle infecting marsupial as well as placental mammals, while *T. cruzi* II group parasites are mostly associated to domestic transmission cycle, infecting mainly placental mammals. There are related cases of hosts infected by TcI/ TcII mixture strains, which reinforces parasite epidemiology complex scenario. Characterizing field strains is crucial. Our group is interested in *T. cruzi* species diversity. By analyzing protease expression general profile, we showed a TcI expression pattern distinct from TcII, being the latter more complex (Fampa et al. Chagas 2006). At the present work we also assayed natural and *in vitro* induced TcI/ II mixtures, in order to associate proteases activity to the dynamics of these mixed populations. Little is known about competition of TcI and II populations in natural mixed infections, and its effect to host well succeeded infection. Our goal is also: (i) to identify the class of the proteases differentially expressed among TcI and II strains, by utilizing proteases inhibitors; (ii) to compare the expression of well studied crupizipain and gp 63, virulence factors proteases, between TcI and II groups. Preliminary results showed that parasite field strains active proteases in acidic pH are cysteine-proteases, and those active in alkaline pH are metallo-proteases. It was also observed that TcI strains express cruzipain in higher levels than TcII. Further, we intend to perform assays to determine differentially expressed proteases role in interaction with hosts. We expect to identify new pathogenicity markers that contribute to the definition of *T. cruzi* I and II phylogenetic groups and to *T. cruzi*-hosts relationship understanding. Supported by: CNPq, FIOCRUZ& FAPERJ.

BQ06 - CHARACTERISATION OF THE SERINE PEPTIDASE INHIBITOR (ISP2) OF *Trypanosoma cruzi.*

Abreu, M. F. *(Universidade Federal do Rio de Janeiro)*; Reis, F. C. G. *(Universidade Federal do Rio de Janeiro)*; Garcez, T. C. *(Universidade Federal do Rio de Janeiro)*; Coombs, G. *(University of Glasgow)*; Mottram, J. *(University of Glasgow)*; Lima, A. P. C. A. *(Universidade Federal do Rio de Janeiro)*

Ecotins are high affinity competitive inhibitors of clan PA, family S1 serine peptidases that are found in the periplasm of several bacteria species. They present a broad range of

inhibition inactivating trypsin, chymotrypsin, cathepsin G, neutrophil elastase (NE), and members of the coagulation cascade with moderate to high potency. In *E. coli*, ecotin was found to exert a protective role against the deleterious effect of NE. Genes presenting similarity to ecotins were identified in the genomes of *T. cruzi*, *T. brucei* and *Leishmania*, and have been designated Inhibitors of Serine Peptidases (ISPs). No clan PA peptidases are encoded in the *T. cruzi* genome raising the possibility that ISP could function to modulate the activity of host enzymes. We cloned the ISP2 gene of *T. cruzi* Dm28c into pQE-30 and expressed it as a fusion protein with an N-terminal 6Xhistidine tag. His-tagged proteins were purified using Ni-NTA affinity resin under native and denaturing conditions. Soluble recombinant ISP2 was functional, as assessed by the inhibition of trypsin activity. Purified denatured ISP2 was injected into mice for the production of anti-ISP antisera. Western blot analysis of parasite lysates revealed that ISP2 expression is developmentally regulated, being higher in epimastigotes and lower in trypomastigotes and amastigotes. A possible role of ISP2 in the host-parasite interaction was addressed by performing invasion assays of epithelial or smooth muscle cell lines by tissue culture trypomastigotes. We observed that rec-ISP2 significantly reduced host cell invasion, suggesting that host serine peptidases might play a role in parasite invasion.

BQ07 - SPECIFICITY STUDIES ON SERINE PROTEASES FROM LEISHMANIA AMAZONENSIS: SUBSTRATES, INHIBITORS AND DISCOVERY OF NEW ENZYMES

Moreira, C.S *(Fundação Oswaldo Cruz)*; Anobom, C.D *(Fundação Oswaldo Cruz)*; Gomes, B.S.F.B *(Fundação Oswaldo Cruz)*; De-Simone, S.G *(Universidade Federal Fluminense)*

Leishmaniasis is a disease caused by trypanosomatidae protozoa, endemic in tropical and subtropical regions of the world. The high-cost, toxicity and resistance issues associated with the current treatment turns necessary the discovery of new drugs. Serine proteases are important targets for immunoprophylaxis and chemotherapy due to their involvement in host-parasite interaction. The goal of this work is improving our knowledge about serine protease of *L. amazonensis* to design specific inhibitors. Recently, our group has identified three serine proteases: LSP-I, II and III in *L. amazonensis* fractions. We have obtained affinity chromatography fractions of the aqueous- (LAAE) and detergent-soluble cellular extracts (LADE) and from the culture supernatant (LACS) of *L. amazonensis*. Zymography indicated gelatin-degrading serine proteases other than LSPs in LADE (75kDa), LAAE (116kDa) and LACS (73 and 80kDa). From gel filtration of LACS we have isolated a new oligopeptidase (120kDa) that was not described in literature yet. All the fractions were active over synthetic substrates carrying arginine in P1. We are now testing substrates with different residues in P1-P4. To find inhibitors for these enzymes we are screening a panel of pseudo peptides where the affinity constants for the most active will be analyzed using NMR and enzyme kinetics. These data will provide the design of new peptidomimetics for pursuing the future development of new antileishmanial drugs.

BQ08 - Proteolytic activation of leishporin by an intrinsic serine-protease and evidences that the smallest cytolytic molecule is a proteolytic-resistant peptide.

Giullianetti-Almeida, M. P. *(Universidade Federal de Minas Gerais)*; Castro-Gomes, T. *(Universidade Federal de Minas Gerais)*; Silva, R. A. *(Universidade Federal de Minas Gerais)*; Noronha, F. S. M. *(Universidade Federal de Minas Gerais)*; Pimenta, A. *(Universidade Federal de Minas Gerais)*; Horta, M. F. *(Universidade Federal de Minas Gerais)*

Leishporin is a pore forming protein present in protozoan of the genus *Leishmania*, which was partially characterized by our group in *Leishmania amazonensis*. Since leishporin is optimally active at pH 5.5 and 37°C, it might act inside the macrophage phagolisosome and be involved in the rupture of the fagolisosome and of the macrophage itself. To cause lysis, leishporin must undergo proteolytic digestion. In this study we verified that a serine-protease presents in membrane extracts of the parasite (ext-ms) can activate the cytolisin. Since leishporin is activated by proteolysis we studied its resistance to this process by treating active ext-ms with proteinase-K. Leishporin was not destroyed by proteolysis. After incubation of the native PAGE of the ext-ms treated with proteinse-K with red blood cells, the hemolytic activity co-localized with a restrict number of proteins. Results from reverse-phase chromatography and SDS-PAGE of the extract treated with proteinase-K showed that, in fact, the smallest molecules with lytic activity are peptides, which seems to have different sizes and be probably derivate from a larger precursor. Financial support: WHO, CNPq, CAPES, FAPEMIG.

BQ09 - Ibuprofen induces oxidative stress in *Trypanosoma cruzi*

Souza, G.G. *(Universidade Estadual de Campinas)*; Regonha, L.F *(Universidade Estadual de Campinas)*; Degasperi, G.R *(Universidade Estadual de Campinas)*; Morales Neto, R. *(Universidade Estadual de Campinas)*; Gadelha, F.R. *(Universidade Estadual de Campinas)*

The drugs available for Chagas'disease treatment have low specificity and are potentially toxic to the host. Ibuprofen has been reported as an inductor of cellular death in neoplasic cells. The aim of this project was to verify if this drug induces oxidative stress in two strains of T. cruzi with different resistance to oxidative stress (Y e Tulahuen 2) since a cytotoxicity effect was observed. Reactive oxygen species (ROS) was determined by flow cytometry using dihidroeti-

dine (DHE, 2μM) as a probe. After incubation of 1 x 10^6 cells/ml in the presence of 250μM ibuprofen during 24 and 48 h an increase in ROS was observed (approximately two times for Tulahuen 2 and 3 times for Y strain). Analysis of total thiols after incubation in the presence of 250μM ibuprofen for 24, 48 and 72h showed no significant differences among controls and treated cells. The activity of glucose-6-phosphate (G6PD) and 6 phosphogluconate dehydrogenases, enzymes of the Pentose Phosphate Pathway responsible for the production of NADPH was also evaluated. Treatment of cells with 250μM Ibuprofen during 48 and 72h lead to an increase in G6PD activity (2 and 1.4 times for Y and Tulahuen 2, respectively for 48h and 3 and 1.8 times for Y and Tulahuen 2 cells, respectively for 72h). In conclusion, cell death induced by ibuprofen appears to involve the generation of ROS. This is corroborated by an increase in G6PD activity, an important enzyme for the detoxification of ROS. Supported by: FAPESP

BQ10 - Identification and metabolic characterization of carotenoids in intraerythrocytic stages *Plasmodium falciparum*

Tonhosolo, R *(Instituto de Ciências Biomédicas II - USP)*; D´Alexandri, F. L *(Instituto de Ciências Biomédicas II - USP)*; de Rosso, V *(Faculdade de Engenharia de Alimentos - UNICAMP)*; Gazarini, G *(Universidade Federal de São Paulo, Departamento de Ciências da Saúde, Baixada Sa)*; Merino, E.F *(Department of Medical Parasitology, NYU School of Medicine)*; Peres, V. J *(Instituto de Ciências Biomédicas II - USP)*; Wunderlich, G *(Instituto de Ciências Biomédicas II - USP)*; Carlton, J.M *(Department of Medical Parasitology, NYU School of Medicine)*; Mercadante, A. Z *(Faculdade de Engenharia de Alimentos - UNICAMP)*; Kimura, E. A *(Instituto de Ciências Biomédicas II - USP)* Katzin, A. M *(Instituto de Ciências Biomédicas II - USP)*

Lethal forms of the malaria are caused by *Plasmodium falciparum* and the spreading resistance of this parasite against virtually all drugs calls for the identification of new therapeutic targets. An important new antimalarial target is the isoprenoid biosynthesis, which occurs in *P. falciparum* via the 2-C-methyl-D-erythritol-4-phosphate (MEP) pathway. By different biochemical and mass spectrometrical analyses, the following isoprenic compounds downstream of the MEP pathway were identified in *P. falciparum*: neoxanthin, neocrome, violoxanthin, lutein, zeaxanthin, phytoene, zeinoxanthin, phytofluene, α-criptoxanthin, cis-α-carotene, α-carotene, α-carotene (all-trans), neurosporene and lycopene. In plants, the phytoene synthesis can be ablated by the herbicide norflurazon, which inhibits the phytoene desaturase. Importantly, this drug inhibited parasite growth in vitro at IC50 150 μM concentrations. In a parallel experiment, we showed that chloroquine treatment promoted an increase of *P. falciparum* carotenoid biosynthesis, suggesting a possible role of plasmodial carotenoids in the protection against oxidative stress. Finally, we cloned and expressed a recombinant protein which has phytoene synthase activity in vitro. The Michaelis constant and the subcellular localization of this *P. falciparum* phytoene synthase enzyme were determined. We suggest that the carotenoid synthesis pathway in *P. falciparum* could be exploited in the screening of novel drugs. Supported by FAPESP and CNPq

BQ11 - FUNCTIONAL CARACTERIZATION OF THE GENE CODING FOR PROLINE OXIDASE OF *T. CRUZI*

Paes, L.S. *(Universidade de São Paulo)*; Suárez, B.A. *(Universidade de São Paulo)*; Magdaleno, A. *(Universidade de São Paulo)*; Silber, A.M. *(Universidade de São Paulo)*

The flagellated parasite *Trypanosoma cruzi* can use either carbohydrates or amino acids as main energy sources. Biochemical evidences support the hypothesis that *T.cruzi* is able to obtain energy from L-proline by oxidizing it through the enzyme proline oxidase (PO) (EC 1.5.1.2) or the enzyme proline dehydrogenase (PD) (EC 1.5.99.8). In some eukaryote organisms, L-proline is involved in stress protection mechanisms. It is relevant for example the fact that proline confers resistance to oxidative stress. The PO activity of the enzyme encoded by the *T. cruzi* gene named as Tc00.1047053510943.50 was recently demonstrated by our group by complementing a null mutant of *Saccharomyces cerevisiae*, which is deficient for the endogenous PO (strain YLR142w). The yeast complemented with the *T. cruzi* PO gene was submitted to oxidative stress in the presence of proline, showing enhanced sensibility to oxidative stress in comparison to the null mutant. Enzymatic activity assays have shown that the highest PO levels in the complemented strain are very similar to those measured in *T. cruzi* epimastigote stage. In addition, the higher the PO activity, the lower the internal free proline concentration was observed in the complemented strain. Thus, our data show that the proline consumption increase the sensibility to oxidative stress in *S. cerevisiae* cells complemented with the *T. cruzi* PO gene involving the regulation of this gene in oxidative stress resistance. Additional analyses will be performed measuring the activity of both catalase and glutathione (GSH) enzymes to better characterize the functional relationship between PO activity and oxidative stress in *T. cruzi*. Support: USP - FAPESP

BQ12 - Inhibition of *Trypanosoma cruzi* growth by the glutamate analog methionine sulfoximine

Magdaleno, A. *(Universidade de São Paulo)*; Ahn, I.Y. *(Universidade de São Paulo)*; Silber, A.M. *(Universidade de São Paulo)*

The trypanosomatids can use amino acids as carbon and energy sources playing besides several roles in biological processes. In particular, proline and glutamate are directly involved in osmoreguation, cell volume control, metacyclogenesis and production of intermediates for the Krebs cycle. Several amino acids analogs have been analyzed on prokaryote and eukaryote cultures, showing inhibitory effects on the amino acids transport and enzymatic function. Methionine sulfoximine (MS) is a glutamate analog that inhibits the enzyme glutamine synthetase and interact competitively with glutamate transporter in pathogenic bacteria and mammalian cells. In the present work, we analyze the effects of MS on the epimastigotes growth in different stress conditions (temperature, pH and starvation). The cells were cultured in LIT medium supplemented with 10 % FBS, at 28, 33 and 37 $^{\circ}$C, pH = 5.5, 6.5 and 7.5. The MS concentrations tested were 5, 10 and 100 mM. The nutritional stress was carried out in PBS, or in the presence of 10 mM glucose, 10 mM glutamate, and 10 mM MS. Taking in account the pH stress, the highest growth inhibition was observed at pH 5.5-10 mM MS (35 % I), and at pH 7.5-100 mM MS (52 % I). The epimastigotes growth was temperature dependent being the highest MS inhibition at 33 and 37 $^{\circ}$C at 100 mM (87 and 73 %, respectively). The effect of nutritional stress on viability was analyzed by MTT assay, showing that the cells could be viable until 72 hrs in the presence of glucose and glutamate with or without MS, but the cell viability highly decreased within 24 hrs in PBS containing only this drug. Our preliminary results show that MS would be proposed as a possible therapeutic drug. Additional studies on intracellular stages are in process to support the therapeutic perspectives of this compound. Support: USP-FAPESP.

BQ13 - Intracellular signaling induced by Platelet-Activation Factor (PAF) during *Trypanosoma cruzi* differentiation

Gomes, M.T. *(Universidade Federal do Rio de Janeiro)*; Zimmermann, L.T. *(Universidade Federal do Rio de Janeiro)*; Portela, M.B *(Universidade Federal do Rio de Janeiro)*; Silva-Neto, M.A. *(Universidade Federal do Rio de Janeiro)*; Mello, F.G. *(Universidade Federal do Rio de Janeiro)*; Soares, R.M. *(Universidade Federal do Rio de Janeiro)*; Lopes, A.H. *(Universidade Federal do Rio de Janeiro)*

Platelet-activating factor (PAF) is a potent phospholipid mediator of several cellular functions in diverse biological and pathophysiological processes, such as cell differentiation, inflammation and allergy. PAF is known to trigger cell differentiation in *T. cruzi* and in *Herpetomonas muscarum muscarum*. In the present study we show that PAF-induced cell differentiation in *T. cruzi*, clone Dm28c, from epimastigotes into trypomastigotes, takes place through a cascade of signal transduction events, leading to enhancement of cAMP activity and inhibition of the expression of a metalloprotease and a cystein protease, involving protein kinase CK2 and protein kinase C (PKC). The results presented in this study suggest that the signaling pathways triggered by PAF are dependent on PKC and CK2.
This work was supported by CNPq, FAPERJ, CAPES and PIBIC/CNPq/UFRJ.

BQ14 - Intracellular signaling in murine macrophages is modulated by bug saliva and lysophosphatidylcholine.

Carneiro, A. B. *(Instituto de Bioquímica Médica - UFRJ)*; Atalah-Santos, M. *(Instituto de Bioquímica Médica - UFRJ)*; Feio-Lemos, R. *(Instituto de Bioquímica Médica - UFRJ)*; Ferreira-Pereira, A. *(Instituto de Microbiologia Professor Paulo de Góes - UFRJ)*; Silva-Neto, M. A. C. *(Instituto de Bioquímica Médica - UFRJ)*

Rhodnius prolixus is a vector of *Trypanosoma cruzi* in South America. The parasite is transmitted by vector feces deposited on human skin during blood feeding. One of the routes of host cell invasion occurs through the wound produced by the insect bite. Parasite thus faces a cell environment within the wound previously stimulated by saliva. *R. prolixus* saliva and feces stores the bioactive lipid lysophosphatidylcholine (LPC). LPC is a powerful modulator of cell signaling in mammalian cells. In the present work we tested the role of bug saliva on intracellular signaling in murine peritoneal macrophages. Saliva and LPC were able to reduce LPS-induced NO production in macrophages in a dose dependent fashion. LPC and saliva greatly altered macrophage intracellular free calcium concentration. Moreover, treatment of macrophages with saliva triggered the phosphorylation of several proteins on phosphotyrosine but specifically of a 45 kDa polypeptide which is readily absent from control cells. Finally, protein kinase-directed antibodies identified the activation of GSK-3 and Akt in saliva-treated macrophages. The above set of results shows that previous exposition to saliva manipulates the intracellular signaling system of host macrophages. The mapping of target phosphoproteins of such intracellular signaling systems is under way in our laboratory and may conduct to novel strategies in the future to block Chagas disease transmission. Supported by CNPq, FAPERJ, IFS, OMS.

BQ15 - A zymographic analysis of protease activities in extracts of first instar larvae of *Oxysarcodexia* sp. (Diptera: Sarcophagidae)

Altoé, E. C. F. *(Instituto Oswaldo Cruz)*; Pires, F. A. *(Instituto Oswaldo Cruz)*; d'Avila-Levy, C. M. *(Instituto Oswaldo Cruz)*; Cuervo, P. *(Instituto Oswaldo Cruz)*; de Jesus, J. B. *(Instituto Oswaldo Cruz)*; Alves, C. R. *(Instituto Oswaldo Cruz)*

Among arthropod diseases affecting animals, larval infections - myiases - of domestic and wild animals have been considered important since ancient times. Besides the significant economic losses to livestock worldwide, myiasis-causing larvae have attracted the attention of scientists because some parasitise humans and are of interest in forensic entomology. *Oxysarcodexia* sp. (Diptera: Sarcophagidae) was collected in Fiocruz campus, Rio de Janeiro, and larvae morphology was analyzed by differential interference contrast microscopy (DIC). The proteases were analyzed and characterized by gelatin-SDS-PAGE. The first instar larvae (L1) presented a proteolytic pattern of 9 proteases bands migrating from 21 to 150 kDa. The enzymes were sensitive to phenylmethylsulphonyl fluoride (PMSF), while the inhibitors E-64, 1,10-phenanthroline and pepstatin showed no effect. The proteases were active over a broad pH range (pH 5.5 to 9.0), being optimally active from pH 7.5 to 9.0, and no activity was detected bellow pH 5.5. This is the first report on the characterization and identification of proteases in this sarcophagid fly. Supported by: MCT-CNPq, FAPERJ, CAPES and FIOCRUZ.

BQ16 - Intact cells of *Trypanosoma rangeli* can hydrolyze polyphosphates

Amora, K. *(Instituto de Bioquímica Médica, Universidade Federal do Rio de Janeiro)*; Fonseca de Souza, A. L. *(Instituto de Bioquímica Médica, Universidade Federal do Rio de Janeiro)*; Dick, C. F. *(Instituto de Bioquímica Médica, Universidade Federal do Rio de Janeiro)*; Meyer Fernandes, J. R. *(Instituto de Bioquímica Médica, Universidade Federal do Rio de Janeiro)*

Trypanosoma rangeli is a digenetic hemoflagelated parasite widely distributed on the Central and South Americas. It is able to infect several animal groups, as well as humans. The life cycle begins with the ingestion of trypomastigote forms present in vertebrate bloodstream by the triatominae. Inside of the vector gut, parasites differentiate to epimastigote forms and pass through the gut epithelium, achieving the hemocoel. Parasites proliferate in the haemolymph or into the haemocytes and migrate to the salivary glands where trypomastigote is formed. This stage is able to infect the vertebrate host during new blood feed. Inorganic polyphosphate chains are formed by phosphate units linked by high-energy phosphoanhydride bonds. Polyphosphates are required for responses to a variety of stresses and stringencies and for the virulence of some pathogens. Moreover, the disruption of phosphoanhydride bonds can generate free phosphate units for the internalization by the cells. They are also involved in the cell proliferation and differentiation by stimulating protein kinases activity. In this work, we showed that intact cells of *T. rangeli* are able to hydrolyze tri- and hexapolyphosphates. In addition, we observed that polyphosphates hydrolysis did not inhibit *p*-NPP hydrolysis, suggesting that the enzymes responsible by *p*-NPP hydrolysis are not the same that hydrolyze the polyphosphates. The polyphosphates hydrolysis is modulated by inorganic phosphate content present at culture medium of parasites, since cells maintained at Pi-starved medium present higher capacity in polyphosphate hydrolysis than the cells maintained at Pi-supplemented medium. Taken together, these data suggest that there is an ecto-polyphosphatase activity in *T. rangeli*. Supported by CNPq, CAPES and FAPERJ.

BQ17 - Identification and characterization of protein kinase CK2 activities in epimastigotes of *Trypanosoma cruzi*.

Souza, J.E.O. *(Instituto de Microbiologia Prof. Paulo de Góes)*; Lima, A. K. C. *(Departamento de Microbiologia, Imunologia e Parasitologia)*; Lopes, A.H.C.S. *(Instituto de Microbiologia Prof. Paulo de Góes)*; Atella, G.C. *(Instituto de Bioquímica Médica)*; Silva-Neto, M.A.C. *(Instituto de Bioquímica Médica)*; Dutra, P.M.L. *(Departamento de Microbiologia, Imunologia e Parasitologia)*

Trypanosoma cruzi undergoes complex morphological changes through its life cycle in both insect vector and vertebrate host. Cell differentiation of these parasites is highly regulated and includes significant changes in signaling pathways. Protein kinase CK2 was directly related to cell differentiation in trypanosomatids. CK2 activities have been described on the cell surface and as secreted enzymes in different trypanosomatids, where these enzymes seem to be involved in cell growth, morphology and infectivity. In the present study, we demonstrate the presence of CK2 activities in *T. cruzi* (Colombiana strain CTC-IOC 004) on the surface, in a membrane-enriched fraction, as a secreted form, and in the cytoplasmic contents of this parasite. Macrophage proteins, inactivated human serum and midgut contents of *Rhodnius prolixus* were able to promote an enhancement (67%, 36% and 84%, respectively) on the secreted CK2 activity. This last enzymatic form showed a specific activity of 16.8 pmoles Pi/mg.min after purification by HPLC. Polyclonal antibodies raised against the mammalian CK2 alfa catalytic subunit were able to recognize, through immunoblotting, a protein from *T. cruzi*, with a compatible molecular mass of 55 kDa. CK2-activators stimulated epimastigotes growth, while CK2-inhibitors reversed this stimulatory effect, which substantiates the meaning of CK2 for the life cycle of trypanosomatids. Supported by: CNPq, FAPERJ, PIBIC-UERJ/CNPq, CAPES.

BQ18 - CrATP is a selective inhibitor of ecto-enzymes of trypanosomatids

Moreira, O. C. *(Instituto de Bioquímica Médica)*; Barrabin, H. *(Instituto de Bioquímica Médica)*

The Trypanosomatidae family has singular importance due to its capacity to infect several organisms, including men. The ecto-enzymes of these parasites are known to be involved in many cellular processes, such as protection of parasites against host immune defense and transport of nutrients and ions. CrATP, a complex formed by the stable binding of ATP and Cr^{3+} ion, has been utilized in several kinetic studies due to its inhibitory properties on ATPases. CrATP inhibits partially and reversibly the Mg^{2+}-dependent ecto-ATPase of Herpetomonas sp., a plant trypanosomatid, with an apparent Ki = 4.8 ± 1.0 μM. DIDS and suramine showed additive effect in this inhibition. The ATP concentration had influence in the inhibition for CrATP: with the increase of the ATP concentration, an ecto-ATPase activity resistant to the CrATP is observed, which possess Km= 3.49 ± 0.19 mM and V_{Max} = 115.6 ± 3.2 nmol Pi / 10^8 cel x h. The ecto-ATPase activity sensible to the CrATP seems to have higher affinity for ATP (K_m = 0.085 ± 0.039 mM) and lower V_{Max} (39.2 ± 3.9 nmol Pi / 10^8 cel x h) that the CrATP resistant activity. Also, the ADPase and *p*-NPPase activities are not sensible to CrATP. The observed data suggest that activities found in the external face of the plasma membrane of Herpetomonas sp. correspond to different enzymatic entities, that can be distinct isoenzymes or aggregated ones. The Mg^{2+}-dependent ecto-ATPases of Tripanosoma rangeli, Tripanosoma cruzi and Leishmania amazonensis, pathogenic trypanosomatids, also had been sensible to CrATP, however with different affinities. The incubation of CrATP with promastigote forms of Herpetomonas sp. inhibited cellular growth in about 25%, after 28 hours of incubation, showing that this compound can be used as chemotherapeutic drug in trypanosomiasis.

BQ19 - *Trypanosoma cruzi* ecto-nucleotidase activity and its relationship with "in vitro"infectivity.

de Freitas Santos, R. F. S. *(UNIVERSIDADE FEDERAL DE OURO PRETO)*; Pereira Moreira, B. P. M. *(UNIVERSIDADE FEDERAL DE VOÇOSA)*; Sousa Pôssa, M. S. P. *(UNIVERSIDADE FEDERAL DE OURO PRETO)*; Silva e Bastos, M. S. B. *(UNIVERSIDADE FEDERAL DE OURO PRETO)*; Marcos da Matta Guedes, P. M. M. G. *(UNIVERSIDADE DE SÃO PAULO)*; Terezinha Bahia, M. T. B. *(UNIVERSIDADE FEDERAL DE OURO PRETO)*; Lopes Rangel Fietto, J. L. R. F. *(UNIVERSIDADE FEDERAL DE VIÇOSA)*

Extracellular nucleotides have been related as regulatory molecules and seen to be involved in some different biological process (cellular differentiation, adhesion, and immune response modulation). The main objective of this study is to evaluate the role of ecto-nucleotidases in *T. cruzi* "in vitro"infectivity. To achieve this objective we infected mouse with different *T. cruzi* strains/clone (Y, CL, Be-62, CL-Brener), recovered blood trypomastigotes and used then to infect VERO cells. After the first infection cycle we collected trypomastigotes and analyzed the "in vivo"ecto-nucleotidase activity and infectivity to VERO cells. "In vivo"ecto-ATPase activity was higher in trypomastigotes showing the following decreasing profile ($Y > CL > CL-Brener > Be-62$). ADP hydrolysis was similar in all parasites except in Y strain that presented lower hydrolysis. The resulting ATP/ADP ratios were quite different in trypomastigotes (17,6:1; 4:1; 3:1; 2,2:1 for Y, CL, CL-Brener, Be-62 respectively). AMP hydrolysis was only detectable in CL-Brener. In vitro differentiated amastigotes showed preference for ATP in all strains. ADP hydrolysis was undetectable (CL) or very slow (Y) and only Y strain presented detectable ecto-AMPase activity. Furthermore we evaluated the ecto-nucleotidase activities in trypomastigotes (Y) recovered after more cellular passages. As we suspected the continue cellular infection (third to fourth passage) generate parasites with higher ecto-ADPase activities leading to decreasing in ATP/ADP hydrolysis. This phenomenon seems to be related with infectivity decreasing (about 80% of total trypomastigotes did not infected VERO cells and differentiated to amastigote-like parasites). These parasites presented very lower ecto-nucleotidase activity when compared with previous passages. These data suggested that the high ATP/ADP hydrolysis ratio could be related with higher infection capacity. The inhibiton of *T. cruzi* ecto-nucleotidase activities using apyrase inhibitors (suramin, gadolinium and ARL67156) and evaluation of their effects in the infectivity assays will be the next steps in this work.

BQ20 - Modulation of Ecto-phosphatase Activity in *Trypanosoma rangeli* by Redox Reaction

Cosentino Gomes, D. *(Universidade Federal do Rio de Janeiro)*; Abrahão, T. *(Universidade Federal do Rio de Janeiro)*; Fonseca de Souza, A. L. *(Universidade Federal do Rio de Janeiro)*; Meyer Fernandes, J. R. *(Universidade Federal do Rio de Janeiro)*

Trypanosoma rangeli is a digenetic haemoflagellate parasite that infects humans as well as domestic and wild animals in Central and South America. The life cycle of this trypanosomatid begins during blood meal of the insect vector, like those of the *Rhodnius* genus. In the midgut, trypomastigote forms differentiate into proliferative short epimastigote forms, pass through intestinal barrier and achieve the hemolymph, where they differentiate to long forms. The development is completed in salivary glands, where metacyclogenesis takes place. Phosphorylation and dephosphorylation play a central role in regulating a variety of fundamental cellular processes. In this context, phosphatases could be key enzymes to control these processes. Among phosphathase

classes are ecto-phosphatases, which have their catalytic site faced to extracellular medium. Several functions are described for ecto-phosphatases such as the process of parasite adhesion to host cell, cell division and nutrient acquisition. It appears that some groups of these enzymes utilize a nucleophilic cysteine residue in catalysis, which is susceptible to oxidation. Recent works have showed that oxidation can be an important regulatory mechanism for diverse members of the phosphatases family and the biological processes that they control. In the present study, we show that an ecto-phosphatase activity present in *T. rangeli* is modulated by oxi-reduction reactions. Previous experiments demonstrated that hydrogen peroxide, 500μM can inhibit ecto-phosphatase activity of short epimastigote forms and this inhibition is reversible when the oxidizing is removed from the medium. Antioxidants agents like cysteine, β-mercaptoethanol and reduced glutathione at 1mM concentration, are able to protect this oxidation. Catalase, an enzyme that dismutate H_2O_2 into water and oxygen, and which are found in vector midgut, stimulated this activity in a dose dependent manner. This work was supported by grants of CNPq and FAPERJ.

BQ21 - Modulation of Ecto-ATPase from *Trypanosoma cruzi* by heat shock

Giarola, N. L. L. *(Universidade Federal do Rio de Janeiro)*; Fonseca de Souza, A. L. *(Universidade Federal do Rio de Janeiro)*; Domenech, C. *(Universidade Federal do Rio de Janeiro)*; Almeida Amaral, E. E. *(Universidade Federal do Rio de Janeiro)*; Junior, I. C. *(Universidade Federal do Rio de Janeiro)*; Meyer Fernandes, J. R. *(Universidade Federal do Rio de Janeiro)*

Ecto-nucleotidases are surface enzymes able to hydrolyze extracellular nucleotides. Some functions are suggested for these proteins: cell adhesion, purine acquisition, protection against cytotoxic effects of extracelular ATP and, recently, MDR phenomenon. A well-known family of enzymes, called heat-shock proteins, is stimulated in response to stress such as heat and can present ATPase activity. In this work, we verified how the ecto-nucleotidase activity from *Trypanosoma cruzi* is modulated by heat-shock stresses. For this purpose, cells were submitted to heat-stress (37 degrees) for two hours. After this pre-incubation time, cells were used to determine Mg^{2+}-dependent ecto-ATPase activity by measuring ^{32}Pi release from the substrate [γ-^{32}Pi]ATP. Our results show that ecto-ATPase from *T. cruzi* was increased gradually until two hours of incubation achieving a stimulation of 155%. in a time-dependent manner. In addition, different development forms or strain of *T. cruzi* showed different stimulation pattern by the heat-stress. Using membrane fraction the ATPase activity was not increased by the heat treatment, suggesting influence of cytosolics components. Moreover, when we used cycloheximide (10μM) the inhibition of protein synthesis blocked the response of Ecto-ATPase. Taken together, these data could be indicating that the synthesis of some protein that would be act in membrane may be involved in the response to the heat shock. Supported by CNPq and FAPERJ.

BQ22 - HEME (FE-PROTOPORPHYIRIN IX) INCREASES CaMK II ACTIVITY

Souza, C.F. *(Universidade do Estado do Rio de Janeiro)*; Carneiro, A.B. *(Universidade Federal do Rio de Janeiro)*; Silveira, A.B. *(Universidade Federal do Rio Janeiro)*; Laranja, G.A.T. *(Universidade do Estado do Rio de Janeiro)*; Silva-Neto, M.A.C. *(Universidade Federal do Rio de Janeiro)*; Gonçalves da Costa, S.C. *(FIOCRUZ - IOC)*; Paes, M.C. *(Universidade do Estado do Rio de Janeiro)*

Trypanosoma cruzi, the ethiologic agent of Chagas disease, is transmitted through triatomine vectors during their bloodmeal on vertebrate host. Since *T. cruzi* epimastigotes live in constant presence of heme, we have investigated the role of heme as a signaling molecule and as a regulator of cell proliferation. We evaluated the effect of several protein kinase inhibitors *in vivo*. Among all inhibitors tested, only KN-93, a classical inhibitor of CaM kinases, reversed heme inducedcell proliferation. When KN-92, an inactive analogue, was tested there was not effect, confirming the specificity of KN-93. In order to identify the CaMK involved, we tested the peptide Myr-AIP, a highly specific inhibitor derived from the CaMK II autoinhibitory domain. We observed that the addition of the inhibitor blocked parasite growth in the presence of heme, confirming the involvement of CaMK II pathway. When CaMK II activity was assayed in *T. cruzi* epimastigotes extracts, we observed that this activity increased in the presence of heme. Interestingly, we are showing through western blotting that the phosphorylation level of CaMK II, and therefore its activation, also increased in the presence of heme, confirming the presence of this enzyme in this process. Finally, we assayed this activity using a recombinant CaMK II. Addition of heme in the reaction medium enhanced up to 10-fold total enzyme activity, suggesting binding sites for heme in CaMK II. Taken together, these results show for the first time the modulation of CaMK II by heme. Future efforts will be directed towards elucidating the mechanism by which heme increases CaMK II activity. Supported by PIBIC/UERJ, FAPERJ, CNPq.

BQ23 - Heme as a signalling molecule in *Trypanosoma cruzi*

Eitler, R.B. *(Universidade do Estado do Rio de Janeiro)* Laranja, G.A.T. *(Universidade do Estado do Rio de Janeiro)*; Breder, L.O. *(Universidade do Estado do Rio de Janeiro)*; Souza, C.F. *(Universidade do Estado do Rio de Janeiro)*; Paes, M.C. *(Universidade do Estado do Rio de Janeiro)*

Heme is an ancient and ubiquitous molecule present in organisms of all kingdoms, composed of an atom of iron linked

to four ligand groups of porphyrin. *Trypanosoma cruzi* epimastigotes (EPI) proliferate inside of the hematophagous insects that usually ingest in a single meal about 10mM heme bound to hemoglobin. We showed recently that EPI acquires extracellular heme from the medium and the addition of heme increases significantly the parasite proliferation in a dose-response manner (Lara *et al.*, 2007). Others previous results show the involvement of CaMK II pathway in this process. In order to investigate whether their nutritional needs is heme, iron, the protoporphyrin chain, or biliverdin (the heme catabolism product), we tested the free iron addition to the medium and we also tested the addition of deferoxamine (an iron chelator). The results show that EPI growth did not increase in the presence or absence of iron. When the protoporphyrin and biliverdin were added to the culture we observed no significant changes in the parasite proliferation. Altogether these results are demonstrating that the whole heme is the responsible for the increase parasite growth suggesting a specific mechanism of cell signalling. Supported by PIBIC/UERJ and FAPERJ

BQ24 - Heme leads to 4-hydroxynonenal (HNE)-protein adducts in *Trypanosoma cruzi* epimastigotes

Nogueira, N. P. A. *(Universidade Federal do Rio de Janeiro - Instituto de Bioquímica Médica)*; Laranja, G. A. T. *(Universidade do Estado do Rio de Janeiro - Departamento de Bioquímica)*; Coelho, M. G. P. *(Universidade do Estado do Rio de Janeiro - Departamento de Bioquímica)*; Dalmau, S.R. *(Universidade do Estado do Rio de Janeiro - Departamento de Bioquímica)*; Oliveira, M. F. *(Universidade Federal do Rio de Janeiro - Instituto de Bioquímica Médica)*; Paes, M. C. *(Universidade do Estado do Rio de Janeiro - Departamento de Bioquímica)*

Trypanossoma cruzi epimastigotes proliferate inside of the hematophagous insects that usually ingest in a single meal about 10mM heme bound to hemoglobin. Recently we showed that epimastigotes acquires extracellular heme from their invertebrate vectors and the addition of heme increases drastically the parasite proliferation (Lara *et al.*, 2007, BBRC). Although heme induces the growth in these cells this molecule is composed of an atom of iron linked to four ligand groups of porphyrin and is a powerful generator of reactive oxygen species (ROS) that promotes an imbalance redox. Exposure of biological membranes to pro-oxidant agents is known to induce peroxidative decomposition of polyunsaturated lipid components. The $alfa$-unsaturated aldehyde 4-hydroxy-2-nonenal (4HNE) has been demonstrated to be a major product of the lipid peroxidation process and the subsequent accumulation of the modified proteins have been found in cells during aging, oxidative stress, and in various pathological states. In order to investigate the oxidative stress induced by heme in *T. cruzi* epimastigotes, were maintained in BHI supplemented with 10% FCS at 28°C without addition of heme for two passages. Afterwards, cells were incubated in the absence or in the presence of different concentrations of heme for different periods. Thus, protein modification using 4-HNE immunoblotting was examined. We observed a non-enzymatic oxidative modification of proteins according to heme addition. The ROS formation analysis through flow cytometry of CMH2DCFDA and microscopy showed that the fluorescent signal is proportional to heme concentration and decreases in a short period of time, indicating that T. cruzi might have selected effective mechanisms against high heme concentration found in its environment and the protein modifications by lipid peroxidation may be an alternative signal for this adaptation. Supported by FAPERJ, CNPq .

BQ25 - Sn-Protoporphyrin IX Inhibits Heme Internalization in *T. cruzi* epimastigotes

Cupello, M.P. *(Universidade do Estado do Rio de Janeiro)*; Souza, C.F. *(Universidade do Estado do Rio de Janeiro)*; Costa e Silva, M.C. *(Universidade do Estado do Rio de Janeiro)*; Laranja, G.A.T. *(Universidade do Estado do Rio de Janeiro)*; Coelho, M.G.P. *(Universidade do Estado do Rio de Janeiro)*; Paes, M.C. *(Universidade do Estado do Rio de Janeiro)*

Heme (iron protoporphyrin IX) is an important molecule in metabolism of all living organisms. Pathogenic protozoa take up heme from the environment to supply their nutritional needs. Since heme is present in the environment of *T. cruzi* epimastigotes (EPI) in high concentrations (about 10 mM) we have been investigating its detoxification mechanism in EPI. The enzymatic heme cleavage in several organisms by heme oxygenase (HO) yields biliverdine, carbon monoxide, iron and the biliverdine is reduced to form bilirubin. We are investigating the metabolism of heme in EPI and the results showed the presence of peaks the same retention times of heme and billiverdine when analyzed by HPLC. It was confirmed through spectrophotometric analysis. In order to investigating the possible Heme Oxigenase involvement in heme catabolism, we evaluated the effect of Sn Protoporphyrin IX (SnPPIX - an inhibitor of Heme Oxigenase) on parasite proliferation. Our previous results showed that the addition of heme increases the parasite proliferation in a dose-dependent manner. Interestingly, the addition of SnPPIX decreased the parasite proliferation in a dose-dependent manner and we did not observed the peak referent to biliverdine when cells were treated with heme and SnPPIX. Furthermore, we also did not observe the peak referent to heme, indicating that SnPPIX and Heme might be competing by the same transporter in *T. cruzi*. Supported by PIBIC/UERJ, FAPERJ.

BQ26 - Overexpression of the Nucleoside Diphosphate Kinase in *Leishmania major* promastigotes

de Oliveira, A. H. C. *(Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto-USP)*; Vieira, P. S. *(Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto-USP)*; Pinto, M. R. *(Faculdade de Medicina de Ribeirão Preto-USP)*; Tosi, L. R. O. *(Faculdade de Medicina de Ribeirão Preto-USP)*; Cruz, A. K. *(Faculdade de Medicina de Ribeirão Preto-USP)*; Ward, R. J. *(Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto-USP)*

The nucleoside diphosphate kinase (EC 2.7.4.6; NDK) is ubiquitous in both prokaryotes and eukaryotes and is involved in the maintenance of nucleotide pools and in other functions in the cell. Extracellular secretion of NDK has been reported in several pathogens, perhaps involving modulation of the macrophage survival by the nucleotide concentration and P2Z purinergic receptor activation at the macrophage surface. Our group has previously shown NDKb as an abundant component of the microsomal fraction of *L. major* promastigotes by subproteomic analysis (de Oliveira et al., Comp. Biochem. Physiol. Part D, vol. 3, 2006). Here we have characterized the NDK overexpression in *L. major* promastigotes, as part of an investigation of the functions of this protein in *Leishmania* species. The region containing the unique NDK coding sequence was amplified from *L. major* genomic DNA by PCR and cloned into pET28a, pX63NEO and pXG/GFP vectors. The recombinant Lm-NDK was expressed and purified from *E. coli* and polyclonal antibodies raised against the native protein from the promastigote extracts. The subcellular localization with fluorescence microscopy demonstrated that the native NDK was localized in the cytoplasm and that the overexpressed NDK-GFP fusion was localized in the cytoplasm, near the flagelar pocket region and accumulated in a large posterior vesicle. Preliminary comparative analysis of 2D-gels protein profile of transfectants revealed differences of promastigote protein expression, including the NDK overexpression. Supported by FAPESP, CNPq, PRP-USP.

BQ27 - Identification of a 48 kDa protein from *Leishmania amazonensis* extracts as a possible responsible for leishporin activity.

Castro-Gomes, T. *(Universidade Federal de Minas Gerais)*; Giulianetti-Almeida, M. P. *(Universidade Federal de Minas Gerais)*; Silva, R. *(Universidade Federal de Minas Gerais)*; Campos, P.C. *(Universidade Federal de Minas Gerais)*; Chapeaurouge, A. *(Fundação Instituto Oswaldo Cruz)*; Parales, J. *(Fundação Instituto Oswaldo Cruz)*; Pimenta, A. *(Universidade Federal de Minas Gerais)*; Frézard, F. *(Universidade Federal de Minas Gerais)*; Andrade, H. *(Universidade Federal de Minas Gerais)*; Horta, M. F. *(Universidade Federal de Minas Gerais)*

We have previously described in *L. amazonensis* a pore-forming protein (leishporin) that lyses cells, including macrophages. Leishporin is optimally active at 37 ^{o} C and pH 5,5, it may act in the mammalian host and be involved in the rupture of fagolisosome and macrophages. We also found this cytolytic activity in others species of Leishmania such as *L. major* and *L. guyanensis*. In previous works we showed that liposomes made of DPPC are capable of removing the lytic activity when they were incubated with active parasites membrane extracts and that all components required for lysis bind to them. In this work, we identified two major proteins, as distinguished by SDS-PAGE, which were co-removed with lytic activity. We show that one protein with 63 kDa was identified by Mass Spectrometry as the surface protease gp63. The second, with 48 kDa, was identified as a hypothetical protein from *Leishmania* and can be leishporin. Searches for similarity showed that this protein was related in *L. major*, *L. brasiliensis* and *L. infantum* with a high score of conservation between them (at least 76 %). One or both proteins can be involved in the lytic activity presents in extracts of these parasites. Financial support: WHO, CNPq, CAPES, FAPEMIG.

BQ28 - Lysis mediated by *Leishmania amazonensis* leishporin is a cholesterol, carbohydrate and protein-independent process.

Castro-Gomes, T. *(Universidade Federal de Minas Gerais)*; Calzavara-Silva, C. E. *(Universidade Federal de Minas Gerais)*; Almeida-Campos, F. R. *(Universidade Federal de Minas Gerais)*; Giulianetti-Almeida, M. P. *(Universidade Federal de Minas Gerais)*; Silva, R. *(Universidade Federal de Minas Gerais)*; Passagli, C. R. *(Universidade Federal de Minas Gerais)*; Frézard, F. *(Universidade Federal de Minas Gerais)*; Horta, M. F. *(Universidade Federal de Minas Gerais)*

Leishporin is a protein from *L.amazonensis* that lyses macrophages by forming pores in their membrane. Because it is optimally active at pH 5.5 and 37^{o}C, we postulate that it may act in the mammalian host cell being involved in the rupture of fagolisosome and the macrophages, amplifying the infection. To lyse cells, some pore-forming proteins require the binding to specific carbohydrates, proteins or lipids. In the present work, we have studied the requirements of leishporin to lyse cells. For this, we have performed lytic assays using erithrocytes or liposomes. Initially, we have treated human erithrocytes with trypsin, pronase or proteinase-K before incubation with *L. amazonensis* promastigotes membrane extract (ext-ms), which contains leishporin. We have found that the treatment with these proteases did not reduced the erithrocytes susceptibility to ext-ms-mediated lysis, indicating that leishporin do not require any erythrocyte surface protein to lyse. To investigate whether carbohydrates are necessary for leishporin-mediated lysis, we have incubated ext-ms with frutose, glucose, galactose, lactose, maltose or manose in an attempt to inhibit the lysis by competition. Our results showed that none of the

carbohydrates used reduced leishporin-mediated lysis, suggesting that, these molecules are also not required for lysis. To confirm the above results, we have constructed calcein-containing liposomes made of dipalmitoilphosphatidilcholine (DPPC). The vesicles were incubated with ext-ms, and lysis was detected by the fluorescence of the released calcein from liposomes. We have found that liposomes were lysed in a temperature-dependent manner and the inclusion of cholesterol in the lipidic composition of the DPPC liposomes did not affect its susceptibility to leishporin-mediated lysis. These results demonstrate that leishporin can lyse cells by binding directly to lipids and allow us to classify leishporin in the family of cholesterol-independent cytolysins. Financial support: WHO, CNPq, CAPES, FAPEMIG.

BQ29 - LIPID COMPOSITION OF THE URINARY TRACT OF THE INSECT VECTOR Rhodnius prolixus

Silveira, J.A. *(Instituto de Bioquímica Médica - UFRJ)*; Ximenes, A.A. *(Instituto de Bioquímica Médica - UFRJ)*; Atella, G.C *(Instituto de Bioquímica Médica - UFRJ)*

Rhodnius prolixus, a blood sucking bug from Hemiptera order confined to drier savannah areas from southern Mexico to northern South America. This insect by transmitting the parasite Trypanosoma cruzi is the second more important vector of Chagas' disease. T. cruzi, the etiological agent of Chagas' disease, is transmitted by R. prolixus and while in the triatomine midgut the parasite differentiates from a non-infective epimastigote stage into the pathogenic trypomastigote metacyclic form. An adult Rhodnius will ingest from two to three times its weight of blood at a single meal, and about three-quarters of the water in this blood is excreted as a clear fluid during the next three or four hours. The triatomine urine components are responsible for the parasite differentiation. The main objective of this work is to determine the lipid composition from the R. prolixus urine and if the lipids from urine can help parasite differentiation. Twenty-six urinary tracts from R. prolixus were dissected, the content was removed and subjected to a lipid extraction. The lipids were analyzed by thin-layer chromatography (TLC). The major phospholipids found were phosphatidylethanolamine (36,4%), phosphatidylcholine (32,8%). We also observe lysophosphatidylcholine (6,9%) phosphatidic acid (10,4%) and phosphatidylserina (13,6%). Apoiado por CNPq, FAPERJ, IFS

BQ30 - Lipid composition of *Triatoma infestans* saliva

Lima, M. S. *(Universidade Federal do Rio de Janeiro)*; Silva Neto, M. A. C. *(Universidade Federal do Rio de Janeiro)*; Atella, G. C. *(Universidade Federal do Rio de Janeiro)*

Triatoma infestans (T. infestans), is a blood sucking bug from subfamily Triatominae. It is widespread in the Southern Cone countries of South America and it is a vector of Chagas' disease. *T. cruzi*, the etiological agent of Chagas' disease, is transmitted by *T. infestans* and while in the triatomine midgut the parasite differentiates from a non-infective epimastigote stage into the pathogenic trypomastigote metacyclic form. An adult *Triatoma* will ingest from two to three times its own weight of blood at a single meal. Blood-sucking insects possess a variety of anti-hemostatic factors in their salivary glands to maintain blood fluidity during feeding. In our lab we demonstrated the anti-hemostatic properties of lysophosphatidylcholine (LPC) isolated from the salivary glands of another kissing-bug, *Rhodnius prolixus*. Here we are have searched for the presence of phospholipids in *T. infestans saliva*. Saliva was collected through an artificial feeder and subjected to lipid extraction and high performance thin-layer chromatography (HPTLC). The major neutral lipid found were fatty acids (41%), cholesteryl-esther (29%) and triacylglycerol (29%) whereas phosphatidylcholine was the major phospholipid found. This result shows that the presence of phospholipids or lipid-derived molecules may be a general feature of triatominae saliva. We are currently searching for the eventual role of such molecules in *T. infestans* saliva and in parasite transmission.

BQ31 - Phosphalipase A_2 from *Rhodnius prolixus* salivary glands

Cardoso, L.S. *(Universidade Federal do Rio de Janeiro)*; Fuly, A.L. *(Universidade Federal Fluminense)*; Silva-Neto, M.A.C. *(Universidade Federal do Rio de Janeiro)*; Atella, G.C. *(Universidade Federal do Rio de Janeiro)*

In hematophagous insects, salivary anti-hemostatic factors are of vital importance in order to maintain blood fluidity during feeding. We have already verified the presence of phospholipids (phosphatidylcholine, PC; and lysophosphatidylcholine, lysoPC) in the lumen of the salivary glands and saliva from *Rhodnius prolixus*, and demonstrated that salivary lysoPC displays anti-hemostatic properties. Phospholipase A_2 (PLA_2) is the enzyme responsible for hydrolyzing phospholipids yielding lysophospholipids and fatty acids. In previous work, we demonstrated the presence of PLA_2 in the lumen of *Rhodnius prolixus* salivary glands, and we now report the further characterization of this enzyme. Two hundred Fifth instar nymphs were dissected, the salivary glands were removed, disrupted and the lumen was separated from the epithelium. The luminal contents was assayed using a Phosphatildylcholine fluorescent substrate and measured by a fluorimeter. *R. prolixus* luminal glands showed that a PLA_2 activity was time- and concentration-dependent, and calcium-dependent. We also demonstrated that PLA_2 preferencially uses phosphatidylcholine and phosphatidylglycerol as substrates. Phosphatidic acid can also be hydrolyzed but with low affinity. This finding correlates with the greater increase in lysoPC concentration observed in the lumen after feeding, as the salivary components are refilled. In addi-

tion to being involved in the production of salivary lysoPC, the PLA_2 may also display direct anti-hemostatic effects itself. This possibility, together with the inhibitor profile and pH-dependence of the enzyme, are currently under investigation.

BQ32 - Cell Death and morphology alterations in *Trypanosoma rangeli* is not caused by alterations in mitochondria

Dick, C. F. *(Universidade Federal do Rio de Janeiro)*; Santos, A. L. A. *(Universidade Federal do Rio de Janeiro)*; Fonseca de Souza, A. L. *(Universidade Federal do Rio de Janeiro)*; Pereira, M. G. *(Universidade Federal do Rio de Janeiro)*; Cunha e Silva, N. L. *(Universidade Federal do Rio de Janeiro)*; Galina Filho, A. *(Universidade Federal do Rio de Janeiro)*; Meyer Fernandes, J. R. *(Universidade Federal do Rio de Janeiro)*

Trypanosoma rangeli is a digenetic hemoflagellate widely distributed in Central and South America, and it is apathogenic for the vertebrate host. This parasite co-exists with *T. cruzi*, and this fact can produce mixed infections in vectors or host, generating crossed serological reactions complicating the specific diagnosis of Chagas infection. Inorganic phosphate plays a significant role in increasing cell resistance to unfavorable environmental conditions and regulating many biochemical processes. So, we observed that phosphate is an important nutrient to maintenance of parasites morphology. Cells maintained at low-Pi content medium presented as spherical forms, showing low motility, inefficient cell proliferation and cell death. We observed that cell proliferation was arrested in low-Pi content medium. At the high-Pi medium, cells exhibit a normal growth curve, achieving the stationary phase at sixth day, while at low-Pi medium, the cells exhibit normal growth curve up to fourth day, from this moment they start to die. In order to verify if cell death induced by phosphate starvation involves mitochondria pathway, we observed the mitochondria at ultra structural and functional levels. The mitochondria at ultra structure level shows typical morphology, with intact borders and normal volume, in both cells maintained at low-Pi content and high-Pi content. The mitochondrial function was observed by oxygen consumption by the cell. The respiratory capacity was similar in both cases. These results indicated that the cell death of *T. rangeli* is not caused by alterations in mitochondria. Supported by CNPq, CAPES and FAPERJ.

BQ33 - Mitochondrial membrane potential and reactive oxygen species generation in *T. cruzi* strains isolated from cardiac and asymptomatic patients

Ribeiro, L.H.G. *(Universidade Estadual de Campinas)*; Mendonça, M.A.G. *(Universidade Estadual de Campinas)*; Carranza, J.C. *(Universidade de São Paulo, Brasil e LIPT. Universidad del Tolima, Colômbia.)*; Zingales, B *(Universidade de São Paulo)*; Gadelha, F.R. *(Universidade Estadual de Campinas)*

Using DNA microarray to compare the transcript profiles of human isolates from asymptomatic and from cardiac patients, the pre-edited maxicircle gene NADH dehydrogenase subunit 7 (ND7) was among the seven signals expressed differentially between the two classes of isolates. The ND7 gene from asymptomatic isolates showed a deletion of 455bp from nt222 to nt677 relative to the CL Brener reference strain (Baptista et al., 2006). The ND7 lesion could produce a truncated product that could impair the function of mitochondrial complex I. The aim of this work was to determine the mitochondrial membrane potential, generation of reactive oxygen species (ROS) and resistance to hydrogen peroxide (H_2O_2) in the two classes of isolates (7 isolates without ND7 deletion and 3 with the deletion). Mitochondrial membrane potential and generation of ROS were evaluated by flow cytometry using $Dioc_6(3)$ and dihydroetidine (DHE) as probes, respectively. Resistance to H_2O_2 (50-100 μM) was determined by the MTT assay. Under our experimental conditions, no significant differences were observed among the parameters studied indicating that ND7 deletion does not influence the mitochondrial membrane potential and ROS generation. Mitochondrial oxygen consumption will be determined in order to establish if mitochondrial function is altered in the isolates with deletion in complex I ND7.
Supported by: FAPESP; MCT/CNPq (Edital Estudo de Doenças Negligenciadas).

BQ34 - *Trypanosoma cruzi* cytotoxicity induced by Ibuprofen does not impair mitochondrial function

Regonha, L.F. *(Universidade Estadual de Campinas)*; Souza, G.G. *(Universidade Estadual de Campinas)*; Degasperi, G. *(Universidade Estadual de Campinas)* Morales Neto, R. *(Universidade Estadual de Campinas)*; Gadelha, F.R. *(Universidade Estadual de Campinas)*

Chagas'disease therapy is insatisfatory due to a low eficacy, high toxicity of drugs and the heterogeneity of strains. Ibuprofen has been pointed out as an inductor of cellular death in different neoplasic cells. Thus, the aim of this project was to study the action of this drug in two differents strains of *T. cruzi*, evaluating mitochondrial transmembrane potential, oxygen consumption, cellular viability and proliferation. Cells (1.10^6 /mL) were incubated for 24, 48 and 72 h in the presence of ibuprofen (100 μM - 2 mM). After 48 h in the presence of 250 μM (a lower concentration than the IC50, 611μM and 879μM for Tulahuen 2 and Y respectively) an inhibition of proliferation of 24% and 18% for Y and Tulahuen 2, respectively was observed. Cellular viability was evaluated by the MTT method after 24 h of incubation in the presence of the drug (250 μM - 2 mM). The results showed a dose and time-dependent effect. Mitochondrial transmembrane potencial was evaluated by flow cytometry using $DioC_6$ (3) as a

probe and oxygen consumption was also determinated after differents times of incubation (24, 48 e 72h) using 250 μM of ibuprofen. In both experiments no significant differences were observed among controls and after treatment . The results suggest that the drug doesn‘ t affect mitochondrial activity, but has cytotoxic effect in *T. cruzi* epimastigotes. The mechanism of cell death induction is under investigation. Supported by: CNPq and FAPESP

BQ35 - Regulatory Mechanism of H^+-release in Acidocalcisomes of *Herpetomonas* sp.

Tesch, K.R.A. *(Instituto de Bioquímica Médica)*; Soares Medeiros, L.C.A. *(Intituto de Bioquímica Médica)*; Barrabin, H. *(Instituto de Bioquímica Médica)*

Acidocalcisomes are acidic calcium storage organelles first described in trypanosomatids and then found in several microrganisms. They are eletron-dense, posses a sorrounding membrane, have variable size, and contain high amounts of Ca^{2+}, Mg^{2+}, Zn^{2+}, Na^+, and short and long chain polyphosphate. They have exchangers and a V-H^+-PPase. This enzyme is similar to those found in plant tonoplast. *Herpetomonas* sp. lysed with glass beads or permeabilized with digitonin shown a pyrophosphate-driven H^+-uptake with acridine orange as an indicator dye. To determine the H^+ release, after 11 minutes of the beginning of the transport this uptake was inhibited by dicyclohexylcarbodiimide (DCCD) and then additions were made. The addition of IDP (imidodiphosphate), a non-hydrolysable PPi analogue, AMDP (aminomethylenediphosphonate), a PPi analogue and specific inhibitor of plant vacuolar pyrophosphatases, ADP, ATP, AMP-PNP, a non-hydrolysable ATP analogue or even high PPi concentrations promoted a fast H^+ release. The greather release was promoted by ATP, IDP and PPi. These results suggest that acidocalcisomes from *Herpetomonas* possess a regulatory mechanism of H^+ release dependent of PPi or compounds that contain diphosphate group. SUPPORTED BY CNPq, CAPES, FAPERJ

BQ36 - Secretion of cruzipain-like molecules in *Phytomonas serpens*

Elias, C.G.R. *(Universidade federal do Rio de Janeiro)*; Pereira, F.M. *(Universidade federal do Rio de Janeiro)*; Padron, T.S *(Universidade federal do Rio de Janeiro)*; D´Avila-Levy, C.M. *(Universidade federal do Rio de Janeiro)*; Branquinha, M.H *(Universidade federal do Rio de Janeiro)*; Santos, A.L.S. *(Universidade federal do Rio de Janeiro)*

Phytomonas serpens is a plant flagellate able to cause injuries in tomatoes. A previous study from our group described the production of two distinct cell-associated cysteine peptidases of 38 and 40 kDa by *P. serpens*. These peptidases possess common features with the cruzipain of *Trypanosoma cruzi*, such as preferential cytoplasmic location, best hydrolytic activity at acidic pH, requirement of a reducing agent and inhibition profile. Interestingly, the 40 kDa cysteine peptidase was also detected in the hydrophobic fraction of *P. serpens*.Additionally, we demonstrated through immunoblotting that anti-cruzipain antibodies recognized two polypeptides in *P. serpens*, including a 40 kDa surface component. Flow cytometry and immunocytochemical analyses confirmed that this molecule has a location on the cell surface. Moreover, gold particles were also detected on flagellar pocket, some of them were free or bound to small membrane vesicles, suggesting a shedding of the enzyme. In the present study, in order to ascertain the presence of cruzipain-like molecules in the extracellular environment, we performed a western blotting analysis using the concentrated culture supernatant obtained after *P. serpens* growth *in vitro*. Our results showed a reactive polypeptide of 40 kDa in the culture supernatant extract. In addition, we evidenced the proteolytic activity in the culture supernatant of *P. serpens* by quantitative measurement. To determine the nature of the *P. serpens* peptidase cleaving the gelatin soluble substrate, we incubated the supernatant in the presence of inhibitors of the four major peptidase classes. Addition of the cysteine peptidase inhibitors powerfully inhibited the extracellular proteolytic cleavage. Conversely, serine, metallo and aspartic peptidase inhibitors did not significantly diminish the proteolytic activity. Furthermore, we have demonstrated that phospholipase C-treated *P. serpens* had a reduction on the binding of anti-cruzipain antibody when compared to the non-treated parasites, suggesting that these molecules are expressed on the cell surface through a glycosylphosphatidylinositol anchor

BQ37 - Metallo-proteinases from *Leishmania (L.) chagasi* and *Leismania (V.) braziliensis* may play a role in parasites binding to glycosaminoglycans

Côrtes, L.M.C. *(FIOCRUZ)*; Rebello, K.M. *(FIOCRUZ)*; da Silva, G.G. *(FIOCRUZ)*; Pereira, B.A.S. *(FIOCRUZ)*; Oliveira, F.O.R.Jr. *(FIOCRUZ)*; Pereira, M.C.S. *(FIOCRUZ)*; Côrte-Real, S. *(FIOCRUZ)*; d‘Avila-Levy, C.M. *(FIOCRUZ)*; Alves, C.R. *(FIOCRUZ)*

During *Leishmania* spp parasite cycle, the promastigote forms of this parasite are transmitted to the mammalian host by the bite of a female phlebotomine sand fly, while the insect is infected by blood contaminated with amastigote forms, which then transform into promastigotes inside the vector gut. The adhesion of the protozoa to gut epithelial cells is an essential step for the maintenance of the parasite life cycle. In this context, we have previously showed the potential of heparin binding proteins (HBPs) from *Leishmania (V.) braziliensis* promastigotes to bind to gut proteinaceous extract obtained from *Lutzomyia intermedia* and *Lutzomyia whitmani* (Azevedo-Pereira et al., 2007). In the present work, we show that the HBPs from promastigotes of *Leishmania (L.) chagasi* and *Leishmania (V.) braziliensis* present bio-

chemical and structural properties of *Leishmania* metalloproteinases. We obtained an enriched fraction of HBPs from promastigotes of *L. (L.) chagasi* and *L. (V.) braziliensis* by affinity chromatography using heparin-sepharose 4B column, following enzymatic assays in gelatin zymography and immunoblotting with anti-gp63 antibody (Ilg et al., 1993). We showed that the 64 kDa and 55 kDa HBPs have gelatinolytic activity in acid pH and are sensitive to 1, 10-phenanthroline, a specific metallo-proteinase inhibitor. In addition, only the 64 kDa protein was recognized by the anti-gp63 antibody. The present results suggest that the HBPs may be characterized as metallo-proteinases of *L. (L.) chagasi* and *L. (V.) braziliensis*, which may be involved in the adhesion process to the gut epithelium from the sand flies via glycosaminoglycans receptors and that a gp63-like enzyme may play a role in such process. Supported by FAPERJ, CAPES and CNPq.

BQ38 - SECRETION OF METALLOPEPTIDASES BY Leishmania amazonensis IS MODULATED BY PROTEIN MOLECULES

Machado Pereira, FMP *(Universidade Federal do Rio de Janeiro)*; Dias-Junior, P F F D J *(Universidade Federal do Rio de Janeiro)*; Souza dos Santos, A L S S *(Universidade Federal do rio de janeiro)*

Infection by *Leishmania* parasites can cause, depending on the parasite species, a variety of disease outcomes in humans, ranging from single self-healing cutaneous lesions to visceral dissemination of the parasite, which may lead to death if not properly treated. Furthermore, New World *Leishmania* species may cause severe cutaneous forms, such as diffuse or mucocutaneous leishmaniasis, widening the range of possible outcomes. For instance, *L. amazonensis* is the causative agent of diffuse cutaneous leishmaniasis, a disease that is characterized by a decreased immune response in infected patients and also often causes disseminated cutaneous leishmaniasis. A number of *Leishmania* molecules have been implicated in parasite virulence, including the 63 kDa surface zinc-metallopeptidase named gp63 or leishmanolysin. During its life cycle, *Leishmania* parasites interact with several host molecules, including different proteinaceous compounds. In the present study, we have incubated live *L. amazonensis* promastigote cells in PBS-glucose supplemented or not with different protein molecules (bovine serum albumim, human serum albumim, immunoglobulin G, hemoglobin, mucin, fetal bovine serum and casein) for 3 h. Then, these mixtures were centrifuged and the supernatants were used to investigate the possible presence of extracellular peptidases through gelatin-SDS-PAGE and western blotting using an anti-gp63 polyclonal antibody. Our results showed that *L. amazonensis* promastigotes secreted a major peptidase of 66 kDa and a minor 120 kDa component in all systems. Both proteolytic activities were completely inhibited by 1,10-phenanthroline, a metallopeptidase inhibitor. Immunoblotting analysis using an anti-gp63 antibody revealed the presence of a reactive polypeptide of 63 kDa. Curiously, hemoglobin, immunoglobulin G and casein stimulated the secretion of the gp63 molecule to the extracellular environment. *Financial support: CNPq, FAPERJ and FUJB.*

BQ39 - INHIBITION OF *Trypanosoma cruzi trans*-SIALIDASE BY 4-N-DANSYL-2-DIFLUOROMETHYLPHENYL-*alpha*-N-ACETYLNEURAMINIC ACID

Carvalho, S.T. *(universidade Federal do Rio de Janeiro)*; Nishimura, S.I. *(Hokkaido University)*; Hinou, H. *(Hokkaido University)*; kurogochi, K. *(Hokkaido University)*; Neves, B. C. *(Universidade Federal do Rio de Janeiro)*; Mendonça-Previato, L. *(Universidade Federal do Rio de Janeiro)*; Todeschini, A. R. *(Universidade Federal do Rio de Janeiro)*; Previato, J.O. *(universidade Federal do Rio de Janeiro)*

Trypanosoma cruzi's trans-sialidase (TcTS) is thought to play an important role in the pathogenesis of Chagas' disease, representing a potential therapeutic target. In contrast to sialidases, which are strict hydrolases, TcTS scavenges sialic acid from host glycoconjugates to sialylate glycoproteins at the parasite surface. Despite of determination of the 3 D structure and extensive mechanistic studies of TcTS, no potent inhibitor is available. Here, we report that the 4-N-dansyl-2-difluoromethylphenyl-alpha-ketoside of N-acetylneuraminic acid inactivates TcTS time dependently. Characterization of inactivated TcTS by MALDI-TOF/TOF mass spectrometry analysis revealed that inactivation of enzyme occurs through a covalent bond formation between two amino acids with the reactive aglycon generated by the hydrolysis of 4-N-dansyl-2-difluoromethylphenyl-N-acetylneuraminic acid. Since one of the amino acid residue has never been identified as members of the active site of TcTS investigated by crystallographic analysis thus this result point it as new target for drug design. Supported by: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ), Mizutani Foundation for Glycoscience and The Millennium Institute for Vaccine Development and Technology (CNPq, 420067/2005-1).

BQ40 - Low expression of α-galactosyl epitopes in the intracellular epimastigotes of *Trypanosoma cruzi*.

Perin, P.M.S. *(Universidade de São Paulo)*; Torrecilhas, A.C.T. *(Universidade de São Paulo)*; Tonelli, R.R. *(Universidade de São Paulo)*; Colli, W. *(Universidade de São Paulo)*; Alves, M.J.M. *(Universidade de São Paulo)*

Intracellular-epimastigotes (CL-14) appear inside the host

cell as a transient stage of *T. cruzi*, prior to differentiation to the trypomastigote stage. These parasites share some characteristics with axenic culture medium-derived epimastigotes (e). A synchronous differentiation of the intracellular cycle can be obtained by controlling temperature and proline concentration (Tonelli et al. Cell. Microbiol 6, 733, 2004), but the differences between epimastigotes (e), trypomastigotes (t) and intracellular epimastigotes (ie) were poorly characterized. Considering that glycoinositolphospholipids (GIPLs) and mucin-like glycoproteins (GPI-mucins) are major surface components of *T. cruzi* and GPI-anchor containing unsaturated fatty acids induce secretion of pro-inflammatory cytokines, these molecules on ie were the object of this study. GPI mucins from e, t and ie, the latter purified from *T.cruzi* - infected cells, were isolated. One important characteristic of tGPI-mucin is the presence of terminal α-galactosyl residues in the O-linked oligosaccharide, recognized by anti-α-gal antibodies from chronic chagasic patients (Pereira-Chiocola, J Cell Sci. 113, 1299, 2000). Thus, purified ieGPI-mucin and tGPI-mucin were compared in immunoblottings as to their reactivity towards anti-α-gal antibodies. As expected, tGPI-mucin revealed a strong band, but no reactivity was detected with ieGPI-mucin. Accordingly, the presence of α-galactosyl epitopes during the intracellular differentiation was detected by immunofluorescence only in a few infected cells at 72 to 144 h post-infection, confirming the low expression of this carbohydrate residue in ie-mucins. Preliminary results also showed that extracts of ie activate the secretion of NO (nitric-oxide) and proinflammatory cytokines (IL-12 and TNF-α) by peritoneal macrophages at the same level as do trypomastigotes, in contrast to the absence of response observed with extracts of epimastigotes. Experiments are under way to compare the activity of ie-GPI-mucins and t-GPI mucins on cytokines and NO production. Supported by: FAPESP and CNPq; PP is a fellow from CNPq-PIBIC; ACTT and RRT are FAPESP post-doctoral fellows.

BQ41 - THERMAL DENATURATION STUDIES OF PROLYL OLIGOPEPTIDASE FROM *Trypanosoma brucei*

Lima, M.M. *(Universidade de Brasília)*; Neves, D. *(Universidade de Brasília)*; Bastos, I.M.D. *(Universidade de Brasília)*; de Freitas, S.M. *(Universidade de Brasília)*; Medrano, F.J. *(Universidade Estadual de Campinas)*; Barbosa, J.A. *(Laboratório Nacional de Luz Sincrotron)*; Grellier, P. *(Muséum National d'Histoire Naturelle)*; Santana, J.M. *(Universidade de Brasília)*

Proteases are considered virulence factors and good targets for the design of new drugs as they are associated to a number of processes relative to the physiology of microorganisms and to the host-parasite interaction. *T. brucei* prolil oligopeptidase (POPTb) may be related to the sleeping sickness pathogenesis as it cuts a number of hormones such as LHRH, TRH, bradicinine, neurotensin and B-endorphin (our unpublished data). The inactivation of these hormones would be linked to the neuroendocrine dysfunction observed during infection. Following our previous studies of chemical denaturation and quenching of the tryptophan intrinsic fluorescence, experiments of thermal denaturation of POP-Tb were carried out. Far-UV CD spectra show secondary structure alteration as a function of pH and temperature with the least loss of signal at pH 7.5 in the presence of DTT. Thermal denaturation curves at different pHs as monitored by CD support a two-state model for the equilibrium. The thermodynamic parameters were calculated and show the protein was stabilized by sorbitol as the transition temperature (Tm $= 50.2 \pm 0.8\ ^oC$ for 0.5 M and $49.7 \pm 2.2\ ^oC$ for 1 M) as well as ΔG^{25} increased. CTAB (cetyl trimethyl ammonium bromide), on the other hand, had an opposing effect as it destabilized the structure of the enzyme leading to Tm $= 35.4 \pm 3.2\ ^oC$ and an intense decrease of ΔG^{25} values, none above 2.6 kcal/mol. POP-Tb is more stable in a buffered solution (pH 7.5, $\Delta G^{25} = 6.3$ kcal/mol) than in water ($\Delta G^{H2O} = 3.6$ kcal/mol). These results provide further insight into the protein structure and behavior, which may help guide the design of specific inhibitors.

BQ42 - INTRA-SPECIES POLYMORPHISMS OF LIPOPHOSPHOGLYCAN (LPG) STRUCTURE IN BRAZILIAN STRAINS OF LEISHMANIA CHAGASI AND PRELIMINARY DESCRIPTION OF L. INFANTUM LPG

Coelho, J.M. *(Fundação Oswaldo Cruz)*; Barron, T. *(University of Kentucky)*; Melo, M.N. *(Universidade Federal de Minas Gerais)*; Silva, S.O. *(Universidade Federal de Minas Gerais)*; Campino, L.M. *(Universidade de Lisboa)*; Turco, S.J. *(University of Kentucky)*; Soares, R.P.P. *(Fundação Oswaldo Cruz)*

The LPG of *Leishmania* is a multivirulent factor that enables the parasites to survive and develop both in vertebrate and invertebrate hostes. Modifications of the basic LPG phosphoglycan (repeating Gal-Man-P units) backbone by side chain sugars play important roles in parasite survival and sand fly specificity. In one strain of *L. chagasi* (PP75), the side chains were previously shown to consist primarily of one β-1,3 glucose residues, being very similar to *L. mexicana* LPG. Interspecific variations in LPG side chain substitutions have been extensively investigated. The significance of intraspecific variations of side chain glucosylation of LPGs from *L. chagasi* has not been studied. *L. chagasi* and *L. infantum* has been the controversial object of taxonomic status and molecular studies have defined them as being the same species. However, a LPG of *L. infantum* from Europe has never been characterized. *L. chagasi* isolates from distinct Brazilian regions: two from Minas Gerais (211 and 268) and two from Bahia (640 and PP75) plus a European strain of *L. infantum* (Portugal) were analyzed regarding their LPG structures. LPGs were purified and subjected to western-blot, fluorophore-assisted carbohydrate electrophoresis (FACE) and capillary electrophoresis (CE) to determine side chain substitutions. The LPG repeat

units derived from Minas Gerais, one strain from Bahia and the *L. infantum* isolates are predominately unsubstituted, whereas the other strain from Bahia (PP75), the first *L. chagasi* strain studied was mono-glucosylated. These data indicate a similarity among the LPGs from Brazilian *L. chagasi* isolates and Portuguese *L. infantum*. However, the polymosphism found in strain PP75 warrant further analyses by that would increase the number of strains from Europe and Brazil. Variations in the side chain sugars may have an impact in the intra-species specificity for vectors and also in the interaction with the immune system during the course of the disease.

BQ43 - Sialylated, mannose-rich and gp63 molecules in *Crithidia deanei* mediate adhesion to insect gut epithelium: influence of the endosymbiont

Uehara, L. A. *(Instituto Oswaldo Cuz-IOC)*; Altoé, E. C. F. *(Instituto Oswaldo Cuz -IOC)*; Santos, L. O. *(Instituto Oswaldo Cuz -IOC)*; Marinho, F.A. *(Instituto de Microbiologia Prof. Paulo de Góes-UFRJ)*; Saraiva, E. M. *(Instituto de Microbiologia Prof. Paulo de Góes-UFRJ)*; Santos, A. L. S *(Instituto de Microbiologia Prof. Paulo de Góes-UFRJ)*; Branquinha, M. H *(Instituto de Microbiologia Prof. Paulo de Góes-UFRJ)*; d'Avila-Levy, C. M. *(Instituto Oswaldo Cuz -IOC)*

Crithidia deanei is a trypanosomatid protozoa of insects that normally contain intracellular symbiotic bacteria. The protozoa can be rid of their endosymbionts by antibiotics, producing a cured cell line. Here, we analyzed the glycoconjugate profiles and gp63 expression of endosymbiont-harboring and cured strains of *C. deanei* by Western blotting and flow cytometry analyses using lectins that recognize specifically sialic acid and mannose-like residues and anti-gp63 antibodies. The absence of the endosymbiont leads to an increased intensity of the lectins binding, but diminished anti-gp63 binding. In addition, wild and cured strain-specific glycoconjugate bands were identified. The role of the surface saccharide residues and gp63 on the interaction with explanted guts from *Aedes aegypti* gut was assessed. The aposymbiotic strains of *C. deanei* presented interaction rates 2-fold lower with the insect gut, when compared with the endosymbiont-bearing strain. The interaction rate of sialidase-, hospholipase- and anti-gp63-treated cells of the wild and cured strains of *C. deanei* was reduced in at least 70% in relation to the controls. The interaction of *C. deanei* (wild strain) with explanted guts was inhibited in the presence of mucin (53%), fetuin (56%), sialyllactose (79%), a-methyl-D-mannoside (34%) and purified gp63 (88%). Collectively, our results suggest a possible involvement of sialomolecules, mannose-rich glycoconjugates and gp63 molecules in the interaction between insect trypanosomatids and the invertebrate host.Supported by: MCT/CNPq, FAPERJ, CAPES, CEPG/UFRJ and FIOCRUZ

BQ44 - Sialylglycoconjugates in *Herpetomonas megaseliae*

Silva, B. A. *(Universidade Federal do Rio de Janeiro)*; Alviano, C. S. *(Universidade Federal do Rio de Janeiro)*; Branquinha, M. H. *(Universidade Federal do Rio de Janeiro)*; Santos, A. L. S. *(Universidade Federal do Rio de Janeiro)*; d'Avila-Levy, C. M. *(Instituto Oswaldo Cruz)*

Herpetomonas megaseliae is a monoxenic trypanosomatid isolated from the phorid fly *Megaselia scalaris*. The expression of cell surface carbohydrates in this parasite was analyzed by Western blotting using the peroxidase-labelled lectins *Limax flavus* (LFA), *Maackia amurensis* (MAA), *Sambucus nigra* (SNA), which specifically recognize sialic acid residues, and concanavalin A (Con A) that recognizes mannose-like residues in glycoconjugates. All lectins showed a sugar-inhibited recognition with the parasite extract. The flagellated presented reactive bands migrating at 60, 45, 40 and 15 kDa with the lectins LFA and Con A. SNA and MAA recognized only the 60, 40 and 15 kDa bands. These results demonstrated the presence of mannose-rich and sialoglycoproteins in *Herpetomonas megaseliae* and indicated that molecules containing alpha2,3- and alpha2,6-sialylgalactosyl sequences are present in the protozoa. Previous results from our group suggest a possible involvement of sialomolecules and mannose-rich glycoconjugates in the interaction between insect trypanosomatids and the invertebrate host. It is possible that the sialoglycoproteins and mannose-rich proteins from *Herpetomonas megaseliae* may have a role in the adhesion to the insect host midgut. Supported by: MCT/CNPq, FAPERJ, CAPES, CEPG/UFRJ and FIOCRUZ.

BQ45 - Biochemical and molecular assays reveal the outer membrane origin in the *Crithidia deanei* endosymbiont

Andrade, I. S. *(Universidade Federal do Rio de Janeiro)*; Rosa, M.S. *(Universidade Federal do Rio de Janeiro)*; Probst, C. M. *(Instituto de Biologia Molecular do Paraná)*; Krieger, M. A. *(Instituto de Biologia Molecular do Paraná)*; De Souza, W. *(Universidade Federal do Rio de Janeiro)*; Mohana-Borges, R. *(Universidade Federal do Rio de Janeiro)*; Motta, M. C. M. *(Universidade Federal do Rio de Janeiro)*

The trypanosomatid *Crithidia deanei* harbours a cytoplasmatic bacterium that is integrated in the host cell metabolism through a mutualistic relationship. This association constitutes a good system to study the interaction between two different genomes in the same cell and the eukaryotic cell evolution. As a Gram-negative bacterium, the endosymbiont exhibits two membranes; however its envelope contains a reduced cell wall, which does not form a septum during the cytokinesis. The origin of the symbiont outer

membrane is controversial, since it could be originated from the host protozoan or from a prokaryotic ancestral. Molecular analysis indicates that this symbiont is closely related to *Bordetella* genus, being classified in the β division of Proteobacteria. Thus, the existence of porins and other typical integral membrane proteins can confirm the Gram-negative character of the endosymbiont. In the present work, in order to analyze the endosymbiont membrane composition, we isolated symbionts from *C. deanei* by cell fractioning and prepared protein samples to 1D and 2D eletrophoresis gels. Electrophoretic assays resulted in gels with distinct profiles of proteins, some of them presenting 33 - 44 kDa, corresponding to the molecular mass of porins. Whole protozoa, isolated symbionts and mitochondria were analyzed by transmission electron microscopy in order to verify the protozoan ultrastructure and the quality of these fractions. In parallel, the endosymbiont genome sequencing revealed the presence of a porin enconding gene. Thus, primers were used to amplify this sequence by PCR, producing a 1.2 Kb fragment corresponding to the amplified *porB* gene. Then, the PCR product was purified and cloned in the plasmid pET21dHis-Tev in order to overexpress the recombinant porin. Our next goal, is to produce antiserum against porin, a valuable tool to study the bacterium relationship with its host protozoa. Supported by: CNPq and FAPERJ.

Epidemiologia - Epidemiology

EP01 - Modeling of Chagas disease using Individual Based Model

Abdalla Jr., M. A. *(Departamento de Engenharia Elétrica da Universidade Federal de São João del-Rei)*; Nepomuceno, E. G. *(Departamento de Engenharia Elétrica da Universidade Federal de São João del-Rei)*

Mathematical Epidemiology is based on mathematical hypotheses that quantify aspects from biological phenomenon of the interaction host-parasite, trying to analyze and to quantify the appearance and the sprouting of illnesses. The Chagas disease is an epidemiologic problem that often occurs in countries from Latin America and has *Triatoma infestans* as main vector. The aim of this work is the modeling of the Chagas disease using the Individual Based Model (IBM). This model co-relates the interaction between vector and host (human), considering some individuals characteristics of both. The observed characteristics are the infection state (infected or susceptible individuals), age and life time of the vector and host, besides making a distinction of infection in different social classes of the hosts. This model was created following the premises: constant population, the deceased number equal to the born ones; the disease has no cure; 80% of the vectors are infected; all contact of infected vector with susceptible host generates a new infected host. Spreading between hosts (blood transfusion) is accidental and has a low probability (1%). The validation of the IBM was made by the Monte Carlo Method, which consists of simulating the model many times, in random situations and observing the obtained data. To validate the model, it was simulated 100 times, with 3000 hosts and 955 vectors, in a period of 10 years. The model did not present jumps nor discontinuities and a deviation standards from 0% to 25%. The mathematical formularization of the IBM is flexible, considering characteristics such as biological and spatial spreading of vectors and hosts. The results were pertinent for simulating the dissemination of Chagas disease in a hypothetical area. This shows that it can be used in real situations in order to study strategies of epidemic control.
Financially supported by CNPq.

EP02 - ISOLATION AND CHARACTERIZATION OF STRAINS OF *TRYPANOSOMA CRUZI* CHAGAS, 1909 (KINETOPLASTIDA, TRYPANOSOMATIDAE) ISOLATED FROM DOMESTIC CAT, ORIGINATED FROM SANTO INÁCIO, BAHIA, BRAZIL.

Bassi, M. *(Faculdade de Ciências Farmacêuticas)*; Rosa, J. A. *(Faculdade de Ciências Farmacêuticas)*

With the objective of bringing additional subsidies to the epidemiology of this disease, samples of blood were collected

from residents of Santo Inácio, a district of the small country town Gentio do Ouro, in the state of Bahia, Brazil. The samples of human blood were taken by finger prick, with disposable lancets the dsop of blood being absolved in filter paper Whatman n°1, laboratory diagnosis was done by means of the Indirect Fluorescent Antibody Test (IFAI) and PCR. Feces of insets used for xenodiagnosis were examined by abdominal compression and the ones contaminated with Trypanosomatidae, were diluted in saline and inoculated intraperitoneally in five albino " Swiss "mice and in LIT middle (Liver Infusion Tryptose) to isolate the parasites. They were isolated four strains of *Trypanosoma cruzi*, S.I.G.R2; S.I.G.R3; S.I.G.R5; S.I.G.R6, were isolation from a cat in the same area for these, the evolution of parasitemia was followed. The results showed that strains S.I.G. R2 had an incubation period of 11 days, peaking with 9 forms on the 28th day and these disappeared on 33rd day. For the strain S.I.G. R3, the incubation period was 14 days and parasitemia peak occurred on the 38th day, with 80 forms, and these disappeared on the 52nd day while for S.I.G. R5, the incubation period lasted 14 days, the parasitemia peaking on the 33rd day, with 31 forms, and these disappearance on the 54th day. The parasitemia curve of the strain S.I.G. R6 is still being study. The results show that differences exist among the profiles of parasitemia curves, even though all the strains were isolated from a single source. Project approved by the Ethics Committee of the Health Department of the State of Bahia. Permit 09/2006 in 19/04/2006. Support: PADC/FCF/Unesp/Araraquara.

EP03 - An improved use of PCR to detect and characterize pathogenic trypanosomatid species in field-collected triatomine feces

Bayer-Santos, E. *(Universidade Federal de Santa Catarina)*; Cáceres, A. G. *(Universidad Nacional Mayor de San Marcos)*; Gonzáles-Pérez, A. *(Centro de Salud Bagua Grande)*; Guarneri, A. A. *(Centro de Pesquisa René Rachou)*; Stoco, P. H. *(Universidade Federal de Santa Catarina)*; Rodrigues, J. B. *(Universidade Federal de Santa Catarina)*; Huiz, A. F. *(Universidad Nacional Mayor de San Marcos)*; Sevilla, C. R. A. *(Universidad Nacional Mayor de San Marcos)*; Cordoba, E. *(Universidad Nacional de San Agustín)*; Grisard, E. C. *(Universidade Federal de Santa Catarina)*

Chagas disease affects around 670 thousand people in Peru, being the northern and northwestern regions considered critical due the occurrence of *Trypanosoma cruzi* and/or *T. rangeli* in humans, triatomines and mammals. Since misdiagnosis of Chagas disease may occur in such areas, we have tested a PCR method for detection of trypanosomatids in field-collected triatomine feces. A total of 114 samples collected from triatomines (*Triatoma carrioni*, *T. infestans*, *Panstrongylus herreri* and *Rhodnius pictipes*) captured inside human dwellings in five Peruvian Departments located in both extremes of the country between 2002 and 2006 were used. The insects' feces were spotted onto sterile filter papers and stored at room temperature. Prior PCR analysis, sample papers were individually mixed with ultra pure water, crushed with disposable pestles, boiled for 15 minutes and left cooling to room temperature. After a short spin, the supernatant was submitted to PCR either pure or diluted. Reactions were initially performed using primers directed to the kDNA mini-circles (S35/S36) and, when negative, to another PCR using primers to the 24Sα rDNA gene (D75/D76). In 100 out of 114 samples tested (87.72%) it was possible to clearly identify the presence of *T. cruzi* by kDNA PCR. Further testing of the negative samples by PCR using primers D75/D76 revealed 10 positive for *T. cruzi* infections (four TcI, five TcII, one mixed TcI/TcII) and a single *T. rangeli* infection. Two samples showed a distinct band pattern not allowing specific characterization and one remained negative possibly due to presence of PCR inhibitors. In conclusion, comparing to the microscopical examination 99.1% of the samples were positive by PCR which was also able to type the *T. cruzi* strains. Thus, the results reinforced the efficacy of this methodology to detect and characterize field samples even for samples stocked for long time periods. Supported by CNPq/UFSC/UNMSS/INS.

EP04 - Epidemiological aspects, Diagnosis and Treatment of American Cutaneous Leishmaniasis in South and Southwestern regions in the State of Minas Gerais, Brazil

Souza, L.B. *(Universidade Federal de Alfenas)*; Peloso, E.F. *(Universidade Federal de Alfenas)*; Silva, T.M. *(Universidade Federal de Alfenas)*; Franco, M.C. *(Universidade Federal de Alfenas)*; Faria e Silva, P.M. *(Universidade Federal de Alfenas)*; Marques, M.J. *(Universidade Federal de Alfenas)*

American Cutaneous Leishmaniasis (ACL) is considered a disease in expansion in Brazil and it represents an important cause of morbidity to people who live in endemic areas. Epidemiological profile and services of diagnosis and treatment evaluations are necessary for the establishment of strategies for the disease control. The objective of the present work was the evaluation of epidemiological aspects, diagnosis and treatment of patients with ACL from different regions of the State of Minas Gerais, Brazil. In the period from 2002 to 2006, 261 cases of ACL were registered by Regional Health Authorities (RHA) from the cities of Alfenas, Passos, Pouso Alegre and Varginha, which involve the South and Southwestern regions of the referred State. In the period, there were decreases in the number of ACL cases (59,7%, $p < 0.0001$) registered in the 74 cities belonging to these regions. Cutaneous leishmaniasis was the predominant clinical form among the 261 related patients reported (91.9%, $p < 0.0001$). Moreover, the data showed that the majority of the patients ($p < 0.0001$) live in urban area (51.7%), are male (64.4%) and are between 21 to 50 years old (55.2%). Positivity index of the ACL cases by Montenegro Skin Test, parasitological and histopatological exams were 55.6%, 10.7% and 19.5%, respectively. The cure after of the medication used for

ACL treatment varied from 71,8% to 92,0%, as the studied RHAs. The cases can be correlated to the domiciliary and peridomiciliar way of ACL transmission and decreased significantly in the studied period. However, it is necessary to implement actions which optimize the use of files of notification, as well as an ACLs Diagnosis and Treatment Reference Center in South and Southern regions of Minas Gerais. Supported by Capes, CNPq, Fapemig, Finep and Unifal-MG.

EP05 - Human Cutaneous Leishmaniasis in Santa Catarina State is caused by *Leishmania braziliensis* and *Leishmania amazonensis* as demonstrated by PCR-RFLP and Southern blot analysis

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

Human cases of American Cutaneus Leishmaniasis (ACL) due both *L.* (*L.*) *amazonensis* and *L.* (*V.*) *braziliensis* were previously reported at the western region of the state of Santa Catarina (SC), southern Brazil. During the last decade, an increasing number of ACL cases were reported in several municipalities at the Vale do Rio Itajaí, norteasthern region of SC. In the present study we have identified the etiological agents of human ACL cases using 99 skin biopsies and parasites isolated from 17 of these patients using PCR-RFLP and Southern blot analysis. DNA from biopsies and culture parasites was extracted by the phenol-chloroform method and PCR was performed with primers 150/152 directed to the conserved region of the Leishmania sp. kDNA mini-circle. The amplified fragments of 120bp were then digested with 1U of *Hae*III and *Ava*I restriction enzymes. For southern blot assays, the same amplicons of *L.* (*V.*) *braziliensis* and *L.* (*L.*) *amazonensis* standard strains were labelled with horseradish peroxidase (ECL, GE Healthcare) and used as probes. The amplified products were then resolved in 2% agarose gels, blotted onto a nylon membrane, UV cross-linked, hybridized at 42°C in Gold Hybridization buffer and developed using the ECL detection reagents. After exposed to the blot for 15 minutes, the X-Ray film was developed and digitally recorded. All samples revealed the 120bp amplicon and, among these, 98 skin biopsies and 16 cultures revealed by PCR-RFLP to be *L.* (*V.*) *braziliensis* as confirmed by Southern Blot. A single skin biopsy and the correspondent culture showed a *L.* (*L.*) *amazonensis* profile in both PCR-RFLP and Southern Blot analysis. These results confirm the presence of both *L.* (*V.*) *braziliensis* and *L.* (*L.*) *amazonensis* in SC and shows that *L.* (*V.*) *braziliensis* is the major species causing ACL in humans in that region. Supported by CNPq and UFSC.

EP06 - Genetic polymorphisms of *crt-o* and *mdr1* genes of *Plasmodium vivax* among chloroquine resistant isolates from the Brazilian Amazon region.

Orjuela, S. P. *(Departamento de Parasitologia, ICB - USP)*; de Santana Filho, S. F. *(FundaÃ§Ã£o de Medicina Tropical do Estado do Amazonas)*; Paquola, C. M. A. *(Instituto de MatemÃ¡tica e EstatÃstica - Universidade de SÃ£o Paulo)*; Machado-Lima, A. *(Instituto de MatemÃ¡tica e EstatÃstica - Universidade de SÃ£o Paulo.)*; Chechuan, Y. *(FundaÃ§Ã£o de Medicina Tropical do Estado do Amazonas)*; Costa, M. M. *(FundaÃ§Ã£o de Medicina Tropical do Estado do Amazonas)*; Alecrim, G. *(FundaÃ§Ã£o de Medicina Tropical do Estado do Amazonas)*; del Portillo, A. H. *(Departamento de Parasitologia, ICB - USP)*

The development of chloroquine (CQ) resistance is threatening the use of this cheap and non-toxic antimalarial drug as the first-line treatment against *Plasmodium vivax*, the most widely distributed human malaria parasite. CQ resistance in *pfmdr1* has 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*, the most prevalent species, but to date few studies associating mutations in these genes with the CQ resistance phenotype have been conducted. In this work we report a single-nucleotide polymorphisms (SNPs) analysis of *pvmdr1* and *pvcrt-o* among CQ resistance isolates from the Brazilian Amazon region. Besides coding regions, our analysis included introns and 5‘ untranslated regions (UTRs) in order to test the possibility that mutations in regulatory regions of these genes may be associated with CQ resistance. Three CQ sensitive and three CQ resistant isolates were obtained from WHO 28-day control study conducted at FMT/AM during September 2004 - February 2005 in Manaus, Brazil. Analysis of 5‘ UTR of *pvmdr1* and *pvcrt-o* showed that these regions are highly conserved suggesting functional constraints at regulatory regions. None of *pfcrt* mutations previously correlated with CQ resistance in *P. falciparum* were found in *pvcrt-o* SNP analysis. Interestingly, some of the polymorphisms found in *PvMDR1* were already described in samples from Southeastern Asia and Middle East, including the double mutant haplotype Y976F/F1076L, previously suggested as molecular a marker for *P. vivax* CQ resistance surveillance. Neutrality tests using McDonald & Kreitman test and dN/dS analyses of *PvMDR1* e *PvCRT-O* are currently being conducted in order to confirm if domains of these proteins are under positive selection by the use of CQ.

EP07 - The first record of American Visceral Leishmaniasis in domestic cats from Rio de Janeiro, Brazil.

Silva, AVM *(Fundação Oswaldo Cruz)*; Jesus, CMM *(Fundação Oswaldo Cruz)*; Brazil, RP *(Fundação Oswaldo Cruz)*; Carreira, JCA *(Fundação Oswaldo Cruz)*

In Brazil, besides being a rural zoonosis, American Visceral Leishmaniasis is becoming a peri-urban and even urban zooanthroponosis. Originally, dogs were considered to be as the only domestic reservoirs of *Leishmania (infantum) chagasi* in Brazil. However, the increasing number of cases of feline leishmaniasis described in the literature since 1990 suggest cats may also have some role in the epidemiology of visceral leishmaniasis. In the present study eight cats from an endemic area in Rio de Janeiro city were tested by IFA. The animals comprised mainly domestic shorthair adults with little access to veterinary care. A relatively high seroprevalence of 25% was observed among the examined cats (2/8). Positive serological titers were 1:40 and 1/320 and none of the seropositive animals showed any symptoms of VL. Sera from cats of a non-endemic area from Rio de Janeiro were negative or reactive at a dilution of 1/10 and negative controls from the study area did not exceed reactivity at the dilution 1/20. This is the first to report visceral leishmaniasis in domestic cats (*Felis catus domesticus*) from an endemic area in Rio de Janeiro state - Brazil. This work received financial support from CNPq/Fiocruz Papes IV and from Instituto Oswaldo Cruz.

EP08 - Evidence for highly similar - of possibly single origin - Plasmodium falciparum genotypes in a limited outbreak in Candelaria suburb - Porto Velho

Albrecht, L. *(Instituto de Ciências Biomédicas)*; DallaMartha, R. *(Cepem)*; Fischer, W. *(Instituto de Ciências Biomédicas)*; Tada, M. *(Cepem)*; Pereira da Silva, L. H. *(Cepem)*; Wunderlich, G. *(Instituto de Ciências Biomédicas II)*

In riverine areas of the Amazon, a major part of the population is frequently infected asymptomatically with *P. falciparum* or *P. vivax* and thus shows a certain degree of immunity to symptomatic infection. We monitored a limited outbreak of symptomatic *P. falciparum* infections in a previously treated riverine population in order to i) genetically differentiate the infecting parasites by their microsatellite markers and repertoires of variant antigens ii) monitor how much the infecting parasite changes after one natural human-mosquito-human passage. Our results indicate that the genotypes in infections occurring in the same time frame are very similar in both microsatellites and variant gene repertoires, but differ from the parasite isolated from the probable infection source and also from parasites obtained in the same location two months after the outbreak. We are currently analyzing the complete repertoire from the *P. falciparum* of the possible index case and results are presented. Supported by FAPESP

EP09 - Microsatellite marker development and strain genotyping of *Eimeria maxima*

Noves, J. *(Instituto de Ciências Biomédicas - USP)*; Sobreira, T.J.P. *(Instituto do Coração)*; Solis, J. *(Laboratório Biovet S/A)*; Gruber, A. *(Instituto de Ciências Biomédicas - USP)*

Chicken coccidiosis is caused by seven different species of the genus *Eimeria*. *E. maxima* is the most immunogenic species, and birds infected with very small doses develop an almost complete protection against a homologous challenge. However, this species exhibits a high intra-specific antigenic diversity, characterized by a usually incomplete cross-strain protection, sometimes with a unidirectional character. This fact is highly relevant for devising vaccination strategies, since the correct choice of the strain(s) to compose a vaccine is essential for obtaining a successful protection. The aim of the present work was to develop microsatellite markers for the intra-specific differentiation of *E. maxima*, and to investigate if this genetic variability could be related to antigenic diversity. We used *E. maxima* ORESTES sequences generated in our laboratory, and selected potential candidate *loci* using the programs Tandem Repeats Finder and TRAP (Sobreira *et al.*, *Bioinformatics* 22: 361-362, 2006). In total, we obtained 20 species-specific polymorphic markers, which were used to genotype 27 distinct strains. We used field isolates and commercial vaccine samples, both originated from different geographic sources. Similarly to what our group have previously observed for *E. acervulina* and *E. tenella*, *E. maxima* presented a relatively low genetic variability with an average of 2.9 alleles per locus. Distance analysis revealed a population structure clearly composed by two well-defined groups. Interestingly, all the tested vaccine strains, originated in the USA and Europe, were clustered in the same clade, whereas most of the Brazilian strains were grouped in the second clade. Additional studies are necessary to demonstrate if there is a correlation between genetic distance and cross-protection ability. Nevertheless, preliminary cross-strain protection studies revealed a good correlation with the genetic distance tree.

Financial support: FUSP/Laboratorio Biovet SA.
E-mail: argruber@usp.br

Imunologia - Immunology

IM01 - New recombinant proteins from *Leishmania chagasi* obtained by immunological screening and their potential to the diagnostic of visceral leishmaniasis (VL) using ELISA assays.

Magalhães, F.B. *(Centro de Pesquisas Aggeu Magalhães)*; Nascimento, M.B. *(Centro de Pesquisas Aggeu Magalhães)*; Santos, W.L.C. *(Centro de Pesquisas Gonçalo Muniz)*; Pontes-Carvalho, L.C. *(Centro de Pesquisas Gonçalo Muniz)*; Oliveira, G.G.S *(Centro de Pesquisas Gonçalo Muniz)*; de Melo Neto, O.P. *(Centro de Pesquisas Aggeu Magalhães)*

Visceral Leishmaniasis is a critical infectious disease that can causes death when not treated. An accurate and precocious diagnosis is fundamental to its control and to reduce its impact. In the study described herein, a *L. chagasi* genomic DNA expression library was screened using a pool of sera from VL patients. This step resulted in the identification of several proteins not formerly described. Four of these, designated as Lcg7, Lcg22, Lcg36 and Lcg56, had fragments of their genes subcloned into the pRSET expression plasmid, were expressed in *Escherichia coli* and subsequently purified through affinity chromatography. ELISA assays were performed using these proteins and total parasite extract (TC) in order to investigate their potential use to the diagnosis of human and canine VL. Two others recombinant proteins (Lc9; Lc13), previously identified in a similar approach using infected dog sera and a *L. chagasi* cDNA library, were also included. We have evaluated 107 canine and 157 human sera which were classified into 3 groups: negative, positive and other diseases. ELISA assays using canine sera showed that the sensibility of TC and Lc13 (93%) is superior to that for the others recombinant proteins, which varies between 51 and 90,60%. In addition the highest specificity was found for the Lcg22 (100%). Using the human sera the sensibility of TC was also significantly higher (95,44%) than the recombinant proteins which displayed sensibilities varying from 15,9 to 63,63%. In comparisons with sera from healthy controls or chagasic patients, the specificity for the six recombinant proteins (100%) showed better results when compared to TC (96%; 77,5%) since these did not produce false positive results. When sera from patients with tegumentary leishmaniasis were included, only Lc9 and Lcg56 did not produce false positive results. These results confirm the identification of relevant new antigens for the diagnosis of VL.

IM02 - Study of factors conditioning the time course of infection in different mice strain infected with *Leishmania amazonensis* and the protective effects of a Subcellular Fraction vaccine associated with BCG

Rodrigues, I. A. *(Universidade Federal do Rio de Janeiro)*; Calabrese, K. S. *(Fundação Oswaldo Cruz)*; Alviano, C. S. *(Universidade Federal do Rio de Janeiro)*; Rosa, M. S. S. *(Universidade Federal do Rio de Janeiro)*

In the present work, we decided to investigate the kinetic of *Leishmania amazonensis* infection in mice from different genetic backgrounds. BALB/c, CBA, C57BL/10, DBA/2, C57BL/6 and C3H/HeN mice were infected with 10^2, 10^4 and 10^6 viable amastigotes cells. The time course of infection was then followed during 120 days. A direct correlation between dose and lesion spread was observed. As early as in the 20th day a characteristic nodular lesion was formed in Balb/c, considered the most susceptible, followed by CBA mice. In this early phase of infection, C57BL/6 appears as an intermediate strain while C57BL/6, DBA/2 and C3H/HeN may be considered as resistant, since a regression of the nodular lesion occurs nevertheless without healing. In addition, DBA/2 mice strain showed that female are more resistant than male; age appears also as an important factor. Balb/c and DBA/2 mice, susceptible and resistance to *L. amazonensis*, were vaccinated subcutaneously with 10 μg of the subcellular fractions 21 days after received 1x10^6 BCG vaccine injected into the left hind footpad. Six days after vaccination with Subcellular Fractions, mice were challenge with 10^4 amastigotes in the contralateral footpad and time course infection was followed during 120 days. We observed that the association the fraction and BCG promoted the increasing of the resistance in the Balb/c mice infections by textitL. amazonensis. These results confirmed that of BCG and Subcellular Fractions vaccine to have a protective capacity that could be associated with larger classical cellular immune response.

IM03 - Recombinant adenoviruses induce strong T and B cell responses and are efficient tools for prime-boost immunization against intracellular (*Plasmodium, Leishmania, Toxoplasma and Trypanosoma*) parasites.

Bruna-Romero, O *(UFMG/ICB e FIOCRUZ/IRR)*

Our main efforts during the last years have been devoted to the development of experimental recombinant tools for vaccination against intracellular pathogens. For this, we chose antigens of four parasites with intracellular stages (CS/*Plasmodium*, A2/*Leishmania*, SAG1,2,3/*Toxoplasma* and TS-ASP2/*Trypanosoma*) that had been described to induce protective immune responses. Side-by-side comparisons showed that recombinant adenoviral vectors encoding some of these antigens were the most efficient vaccines both in terms of immunogenicity and protective levels after a single immunization. However, complete protection after a single inoculation with a given adenovirus was achieved in only a minority of cases. Thus, we have also studied possible prime-boost protocols that could improve primary vaccine-induced immune responses by using repeated administration of those adenoviruses or combining them with other recom-

binant vectors. By doing this, a much higher degree of protection could be elicited in all cases against a challenge with the corresponding live parasites. Not only the numbers of IFNγ-producing cells or the antibody titers, but also the in vivo cytolytic capacity of the vaccine-induced T lymphocytes was augmented in this situation. Curiously, although not an isolated finding when considered recent reports found in the literature, the prime-boost protocol that induced the highest levels of Th1-related immune memory was the one that not only induced IFNγ and TNFα but also induced the highest levels of interleukin 4 (IL-4) after the boost. The conclusions drawn from these experiments and their future applicability in a real clinical setting will be discussed during our presentation.

IM04 - BALB/c MICE VACCINATED WITH ADJUVANT-FREE SERINE PROTEASES OF *Leishmania amazonensis* ARE DIFFERENTIALLY PROTECTED AGAINST *Leishmania major* AND *Leishmania amazonensis.*

Guedes, HLM *(UFRJ)*; Chaez, SP *(UFRJ)*; De-Simone, SG *(FIOCRUZ)*; Rossi-Bergmann, B *(UFRJ)*

Leishmania amazonensis is the main agent of the anergic diffuse cutaneous leishmaniasis in man. We have shown that the L. amazonensis promastigote antigens (LaAg) induce T-cell anergy in vitro and exacerbated cutaneous leishmaniasis in mice. Here, we compared the immunogenicity of serine proteases of *L. amazonensis* promastigotes with LaAg in vitro, and the effect of vaccination of BALB/c mice on *L. amazonensis* or *L.major* infection. Serine proteases were purified from detergent-solubilized extract of L. amazonensis promastigotes using aprotinin-agarose affinity chromatography, and were herein named LSPI. To compare the capacity of immune T cells to respond to LSPI and LaAg in vitro, lymph node cells from 7-day infected (L. amazonensis) BALB/c mice were re-stimulated with 50 ug/ml of LSPI and 50 ug/ml of LaAg. Cell proliferation was measured by MTT and the production cytokines was measured in the supernatants by ELISA. We observed that contrary to LaAg, LSPI activated T cell proliferation and inhibited the spontaneous production of both TGF-b and IL-10. In vivo, mice received two intramuscular injections with 25ug of LSPI with no adjuvants, with a 7-day interval. Seven days later, they were infected in the footpads with either L. amazonensis or *L. major*. We found that vaccination increased mouse resistance to infection with *L. major*, but not with *L. amazonensis*. Only when saponin was used as an adjuvant during vaccination were mice protected against *L. amazonensis*. Protection was accompanied by increased T cell activation in vivo, as measured by higher spontaneous cell proliferation ex vivo. These results show that the disease-promoting effect of LaAg is unlikely due to the presence of LSPI. Also, they show that L. amazonensis and L. major respond in a different manner to vaccination with serine proteases of *L. amazonensis*, possibly because of the higher anergenic feature of the later.

IM05 - Difference in intestinal response of C57BL/6J and BALB/c mice immunized with irradiated tachyzoites of *Toxoplasma gondii* RH strain

Galisteo Jr, A.J. *(Instituto de Medicina Tropical de São Paulo)*; Zorgi, N.E. *(Instituto de Medicina Tropical de São Paulo)*; de Andrade Jr, H.F. *(Instituto de Medicina Tropical de São Paulo)*

Toxoplasma gondii, a worldwide protozoan disease, causes severe disease, in fetus of pregnant women and immunocompromised hosts. Infection with *T. gondii* naturally occurs through ingestion of raw or undercooked meat containing cysts or through contact with oocysts from cat feces. Vaccines could results in low dissemination specially if orally administered to cats. Oral vaccines depend of good development of intestinal immunity. Irradiated *T. gondii* tachyzoites induce significant protection to mice, similar to chronically infected animal. We studied mucosal immune response of C57Bl/6j and BALB/c mice, immunized with 10^7 tachyzoites radiation sterilized (255Gy/60Co) *T. gondii* RH strain (oral or parenteral route), with 3 biweekly doses. Anti-*T. gondii* antibody specific for IgG and S-IgA were detected in feces of the immunized mice, by ELISA. We evaluated the intestinal epithelial of immunized mice, to assess the integrity and penetration of irradiated parasites *in vivo* by electron microscopy. Fecal S-IgA and IgG secretion was found to be more expressive in oral immunized mice. Intraperitoneal immunization induced differences in production of fecal S-IgA and IgG between mice strains, with higher levels in BALB/c mice. Electron microscopy studies showed intact parasites invading the inner intestinal mucosal, with adequate morphology and mechanisms of invasion, probably due to parasite resistance and alum hydroxide reduction of acid condition of stomach. Our results had demonstrated that irradiated parasites maintain their cell biology and immunogenic properties, adequate for the development of an oral immunogen, to be used in animals, through possibly attractive baits. Galisteo Jr., A.J. is a fellow of CNPq (141404/2004-3). This work was supported by CNPq and LIMHCFMUSP-49

IM06 - EFFECTS OF AMMONIUM MOLYBDATE ON THE DEVELOPMENT OF CUTANEOUS LESIONS AND THE IMMUNE RESPONSE TO *Leishmania (V.) braziliensis*

Figueiredo, A. B. *(Universidade Federal de Ouro Preto)*; Marques-da-Silva, E. A. *(Universidade Federal de Ouro Preto)*; Oliveira, J. C. *(Universidade Federal de Ouro Preto)*; Fietto, J. L. R. *(Universidade Federal de Viçosa)*; Afonso, L. C. C. *(Universidade Federal de Ouro Preto)*

Infection of mice with *Leishmania (V.) braziliensis* results in self-heling cutaneous lesions. The membrane of this parasite contain enzymes (NTPDase and 5'-nucleotidase) that

degrade the extracellular-ATP to adenosine, an importante molecule to parasite survival and to host immune response modulation. The ammonium molybdate is a potent inhibitor of 5'-nucleotidase. To investigate the effect of molybdate on the infection with *L. braziliensis*, C57BL/6 mice were inoculated in the footpad with 1.0x10^5 metacyclic promastigotes, with or without 5μM molybdate, and the lesion size was measured weekly. Moreover, the number of parasites in the lesion was estimated by limiting dilution assay. Our results showed that the molybdate reduces the lesion size and the footpad parasitism. In addition, we examined the response of peritoneal macrophages stimulated with 50μM ATP and infected with promastigotes (1:10 cell to parasite ratio), in the presence or absence of 5μM molybdate. The production of IL-10 was investigated by ELISA and molybdate treated macrophages produced smaller levels of this cytokine. The infection level of the macrophages was evaluated through the number of infected cells and the number of amastigotes/cell. We observed that the molybdate treatment reduces the ratio of infected cells but does not influence the number of amastigotes/cell. To investigate the long term effect of the molybdate on the parasite, we treated *L. braziliensis* cultures with this inhibitor for 5 days. An increase in AMP hydrolisis by treated parasites was observed, suggesting an increase in 5'-nucleotidase expression. C57BL/6 mice inoculated in the footpad with ammonium molybdate-treated parasites showed an increased lesion size and footpad parasitism. In conclusion, ammonium molybdate have a significant influence on the *L. braziliensis* infectivity, probably due to 5'-nucleotidase activity modulation, suggesting that this enzyme can contribute to the *L. braziliensis* pathogenesis.
Financial Support: FAPEMIG, CNPq

IM07 - Development of delivery system in glyceraldehyde-crosslinked microparticles of chitosan for intranasal vaccination with antileishmanial LACK DNA

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

We have previously shown the effectiveness of intranasal vaccination against cutaneous leishmaniasis in mice using LACK DNA (Pinto et al, 2004). In this work we used chitosan microparticles to optimize vaccine efficacy. Chitosan is a mucoadhesive biopolymer that is potentially suitable for mucosal delivery of DNA vaccines due to its easy complexation with negatively charged DNA plasmids, and for protecting DNA from nuclease degradation. Firstly, we prepared and tested the safety of glyceraldehyde-crosslinked chitosan microspheres to be used as a non-intumescent delivery system. When a total of 24 mg of microparticles were injected into mouse peritoneal cavity, no systemic allergy was induced as seen by unaltered IgE serum levels and absent eosinophilia and neutrophilia as measured 4 days later. No local cell influx was observed, despite a small increase in nitric oxide levels in the peritoneal exudate. The unaltered levels of TGP, TGO and creatinine serum levels confirmed lack of systemic toxicity. Crosslinked chitosan microparticles were then tested for LACK DNA superficial absorption and release kinetics in simulated biological medium. A complexation rate of 86% between LACK DNA and microparticles was observed. BALB/c mice was immunized twice by the nasal route with LACK DNA-chitosan complex and challenge with *L. amazonensis*. The immunization delayed the grow of lesions and reduced the parasite burden. These results demonstrate that glyceraldehyde-crosslinked microparticles of chitosan have a high yield of complexation with LACK DNA, are potentially safe in mice, display acceptable release kinetics and efficient in improving vaccination with intranasal LACK DNA against cutaneous leishmaniasis. Supported by: CNPq

IM08 - INTRANASAL IMMUNIZATION WITH LEISHMANIAL ANTIGENS: AN EFFECTIVE PROPHYLACTIC STRATEGY AGAINST MURINE VISCERAL LEISHMANIASIS

Schwedersky, R.P.S *(Universidade Federal do Rio de Janeiro)*; Gomes, D.O.G *(Universidade Federal do Rio de Janeiro)*; Rossi-Bergmann, B.R.B *(Universidade Federal do Rio de Janeiro)*

Intranasal vaccination is an effective way to promote local and systemic specific responses against not only respiratory but also systemic infectious diseases. Easiness of 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 a protective response against murine cutaneous leishmaniasis caused by *L. amazonensis*. In the present work, we investigated the effect of the intramuscular (i.m.) and intranasal (i.n.) administration of total *L. amazonensis* (LaAg) and *L. chagasi* (LcAg) promastigotes antigens as an attempt to induce protective immune responses 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. As determined by limiting dilution assay 1 month after infection, LaAg and LcAg-i.n. vaccinated mice displayed significantly lower parasite loads in the spleens as compared with non-vaccinated or LcAg i.m.-vaccinated mice. Splenocytes of LaAg or LcAg i.n.- vaccinated mice showed a significant increase in nitric oxide (NO) production, as determined by Griess assay, in relation to controls (180% vs 263%), or in relation to LcAg i.m vaccinated mice (175% vs 258%). In addition, spleen cells of LaAg or LcAg i.n.- vaccinated groups produced higher amounts of IFN-γ after in vitro recall with LaAg or LcAg

respectively, as compared with i.m.-vaccinated LcAg or control groups. Our results suggest that intranasal immunization with whole leishmanial antigens can be an important strategy to induce a protective responses not only against cutaneous but also against visceral leishmaniasis. Financial support: CNPq

IM09 - INTRANASAL DELIVERY WITH LACK-DNA INDUCE LONG TERM RESPONSE AGAINST MURINE VISCERAL LEISHMANIASIS AND SYSTEMIC mRNA TISSUE EXPRESSION

Gomes, D.O.G *(Universidade Federal do Rio de Janeiro)*; Rossi-Bergmann, B.R.B *(Universidade Federal do Rio de Janeiro)* Schwedersky, R.P.S *(Universidade Federal do Rio de Janeiro)*; De Melo, L.D.B.M *(Universidade Federal do Rio de Janeiro)*; Lopes, U.G.L *(Universidade Federal do Rio de Janeiro)*

LACK (Leishmania analogue of the receptor kinase C) is a conserved protein of all Leishmania species. We demonstrated that intranasal immunization with a plasmid carrying the LACK gene of *Leishmania infantum* (LACK-DNA) promotes protective immunity against *Leishmania amazonensis*. In the present study, we investigated the systemic expression of intranasally administered LACK-DNA and its ability to induce protection against murine visceral leishmaniasis. By using RT-PCR we found that BALB/c mice doubly vaccinated intranasally with 30 mg of LACK-DNA expressed LACK mRNA in the spleen, brain, cervical and popliteal lymph nodes, 4 weeks later. Elevated levels of Leishmania-especific IgG and lower amounts of TNF-α were detected in the serum of LACK-DNA vaccinated group. Mice vaccinated with i.n. LACK-DNA and challenged i.v. with 10^7 *Leishmania chagasi* promastigotes 7 days or 3 months after the second immunization dose displayed significant lower parasite loads in the liver and spleen one month after infection. Vaccinated infected animals 7 days after booster produced higher amounts of IFN-γ but reduced levels of IL-10 during recombinant LACK recall in the spleen as compared with infected controls. Spleen cells of vaccinated infected animals 3 months after booster showed significant proliferative response to specific *Leishmania* antigens and Jones-Mote DTH type reduction as compared with infected controls. Together, these data show that intranasal vaccination with LACK-DNA promotes systemic expression of the antigen that induces a strong protective long-term immunity against visceral leishmaniasis. Financial support:CNPq

IM10 - DEVELOPMENT OF AN EDIBLE VACCINE FOR LEISHMANIASIS USING LACK-TRANSGENIC TOBACCO.

Gomes Filho, J.S. *(Universidade Federal do Rio de Janeiro)*; Pinto, E. F. *(Universidade Federal do Rio de Janeiro)*; Hansen, E. *(Universidade Estadual do Norte Fluminense Darcy Ribeiro)*; Rossi-Bergmann, B. *(Universidade Federal do Rio de Janeiro)*

Induction of oral tolerance against disease-inducing antigens has emerged as a feasible strategy to prevent immunopathologies. Previously, we found that oral immunization with 100 ug of whole *Leishmania amazonensis* antigen (LaAg) protects BALB/c mice against cutaneous leishmaniasis. Likewise, a DNA plasmid encoding LACK, a disease-promoting LaAg component, induced protective immunity when administered in the nasal mucosa. In this work we attempted to develop an edible vaccine against leishmaniasis using transgenic tobacco expressing LACK. *Nicotiana tabacum* plants were transformed by the plant pathogen *Agrobacterium tumefaciens* containing the *LACK* gene plasmid. The presence of the transgene was confirmed by PCR and the production of LACK protein was confirmed by dot-blot. LACK expression was 0.18% of total dry leaf tissue. For vaccination, BALB/c mice were fasted for at least 3 h before receiving each of two doses of 20 mg of tobacco leaf extract (36 ug LACK) by intragastric gavage. Seven days after the second dose the animals were challenged in the footpad with *L. amazonensis*, and on day 7 of infection the peripheral hypersensitivity response to LaAg was evaluated. We found that whereas animals receiving wild-type tobacco or saline mounted a strong TH2-related Jones-Mote reaction that peaks in 18 h, the reaction was insignificant in animals receiving LaAg or LACK-expressing tobacco. To evaluate the protective effect of the transgenic plant, BALB/c mice received 5 oral doses of 20mg of plant extracts prior to infection with *L. amazonensis*. Evaluation of infection control is in progress. This preliminary result indicates the potential of this edible vaccine, where ingestion of a LACK-expressing plant extract can down-regulate peripheral antileishmanial responses in a manner that may favor protective immunity against cutaneous leishmaniasis.

IM11 - Cross-protective efficacy of the prophylactic VR1012-NH36 Leishmania donovani-DNA vaccine against cutaneous murine leishmaniasis by Leishmania amazonensis

Olegario Pereira de Souza, L.O.P.S *(Universidade Federal do Rio de Janeiro)*; Nico, N.D *(Universidade Federal do Rio de Janeiro)*; Palatnik de Sousa, CBPS *(Universidade Federal do Rio de Janeiro)*

The fucose-mannose ligand (FML) is a major antigenic complex of Leishmania donovani, and its most immunogenic fraction, a glycoprotein of 36 kDa has a proteic moiety identified as a nucleoside hydrolase (NH) of 36 kDa. The VR1012NH36 DNA vaccine was immunoprophylactic and immunotherapeutic against mice visceral leishmaniasis by L. chagasi and cutaneous leishmaniasis by L. mexicana. In the present study, we evaluated the immune response and protection induced by three sc doses of FML (150ug) + saponin

(100ug), or two im doses of VR1012 empty plasmid (100ug) or VR1012NH36 (100ug) DNA vaccines, against the footpad L. amazonensis infection with 1 million promastigotes. At week 26 after infection, significant differences were found between groups in the footpad swelling (ANOVA; $p = 0.004$). The DNA vaccine induced a 80.4% reduction different from the empty plasmid group ($p < 0.05$) which only induced 26.5% of reduction, indicating that protection was specifically due to the Nucleoside hydrolase antigen. Significant differences were still observed at week 31 ($p < 0.0001$) when the saline treated animals showed higher footpad swelling than both, the VR1012 plasmid ($p < 0.05$) and the VR1012-NH36 treated group ($p < 0.05$). On week 33, 90% of survival was observed in mice treated with the DNA vaccine while survival was 70% for the empty plasmid, FMLSAP vaccinated and saline treated animals. Of note, while deaths begun at week 13 in other groups, the only obit in the DNA vaccinated animals occurred by the end of the assay, at week 33. Our results point out that the NH36 gene of L. donovani, as a DNA vaccine, induces significant cross-protection against tegumentar leishmaniasis induced by L. amazonensis. The rates of protections are similar to those previously described against cutaneous infection by L. mexicana (88%) confirming the potential use the NH36 DNA vaccine in a bivalent vaccine tool for the control of both endemies.

IM12 - Nucleoside hydrolase DNA vaccine against canine visceral leishmaniasis

Borja Cabrera, G.P.B.C *(Universidade Federal do Rio de Janeiro)*; Nunes Santos, F.N.S *(Universidade Federal do Rio de Janeiro)*; Moreira Myashiro, L.M.M *(Universidade Federal do Rio de Janeiro)*; Bauer Santos, F.B.S *(Universidade Federal do Rio de Janeiro)*; Palatnik de Sousa, C.B.P.S *(Universidade Federal do Rio de Janeiro)*

The Leishmania donovani Nucleoside hydrolase is the main antigen of the FML complex (Leishmune vaccine against canine visceral leishmaniasis). In Balb/c, the DNA formulation (VR1012-NH36) induced 88% of protection, 91% of curative potential against visceral and 65% of protection against cutaneous leishmaniasis. In this work we immunized 6 mongrel dogs with 3 doses of 750 ug of the VR1012-NH36 plasmid. Control dogs (n=13) received saline. Dogs were challenged with 7 x 10 8 L (L.) chagasi amastigotes. On day 93, 6/13 seropositive and symptomatic controls were treated with 3 doses of the VR1012NH36 vaccine (immunotherapy). On month 5 after infection, anti-FML IgG antibodies were high in all groups. One control dog died from visceral leishmaniasis on day 76 after infection while another dog of the prophylaxis group died on day 186. Protection was evident in the number of positive DTH reactions to L chagasi antigen that was higher ($p < 0.001$) in prophylactically (6/6) and immunotherapy (5/6) dogs than in controls (1/7) and in the mean size of skin test reactions of prophylaxis group (8.5mm) that fell outside that of controls (3.94mm; IC95%, 0.79-7.10mm). An increased average of CD4 Leishmania-specific lymphocyte proportions was found in the prophylaxis group (mean= 40.03%) that fell outside the IC95% of the untreated controls (28.45%, CI95% ; 20.33-36.57), but not in the immunotherapy group (mean= 32.89), while decreased accumulated scores of clinical symptoms were found in prophylaxis group (7.66%) that fell outside the IC95% of the untreated control dogs (10.5%, CI95%, 8.04-12.96) but not in the immunotherapy group (8.16%). The ratio of parasites to lymphnode cells and of the loss of corporal weight were not significantly higher in untreated controls, than in the immunotherapy or the prophylaxis group. We conclude that the VR1012-NH36 vaccine induces strong prophylactic protection against a high dose canine infection with L. chagasi.

IM13 - Killed *Leishmania* vaccine with saponin adjuvant as promising candidate against canine visceral leishmaniasis

Giunchetti, R.C. *(Universidade Federal de Ouro Preto)*; Corrêa-Oliveira, R. *(Instituto René Rachou)*; Martins-Filho, O.A. *(Instituto René Rachou)*; Teixeira-Carvalho, A. *(Instituto René Rachou)*; Roatt, B.M. *(Universidade Federal de Ouro Preto)*; Aguiar-Soares, R.D.O. *(Universidade Federal de Ouro Preto)*; Braga, S.L. *(Universidade Federal de Ouro Preto)*; Abreu, R.T. *(Universidade Federal de Ouro Preto)*; Lana, M. *(Universidade Federal de Ouro Preto)*; Reis, A.B. *(Universidade Federal de Ouro Preto)*

Canine visceral leishmaniasis (CVL) is the major veterinary and public health problem caused by *Leishmania chagasi* (syn. *Leishmania infantum*) in endemic areas of the New World and the Mediterranean basin. Although the current strategies for vaccination against leishmaniasis are based on use of recombinant antigens, killed vaccines are still attractive in terms of stability of their biochemical composition and antigenicity, cost, and safety requiring fewer tests on bulk intermediates and finished products. Herein, cellular and humoral immune responses of dogs to a candidate vaccine have been investigated as a pre-requisite to understanding the mechanisms of immunogenicity against CVL. In this sense, twenty five dogs were divided into distinct groups: control (C, n=10), saponin (Sap, n=5), killed *Leishmania* vaccine (kLvac, n=5), kLvac plus saponin (kLvac-Sap, n=5). The candidate vaccine elicited strong antigenicity related to increased immunoglobulin isotypes, together with higher levels of lymphocytes, particularly of circulating CD8+ T-lymphocytes and *Leishmania chagasi* antigen-specific CD8+ T-lymphocytes. As indicated by the intense cell proliferation and increased nitric oxide production during in vitro stimulation by *L. chagasi* soluble antigens, the candidate vaccine elicited a potential immune activation status indicating an effective control of the etiological agent of CVL. Financial support: CNPq, FAPEMIG, UFOP/UFMG.

IM14 - USE OF A GENE ENCODING A CYSTEINE PROTEINASE FROM *Leishmania (Leishmania) chagasi*, *Ldccys1*, FOR VACCINATION OF HAMSTERS AGAINST HOMOLOGOUS INFECTION

Ferreira, J. H. L. F. *(Faculdade de Saúde, Ciências Humanas e Tecnológicas do Piauí and Unifesp)*; Gentil, L. G. G. G. *(Universidade Federal de São Paulo/Escola Paulista de Medicina)*; Dias, S. S. D. *(Universidade Federal de São Paulo/Escola Paulista de Medicina)*; Katz, S. K. *(Universidade Federal de São Paulo/Escola Paulista de Medicina)*; Barbiéri, C. L. B. *(Universidade Federal de São Paulo/Escola Paulista de Medicina)*

The present work evaluated the ability of the gene encoding a cysteine proteinase of 30 kDa from *Leishmania (L.) chagasi*, *Ldccys1*, to induce protective responses in hamsters against *L. (L.) chagasi* infection. We have previously reported that the gene *Ldccys1*, as well as the recombinant cysteine proteinase rLdccys1, conferred protective immunity against homologous infection in BALB/c mice. Hamsters were immunized intramuscularly with three doses of 100 μg of a plasmid carrying the *Ldccys1*gene (pcDNA3+*Ldccys1*) plus CpG ODN as the adjuvant, followed by a booster with 25 μg g rLdccys1 plus CpG ODN. Controls animals received PBS or empty pcDNA3 plus CpG ODN. Two weeks after the last dose all animals were challenged with 1x10^8 *L. (L.) chagasi*amastigotes by intraperitoneal route. The immunized animals presented a strong humoral response after second dose. The highest degree of protection was observed in hamsters immunized with the *Ldccys1* gene, which exhibited a parasite load approximately 4 orders of magnitude lower than controls immunized with the empty vector or PBS. Preliminary results on lymphokine production evaluated by RT-PCR from spleen cells of immunized animals showed a significant level of IFN-γ and IL-10 transcripts in hamsters immunized with the *Ldccys1* gene, indicating that there was induction of a mixed Th1/Th2 response. These results confirmed the ability of the *Ldccys1* gene to confer protective immunity against *L. (L.) chagasi* infection, opening perspectives to extend this immunization schedule to the dog, the main reservoir of canine visceral leishmaniasis in Brazil. Supported by FAPESP and NOVAFAPI.

IM15 - PROTEIN DEFICIENCY DECREASES RESPONSE TO *LEISHMANIA CHAGASI* VACCINE IN BALB/C MICE

Pinto, G.M. *(Universidade Federal de Ouro Preto)*
Serafim, T.D. *(Universidade Federal de Ouro Preto)*; Perdosa, M.L. *(Universidade Federal de Ouro Preto)*; Rezende, S.A. *(Universidade Federal de Ouro Preto)*

Protein-energy malnutrition is the most frequent cause of human immunodeficiency. Human malnutrition is complex, typically involving deficiency of protein and energy with deficits of other nutrients. Zinc deficiency is usually associated with protein-energy malnutrition as well as with iron deficiency. Vaccine efficacy depends on the ability of the individuals to exhibit an adequate immune response and may be weak in malnutrition. Thus, in this study we used a mouse model to study the effect of combined protein, iron and zinc deficiency in the response to a *Leishmania chagasi* Ag vaccine. BALB/c mice were fed initially with a standard diet and then half of the animals were fed with a diet deficient in casein (3%), iron and zinc. Control diet contained 14% casein and had normal content of zinc and iron. Total body weight was analyzed weekly and, after malnutrition was established, mice were vaccinated subcutaneously with L. chagasi Ag and saponin. After vaccination, mice were nutritionally recuperated and were challenged intravenously with promastigotes of *L. chagasi*. Four weeks later, mice were sacrificed and liver and spleen parasite loads were evaluated. Our data show that *L. chagasi* vaccine caused a great reduction in parasite load in spleen and liver from control diet mice. Furthermore, spleen parasitism was greatly increased in mice fed with deficient diet and these mice presented a weaker response to the vaccine. Therefore, these data suggest that malnutrition may alter immune response to *L. chagasi* vaccine in BALB/c model of infection.
This research is sponsored by: FAPEMIG, UFOP

IM16 - The effect of malnutrition in the immune response of BALB/c mice in *Leishmania chagasi* infection

Serafim, T.D. *(Universidade Federal de Ouro Preto)*; Pinto, G.M. *(Universidade Federal de Ouro Preto)*; Perdosa, M.L. *(Universidade Federal de Ouro Preto)*; Rezende, S.A. *(Universidade Federal de Ouro Preto)*

Nutrition is a critical factor in modulating immune homeostasis and thereby the outcome of host microbe interactions. Protein-energy and micronutrient deficiencies produce a wide spectrum of effects which could significantly influence the health and survival of the population. It is now well established that these deficiencies down-regulate immune responsiveness and increase morbidity and mortality due to infections. A currently and important problem for public health, especially in underdeveloped countries, is a broad spectrum of disease manifestations collectively known as Leishmaniasis, especially Visceral Leishmaniasis. Visceral Leishmaniasis is caused in New World by *Leishmania chagasi* and it is characterized by a progressive debility, being fatal if patients are not specifically treated. In this study, we used a mouse model of malnutrition to study the effect of protein, iron and zinc deficiency in the response to *Leishmania chagasi* infection. BALB/c mice were fed initially with a standard diet and then half of the animals were fed with a diet containing 3% protein and zinc and iron deficient. Control diet contained 14% of protein and was sufficient in zinc and iron. Total body weight was analyzed weekly and, after malnutrition was detected, mice were inoculated with promastigotes forms of *L. chagasi* by endovenous route. Four weeks latter, mice were sacrificed, liver and spleen parasite load were evaluated and

biochemical aspects were analized. We observed a significant decrease in glucose and total protein serum concentration in malnourished mice compared with control animals. Furthermore, malnourished animals exhibited a significant decrease in liver and spleen weight and a significant increase in parasite load in these organs, if compared with control animals. Therefore, these data suggest that malnutrition may alter the immune response to *L. chagasi* in BALB/c model of infection.

This research is sponsored by: FAPEMIG, PIBIC/CNPq, Rede Mineira de Bioterismo.

IM17 - Evaluation of the effect of Lipophosphoglycan (LPG) from *Leishmania braziliensis* and *L. amazonensis* in *Leishmania* infection

Passamani, R. *(Universidade Federal de Ouro Preto)*; Pacheco, L.M. *(Universidade Federal de Ouro Preto)*; Afonso, L.C.C. *(Universidade Federal de Ouro Preto)*

In this work, we evaluated the effect of Lipophosphoglycan (LPG) from *L. braziliensis* and *L. amazonensis* in *L. braziliensis* infection. C57BL/6 mice were inoculated intradermally in the ear with high and low dose of *L. braziliensis* metacyclic promastigotes alone or in the presence of LPG from *L. braziliensis* or *L. amazonensis*. The course of lesion development was monitored for 8 weeks. In the infection with low dose of parasites no effect of LPG on lesion development was observed. However, in animals inoculated with a high dose of parasites, addition of LPG from *L. braziliensis* or *L. amazonensis* induced an increase in lesion development during the first weeks of infection. Analysis of cytokine production 3 days after inoculation showed increased IL-10 and decreased IFN-γ production by antigen stimulated spleen and lymph node cells from mice inoculated with high dose of *L. braziliensis* in the presence of LPG. Intraperitoneal macrophages activation with LPG from *L. braziliensis* or *L. amazonensis* did not demonstrate NO and IL-10 production. Our data demonstrate that LPG can have an important role in the immune response during the early of phase of interaction of parasite with the cells of the host.

Financial Support: CNPq, PRONEX-FAPEMIG/CNPq, UFOP

IM18 - Human anti-saliva immune response following exposure to the visceral leishmaniasis vector, *Lutzomyia longipalpis*

Vinhas, V. S. F. *(Centro de Pesquisa Gonçalo Moniz)*; Andrade, B. B. A. *(Centro de Pesquisa Gonçalo Moniz)*; Paes, F. P. *(Centro de Pesquisa Gonçalo Moniz)*; Bomura, A. B. *(Centro de Pesquisa Gonçalo Moniz)*; Clarêncio, J. C. *(Centro de Pesquisa Gonçalo Moniz)*; Miranda, J. C. M. *(Centro de Pesquisa Gonçalo Moniz)*; Báfica, A. B. *(Universidade Federal de Santa Catarina)*; Barral, A. M. P. B. *(Centro de Pesquisa Gonçalo Moniz)*; Barral-Netto, M. B. N. *(Centro de Pesquisa Gonçalo Moniz)*

Experiments performed in animal models have demonstrated that phlebotomine saliva enhances *Leishmania* infection, and vaccinationwith salivary gland components protects against parasite-mediated disease. We have previously shown that individuals living in an endemic area of visceral leishmaniasis/ *L. chagasi* displayed a robust antibody response to saliva from the vector *Lutzomyia longipalpis*, which correlated with an antiparasite cell-mediated immunity. In attempet to explore anti-saliva immune responses following exposure to sand flies in humans, we have utilized an in vivo bite model in which human subjects were exposed to the laboratoy-reared *L. longipalpis*. Normal volunteers (NV, n=6) were exposed four times (15-day intervals) to 30 uninfected sand flies. Following the third exposure, NV were found to present diverse dermatological reactions at the site of insect bite. Serum from 5 out of 6 NV displayed high levels of anti-salivary gland sonicate (SGS) IgG1, IgG4 and IgE antibodies as well as detection of several salivary gland protein by means of western blot. In addition, following in vitro SGS-stimulation, there was an increased in the frequencyof CD4+CD25+ and CD8+CD25+ T cells as well as induction of cytokine synthesis such as IFN-g and IL-10. Strikinly, after one year of the first exposure, PBMC from *L. longipalpis*-bitten subjects displayed recall IFN-g responses that correlated with a significant reduction in infection rates using a macrophage-lymphocyte autologous culture system. Together, these data suggest that human immunization against sand fly saliva is feasible and recall responses are obtained even one year after exposure, opening perspectives for vaccination in man.

IM19 - Histological analysis of dermal cellular infiltrates in dogs vaccinated with Leishmania-vaccine, saponin and saliva from Lutzomyia longipalpis

Vitoriano-Souza, J. *(Universidade Federal de Ouro Preto)*; Moreira, N. D. *(Universidade Federal de Ouro Preto)*; Vieira, P.M.A. *(Universidade Federal de Ouro Preto)*; Gontijo, N.F. *(Universidade Federal de Minas Gerais)*; Corrêa-Oliveira, R. *(IRR - FIOCRUZ)*; Giunchetti, R.C. *(Universidade Federal de Ouro Preto)*; Reis, A.B. *(Universidade Federal de Ouro Preto)*; Carneiro, C.M. *(Universidade Federal de Ouro Preto)*

Efforts have focused on understanding the early events that influence effectiveness of vaccine antigens. In the present study, we investigated kinetics of dermal cellular infiltrates in dogs vaccinated with killed Leishmania vaccine, Saponin and saliva of Lutzomyia longipalpis. Based on cell migrations, we have developed a new experimental model to study some aspects of the inflammatory immune response. In this sense, intradermal injections into the dorsal region of dogs were

performed at different times (1, 12, 24, 48, 96 hours). Stimuli with distinct vaccine antigen components were evaluated, such as saponin (Sap), sand fly saliva (Sal), killed Leishmania vaccine (kLvac), and kLvac associated with Sap (kLvac-Sap). Those stimuli were compared with control group saline inoculum. Skin samples were fixed in 10% neutral buffered formalin for routine histopathological examination of sections by subsequent haematoxylin-eosin (HE) staining. Macroscopic observations (local swelling, hyperemia and necrosis) were reported during the course of the experiment. Dogs inoculated with Sap or kLvac-Sap presenting local swelling and hyperemia after 12hs. However, no macroscopic alterations were observed in dogs inoculated with Sal. Intense dermal cellular infiltrates hallmarks of Sap and kLvac-Sap frequently maintained throughout the time. The cellular counts in dermal compartment (upper and deeper dermis) showed the higher number of lymphocytes and neutrophils in kLvac-Sap group during 12-48h and 24-48h, respectively. Our data demonstrated that lymphocytes and neutrophils are the major cells populations into infiltrate focus observed in kLvac-Sap group. Besides the edematous reactions have frequently been observed after saponin injection or in combination with kLvac-Sap, the induction of a strong cellular response, simple formulation, safety, and the low costs, allow its use as alternative adjuvant in veterinary medicine. In this sense, kLvac-Sap presented as promising candidate against canine visceral leishmaniasis.Supported by FAPEMIG, CNPq and UFOP

IM20 - Dynamics of cells, levels of serum nitric oxide and iNOS expression in dermal tissues in two different anti-canine visceral leishmaniasis vaccines schedules using the hamster model

Moreira, N.D. *(Universidade Federal de Ouro Preto)*; Vitoriano-Souza, J. *(Universidade Federal de Ouro Preto)*; Vieira, P.M.A *(Universidade Federal de Ouro Preto)*; Roatt, B.M. *(Universidade Federal de Ouro Preto)*; Giunchetti, R.C. *(Universidade Federal de Ouro Preto)*; Malaquias, L.C.C. *(Universidade Vale do Rio Doce)*; Carneiro, C.M *(Universidade Federal de Ouro Preto)*; Reis, A.B. *(Universidade Federal de Ouro Preto)*

Hamsters have been intensively used as a model for understanding the mechanisms of immunogenicity for vaccines against canine visceral leishmaniasis (CVL). Since different antigens may have distinct immunogenicity, we test two vaccines schedules: a commercial Leishmune ® (Lh) vaccine and a killed Leishmania antigen plus saponin (kLvac-Sap). Dermal inflammatory infiltrate profiles, circulating leukocytes and the role of NO/iNOS during intradermal injections in abdominal area at different timetables (1, 12, 24, 48, 96, 168 hours) were assessed. Saline (Sn), saponin (Sap) and killed Leishmania antigen (kLvac) were used as a control groups. Our results showed that dermis and adjacent muscle were the major inflammatory sites for Sap, kLvac and kLvac-Sap groups. Intense dermal inflammatory infiltrate in response to kLvac-Sap were observed during the early phase (1h) and sustained until 24h, whereas the inflammatory response was delayed to Lh. Similarly, inflammatory infiltrates in muscle tissue at 12 and 24h were the hallmark of kLvac-Sap. Additionally, kLvac-Sap presented enhanced expression of iNOS in dermis at both early (12h) and late (168h) phases. Treatment led to highest NO levels at 48h. The counts of circulating leukocytes showed a decrease of lymphocytes in Sap and Lh compared with Sn after 12 and 24 hours and an increase in neutrophil counts (24h-Sap and 168h in Sap, kLvac, kLvac-Sap and Lh compared with Sn). While elevated lymphocyte counts were observed during the initial phase (1h) in Lh a decrease was observed at 12 and 24 hours, followed an enhancement in lymphocytes counts after 96 hours. Higher lymphocytes numbers in kLvac-Sap were observed during late phase (96h). Our data suggest that kLvac-Sap may be a promising candidate vaccine against CVL, eliciting an intense dermal inflammatory response associated with higher NO levels. Supported by FAPEMIG, CNPq and UFOP

IM21 - Systemic and compartmentalized participation of nitric oxide during acute phase of *Trypanosoma cruzi* infection in dogs

Vieira, P.M.A. *(Laboratório de Imunopatologia - NUPEB)*; Francisco, A.F. *(Laboratório de Imunopatologia - NUPEB)*; Malaquias, L.C.C. *(Núcleo de Pesquisa em Imunonogia)*; Giunchetti, R.C. *(Laboratório de Imunopatologia - NUBEP)*; Reis, A.B. *(laboratório de Imunopatologia - NUPEB)*; Tafuri, W.L. *(Laboratório de Imunopatologia - NUPEB)*; Carneiro, C.M. *(Laboratório de Imunopatologia - NUPEB)*

Immune control of many intracellular pathogens, including *T. cruzi*, is reported to be dependent on the nitric oxide (NO) production. The inducible nitric-oxide-synthase (iNOS) is assumed to be responsible for the NO increase after several infections. This study aimed to evaluate the NO participation during acute phase (42 d.p.i.) of *T. cruzi* infection. Twelve dogs had been divided in three groups: non-infected (NI), infected with blood (BT) or metacyclic forms (MT) of Berenice-78 T. cruzi strain. Parasitaemia was followed from the 10th day of infection up to negativation by fresh blood collected from the marginal ear vein. Serum was collected weekly to determine the NO concentration. Heart and spleen fragments were collected during necropsy to histopathological and immunohistochemistry study of parasitism and iNOS expression. The MT group presented an increase on NO levels along the infection and a delay in the parasitemia peak compared to BT group. The early enhanced NO serum level observed in MT probably is related to a better control on tissue parasitism in this group. The same pattern was observed in the spleen, where MT presented an intense expression of iNOS while the BT group demonstrated a moderated expression. In the heart the iNOS expression were similar between MT and BT, higher than NI group. On the other hand, histopathological evaluation demonstrated more intense alterations and elevated tissue parasitism in BT group in heart. Taken together these data suggests that NO participation

in systemic and compartmentalized response during acute phase of Chagas disease were related to protection. While MT group was characterized by a better immunopathological profile, BT group presented an extreme level of histopathological alterations associated to a lower NO levels. Supported by FAPEMIG, CNPq AND UFOP

IM22 - The levels of IL-17 production in *Trypanosoma cruzi* infected mice correlates with the myocarditis

Guedes, P.M.M. *(Faculdade de Medicina de Ribeirão Preto / USP)*; Gutierrez, F.R.S *(Faculdade de Medicina de Ribeirão Preto/USP)*; Pavanelli, W.R. *(Faculdade de Medicina de Ribeirão Preto/USP)*; Maia, F.L. *(Faculdade de Medicina de Ribeirão Preto/USP)*; Silva, J.S. *(Faculdade de Medicina de Ribeirão Preto/USP)*

IL-17 is involved in the pathogenesis of several autoimmune diseases (rheumathoid arthritis, encephalitis), and parasitary diseases (toxoplasmosis, schistosomiasis, tuberculosis). As the infection with *T. cruzi* results in a heart disease, with a possible autoimmune environment, the aim of this study is to evaluate the IL-17 production in to the heart and sera and to correlate the data with cardiac inflammation and fibrosis in mice infected with *T. cruzi*. Thirty Balbc mice were infected with 100 trypomastigotes of the Y *T. cruzi* strain, and euthanazied 14 and 21 days later. For histopathological analysis heart fragment were processed by hematoxylin-eosin and picrocirius red. IL-17, IL-10 and IFN-γ production were determined in heart (immunohistochemistry and ELISA) and serum (ELISA). The peak of parasitemia was observed 10 days after infection, and animals survived until 26 days after infection. Severe heart inflammation was observed in all animals examined 14 and 21 days after infection. In serum was observed only IFN-γ and IL10 production in infected animals, and IL-17 levels was similar in infected and uninfected animals. The IL-17, IFN-γ and IL-10 production in the heart was similar in the animals at 14th and 21th day of infection. The cytokine production accompanied the inflammatory process of the heart. Our results indicate that IL-17 participates in myocarditis induced by *T. cruzi* infection, and this cytokine could be involved with immunopathological mechanism of Chagas disease. Modulation of IL-17 production and your influence in the cardiac lesions genesis in Chagas disease are currently under investigation. Supported by: CNPq and USP.

IM23 - Evaluation of the participation of IL-10 and cells T regulatory (T CD4$^+$CD25$^+$) during the infection of IL-12 p40 deficient mice by *Leishmania braziliensis*

Lima Junior, D.S *(Universidade Federal de Ouro Preto)*; Marques-da-Silva, E. A *(Universidade Feferal de Ouro Preto)*; Carregaro, V *(Faculdade de Medicina de Ribeirão Preto/USP)*; Silva, J.S *(Faculdade de Medicina de Ribeirão Preto/USP)*; Vieira, L. Q *(Universidade Federal de Minas Gerais)*; Afonso, L. C. C *(Universidade Federal de Ouro Preto)*

It is well established that IL-10 deficiency leads to decreased lesion development and parasite load in mice infected buy several *Leishmania* species. On the other hand, in mice resistant to *L. major* infection, it has been demonstrated that the delayed production of IL-10 impairs full elimination of the parasite and interrupts inflammatory reaction, diminishing possible damages to the organism. Initial results showed large amounts of IL-10 produced by NK 1.1$^+$ and dendritic cells present in lesions of mice deficient in the IL-12p40 subunit (IL-12p40$^{-/-}$), after 3 and 6 weeks of infection with *L.braziliensis*. In this context, we investigated the participation of IL-10 and regulatory cells T regulatory (T CD4$^+$CD25$^+$) during the infection of IL-12 p40 deficient mice by this parasite. Thus, IL-12p40$^{-/-}$ mice were infected with *L. braziliensis* promastigotes (1x 10^7/footpad) and five weeks after, received three i.p. treatments (seven days intervals) with anti-IL-10 receptor (α-IL-10r) or anti-CD25 (α-CD25) antibodies. Lesion development was evaluated weekly. At 8^{th} week of infection, the parasite load (limiting dilution assay) and the cytokine production (ELISA) were evaluated. Lesion development was stabilized from 5 or 6 weeks of infection onwards in the control group (rat-IgG). However, parasite dissemination to the spleen was observed. The α-IL-10r treatment induced a lower lesion development as well as lower parasitism. Conversely, no effect in lesion development and parasite burden was observed in α-CD25 treated mice. Regarding the cytokine production, high production of IL-4 and low production of IFN-γ was observed in all groups analyzed. Taken together, the results suggest that IL-10 contributed for the increase in the development of the lesion and the parasite load in these animals. However, CD4$^+$CD25$^+$ T cells do not appear to be involved in the production of this cytokine in this experimental setting. Financial Support: CNPq, FAPEMIG,UFOP, FMRP/USP

IM24 - Double-negative T cells display a distinct functional profile between chagasic patients with different levels of cardiomiopathy

Amaral, F. N. *(Universidade Federal de Minas Gerais)*; Santos, J. S. C. *(Universidade Federal de Minas Gerais)*; Antonelli, L. R. V. *(National Institutes of Health)*; Rocha, M. O. C. *(Universidade Federal de Minas Gerais)*; Gollob, K. J. *(Universidade Federal de Minas Gerais)*; Dutra, W. O. *(Universidade Federal de Minas Gerais)*

Chronic Chagas disease cardiomiopathy is the most severe outcome of human infection with *Trypanosoma cruzi*, leading to heart failure and death. Many studies have shown that morbidity in chagasic patients is related to parasite factors as well as the hostś immune response. We have previously shown that the frequencies of activated CD4$^+$ and CD8$^+$ T cells are increased in the peripheral blood of Chagas disease patients and that these cells are capable of producing

cytokines, specially IFN-γ in cardiac patients. We have recently observed that $CD4^{-}CD8^{-}$ (DN T cells) are an importat source of IFN-γ in cardiac chagasic patients. Morevoer, it has been shown that DN T cells are increased in rats infected with *T. cruzi*. Considering the possible role of these cells in the immunopathology of human Chagas disease, we determined the activation state and the expression of immunoregulatory cytokines by the DN T cells expressing either alpha-beta ($\alpha\beta$TCR) or gamma-delta TCR ($\gamma\delta$TCR) freshly isolated from cardiac chagasic patients with non-dilated (NDC) or dilated (DC) cardiomiopathy. We observed that NDC displayed a higher frequency of circulating $\alpha\beta$ DN T cells, as compared to DC, while the frequency of $\gamma\delta$ DN T cells was similar between groups. However, expression of the activation marker HLA-DR was higher in $\gamma\delta$ DN T cells from NDC, as compared to DC. Expression of CD28 and CD45RO did not differ among groups. Activated $\gamma\delta$ DN T cells from NDC expressed more IL-10 than DC, suggesting an important regulatory role of these cells in chagasic cardiomiopathy. Expression of pro-inflammatory cytokines, IFN-γ and TNF-α, by DN T cell subpopulations was similar between groups. These results provide the first information about DN T cells human Chagas disease and suggest that these cells may carry out an important modulatory role in chagasic cardiomiopathy.

IM25 - EFFECTS OF ANGIOTENSIN CONVERTING ENZYME (ACE) INHIBITOR AND SUBSTANCE P DURING THE CARDIOMYOPATHY INDUCED BY *Trypanosoma cruzi*

Pedrosa, M.C *(Universidade Federal de Ouro Preto)*; Costa, G.P. *(Universidade Federal de Ouro Preto)*; Silva, M.C. *(Universidade Federal de Ouro Preto)*; Caldas, S. *(Universidade Federal de Ouro Preto)*; Melo, L. *(Universidade Federal de Ouro Preto)*; Martins, R.F. *(Universidade Federal de Ouro Preto)*; Azevedo, M.A. *(Universidade Federal de Ouro Preto)*; Bahia, M.T. *(Universidade Federal de Ouro Preto)*; Talvani, André *(Universidade Federal de Ouro Preto)*

Chagasic Chronic Cardiomyopathy (CCC) is an inflammatory disturbance associated with Trypanosoma cruzi infection and affects around 17 million people in Latin American. During chronic phase, 30% of infected individuals will develop mild or severe cardiomyopathy. Angiotensin Converting Enzyme (ACE) inhibitor is an important drug used to ameliorate heart functional capacity and its remodeling in individuals presenting CCC. During its biological degradation, ACE inhibitor promotes inhibition of Kininase III, an enzyme whose function is to block the degradation of some kinines, in special, the Substance P (SP). Interestingly, SP exerts important role during activation of immune cells in different inflammatory diseases. Our goal here is investigate the role of ACE inhibitor and SP during acute and chronic phases focusing whether those stimuli might be contributing to clinical evolution of CCC. Our previously results indicated an elevation of circulating leukocytes and heart mast cells in C57BL6 mice treated with ACE inhibitor (25 mg/Kg), in association or not with infection with 50 trypomastigotes forms of T. cruzi (Colombiana strain). Besides, ACE treatment reduced drastically the peak of blood parasitamia and avoided mortality among all animals for 60o days. Until now, our data suggest an anti-parasitism effect to ACE inhibitor in blood secondarily to leukocytes increased. However, further studies are needed to clarify if this mechanism is based directly on ACE inhibitor or, secondarily on SP and its repercussion in experimental CCC. Supported by CNPq, FAPEMIG and FIP-PUC MG.

IM26 - Phenotypic Evaluation of Lymphocytes and Polymorphonuclear Cells in Brazilian Dogs Naturally Infected by Leishmania (Leishmania) chagasi

Guerra, L.L. *(Fundação Oswaldo Cruz/Instituto René Rachou)*; Souza, D.M. *(Fundação Oswaldo Cruz/Instituto René Rachou)*; Teixeira-Carvalho, A. *(Fundação Oswaldo Cruz/Instituto René Rachou)*; Martins-Filho, O.A. *(Fundação Oswaldo Cruz/Instituto René Rachou)*; Giunchentti, R.C. *(Universidade Federal de Ouro Preto)*; Corrêa-Oliveira, R. *(Fundação Oswaldo Cruz/Instituto René Rachou)*; Reis, A.B. *(Universidade Federal de Ouro Preto)*

Canine Visceral Leishmaniasis (CVL) is one of the most important emerging diseases with high prevalence in Latin American countries. Considering the putative role of dogs in the transmission of the disease, we investigated the importance of lymphocytes and polymorphonuclear cells in Leishmania infections. We proposed to evaluate the expression of different cell markers (Thy-1, CD5, CD4, CD8, CD21 and CD14 in lymphocytes and MHC-II, CD45RA and CD45RB in granulocytes) in dogs naturally infected by L. chagasi. Herein, 30 dogs were subdivided into three groups according to spleen or skin parasitism: Low Parasitism (LP; n=10), Medium Parasitism (MP; n=10) and High Parasitism (HP; n=10). The criteria used for parasite load was the LDU values ("Leishman Donovan Units"), defined such as number of amastigotes per 1000 nucleated cells. Twenty non-infected dogs (NI), used as control group, were serologically and parasitologically negative for L. (L.) chagasi. Our data demonstrated a decrease in the absolute counts of T lymphocytes (Thy-1+ and CD5+) cells in dogs with high spleen parasitism in comparison to LP and MP dogs. An increase of T CD8+ cells was observed in LP and MP dogs when compared to NI dogs. In the evaluation of granulocytes, the eosinophils demonstrated an increase of MHC-II (Medium Fluorescence Channel - MFC), in HP group in comparison to NI dogs. The decrease in the expression of Thy-1+ and CD5+ T cells in LP and MP dogs, demonstrate the importance of these populations in the maintenance and establishment of the parasite/host relation. This data reflects the increase of CD8+ subpopulation in LP and MP dogs, reinforcing the important role of these cells in the protection mechanism in CVL.

The increase in MHC-II+ eosinophils in HP dogs suggest the participation of them as antigen presenting cells, trying to eliminate the parasites through phagocytic mechanisms.

IM27 - Phenotypic and functional study of T cells expressing distinct TCR Vβ regions from human cutaneous leishmaniasis patients

KEESEN, T. S. L. *(Universidade Federal de Minas Gerais)*; ANTONELLI, L. R. V. *(National Institutes of health)*; GUIMARÃES, L. H. *(Universidade Federal da Bahia)*; BACELLAR, O. *(Universidade Federal da Bahia)*; CARVALHO, E. *(Universidade Federal da Bahia)*; DUTRA, W. O. *(Universidade Federal de Minas Gerais)*; GOLLOB, K. J. *(Universidade Federal de Minas Gerais)*

Leishmaniasis is an endemic parasitic disease affecting 12 million worldwide. The establishment of an effective immune response directed against *Leishmania* is critical for the disease control. To better understand the role that subpopulations of $CD4^+$ T cells expressing distinct Vβ usage may have in the human immune response against *Leishmania braziliensis*, a detailed study defining the activation state and the cytokine profile was performed with a group of well-defined cutaneous leishmaniasis patients and a group of normal individuals. Using flow cytometry, we evaluated in vitro the frequency of T $CD4^+$ cells expressing the following Vβ: Vβ 2, 3, 5.1, 5.2, 8, 11, 12, 17 and 24 in the different groups. The frequency of Vβ positive, $CD4^+$ within $CD4^+$ T cells was calculated without stimulus, as well as after culture with soluble *Leishmania* antigen (SLA). Our results show that: (1) $CD4^+$ T cells expressing distinct Vβ usage, increased expression of Vβ 5.2 and 24 in cutaneous leishmaniasis as compared with normal individuals; (2) cutaneous leishmanisis patients demonstrate and increase in $CD4^+$ T cells expressing Vβ 5.2, 11, 12 and 17, after stimulus with SLA; (3) Distinct populations of Vβ expressing $CD4^+$ T cells are found to expressing activation markers (HLA-DR), memory markers (CD45RO), as well as pro-inflammatory (IFN-γ, TNF-α fw, and anti-inflammatory (IL-10), cytokines after stimulus with SLA; (4) Positive correlations between $CD4^+$ T cells expressing Vβ 5.2, 11 and 17, producing pro-inflammatory (IFN-γ, TNF-αfw, and anti-inflammatory (IL-10), cytokines are seen after stimulus with SLA. Given that cutaneous leishmaniasis is a form of leishmaniasis that is often followed by spontaneous cure, the activation of specific subpopulations during this disease could allow for the formation of an effective cellular response. This study might lead us to the discovery of immunodominant *Leishmania* antigens important for triggering efficient host responses against the parasite.

IM28 - EXPRESSION OF THE INDUCIBLE NITRIC OXIDE SYNTHASE IN HUMAN CUTANEOUS LEISHMANIASIS CAUSED BY *Leishmania (Viannia)* sp.

LIMA, A.C.L. *(Universidade de São Paulo)*; GOMES, C.M.C. *(Universidade de São Paulo)*; SILVEIRA, F.T. *(Instituto Evandro Chagas-PA)*; GAMA, M.E.A. *(Universidade Federal do Maranhão)*; FERNEZLIAN, S.M. *(Universidade de São Paulo)*; TOMOKANE, T.Y. *(Universidade de São Paulo)*; CORBETT, C.E.P. *(Universidadede São Paulo)*

The production of nitric oxide (NO) from amino acid L-arginine by NO synthase (iNOS or NOS_2) is one of the key defence mechanisms of mammalian phagocytes. The expression of the inducible nitric oxide synthase (iNOS) and generation of nitric oxide in response to IFN-gamma and TNF-alpha stimulus are important in control of Leishmania infection. Studies in murine model have shown that mice with a deleted inducible nitric oxide synthase (iNOs) gene were unable to contain L. braziliensis in the skin. The aim of this study was to determine the iNOS expression in the cutaneous lesions of localized cutaneous leishmaniasis patients (LCL), and the possible correlation between the level of iNOS expression and the disease duration.Twenty-two LCL patients were diagnosed in the municipal district of Buriticupu, in the pré-Amazonian of Maranhão State, Brazil, based on the clinical and parasitological criteria. Immunohistochemistry staining was carried out by incubating histological sections with polyclonal rabbit anti-human iNOS antibody (N-20, Santa Cruz 651). An image analysis system was used for counting stained cells. Paraffin-embedded sections were also analyzed by PCR-RFLP and semi-Nested PCR for the identification of *Leishmania* parasites. All *Leishmania* strains were identified as belonging to the *Viannia* sugenus: 7 as *L. (V.) braziliensis* and 15 as *L. (V.)* sp. The time of evolution of the disease ranged from 1 month to 1 year, with predominance of the 3-4 months period. There was a strong iNOS expression (average of 725 cells per $^{mm}2$) in skin lesion of all patients. There was no significant correlation between iNOS reactivity and either the time of disease or the Leishmania species. These findings suggest that iNOS expression may has an important role in the resolution of infection in the LCL-patients due to *L.(V.)* sp. Supported by: LIM-50/HCFMUSP, FAPESP

IM29 - IgG DEPOSIT AND T CELLS IN GLOMERULONEPHRITIS IN EXPERIMENTAL VISCERAL LEISHMANIASIS

SIGNORE, F.S. *(Instituto de Medicina Tropical)*; PRIANTI, M.G.P. *(Instituto de Medicina Tropical)*; GOTO, H.G. *(Instituto de Medicina Tropical)*

Introduction: Glomerulonephritis (GN) is frequent in visceral leishmaniasis (VL), it has been associated with immune complex deposition, but in previous studies in hamsters, dogs

and mice with VL, we observed transient IgG and scarse C3b deposit in glomeruli, but significant presence of CD4+ T cells in the glomeruli. To search the pathogenesis, we studied the GN using mice intraperitoneally infected with Leishmania (L.) chagasi that suggested also participation of CD4+ T cells. Aim: To extend the study of pathogenesis, since in mice the infection with L. (L.) chagasi is self controlled, we searched the effect of prolonged antigen stimulation on T cell participation after infection and repeated challenges. Methods: BALB/c mice were infected through intraperitoneal route with 2x107 purified Leishmania (L.) chagasi amastigotes and were reinfected twice with the same inoculum after 20 and 40 days post infection (PI). In different time periods, we analyzed the histopathological alterations by morphometry, and expression of IgG, CD4+, CD8+ T cells by immunohistochemistry. Results: We observed glomerular hipercellularity from 7 and 15 days after the second reinfection. Moderate IgG deposits were observed from 7 days after infection that decreased afterwards and CD4+ and CD8+ T cells were present in glomeruli in greater amount at 7 days after second infection, and decreasing afterwards. After first and second challenges progressively increased the amount of glomeruli expressing CD4+ and CD8+ T cells. The deposit of IgG in glomeruli was seen transiently in the initial phase in infected animals, and the late phase in following reinfections, but C3b deposit was absent. Conclusion: Our data suggest important time-dependent participation of T cells and immunoglobulin in pathogenesis of GN in infected and reinfected mice with leishmania (L.) chagasi Supported by, FAPESP, LIM-38 (HC-FMUSP), CAPES, CNPq and FINEP.

IM30 - HISTOPATHOLOGICAL STUDY OF LIVERS OF DOGS NATURALLY AND EXPERIMENTALLY INFECTED WITH *Leishmania (Leishmania) infantum chagasi*

Martins, G D C *(Universidade Federal de Minas Gerais)*; Carvalho, E.S. *(Universidade Federal de Minas Gerais)*; Moura, E.P. *(Universidade Federal de Minas Gerais)*; Melo, F.A. *(Universidade Federal de Minas Gerais)*; Figueiredo, M.M. *(Universidade Federal de Minas Gerais)*; Michalick, M.S.M. *(Universidade Federal de Minas Gerais)*; Fernandes, A.P.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 is a zoonosis and a chronic disease caused by a protozoan of the genus *Leishmania*. In the New World the causative agent is *Leishmania (Leishmania) infantum chagasi* (Shaw 2006) and dogs are the domestic reservoir for human visceral leishmaniasis. In this study we carried out a histological analysis of livers of naturally (CNI) and experimental (CEI) dogs infected with *Leishmania chagasi*. Seventy one infected animals with positive serological exams to *Leishmania* (IFAT and ELISA) were divided in two groups: 61 mongrel dogs (CNI) obtained from metropolitan area of Belo Horizonte-MG; 10 beagles dogs (CEI) obtained from Hertape-Calier, Betim-MG. After sacrifice with of Sodic Thiopental 2,5% and T-61, liver fragments were processed for histological study. The most important lesion was the hepatic intralobular granuloma formation (69/71 of cases). It was characterized by the presence of epithelioid macrophages, plasma cells, lymphocytes and rare neutrophils. Vacuolar hepatocyte degeneration was the second most lesion observed (66/71 of cases) followed by the chronic portal inflammatory reaction and sinusoidal congestion (60/71 of cases). A chronic inflammatory reaction of the capsule (Glisson's capsule) was found in 54 of 71 cases, but this lesion was discrete in the majority of the cases (36/54 of cases). A semi-quantitative study of the granulomas was carried out using a optical microscope analysis. Besides, the average of the granulomas number was lower in CNI than CEI, this difference was not statistical significant. However, the other lesions did not show any correlation between CNI and CEI. We can conclude that the hepatic intralobular granulomas were the fundamental lesion and it could be consider as an important alteration to study the natural and experimental pathogenesis of the *Leishmania* infection.

IM31 - Imunohistochemistry identification of *Leishmania (Leishmania) infantum chagasi* in canine testicles, epididymis and prepuce indicating the potential venereal transmission of the disease

Silva, L.C. *(Medicina Veterinária PUC Minas - Betim)*; Silva, B.C. *(Medicina Veterinária PUC Minas -Betim)*; Toledo Júnior, J.C. *(Medicina Veterinária PUC Minas -Betim)*; Rachid, M.A. *(Medicina Veterinária PUC Minas -Betim)*; Tafuri, W.L. *(Universidade Federal de Minas Gerais)*; Cordeiro, R.L.R. *(Centro de Zoonoses de Betim-MG)*; Viegas, F.M.V. *(Centro de Zoonoses de Betim-MG)*; Valle, G.R. *(Medicina Veterinária PUC Minas -Betim)*

The canine visceral leishmaniasis, zoonosis caused by *Leishmania (Leishmania) infantum chagasi* (Shaw 2006) has been responsible for the euthanasia of dogs in endemic areas in Brazil although the efforts to retain the spread of the disease through vector elimination. However, the recent PCR identification of its DNA in canine semen has been sustained the hypothesis of venereal transmission of the disease, as by coitus as by artificial insemination. The goal of this work is to examine the presence of amastigote forms of *L. chagasi* in male genital organs and penile and prepuce surfaces of sick dogs and study the probable parasite origin in semen to estimate the potentiality of the venereal transmission by coitus, serching for the parasite presence in penile and prepuce surfaces. Eleven mongrel male dogs with positive parasitological test by bone marrow and/or lymph nodes cytology were euthanized with Thiopental 2,5% (25mg/Kg). At the necropsy fragments of testicles, epididymis, deferent ducts, prostate gland, penian gland and internal prepuce skin were collected and processed to obtain sections for histological and parasite tissue load immunohistochemical evaluation. It was

observed nonspecific inflammation (46% of testicles; 73% of epididymis; 36% of deferent ducts; 55% of prostate glands; 64% penian glands; and 91% of internal prepuce skins). The immunohistochemical analysis was positive in the testicles (9%), epididymis (9%) and prepuce (27%). Thus, it was concluded that there is a potential venereal transmission as by coitus as by artificial insemination because the parasite is present not only in canine copulatory surface (penis/prepuce) but also in testicles and epididymis. Supported by: PUC Minas; CCZ Betim-MG; NIPE-ICB/UFMG

IM32 - THYMIC ALTERATIONS IN *Plasmodium berghei* NK65-INFECTED MICE

Andrade, C.F. *(Universidade Estadual de Campinas)*; Gameiro, J. *(Universidade Estadual de Campinas)* Carvalho, B.O. *(Universidade Estadual de Campinas)*; Talaysis, R.L. *(Universidade Estadual de Campinas)*; Nagib, P.R.A. *(Universidade Estadual de Campinas)*; Verinaud, L. *(Universidade Estadual de Campinas)*; Costa, F.T.M. *(Universidade Estadual de Campinas)*

Malaria represents the most important parasitic disease of humans, affecting annually 300 - 500 million people, and leading to death of more than 1 million individuals; especially children and pregnant women. In Brazil, mainly in the Amazon basin, 600,000 cases of malarial infections were reported in 2005. Despite intense research in the last decades, the development of an effective vaccine has been hampered by the complexity in inducing a sterile long-lasting immunity. T cell maturation in the thymus is a process dependent on various factors, including matrix components that form a meshwork to provide mechanical support and molecular stimuli for complete T cell development. Nevertheless, thymus functions can be altered due to the invasion of several pathogens, such as *Trypanosoma cruzi* and *Paracoccidioides brasiliensis*, which provoke severe atrophy and deep disorganization of thymic architecture. Herein, we investigated thymic alterations in *Plasmodium berghei*-infected mice. Thus, groups of 5 week-old BALB/c male mice were infected intraperitoneally with 10^6 blood forms of *P. berghei*-NK65, a non cerebral malaria line. Three, seven and fourteen days after infection, mice were extensively perfused intracardiacally; thus thymuses were collected and processed for DNA extraction (used for PCR analysis) and for histopathological analysis. Preliminary findings showed parasite sequestration in this organ associated to severe atrophy and to the increasing of parasite load. Moreover, histopathological analyses revealed thymic architecture alterations with loss of cortico-medullary delimitation. The data provided by us demonstrated that *P. berghei* NK65, despite not able to induce imunopathological complications as cerebral malaria, is capable of invading thymus provoking structural alterations that might alter the normal function of this organ.

IM33 - Comparison of the chronic inflammatory reaction and the parasite load of ears skin biopsies of dogs naturally and experimentally infected with *Leishmania (Leishmania) infantum chagasi*

Figueiredo, M.M. *(Universidade Federal de Minas Gerais)*; Moura, E.P. *(Universidade Federal de Minas Gerais)*; Melo, F.A. *(Universidade Federal de Minas Gerais)*; Sampaio, W.M. *(Universidade Federal de Minas Gerais)*; Ferreira, V.M. *(Universidade Federal de Minas Gerais)*; Michalick, M.S.M. *(Universidade Federal de Minas Gerais)*; Melo, M.N. *(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 severe systemic disease caused by *Leishmania (Leishmania) infantum chagasi*(Shaw, 2006). The aim of this study was to evaluate the intensity of inflammatory process and the parasite load of 116 skin biopsies of naturally and experimentally infected animals with *Leishmania infantum chagasi*. They were divided in four groups: group I (controls), 14 animals with serological and parasitological negative exams for Leishmania infection. Group II, 42 mongrel dogs naturally infected with *L. chagasi* obtained from Santa Luzia-MG; Group III, 10 beagles experimentally infected with 1x 107 i.v. promastigotes, from Hertape-Calier, Betim-MG; Group IV, 50 skin biopsies of animals naturally infected with L. chagasi receipted by Departamento de Patologia Geral (ICB/UFMG). All tissues were fixed in formalin (10%) for histological (H&E) and immunohistochemical analysis. The streptoavidin-peroxidase immunohistochemistry method was carried out for tissue amastigotes detection by optical microscopy (Tafuri et al., 2004). The most part (95%) of the 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. However, apposing to what was observed by Sollano-Gallego et al. (2004), we did not observe the presence of giant cells. There are a straight relation between the intensity of the inflammatory reaction and the parasitism among of all groups, except of group III (Kruskal Wallis tests). It means that the parasitism determines an inflammatory reaction, but not necessarily an intense inflammatory process because we have found a normal histological picture and presence of parasites in the skin. In relation of group III results, besides of using higher dose of *Leishmania* parasites for experimental infections, animals did not harbor higher parasite load in organs as naturally infected ones (Sampaio et al. 2007).

IM34 - Characterization of mast cells in a chronic inflammatory reaction of ears skin biopsies of dogs naturally and experimentally infected with *Leishmania (Leishmania) infantum chagasi*: a preliminary study

Figueiredo, M.M. *(Universidade Federal de Minas Gerais)*; Moura, E.P. *(Universidade Federal de Minas Gerais)*; Sampaio, W.M. *(Universidade Federal de Minas Gerais)*; Fernandes, A.P.S.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 severe systemic disease caused by *Leishmania (Leishmania) infantum chagasi* (Shaw, 2006). Off all cell types (neutrophils, eosinophils, macrophages, lymphocytes), the differential roles played by mast cells in *Leishmania* infection remain unknown. The aim of this study was carry out a qualitative and semiquantitative study of mast cells during the *Leishmania* infection. Naturally and experimentally infected animals with *Leishmania infantum chagasi* were divided in four groups: group I (controls), 14 animals with serological and parasitological negative exams for *Leishmania* infection. Group II, 42 mongrel dogs naturally infected with *L. chagasi* obtained from Santa Luzia-MG; Group III, 10 beagles experimentally infected with 1x 107 i.v. promastigotes, from Hertape-Calier, Betim-MG; Group IV, 50 skin biopsies of animals naturally infected with *L. chagasi* receipted by Departamento de Patologia Geral (ICB/UFMG). All tissues were fixed in formalin (10%) for histological (H&E) and immunohistochemical analysis. The streptoavidin-peroxidase immunohistochemistry method was carried out for tissue amastigotes detection by optical microscopy (Tafuri et al., 2004). For mast cell histological characterization we carried out a Dominici staining. The most part (95%) of the 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 general, metacromatic mast cells were observed mainly around the vessels of the upper and deep dermis. The number of mast cells was estimated considering 20 fields of the ear skin fragments tissues stained by Dominici under light microscope analysis. There was any correlation among mast cells numbers, intensity of the inflammatory reaction or the parasitism among of all groups. Other techniques for mast cells identification as Giemsa with modifications (Behmer et al. 1976) and alcian-blue safranin will be considered.

IM35 - Histopathological and immunohistochemical study of type 3 complement receptor (CD11b/CD18) in organs of naturally infected dogs with *Leishmania (Leishmania) infantum chagasi* treated with liposome encapsulated meglumine antimoniate

Moura, E.P. *(Universidade Federal de Minas Gerais)*; Ribeiro, R.R. *(Universidade Federal de Minas Gerais)*; Melo, F.A. *(Universidade Federal de Minas Gerais)*; Sampaio, W.M. *(Universidade Federal de Minas Gerais)*; Lima, W.G. *(Universidade Federal de Minas Gerais)*; Alves, C.F. *(Universidade Federal de Minas Gerais)*; Figueiredo, M.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 caused by *Leishmania (Leishmania) infantum chagasi* (Shaw 2006) and dogs are the domestic reservoir for the human disease. Alternative therapies for CVL have resulted in the development of liposome-entrapped drugs, with enhanced efficacy and therapeutic indices. A novel liposomal formulation of meglumine antimoniate, consisting of vesicles of reduced size has been successful employed. Macrophage complement receptor CR3 (CD11b/CD18) has been implicated in the interaction of both human and murine macrophages with serum-opsonized promastigotes. Histological study of spleens, livers, bone marrow, lymph nodes and skin samples were carried out. Twenty-nine naturally infected dogs with *L. chagasi* from Santa Luzia-MG were used as follow: 9 animals received 4 intravenous doses of Liposome Meglumine Antimoniate (LMA -each corresponding to 6,5 mg Sb/kg) at 4 days intervals; 10 animals received empty liposomes (at the same lipid dose as LMA); 9 untreated animals. Dogs were sacrificed 150 days after treatment with of Sodic Thiopental 2,5% and T-61. Samples of all tissues were fixed in 10% formalin solution for histological and parasitological studies. Frozen sections of fragments of spleen, liver, lymph nodes and skin were processed for CD11b antigens characterization. A semiquantitative study of histological alterations and a quantitative study of parasite load and CD11b expression was carried out under optical microscope analysis. A parasite load was statistical lower than controls in all organs, excepted in the skin. The spleens of treated dogs showed more reactive and the CD11b expression was higher than untreated dogs. We did not find any statistical difference related of the parasite load and CD11b expression of livers. However, treated animals showed less histological alterations than controls. These results showed that this treatment protocol is able to reduce the tissues parasite load. In addition, our results could indicate that treatment protocol might provoke an organ-specific immune response.

IM36 - QUANTITATIVE EVALUATION OF TISSUE PARASITE LOAD IN MICE EXPERIMENTALLY INFECTED WITH *Leishmania major* DURING THE IMPLANTATION OF PARAFFIN TABLETS

Ferreira, V.M. *(Universidade Federal de Minas Gerais)*; Noviello, M.L.M. *(Universidade Federal de Minas Gerais)*; Vieira, L.Q. *(Universidade Federal de Minas Gerais)*; Tafuri, W.L. *(Universidade Federal de Minas Gerais)*; Tafuri, W.L. *(Universidade Federal de Minas Gerais)*

Leishmaniasis is important parasitic infections of humans and produces a wide clinical spectrum of cutaneous, mucocutaneous and visceral involvement. Its well documented that Th1 immune response is essential to prevent *Leishmania* infection. It is becoming increasingly clear that chemokines, which are chemotactic cytokines produced by leukocytes and tissue cells, play a major role in *Leishmania* infections. The *Leishmania major* infection induces the expression de various chemokines like CCL2 (MCP-1), CCL3 (MIP-1 α), CCL4 (MIP-1 β), CCL5 (RANTES), CXCL2 (MIP-2 α) and CXCL10 (γ IP-10).The paraffin is an inert agent that induces the sustained recruitment of monocytes to the site of inflammation and a local immobilization of monocytes-macrophages (Tafuri et al. 2000). The aim of the study was carried out by means of implanting paraffin tablets under the skin of BALB/c or C57BL/6 mice, experimentally infected with *Leishmania major*. The animals were sacrificed with 7, 21 and 30 days after infection, and skin and inflammatory capsule formation paraffin-induced were collected for histological and parasite load analysis. An intense chronic inflammatory reaction in both animals groups was found in skin and the capsule during the implantation of paraffin tablets associated to *L. major* infection. In addition, the parasitism load was higher in capsule than the skin sections. Thus, we quantified the parasite load using limiting dilution (Vieira, 1996). There was no statistical difference in 7 and 30 days after *Leishmania* infection, but at 21 days we note a statistical difference where C57BL/6 had higher parasitism load than BALB/c capsule. We can conclude that the capsule formation induced by the implantation of paraffin tablets showed a conspicuous histological picture characterized by a exuberant granulation tissue formation with high number of monocytes-macrophages parasitized with innumerous forms of *Leishmania*. We are looking forward to carry out RT-PCR analysis for the CCL2 and CCL5 chemokines.

IM37 - Genetic diversity influences the profile of immunoglobuline during experimental *Trypanosoma cruzi* infection

Santos, D.M. *(Universidade Federal de Ouro Preto)*; Talvani, A. *(Universidade Federal de Ouro Preto)*; Guedes, P.M.M. *(Faculdade de Medicina de Ribeirão Preto - Universidade de São Paulo)*; Veloso, V.M. *(Universidade Federal de Ouro Preto)*; Silva, R.R. *(Universidade Federal de Ouro Preto)*; Martins, T.A.F. *(Universidade Federal de Ouro Preto)*; Lana, M. *(Universidade Federal de Ouro Preto)*; Bahia, M.T. *(Universidade Federal de Ouro Preto)*

Phylogenetic diversity of *Trypanosoma cruzi* natural clones and their relevant biological properties has been suggested to explain, at least in part, the variability and complexity behind clinical course of Chagas disease. A large number of laboratory-cloned stocks have been described as a representative amount of this phylogenetic diversity of *T. cruzi*, including genotypes 19 and 20 (*T. cruzi* I lineage), genotype 32 (*T. cruzi* lineage) and genotype 39 (*T. cruzi* II lineage), each one maintaining intrinsic biological properties to host and parasite interaction. Here, we demonstrated that levels of specific humoral response (IgG, IgG1, IgG2a, IgG2b) in infected mice has been droved by genotypes 19, 20, 32 and 39 during acute and chronic phase of infection. In general, infection with *T. cruzi* I lineage have conducted high levels of all subclasses of IgG in both phases of disease. Each genotype has revealed a close relation with intensity of immunoglobulin response, in special, genotype 20 of *T. cruzi*. In addition, after treatment with benznidazole during acute and chronic phases, a significantly reduction in all IgG-subclasses was also conducted by specific genotypes of *T. cruzi*. So, this dramatic reduction of immunoglobulin levels, especially during chronic phase could be interfering in sensitivity of serological tests to specific IgG antibodies anti-*T. cruzi*. Together, our data have shown that genotype of *T. cruzi* were able to drive levels and subclasses of specific-IgG suggesting a new care about serological trials to human Chagas disease. Supported by UFOP, PROBIC/FAPEMIG.

IM38 - The Environment of Clinical Status and Parasite Load in the Erythropoiesis and Leucopoiesis of Dogs Naturally Infected by *Leishmania (Leishmania) chagasi*

Abreu, R.T. *(Universidade Federal de Ouro Preto)*; Ker, H.G. *(Universidade Federal de Ouro Preto)*; Carvalho, M.G. *(Universidade Federal de Minas Gerais)*; Giunchetti, R.C. *(Universidade Federal de Ouro Preto)*; Carneiro, C.M. *(Universidade Federal de Ouro Preto)*; Aguiar-Soares, R.D.O. *(Universidade Federal de Ouro Preto)*; Reis, A.B *(Universidade Federal de Ouro Preto)*

Canine visceral leishmaniasis (CVL) manifests itself as a broad clinical spectrum ranging from asymptomatic to patent severe disease. Herein, bone marrow (BM) smears stained by Giemsa were evaluated considering three clinical groups [i.e. asymptomatic (AD,n=34), oligosymptomatic (OD,n=22) and symptomatic (SD,n=29)] compared with non-infected dogs (NID, n=20). Parasite density was performed in bone marrow and the results expressed as "Leishman Donovan Units"(LDU index), and classified into tertiles as low (LP), medium (MP) or high (HP) parasitism. No significant differences were observed in relation to ery-

thropoiesis considering proerythroblast, basophilic, polychromatic and orthochromatic erythroblasts. However, leucopoiesis has presented some alterations in infected dogs. To date, eosinophilic number has shown a significant decrease in the different clinical groups compared to control. Neutrophilic number show slight fluctuations in the different clinical groups, however, no difference was found among them. Related to mononuclear cells, it was observed for lymphocytes number a significant increase in OD and SD groups when compared with AD group. Similar results were found for plasma cell number showing a clear tendence to a gradual increase according to the severity of the infection. By other hand, monocytes cell number has significantly decreased in all clinical groups compared to control. Parasitological assessment showed higher LDU index in SD compared with AD. Additionally, LDU values showed significant difference in HP compared with LP or MP. Moreover, our data demonstrated a positive correlation among clinical status (AD,OD,SD) and parasite density (LP,MP,HP). Our study showed that the clinical evolution of CVL promotes clear alterations in bone marrow cells. Moreover, the progression of the disease from asymptomatic to symptomatic clinical form was accompanied by intense parasitism in the bone marrow. Finally, We believe that the follow-up of these parameters could be a relevant approach when dealing with diagnosis and prognosis features. Financial support: CNPq, FAPEMIG, UFOP/UFMG.

IM39 - OXIDIZED ATP DECREASES ADHESION/INTERNALIZATION OF *Leishmania amazonensis* IN VITRO AND DISPLAYS ANTILEISHMANIAL ACTIVITY IN MICE INDEPENDENT OF THE P2X7 PURINERGIC RECEPTOR.

Figliuolo, V.R. *(Universidade Federal do Rio de Janeiro)*; Chaves, S.P. *(Universidade Federal do Rio de Janeiro)*; Guedes, H.L.M. *(Universidade Federal do Rio de Janeiro)*; Persechini, P.M. *(Universidade Federal do Rio de Janeiro)*; Coutinho-Silva, R. *(Universidade Federal do Rio de Janeiro)*; Rossi-Bergmann, B. *(Universidade Federal do Rio de Janeiro)*

P2X7 receptors (P2X7R) belong to the family of purinergic receptors activated by ATP. They have activities in physiological events like apoptosis, release of IL-1β, and a role in diseases caused by intracellular microorganisms. A common antagonist of P2X7R is oxidized ATP (oATP), that possibly affect the intracellular machinery. Previous results of our group demonstrated that treatment with oATP decreased the parasite loads in BALB/c mice infected with *L. amazonensis* -GFP. Here we went on to study its effect in cutaneous leishmaniasis using mice deficient in P2X7R . Peritoneal macrophages from BALB/c were incubated in triplicate with oATP (500 uM) for 1 h before the infection with fluorescent *L. amazonensis*-GFP promastigotes for 4 hours at 34°C. At this time, the monolayers were washed to remove free parasites and adherent/internalized parasites were quantified using a plate fluorimeter. oATP partially inhibited the adhesion/internalization of parasites when compared to untreated cells (21.377 $\pm$ 623 UF and 23.257 $\pm$ 295 UF, respectively). *In vivo*, C57Bl/6 mice deficient in P2X7R and wild-type controls were infected with promastigotes of *L. amazonensis* in the footpad, and at day 62 they were treated intralesionally twice a week, total of 6 doses with oATP 1 mM / 20 ul, and lesions sizes measured for up to 134 days. Before initiation of oATP treatment, lesions of P2X7R $^{-}/^{-}$ mice were smaller than in P2X7R $^{+}/^{+}$ mice, but increased with treatment and at the end of treatment both groups were similar. However, when the parasite loads were evaluated by limiting dilution analysis, it was revealed that oATP had antileishmanial activity in both P2X7R $^{+}/^{+}$ (8022 times) and P2X7R $^{-}/^{-}$ (865 times) mice compared to untreated controls. These results demonstrated that oATP interferes with the adhesion/internalization of *L. amazonensis in vitro* and displays antileishmanial activity in vivo in a manner independent of P2X7 receptor blockade.

IM40 - Genetic diversity influences the reactivation of experimental *Trypanosoma cruzi* infection after immunusuppression with cyclophosphamide

Santos, D.M. *(Universidade Federal de Ouro Preto)*; Silva, J.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)*

Reactivation during chronic Chagas' disease has been observed in some immunosuppressed individuals infected with *T.cruzi*. Susceptibility of recrudescence is variable among chagasic individuals and it may be related with host immune deficient or parasite genetic diversity. Our goal is evaluate the role of parasite genetic during experimental *T.cruzi* reactivation in Swiss mice with two parasite clonal stocks: *T.cruzi* I (clones named Cuicacl1, P209cl1, Gambacl1, SP104cl1) and *T.cruzi* II (clones named Bug2148cl1, MNcl2, IVVcl4, MVBcl8). These animals were treated with Cyclophosphamide (Cy) during subpatent parasitemia in acute phase (AP) and in chronic phase (CP) followed by treatment with Benznidazole (Bz). *T.cruzi* I stocks were able to induce parasitemia reactivation during AP (77,5%) and CP (51,25%) while *T.cruzi* II do it only during AP (4.7%). Capacity of reactivation was evaluated individually and percentage of AP and CP in *T.cruzi* I was: Cuicacl1 (100% and 50%), P209cl1 (100% and 20%), Gambacl1 (100% and 40%), SP104cl1 (0% and 80%). In addition, encephalic lesions were observed during CP, but not in heart or skeletal muscle after infection with clones of *T.cruzi* I and Cy treatment. Curiously, Bug2148cl1 clone of *T.cruzi* II did not affect reactivation, but induced inflammation only in heart and skeletal muscle. At least, we also observed that Bz treatment was able to prevent reactivation in mice infected with clone Cuicacl1 during CP (10%). In conclusion,

we demonstrated a direct correlation between genetic diversity of *T.cruzi* and reactivation after Cytreatment. Our data corroborate the hypothesis of clonal theory associated with *T.cruzi* and open new opportunities to clinical investigation focusing Bz as a drug to prevent reactivation. Supported by CNPq, FAPEMIG and UFOP.

IM41 - Ecto-nucleotidase activity of *Trypanosoma cruzi* increases the in vitro infection of macrophages in the presence of extracelular ATP.

Couto, W. F. *(Universidade Federal de Ouro Preto)*; Caldas, S. *(Universidade Federal de Ouro Preto)*; Bahia, M. T. *(Universidade Federal de Ouro Preto)*; Fietto, J. L. R. *(Universidade Federal de Viçosa)*; Afonso, L. C. C. *(Universidade Federal de Ouro Preto)*

Given the fact that ATP may be released from injured cells during *T. cruzi* transmission, we decided to evaluate the effect of extracellular ATP on macrophage response to infection by this parasite. Thus, thyoglicolate elicited C57BL/6 mice peritoneal macrophages were kept in culture for 7 days and were infected by amastigote and trypomatigote forms obtained from Vero cell culture at a parasite ratio of 5 parasites per macrophage. Two hours later these cells were washed and ATP was re-added. Coverslips were analyzed and the nitrite concentration was measured by the Greiss'method 72 hr after infection. ATP, at a concentration of 100 μM, triggered a decrease in NO production that was related to an increase in the percentage of infected cells and to the ratio of parasites per infected cell. In order to verify if the increase in infectivity was only due to the low production of NO, peritoneal macrophages from iNOS knockout mice were also used. In this assay, no significant effect of ATP on the infectivity of the parasite was observed regardless of the concentration used. Enzymatic assays were carried out to verify the capacity of parasite to hydrolyze extracellular nucleotides and our results showed they are able to hydrolyze ATP, ADP and AMP. To verify the possibility that the decreased NO production was due to an increase in adenosine production by the parasite's ectonucleotidases, ammonium molybdate, an inhibitor of 5'-nucleotidase, was added to the cultures at the moment of infection. The presence of 10 μM of ammonium molybdate significantly increased macrophage resistance to infection as well as NO production, indicating that the results observed by the addition of extracellular ATP were, probably, due to enzymatic conversion of ATP to adenosine, a known immunomodulatory molecule.
Financial Support: CNPq, FAPEMIG, SESu-MEC.

IM42 - MIF REGULATES MICROBICIDAL ACTIVITY OF MURINE MACROPHAGES AGAINST Toxoplasma gondii IN VITRO.

Mesquita, JS *(Universidade Federal Fluminense)*; Feijó, DF *(Universidade Federal do Rio de Janeiro)*; Bozza, MT *(Universidade Federal do Rio de Janeiro)*; Cavalcanti, MG *(Universidade Federal do Rio de Janeiro)*

Introduction: In Toxoplasmosis, host resistance is mediated by type 1 immune response which controls parasite multiplication in host tissues. Macrophage-migration inhibitory factor (MIF)is a pro-inflammatory cytokine involved in anti-parasitic immune responses. MIF can be produced by macrophages (MØ) and regulates the growth/multiplication of intra-cellular pathogens. **Objective**: To study the role of MIF in the multiplication of *T.gondii* in activated MØ. **Material /Methods**: Elicited peritoneal MØ from wild type (B6xSv129, wt) and MIF-deficient (MIFKO) mice were stimulated with LPS and IFNγ and infected with RH tachyzoits at parasite to host cell ratio of 5:1. Also, infected cells were treated with or without indomethacin . Then, cells were fixed and stained by Giemsa and cell culture supernatants were collected to determine nitrite levels. Results: Lower numbers of MIFKO MØ were infected with *T.gondii* when compared to than wt MØ (2$\pm$1 versus 20$\pm$1/100 cells, p$<$0.01). Also, decreased numbers of tachyzoits were observed in MIFKO MØ vs wt MØ (5$\pm$2 vs 62$\pm$11/100 cells, p=0.016). Indomethacin reduced both the number of infected cells and the number of tachyzoits in MØ (4 $\pm$ 2 vs 11 $\pm$ 3infected MØ /100 cells, p$<$0.01; 47$\pm$13 vs 12$\pm$ 9 parasites/100 cells, p$\pm$0.01, respectively). Reactive nitrogen intermediates (nitric oxide, RNI) production was reduced in MIFKO MØ when compared to wt MØ (22.50 $\pm$ 4.56 vs 27.01$\pm$ 4.82uM/ml, p$<$0.01).**Conclusion:** Our results suggest that MIF interferes with *T.gondii* multiplication inside macrophages by regulating eicosanoid products. Though MIF augment RNI production it fails to improve microbicidal macrophage activity against protozoa infection.
Supported by CNPq and Capes.

IM43 - DECREASE IN INFECTIVITY DUE TO MAINTENANCE OF *Leishmania (L.) amazonensis* IN CULTURE IS ASSOCIATED WITH DECREASED ECTO-NUCLEOTIDASE ACTIVITY AND IFN-γ PRODUCTION

Assis, E. A. *(Universidade Federal de Ouro Preto)*; Marques-da-Silva, E. A. *(Universidade Federal de Ouro Preto)*; Oliveira, W. S. *(Universidade Federal de Ouro Preto)*; Fietto, J. L. R. *(Universidade Federal de Ouro Preto)*; Afonso, L. C. C. *(Universidade Federal de Ouro Preto)*

It is common knowledge that maintenance of *Leishmania* parasites over many passages in culture results in decreased infectivity of the parasite. In addition, it has been documented that avirulent *Leishmania (L.) amazonensis* have decreased apyrase activity when compared to virulent parasites (Meyer-Fernandes et alli Arch. Biochem. Biophys. 341: 40, 1997). In the present study, we maintained the PH8 strain of *L. amazonensis* in culture by serial passage every 3 to 4 days for at least 100 passages. Our results

show that this procedure led to a decrease in the metacyclics yield. Furthermore, metacyclic promastigotes of high number of passages (HP) showed decreased hydrolytic activity over ATP and ADP when compared to parasites maintained for less than 15 passages (LP). When inoculated in the footpad of C57BL/6 mice, HP promastigotes induced smaller lesions than LP promastigotes. This was associated with decreased tissue parasitism and IFN-γ production by stimulated lymph node and spleen cells. No differences in IL-4 production was observed. To further characterize the involvement of ecto-nucleotidases in lesion development, we added adenosine to LP promastigotes culture, a treatment that led to decreased ATP hydrolytic activity. Adenosine-treated LP promastigotes developed smaller lesions than control parasites, confirming the involvement of this enzyme on lesion establishment. Curiously, however, this treatment did not influence the tissue parasitism after 8 weeks of infection. We speculate that the lack of effect on parasite growth might be associated with a lack of effect of adenosine treatment in the promastigote culture on the ecto-nucleotidase activity of the amastigote in the host. Finally, re-isolation of parasites from mice inoculated with HP promastigotes did not lead to a recovery of ecto-nucleotidase activity, indicating that maintenance of promastigotes in culture may select for parasites with low ecto-nucleotidasic activity.

Financial Support: FAPEMIG, CNPq, CAPES

IM44 - A murine experimental model of infection with nitric oxide and antimonium treatment resistant or susceptible *Leishmania braziliensis*.

Costa, D. L. *(Faculdade de Medicina de Ribeirão Preto)*; Carregaro, V. *(Faculdade de Medicina de Ribeirão Preto)*; Lima-Júnior, D. S. *(Faculdade de Medicina de Ribeirão Preto)*; Silva, N. M. *(Universidade Federal de Uberlândia)*; Milanezi, C. M. *(Faculdade de Medicina de Ribeirão Preto)*; Giudice, A. *(Universidade Federal da Bahia)*; Carvalho, E. M. *(Universidade Federal da Bahia)*; Almeida, R. P. *(Universidade Federal da Bahia)*; Silva, J. S. *(Faculdade de Medicina de Ribeirão Preto)*

Leishmania braziliensis is the main causative agent of cutaneous leishmaniasis in Brazil. The Th1 response, leading to nitric oxide (NO) production by macrophages results in parasites death. Th2, regulatory T cells, IL-10 and TGF-β are considered to favor parasite survival. More recently we have shown that *L. braziliensis* isolates may have different behavior in human macrophages. In order to understand the resistance mechanisms requested by *L. braziliensis*, we developed an experimental model of infection with antimonium and NO resistant or susceptible strains. Thus, BALB/c mice ears were challenged intradermally with $1x10^6$ promastimogotes of LTCP393 (resistant) or LTCP15171 (susceptible) isolates that had different behavior in human macrophages. The ear lesion size, histopathological analyses as well as cellular phenotype from inflammatory infiltrate were evaluated in different time points of infection. The results showed that, despite of infection with both *Leishmania* strains healed spontaneously, LTCP393 strain caused a larger lesion, and also took longer time to heal. Furthermore, the histopathological and flow cytometer analysis of lesions harvested from mice until 3 weeks of infection with LTCP15171 showed more granulocytes, macrophages, and lymphocytes compared with infected with LTCP393. On the contrary after the 5^{th} week of infection, the inflammatory infiltrate is higher in the lesions caused by LTCP393 reflecting a different kinetics of lesion development. In conclusion, this study shows that isolates of *L. braziliensis*, resistant or susceptible to NO and antimonium treatment, may induce different immune response and pathology in mice, as it is seem in humans, and that these characteristics have influence in the virulence of the parasite.

IM45 - Protection induced by A2 DNA, but not by NH DNA, against experimental infection with *Leishmania amazonensis*

AUAREK CAMPOS, A.C.F. *(UNIVERSIDADE FEDERAL DE MINAS GERAIS)*; ZANIN, F.H.C. *(UNIVERSIDADE FEDERAL DE MINAS GERAIS)*; MARQUES DA SILVA, E.A. *(UNIVERSIDADE FEDERAL DE OURO PRETO)*; REZENDE, S.A. *(UNIVERSIDADE FEDERAL DE OURO PRETO)*; TAVARES, C.A. *(UNIVERSIDADE FEDERAL DE MINAS GERAIS)*; CHAVEZ-OLORTEGUI, C. *(UNIVERSIDADE FEDERAL DE MINAS GERAIS)*; COELHO, E.A.F. *(UNIVERSIDAE FEDERAL DE MINAS GERAIS)*; FERNANDES, A.P. *(UNIVERSIDADE FEDERAL DE MINAS GERAIS)*

In the Americas, Leishmaniasis are caused by at least eight species of *Leishmania* and many of them have commons areas of transmission. Because the lack of effective and low-cost treatments, efforts have been employed to the development of vaccines against Leishmaniasis. Although antigenic preparations have been tested, few defined antigens were shown to be efficient against *Leishmania*. A2 antigen was identified in *L. donovani*. Karyotype analysis revealed that A2 genes are conserved in the *L. donovani, L. chagasi, L. amazonensis* and *L. mexicana* species. In vaccination protocols, it was demonstrated that immunization with recombinant A2 protein protects BALB/c mice against *L. donovani* and *L. amazonensis* infection. Nucleoside hydrolase (NH) was identified in *L. donovani*. This surface glycoprotein is expressed in *L. donovani, L. chagasi, L. major* and *L. amazonensis* species. Paraguai-de-Souza et al. (2001) demonstrated that immunization with recombinant NH protein was able to induce protection in BALB/c mice against *L. donovani* and a partial protection was obtained against *L. chagasi* and *L. mexicana*. In this work, we investigated the protective effect of A2 DNA and NH DNA, in BALB/c mice, against *L. amazonensis* challenge infection. Immunization with either A2 DNA or NH DNA was able to induce an elevated Th1 immune response (IFN-g production) prior to challenge infection; however, only mice immunized with A2 DNA were

protected against *L. amazonensis*. An elevated IFN-g production, low IL-4 cytokine and anti-SLA *L. amazonensis* antibodies levels were detected in these mice, after challenge infection.

IM46 - Insulin-like growth factor (IGF)-I expression is affected by Th1 and Th2 cytokines in Leishmânia (L.) major-infected macrophages

Petitto-Assis, F.P.A. *(Instituto de Medicina Tropical)*; Tonon, G.S.T. *(Instituto de Medicina Tropical)* Goto, H.G. *(Instituto de Medicina Tropical)*

IGF-I induces proliferation and differentiation of different cells and we have previously observed its in vitro and in vivo effect on Leishmania growth induction and decreased nitric oxide (NO) production by Leishmania-infected macrophages. Since it is known that macrophage IGF-I production is down-regulated by IFN-gamma but increased by IL-4 and IL-13, cytokines related respectively to resistance and susceptibility in experimental leishmaniasis, we analyzed whever their effect on parasitism is related to IGF-I expression (evaluated by Real-Time PCR) in BALB/c peritoneal macrophages. 2x105/well macrophages were infected with L. (L.) major amastigotes or stationary phase promastigotes (parasites/macrophages = 2:1) and were incubated with rIFN-gamma (200U/mL) or rIL-4 (2ng/mL) and rIL-13 (5ng/mL). IGF-I expression was significantly increased with infection of macrophages even in the absence of cytokines. Cytokines had diverse effects depending on mouse strains. IFNgamma induced significantly increased NO production by macrophages. IL-4 and IL-13 induced increased expression of IGF-I in macrophage as well as its increased parastisim. The data suggest interference of IGF-I expression on the effect of TH1 and TH2 cytokines in Leishmania-infected macrophages. Supported by: FAPESP, CNPq, FINEP and LIM-38 (HC-FMUSP)

IM47 - The role of CCR2 in the oral infection by *Toxoplasma gondii*

Benevides, L. *(Faculdade de Medicina de Ribeirão Preto-USP)*; Yamauchi, L.M. *(Universidade Estadual de Londrina)*; Milanezi, C.M. *(Faculdade de Medicina de Ribeirão Preto-USP)*; Canetti, C. *(Universidade Federal da Rio de Janeiro)*; Silva, J.S. *(Faculdade de Medicina de Ribeirão Preto-USP)*; Silva, N.M. *(Universidade Federal de Uberlândia)*

Introduction and Objectives: *Toxoplasma gondii* is an obligate intracelular protozoan. It is estimated that about one-third of world population is infected with the parasite. C57BL/6 mice orally infected with a high parasitic load develop serius intestinal lesions, whose injuries are similar to those observed in Inflammatory Bowel Disease. These injuries depend on the activation of the exacerbated Th1 immune response induced by the parasite. Morever, chemokines produced by intestinal epithelial cells are involved in the migration and activation of inflammatory cells. In order to verify the role of CCR2 in the oral *T. gondii* infection, we inoculated 5 ME-49 *T. gondii* cysts by gavage, and the survival was monitored daily and histopathological changes, tissue parasitism and immune parameters were analyzed at day 8 post-infection. Methods and Results: The CCR2$^{-/-}$ mice were highly susceptible to infection and the majority of them died at acute phase. The CCR2$^{-/-}$ mice presented a higher parasite burden in brain, lung, and small intestine on day 8 pos-infection compared to C57BL/6 (WT) mice. The WT mice presented severe inflammatory alterations in the lung and liver. In addition, the small intestine of these animals presented an intense inflammatory infiltration in the lamina Propria and submucosa and in some areas a reduced length and increased thickness of the villi were verified. In contrast, *T. gondii* infection induced only mild inflammatory lesions in the lung, liver and small intestine in CCR2$^{-/-}$ mice. In accordance a higher CD4^{+} and MAC-1^{+} cells and decrease CD8^{+} cells migration were observed in the small intestine of WT compared to CCR2$^{-/-}$ mice. Conclusion: The CCR2 are crucial to control *T. gondii* infection, despite involved in inflammatory reaction induced by the parasite that could be detrimental to the host. Financial support: CAPES

IM48 - Role of CCR2 in Resistance to Oral Infection with *Toxoplasma gondii*

Fux, BF *(Washington University School of Medicine in St. Louis)*; DaMatta, RD *(Washington University School of Medicine in St. Louis)*; Dunay, ID *(Washington University School of Medicine in St. Louis)*; Sibley, LDS *(Washington University School of Medicine in St. Louis)*; Presti, RP *(Washington University School of Medicine in St. Louis)*; Colonna, MC *(Washington University School of Medicine in St. Louis)*

Toxoplasma gondii is an important foodborne pathogen that infects a wide range of animals following oral ingestion of tissue cysts from chronically infected animals. We have used oral challenge of C57BL/6 mice to study the immune response following the natural route of infection. In the present study, we compared the survival of MCP1-/- and CCR2-/- mice after peroral infection with in vivo produced bradyzoites of T. gondii. While the majority of C57BL/6 mice survive oral challenge with a low dose of cysts from the Type II strain (ME49-B7), MCP1-/- and CCR2-/- mice rapidly succumb to infection by 15 days. CCR2-/- and MCP1-/- animals developed normal levels of serum IL-12 and IFN-gamma like controls and only modest differences in parasite burdens in the spleen were noted (2-3 fold higher in knock out mice). These data suggest that induction of systemic immunity, and dissemination of the parasite from the gut, are not significantly affected in the absence of this chemokine-receptor pair. In contrast to wild type mice, CCR2-/- and MCP1-/- mice developed severe intestinal necrosis, characterized by influx of neutrophils, high levels of parasites, and tissue destruction. These studies indicate a critical role for

cellular recruitment by CCR2-/- in protecting against oral infection with *T. gondii*.

IM49 - BYSTANDER SUPPRESSION IN LEISHMANIA AMAZONENSIS INFECTED MICE. MODEL: MICE GENETICALLY SELECTED ACCORDING TO ORAL TOLERANCE SUSCEPTIBILITY

Tavares, D *(Universidade do Estado do Rio de Janeiro)*; Pereira, A. *(Centro Universitário Celso Lisboa)*; Xavier, A. *(Universidade Federal Fluminense)*; da Silva, A. C. *(Universidade do Estado do Rio de Janeiro)*

Two strains of mice selected according to extreme phenotypes of susceptibility and resistance to oral tolerance (TS and TR mice, respectively) have inflammatory lesion and parasite load inversely associated in Leishmania amazonensis infection. TS mice developed a minor pathology while permitting parasite growth, in contrast, in TR mice, footpad swelling was increased ($P < 0.05$ after the fourth week) but parasite growth was reduced (4x). To directly address the influence of oral tolerance on infection, mice were submitted to a different bystander suppression protocols. Mice were gavaged with OVA, and 7 days afterwards were infected with 1 x 107 Leishmania amazonensis promastigotes and challenged to bystander suppression with OVA in the same footpad. In TR mice gavaged with 25 mg OVA the inflammatory lesion was largely enhanced ($p = 0{,}001$ in the eighth week), while with 5 mg OVA the lesion was diminished ($p = 0{,}015$ in the eighth week). The bystander effect did not modify the establishment of infection; and similarly to the control non-bystander mice, parasite clearance was maintained in TR mice. In TS mice, the footpad swelling was always lower, wherever, the parasite burden was diminished in TS mice gavaged with 5 mg OVA (32x). A better comprehension of immunoregulation of innate and adaptive immunity in the early stages of infection is necessary for the development of protocols preventing inflammation and contributing to the elimination of parasites. Financial support: grants from FENORTE and FAPERJ.

IM50 - IMMUNOPATHOLOGICAL STUDY IN LYMPH NODES AND SPLEEN OF DOGS NATURALLY INFECTED WITH *L. (L.) chagasi.*

Ibarra, M.C.F. *(Faculdade de Medicina da USP)*; Chassot, C. *(Faculdade de Medicina da USP)*; Larangeira, D.F. *(Faculdade de Medicina da USP)*; Rizzardi, R.L. *(Faculdade de Medicina da USP)*; Tomokane, T.Y. *(Faculdade de Medicina da USP)*; Corbett, C.E.P. *(Faculdade de Medicina da USP)*; Laurenti, M.D. *(Faculdade de Medicina da USP)*

The objective of this study was to correlate the clinical, serological and parasitological diagnosis with the expression of T $CD3^+$ cells and macrophages in lymph nodes and spleen of dogs naturally infected with *L. (L.) chagasi*. Ninety-eight dogs from the Zoonosis Control Center of the city of Araçatuba, sacrified during 2005, were used. Clinical features, direct parasite search in lymph nodes and spleen smears, as well as antibody detection by ELISA were performed for the diagnosis. According to the clinical signs, the dogs were divided into two groups: symptomatic and asymptomatic. Paraffin-embedded lymph nodes and spleen tissues stained by HE were used for histopathological analysis; parasitemia and the expression of macrophages and T $CD3^+$ cells was evaluated by immunohistochemistry using the avidin-biotin method. The quantitative analysis of macrophages and T $CD3^+$ cells was performed with the image analysis system (Axyovision-Zeiss). According to the clinical signs, 45 dogs were classified as asymptomatic and 53 as symptomatic; 42% of asymptomatic and 77% of symptomatic dogs showed positive serological and/or parasitological diagnosis. Histopathologically, lymph nodes showed macrophage hyperplasia and hypertrophy in the medullary area and in many cases granulomatous lymphadenitis. In the white pulp of the spleen, follicular hyperplasia was observed and in a few cases hypoplasia and atrophy was present; and the red pulp showed granulomas. Immunohistochemistry showed increase of macrophages and T $CD3^+$ cells in the symptomatic and asymptomatic infection, but macrophage density was higher in the lymph nodes of symptomatic dogs. The ratio of T $CD3^+$ cells/macrophages was higher in asymptomatic dogs. The results showed more evident inflammatory reaction and parasitemia in lymph nodes and spleen of symptomatic dogs as well as macrophage expression. On the other hand, cellular immune response characterized by T $CD3^+$ cells was higher in asymptomatic dogs. Supported by FAPESP and LIM50 HC-FMUSP.

IM51 - CR3 cell expression in lymph nodes of dogs naturally infected with *Leishmania (Leishmania) chagasi* and its correlation with the parasite tissue load and animal clinical status

Lima, W.G. *(Universidade Federal de Minas Gerais)*; Caliari, M.V. *(Universidade Federal de Minas Gerais)*; Moura, E.P. *(Universidade Federal de Minas Gerais)*; Melo, F. A. *(Universidade Federal de Minas Gerais)*; Michalick, M. S. M. *(Universidade Federal de Minas Gerais)*; Tafuri, W. L. T. *(Universidade Federal de Minas Gerais)*; Tafuri, Wg. L. *(Universidade Federal de Minas Gerais)*

Visceral leishmaniasis (VL) is caused by a protozoa of the *Leishmania* genus. In canine visceral leishmaniasis (CLV) the success of the parasite infection is dependent of this early interaction of the parasites and dog mononuclear phagocytes cells. Complement receptors type 3 (CR3) appears to make quantitatively greater contribution to this interaction. Recently Lima et al (2007) demonstrated a higher expression of CR3 in liver asymptomatic naturally infected dogs associated

a lower parasitism tissue load. In another hand, the same author demonstrated a higher expression of CR3 in spleen of symptomatic animals associated with a higher parasite tissue load. The aim of this work is evaluate the CR3 expression in lymph nodes of naturally infected dogs and its association with the parasite tissue load and a defined animal clinical status. Asymptomatic and symptomatic animals obtained from municipality of Sabará/MG were sacrificed with lethal dose of Sodic Thiopental 2,5% and T61 (Intervet). Axillary, cervical and popliteal lymph nodes samples were collected and these samples were fixed in buffer formalin 10% solution for histological study and immunocytochemical *Leishmania* amastigotas quantification. For the CR3 assessment we used the immunocytochemical method in frozen lymph node tissue sections to determinate the CR3 cell expression. Immunolabeled amastigotas and CR3 cell expression were quantified by morphometrical analysis using a KS300 software. Our results indicate a higher tissue parasitism in symptomatic animals for all studied lymph nodes (statistical difference in cervical lymph node: $p = 0{,}0421$; kruskall-waills). Moreover the CR3 cell expression was higher in symptomatic dogs that asymptomatic ones for all lymph nodes. Thus, CR3 expression has a positive correlation with a tissue parasite load. Our study expose to lymph nodes a similarly spleen CR3 expression and tissue parasitism load. These results can indicate that primary lymphoid organs could be maintained the infection by *Leishmania* in dogs.

IM52 - Immunopathological assessment of the popliteal lymph node in canine visceral leishmaniasis

Giunchetti, R.C. *(Universidade Federal de Ouro Preto)*; Martins-Filho, O. *(Instituto René Rachou)*; Carneiro, C.M. *(Universidade Federal de Ouro Preto)*; Mayrink, W. *(Universidade Federal de Minas Gerais)*; Marques, M.J. *(Universidade Federal de Alfenas)*; Tafuri, W.L. *(Universidade Federal de Ouro Preto)*; Roatt, B.M. *(Universidade Federal de Ouro Preto)*; Aguiar-Soares, R.D.O. *(Universidade Federal de Ouro Preto)*; Corrêa-Oliveira, R. *(Instituto René Rachou)*; Reis, A.B. *(Universidade Federal de Ouro Preto)*

Although enlargement of popliteal lymph nodes (LN) is frequently described in canine visceral leishmaniasis (CVL), there are only a few histopathologic studies of lymph nodes during these chronic immunopathological condition. Besides a detailed histopathologic analysis, herein we have characterized the parasite load and major immunophenotypic features of the LN in *Leishmania (Leishmania) chagasi* infected dogs. Our major histopathological findings highlighted the hypertrophy/hyperplasia of LN cortical zone as the principal characteristic observed in asymptomatic dogs (AD), whereas atrophy of LN cortical zone was predominant in symptomatic animals (SD). Moreover, hypertrophy/hyperplasia of LN medullary zone was also the hallmark of asymptomatic disease. The LN parasite density detected by anti-*Leishmania* immunohistochemical assay or expressed as Leishman Donovan Units was highly correlated with also the skin parasitism, the most reliable to decode the clinical status of CVL. The major LN immunophenotypic changes during ongoing CVL were represented by increased frequency of T-lymphocytes, particularly CD8+ T-cells, besides up-regulation of MHC-II expression by lymphocytes and the decreased levels of CD21+ B-cells. Our findings further demonstrated that changes in the LN B-lymphocyte compartment exhibited a negative correlation with the skin parasite load. On the other hand, our findings showed evidences for a positive association between the skin parasitism and the LN T-cell mediated immunity, suggesting that T-cells, mainly CD8+ lymphocytes, may have a distinct role in this lymphoid tissue in response to CVL. Financial support: CNPq, FAPEMIG, UFOP/UFMG.

IM53 - PLASMODIUM VIVAX: ROLE OF THE MALARIAL VACCINE CANDIDATES PV-AMA-1 AND PV-MSP-119 ON INNATE IMMUNE SYSTEM

Bueno, L. L. *(Universidade Federal de Minas Gerais)*; Fujiwara, R. T. *(Instituto Rene Rachou)*; Morais, C. G. *(Universidade Federal de Minas Gerais)*; Soares, I.S. *(Departamento de Análises Clinicas e Toxicológicas, USP)*; Braga, E.M. *(Universidade Federal de Minas Gerais)*

Malaria is one of the main causes of morbidity and mortality in the tropics and subtropics areas of the world. An effective vaccine against this parasitic disease is needed as its dissemination tends to increase. AMA-1 and MSP-1_19 are considered the two main targets of a protective vaccine. Although the protective effect of these antigens has been demonstrated in animal models, little is known about its role on the innate immune response. In the present work, we aimed to assess the role of Pv-AMA-1 and Pv-MSP-1_19 in the cytokine/chemokine production from peripheral blood mononuclear cells (PBMCs) and on in vitro differentiation of dendritic cells (DCs) from healthy non-exposed donors. PBMCs were isolated and cultivated in the presence of Pv-AMA-1 or Pv-MSP-1_19. Levels of IFN-g, TNF-a, IL-10, IL-4, IL-5 and IL-2 were quantified by cytometric bead array. Chemokines production (TARC/CCL17 and MIP-1a /CCL3) were evaluated by sandwich ELISA. To differentiation of DCs, monocytes were purified from PBMCs using magnetic cell sorting technique and incubated with IL-4/GM-CSF in the presence of Pv-AMA-1 or Pv-MSP-1_19. The phenotypic profile of cell surface markers on DCs (CD11c, CD1a, HLA-ABC, HLA-DR, CD80, CD86, CD40, CD64, CD16 e CD14) was characterized by flow cytometry. Pv-AMA-1 induced a significant production of IFN-g,TNF-a, IL-10, CCL17 and CCL3. In addition, the differentiation of DCs with Pv-AMA-1 elicited the upregulation of CD80 and CD64 expression while it modulated the expression of CD11c, CD40 and CD86. No significant production of cytokines/chemokines and alteration on DC phenotype were observed on cultures stimulated with Pv-MSP-1_19 when compared to the controls. Pv-AMA-1 could play a direct role on innate immune system inducing cytokine and chemokine

production that might be involved in the parasite destruction. Moreover, it regulates the expression of cell surface molecules on antigen presenting cells.

IM54 - Adaptive immunity in subcutaneous *Trypanosoma cruzi* infection is modulated by an innate pathway involving interdependent signaling of dendritic cells by C5a anaphylatoxins and bradykinin.

Schmitz, V. *(Instituto de Biofísica Carlos Chagas Filho)*; Monteiro, A. C. *(Instituto de Biofísica Carlos Chagas Filho)*; Svensjo, E. *(Instituto De Biofísica Carlos Chagas Filho)*; Kohl, J. *(Cincinnatti Children's Hospital Medical Center)*; Scharfstein, J. *(Instituto de Biofísica Carlos Chagas Filho)*

The parasitic protozoan *T. cruzi* activates the kinin system through the activity of its major cysteine protease, cruzipain (CZP). Using a subcutaneous model of infection in mice, we recently showed that endogenously released kinins activate immature dendritic cells via B_2-bradykinin receptors (B_2R), thus linking innate to adaptive immunity (Monteiro et al., 2006). Here we report that tissue culture trypomastigotes (TCT) are able to proteolytically liberate the C5a anaphylatoxins from native C5 via CZP. Analysis of splenic $CD11c^+$ DCs incubated with TCT showed that IL-12p70 were either blocked by B_2R antagonist (HOE-140) or by C5aR antagonist (C5aRA), suggesting that DC activation depends on cross-talk between these two GPCRs. We then asked if C5aRA could mitigate kinin/B_2R function in vivo. Analysis of TCT-induced vascular permeability responses revealed that C5aRA reduced plasma leakage. Studies in the mice infected by the s.c. route showed that C5aRA blocked the paw edema responses induced by TCT, irrespective of presence/absence of inhibitors of ACE, a kinin-degrading metallopeptidase. Combined, these data suggested that activation of the C5a/C5aR signaling pathway favors parasite-mediated generation of kinins in peripheral tissues. We then checked if C5aRA injection interfered with the vigorous type-1 responses otherwise induced by the innate kinin/B_2R-dependent pathways. Indeed, recall assays showed that C5aRA treatment led to decreased IFN-γ production by Ag-specific T cells, this effect being coupled to upregulated IL-4 production. In summary, we show evidence for interdependent roles of C5aR and the B_2R in the regulation of innate/adaptive immunity in T. cruzi infection. Support: FAPERJ, CNPq

IM55 - Activation of the innate kinin pathway is critical for development of host resistance to acute infection with T. cruzi

Monteiro, A.C.M. *(Instituto de Biofísica carlos Chagas Filho)*; Schmitz, V.S. *(Instituto de Biofísica carlos Chagas Filho)*; Arruda, L.A. *(Universidade Federal do Rio de Janeiro)*; Granato, A.G. *(Instituto de Biofísica carlos Chagas Filho)*; Morrot, A.M. *(NIAID)*; Pesquero, J.P. *(Departamento de Biofísica)*; Tanowitz, H.T. *(Albert Einstein College of Medicine)*; Muller-Esterl, W.ME. *(University of Frankfurt)*; Scharfstein, J.S. *(Instituto de Biofísica Carlos Chagas Filho)*

The concept that bradykinin drives Th1 polarization by stimulating dendritic cell differentiation through B_2 R (Aliberti et al., 2003) was recently validated in a mouse model of subcutaneous *T. cruzi* infection (Monteiro et al., 2006). Here we investigated the role of the innate kinin pathway in host resistance mechanisms. Intraperitoneal injection of *T. cruzi* trypomastigotes into C57BL/6 WT ($B_2R^+/+$) and $B_2R^-/-$ mice (i.p. route) showed that $B_2R^-/-$ mice displayed a higher blood parasitemia and accelerated mortality. Real time PCR showed that parasite burden was sharply increased in $B_2R^-/-$ mice. Consistent with this, analysis of Ag-specific recall responses of T cells isolated from heart showed that IFN-γ production was severely decreased in $B_2R^-/-$ mice. In contrast, splenic T cells ($CD4^+$and $CD8^+$) from both mice strains initially responded vigorously, however, the type-1 response of $B_2R^-/-$ mice decayed sharply in spleen as the infection progressed. Considering that *T. cruzi* drive DC maturation in vitro through B_2R, we then asked if the increased susceptibility of $B_2R^-/-$ mice resulted from primary deficiency in DC signaling by kinins. Indeed, adoptive transfer of splenic $B_2R^+/+$ $CD11c^+$ DCs into susceptible $B_2R^-/-$ mice conferred a resistant phenotype to the recipient $B_2R^-/-$ mice and the reversal of $B_2R^-/-$ phenotype was associated with fully restored INF-γproduction by both $CD4^+$ and $CD8^+$ T cells. Collectively, these results demonstrate that the susceptible phenotype of $B_2R^-/-$ mice is caused by deficient sensing of endogenously released kinins by DCs. Ongoing studies should clarify if kinins, here defined as damage-associated signal, influence differentiation, maintenance and/or migration of effector/memory T cells.

IM56 - Innate immunity against Trypanosoma cruzi

Lima, BHF *(Instituto René Rachou)*; Lima, GK *(Universidade Federal de Minas Gerais)*; Mansur, DS *(Universidade Federal de Minas Gerais)*; Brandi, MC *(Instituto René Rachou)*; Gazzinelli, RT *(Instituto René Rachou)*; Campos, MA *(Instituto René Rachou)*

It is well documented the high levels of cytokines produced, as TNF alpha and IFN gamma, during acute episodes of infection caused by strain Y of Trypanosoma cruzi. We have previously studied the role of TLR-2 and MyD88 in the immune response of mice infected with T. cruzi. TLR-2 works as heterodimer with TLR-1 or TLR-6. Here, we evaluate the parasitemia and mortality, as well the cytokines levels produced by TLR-deficient mice after infection with Colombiana strain of Trypanosoma cruzi. TLR4 or TLR-6 deficient mice infected by T. cruzi have a similar parasitemia and mortality as wild type mice. The levels of cytokines (IL-12,

TNF alpha and IFN gamma) from splenocytes supernatants of TLR4 knockout mice were also similar to the levels from cytokines of wild type mice, but the TLR-6 knockout mice have higher levels of IFN gamma when compared to wild type mice. TLR9 knockout mice have a statistic higher mortality and lower levels of cytokines in supernatants of splenocytes when compared to wild type mice. Further, production of cytokines in mice heart tissue from wild type or TLR-deficient mice infected by T. cruzi will help us to understand better the Chagas's Disease pathology.

IM57 - TLR2-induced secretion of CXC chemokine by macrophages governs *Trypanosoma cruzi* ability to activate dendritic cells via the B_2-bradykinin receptor

Schmitz, V. *(Instituto de Biofísica Carlos Chagas Filho)*; Almeida, I. *(University of Texas)*; Teixeira, M.M. *(Universidade Federal de Minas Gerais)*; Scharfstein, J. *(Instituto de Biofísica Carlos Chagas Filho)*

Using a subcutaneous mouse model of infection, we recently documented that kinins generated by *T. cruzi* trypomastigotes (TCT) stimulate dendritic cell maturation through the activation of B_2-bradykinin receptors (B_2R) (Monteiro et al., 2006). Analysis of the dynamics of inflammation showed that parasite-mediated release of kinins in peripheral tissues depends on the availability of kininogens in the inflamed tissues. We further reported that TLR2-driven inflammation induced by microbial signatures (eg. GPI) induces plasma leakage, allowing for cruzipain-mediated processing of the kininogen, further downstream in the dynamics of inflammation. In the present work, we investigated the possibility that kinin generation might depend on earlier events, such as CXC chemokine secretion by innate cells activated by TCT. Our results show that wild type resident macrophages, but not their TLR2$^{-/-}$ counterparts, promptly secrete CXC chemokines KC and MIP-2 upon incubation with TCT. Controls showed that parasite developmental forms that lack the TLR2 ligand, tGPI-mucin, failed to induce CXC chemokines by WT macrophages. Addition of Captopril (inhibitor of ACE, a kinin-degrading metallopeptidase) mildly increased the KC/MIP-2 response evoked by TCT, and the effect was blocked by B_2R antagonist. In vivo experiments revealed that CXCR2 antagonist blocked the kinin-driven edema that TCT otherwise evoke in captopril-treated mice, or in normal mice. Furthermore, the paw edema induced by TCT was nullified in Captopril-treated mice depleted of neutrophils. Collectively, our data suggest that CXC chemokine secretion by TLR2-activated macrophages favor generation of kinins, a potent effector of innate immunity in *T. cruzi* infected mice. Support: CNPq and FAPERJ

IM58 - Targeting of dendritic cells with bradykinin in vivo: a novel strategy for type-1 adjuvant development in vaccines

Monteiro, A.C. *(Instituto de Biofísica Carlos Chagas Filho)*; Schmitz, V. *(Instituto de Biofísica Carlos Chagas Filho)*; Arruda, L.B. *(Universidade Federal do Rio de Janeiro)*; Muller-Esterl, W. *(University of Frankfurt)*; Morrot, A. *(4Intracellular Parasite Biology Section, Laboratory of Parasite Disease, NIAID)*; Scharfstein, J. *(Instituto de Biofísica Carlos Chagas Filho)*

We have recently demonstrated that host resistance to lethal *T. cruzi* infection depends on dendritic cell activation by endogenously released kinins, this innate response being critically required for development of Ag-specific $CD4^+$ and $CD8^+$ memory/effectors T cells (Monteiro et al., submitted). Here we evaluated if inclusion of synthetic bradykinin in alum-based vaccine formulations might likewise target B_2R of immature DCs, inducing protective immunity ($CD4^+$/$CD8^+$ T cell-dependent) against intracellular pathogens. Turning to the i.p. mouse model of *T. cruzi* infection, we chose to use soluble extracts derived from epimastigotes (Epi) (i.e., avirulent *T. cruzi* stages) as the vaccine immunogen because these Ags fail to induce protective immunity by conventional vaccination protocols andlack proinflammatory TLR ligands (i.e, do not possess intrinsic adjuvanticity). BALBc male mice pre-treated or not with the ACE inhibitor, captopril were immunized with alum-based Epi-Ag suspensions containing synthetic BK. After defining booster conditions, we found that mice vaccinated with the optimal formulation were fully protected, the resistance being associated with potent induction of IFN-γ responses by Ag specific $CD4^+$ and $CD8^+$ T cells, coupled to IgG isotype switching (IgG1¿IgG2a). Consistent with our hypothesis, the benefits of BK based vaccine were cancelled by pre-treating mice with HOE 140. Adoptive transfer of IFN-γ producing $CD8^+$/$CD44^+$ T cells or $CD4^+$/$CD44^+$ T cells isolated from the spleen of vaccinated mice into naive recipient BALB/c demonstrated that the $CD8^+$ T subset is the principal effector of protective immunity induced by BK-based vaccine formulations. Support by CNPq, CAPES and FAPERJ

IM59 - EVALUATION OF THE ROLE OF NEUTROPHILS IN A CUTANEOUS LEISHMANIASIS EXPERIMENTAL MODEL OF INFECTION.

Santiago, R. C. *(Centro de Pesquisa Gonçalo Moniz - Fiocruz)*; Novais, F. O. *(Centro de Pesquisa Gonçalo Moniz - Fiocruz)*; de Moura, T. R. *(Centro de Pesquisa Gonçalo Moniz - Fiocruz)*; Clarencio, J. *(Centro de Pesquisa Gonçalo Moniz - Fiocruz)*; Bafica, A. *(Universidade Federal de Santa Catarina)*; Barral-Neto, M. *(Centro de Pesquisa Gonçalo Moniz - Fiocruz)*; Barral, A. *(Centro de Pesquisa Gonçalo Moniz)*; Bradskyn, C. *(Centro de Pesquisa Gonçalo Moniz - Fiocruz)*; de Odliveira, C. I. *(Centro de Pesquisa Gonçalo Moniz - Fiocruz)*

Neutrophils are important components of the immune system and provide the first mechanism of defense against infection. In the dermal experimental model of infection with *L. braziliensis*, we observed that neutrophils are constantly recruited to the lesion site, throughout the infection period. Moreover, it was observed that the interaction between macrophages and neutrophils regulates infection with *L. major*. In the present work we investigated the role of neutrophils in the infection caused by *Leishmania braziliensis* using a mouse intradermal model of infection. Neutrophils were obtained from mouse peritoneal cavities by injection of thioglycolate broth, purity was assessed by flow cytometry using Gr-1 staining. Live neutrophil incubation with *L. braziliensis*-infected peritonial macrophages from BALB/c mice led to a significant decrease in the infection rate as well as in the number of amastigotes per cell when compared to controls. To investigate the role of neutrophils in the *in vivo* infection, BALB/c mice were co-inoculated with *L. braziliensis* and neutrophils. In these experiments, we observed that co-inoculation of live neutrophils and *L. braziliensis* in the ear dermis led to the development of smaller lesions as well as a decrease in parasite load. On the contrary, when mice were depleted of neutrophils by injection of RB6-8C5 antibody, we observed that depleted animals showed an increase in lesion size and in parasite load when compared to animals injected with control rat IgG antibody. According to our data, neutrophils are essencial in the inicial elimination of *L. braziliensis* in BALB/c mice.

IM60 - *Leishmania amazonensis* promastigotes induce, bind to and are killed by neutrophil extracellular trap.

Costa, A.G.B. *(Universidade Federal do Rio de Janeiro)*; Froment, G.S. *(Universidade Federal do Rio de Janeiro)*; Teodoro, C.R.S. *(Universidade Federal do Rio de Janeiro)*; Saraiva, E.M. *(Universidade Federal do Rio de Janeiro)*

Neutrophils upon activation can release fibers formed by granule proteins and chromatin, named neutrophil extracellular traps . This structure binds and kills bacteria and fungi, prevents them from spreading and ensures a high local concentration of antimicrobials, as part of innate immunity. *Leishmania* infection starts with the sand fly injection of promastigotes into a blood pool. Neutrophils are the first leukocytes that encounter the parasites and the first that migrate to the site of infection. Here we investigated the role of NETs in *Leishmania amazonensis* infection. Human neutrophils purified by gradient centrifugation followed by red blood cell lysis, were activated with PMA for 20 min and than *L.amazonensis* promastigotes were added to the culture for more 1h. Slides were then stained with DAPI, anti-elastase or anti-histone antibodies, showing promastigotes bound to the NETs. Next, we studied the leishmanidal activity of NETs. Neutrophils were activated with PMA for 20 min, followed by cytochalasin D or DNAse treatment for more 30 min, and addition of parasites. After 2 hs incubation, fetal calf serum was added and cultures were incubated for 2 days for promastigote growth. Activation with PMA increased promastigote killing by 65% compared to non-activated neutrophils. Phagocytosis inhibition of activated neutrophils by cytochalasin D decreased 45% the parasite killing. NET disruption by DNAse addition to the interaction medium similarly decreased *Leishmania* death. To determine whether these NETs can form in response to *L.amazonensis*, neutrophils were incubated with promastigotes for 1h at different parasite neutrophil ratios, followed by the addition of restriction enzymes for 2h. DNA content determined in culture supernatants by a fluorescent dye, showed a concentration dependent NET induction by *L. amazonensis*. Our results show that *L. amazonensis* promastigotes induce NET‘s formation, are trapped by NETs and are susceptible to NET-mediated killing. Supported by: Capes, CNPq, Faperj.

IM61 - Immunotherapy against canine visceral leishmaniasis with the saponin enriched Leishmune vaccine

Nunes Santos, F.N.S. *(Universidade Federal do Rio de Janeiro)*; Moreira Myashiro, L.M.M *(Universidade Federal do Rio de Janeiro)*; Borja-Cabrera, G.P.B.C. *(Universidade Federal do Rio de Janeiro)*; Menz, I.M. *(Fort Dodge Animal Health, Ltda)*; Palatnik de Sousa, C.B.P.S. *(Universidade Federal do Rio de Janeiro)*

Zoonotic visceral leishmaniasis (ZVL) is a re-emergent canid zoonoses, the epidemiological control of which in Brazil involves the elimination of infected dogs. In order to asses the immunotherapeutic potential on ZVL of the Leishmune®-vaccine, formulated with an increased adjuvant concentration (1mg of saponin rather than 0.5mg), 24 mongrel dogs were infected with Leishmania (L.) chagasi. The vaccine was injected on month 6, 7 and 8 after infection, when animals were seropositive and symptomatic. Control group received saline. Leishmune®-treated dogs showed higher levels of anti-FML IgG antibodies (ANOVA; $p<0.0001$), a higher and stable IgG2 and a decreasing IgG1 response, pointing to a TH1 T cell mediated response. The vaccine had the

following effects: it led to more positive delayed type hypersensitivity reactions against Leishmania lysate in vaccinated dogs (75%) than in controls (50%), to a decreased average of CD4+ Leishmania-specific lymphocytes in saline controls (32.13%) that fell outside the 95% confidence interval of the vaccinees (41.62%, CI95% 43.93-49.80) and an increased average of the clinical scores from the saline controls (17.83) that falls outside the 95% confidence interval for the Leishmune® immunotherapy-treated dogs (15.75, CI95% 13.97-17.53). All vaccinated dogs were clustered, and showed lower clinical scores and normal CD4+ counts, whereas 42% of the untreated dogs showed very diminished CD4+ and higher clinical score. The increase in clinical signs of the saline treated group was correlated with an increase in anti-FML antibodies ($p<0.0001$), the parasitological evidence ($p= 0.038$) and a decrease in Leishmania-specific CD4+ lymphocyte proportions ($p=0.035$). These results confirm the immunotherapeutic potential of the enriched-Leishmune® vaccine. The vaccine reduced the clinical symptoms and evidence of parasite, modulating the outcome of the infection and the dog´s potential infecciosity to phlebotomines. The enriched Leishmune® vaccine was subjected to a safety analysis and found to be well tolerated and safe.

IM62 - Immunomodulatory and pro-inflammatory responses induced by *Trypanosoma cruzi* glycoinositolphospholipid (GIPL).

Oliveira, A. C. *(Universidade Federal do Rio de Janeiro)*; Medeiros, M. M. *(Universidade Federal do Rio de Janeiro)*; Previato, J. O. *(Universidade Federal do Rio de Janeiro)*; Mendonça-Previato, L. *(Universidade Federal do Rio de Janeiro)*; Nóbrega, A. *(Universidade Federal do Rio de Janeiro)* Bellio, M. *(Universidade Federal do Rio de Janeiro)*

We have recently demonstrated that the glycoinositolphospholipid (GIPL) from the surface of the protozoan parasite *Trypanosoma cruzi* is a Toll-like receptor 4 (TLR4) agonist with proinflammatory effects (Oliveira, A-C. *J. Immunol.* 173:5688). Here, we show that GIPL-induced neutrophil recruitment into the peritoneal cavity is mediated by at least two pathways: one where IL-1 β acts downstream TNF- α, and a second, which is IL-1 β- and TNFRI-independent. As a consequence of this inflammatory response, spleen and lymph nodes of GIPL-treated mice have an increase in the percentage of both T and B cells expressing the CD69 activation marker. Cell transfer experiments demonstrate that T and B cell activation by GIPL is an indirect effect that relies on the expression of TLR4 by other cell types. Moreover, while signaling through TNFRI contributed to the activation of B and $\gamma\delta$ T cells, it was not required for increasing CD69 expression on $\alpha\beta$ T lymphocytes. Interestingly, T cells were also functionally affected by GIPL treatment, as spleen cells from GIPL-injected mice showed enhanced production of IL-4 following *in vitro* stimulation by anti-CD3. Together, these results contribute to the understanding of the inflammatory properties of the GIPL molecule and its potential role as a parasite-derived modulator of the immune response during *T. cruzi* infection. **Financial support:** CNPq, PRONEX/FAPERJ, FAPERJ, FUJB and The Millennium Institute for Vaccine Development and Technology (CNPq - 420067/2005-1).

IM63 - Modulation of the antigen-presenting function of dendritic cells by phosphatidylserine exposed on the surface of *Leishmania amazonensis*

Wanderley, J. L. M. *(Instituto Nacional de Cancer)*; Soong, L. *(University of Texas Medical Branch)*; Barcinski, M. A. *(Instituto Nacional de Cancer)*

Leishmania amazonensis (*La*) infection is characterized by an important suppression of immune response against the pathogen, which in some cases can lead to a severe disseminated disease. We had shown that acting by mimicking an apoptotic cell, these parasites are recognized by macrophages through exposed phosphatidylserine (PS) which drives amastigote internalization by macropinocytosis and inactivates macrophage inflammatory activity. Moreover, PS exposure by amastigotes is higher in susceptible mice than in resistant ones which suggest that this feature can be modulated by the infected host and/or participate in the immunological outcome of the disease. Dendritic cells (DC) plays a major role in elicits a specific primary T cell response against the pathogen. Phagocytosis of apoptotic cells can modulate DC functions, generally leading to a tolerogenic response. In this background we observed the phenotype of DCs after infection with parasites displaying different amounts of PS at their surface. We analyzed the expression of CD83, CD80, CD86 and MHC class II and the production of IL-12 and IL-10 by infected DCs. Our results showed an up regulation, mostly in CD83 and MHC class II expression and IL-12 production only upon infection with parasites expressing lower levels of PS. We could not correlate IL-10 production and PS exposure by the parasites. These results suggest that PS exposure by amastigote forms of *La* can participate in the acquisition of the function by DC as professional antigen presenting cells. We intend to further investigate the role of PS in T cell specific response during *Leishmania* infection.

IM64 - Species-specific favoring of *Leishmania* infection promoted by PKR activation

Pereira, R.M.S *(Universidade Federal do Rio de Janeiro)*; Dias-Teixeira, K.L.D *(Universidade Federal do Rio de Janeiro)*; Barreto-de-Souza, V.B *(Fundação Oswaldo Cruz-RJ)*; Vivarini, A *(Universidade Federal do Rio de Janeiro)*; Saraiva, E *(Universidade Federal do Rio de Janeiro)*; Bou-Habib, D.C *(Universidade Federal do Rio de Janeiro)*; Lopes, U.G *(Universidade Federal do Rio de Janeiro)*

Leishmaniasis is a parasitic disease that affects 350 million people around the world and is emerging as a frequent opportunistic infection in AIDS patients. We aimed to investigate anti-viral cellular response involved in the burden of intracellular parasite proliferation. We pursued the possible role of double-strand RNA induced activation of PKR (dsRNA-activated protein kinase R), a host anti-viral protein, in favoring *Leishmania* infection. In assays with differentiated THP-1 cells and human macrophages derived from PBMCs (Peripheral Blood Mononuclear Cell) infected by *L. amazonensis* we verified that the treatment with the synthetic double-strand RNA poly(I:C), a potent PKR inductor, induced PKR phosphorylation and aggravates *Leishmania* infection. In both cell types, the favoring by poly(I:C) was reverted by the treatment with 2-aminopurine, an inhibitor of PKR. Similar to *L. amazonensis*, *L. chagasi* infection was also favored by poly(I:C) treatment, but not infection caused by *L. major*. ELISA assays indicate that IL-10, a suppressor cytokine, is produced by poly(I:C) treatment in cells infected by all *Leishmania* species mentioned, what indicates that its secretion is not the main explanation for differential favoring promoted by poly(I:C). Interestingly, we showed that IFN-α, another cytokine induced by PKR activation, favors *L. amazonensis*, but not *L. major* infection. Taken together, these data indicate that PKR is not only an anti-viral protein, but can also modulate parasitic infection through type I IFN expression.

IM65 - THE LECTIN AND ENZYMATIC ACTIVITY OF *Trypanosoma cruzi trans*-SIALIDASE (TS) PROTEINS HAVE DISTINCT EFFECTS ON CARDIAC INFLAMMATION AND HOST IMMUNE RESPONSES

Freire-de-Lima, L *(Universidade Federal do Rio de Janeiro)*; Alisson- Silva, F *(Universidade Fereral do Rio de Janeiro)*; Saraiva, VB *(Universidade Federal do Rio de Janeiro)*; Costa, MMS *(Universidade Federal de Minas Gerais)*; Nunes, MP *(Instituo Oswaldo Cruz)*; Takiya, CM *(Universidade Federal do Rio de Janeiro)*; Todeschini, AR *(Universidade Federal do Rio de Janeiro)*; Previato, JO *(Universidade Federal do Rio de Janeiro)*; Mendonça-Previato, L *(Universidade Federal do Rio de Janeiro)*; Dos Reis, GA *(Universidade Federal do Rio de Janeiro)*

The study of parasite molecules may help understanding the nature of myocarditis that develops in the course of Chagas disease. Our studies shown that co-stimulation of T cells can be regulated by *trans*-sialidase (TS) proteins. Enzymatically active TS (aTS) desialylates donor glycoproteins while inactive TS (iTS) presents lectin-like properties. Here we analyzed the effects of aTS and iTS on infected Balb/c mice. Mice were treated with both enzymes and infected with blood trypomastigotes (Y strain). Parasitemias were evaluated at days 6-10 post-infection (pi) and the hearts examined at day 15 pi. We observed that aTS but not iTS increased parasitemia when compared with controls. Histopathological and real time PCR analysis of the cardiac tissue showed an increase in the number of amastigote nests and parasite loads respectively, induced by aTS. On the other hand, immunohistochemical analysis showed a reduction in the number of T cells in the cardiac tissue from mice treated with iTS. In agreement, iTS delayed the mortality and reduced the creatine kinase activity in the serum of this experimental group. Analysis of cytokine secretion revealed that aTS but not iTS induced a decrease in IL-4 and an increase in INF-γ secretion. Interestingly, co-injection of iTS inhibited the effect of aTS, suggesting that the two proteins compete *in vivo* by the same epitope during *T. cruzi* infection. Together, our data indicate that, aTS mediates mechanisms of susceptibility to *T. cruzi* and immune cell stimulation. Although both enzymes share conserved sugar binding sites, these molecules might play distinct roles in the pathogenesis of Chagas disease. Supported by: CAPES, CNPq & FAPERJ

IM66 - An immunoregulatory role for *Trypanosoma cruzi* sialoglycoproteins

Calixto, N.O. *(Universidade Federal do Rio de Janeiro)* Koeller, C.M. *(Universidade Federal do Rio de Janeiro)*; Montalvão, F. *(Universidade Federal do Rio de Janeiro)*; Previato, J.O. *(Universidade Federal do Rio de Janeiro)*; Mendonça-Previato, L. *(Universidade Federal do Rio de Janeiro)*; Todeschini, A.R. *(Universidade Federal do Rio de Janeiro)*; DosReis, G.A. *(Universidade Federal do Rio de Janeiro)*; Nunes, M.P. *(Instituto Oswaldo Cruz)*

The protozoan parasite *Trypanosoma cruzi*, the etiological agent of Chagas disease, displays on its surface various glycoconjugates which appear to be involved in the recognition and invasion of mammalian host cells, as well as in establishing and sustaining the chronic infection. The majority of these molecules are attached to the parasite via a post-translational modification of a glycosylphosphatidylinositol anchor. Among these are the mucin-like glycoproteins (sialoglycoproteins), comprising a heterogeneous group of *O*-glycosylated molecules that are rich in Thr and Pro residues. These sialoglycoproteins are essential to control innate and acquired immune responses to infection. The aim of this work is to purify sialoglycoproteins from epimastigote forms from three different strains of *T. cruzi* (Dm28c, Y and Tulahuén),

and to investigate their role in T lymphocyte activation. *T. cruzi* mucins (*Tc*-mucins) were analyzed by 15% SDS-PAGE and gas liquid chromatography-mass spectrometry. Our initial experimental findings showed that *Tc*-mucins were not toxic for immune T cells isolated from spleen of naïve mice and that those molecules drastically suppressed proliferation of enriched or purified splenic $CD4^+$ T cells induced by anti-CD3. In order to evaluate parasite specific T cell responses, BALB/c mice were immunized in the hind footpads with a mix of CFA and *Tc*-mucins. Activation of lymph nodes cells from immunized mice induced by anti-CD3 or *T. cruzi* antigen was also markedly reduced in the presence of *Tc*-mucins and addition of rmIL-2 could partially recover T cell proliferation. These results suggest that *Tc*-mucins have a generalized suppressive effect on T lymphocyte activation. Supported by: CNPq, CAPES and FAPERJ

IM67 - Genetic variants in the chemokine and chemokine receptors in Chagas disease

Zafra, G. *(Grupo Inmunología y Epidemiología Molecular.Universidad Industrial de Santander)*; Morillo, C. *(Grupo de Investigación en Sistema Nervioso. Fundación Cardiovascular de Colombia)*; Martín, J. *(Instituto de Parasitología y Biomedicina López-Neyra. Granada. Spain)* González, C.I. *(Grupo Inmunología y Epidemiología Molecular.Universidad Industrial de Santander)*

Introduction and objective: Clinical symptoms of Chagas disease occur in less than half of the individuals infected with *T. cruzi* and are characterized by heart inflammation and dysfunction. The reason of why some individuals develop the cardiac disease is not clearly understood. Host genetic characteristics, related with the inflammatory process, have been proposed. Chemokines and chemokine receptors control the migration of leukocytes during the inflammatory process and are involved in the modulation of Th1 or Th2 responses. We investigated the possible role of *CCR2* 190, *CCR5*δ32, *CCR5* 29, *CCR5* 208 and *RANTES* -403 gene polymorphisms in determining the susceptibility to T. cruzi infection, as well as in the development of chagasic heart disease. **Results and conclusion:** Our study was realized in an endemic area of Colombia, with 260 seropositive (asymptomatic, $n = 130$; cardiomyopathic, $n = 130$) and 200 seronegative individuals. We found no differences in the distribution of *CCR2* 190, *CCR5*δ32, 208 and *RANTES* -403 genotype or phenotype frequencies between chagasic patients and controls. However, we observed that the *CCR5* 29 A/A genotype was significantly increased in cardiomyopathic with respect asymptomatic patients ($P = 0.021$; $OR = 1.96$, CI 1.06-3.65). In addition, the presence of the *CCR5* 208 G/G genotype was also increased in cardiomyopathic patients with more severe symptoms in relation with less severe symptoms ($P = 0.005$; $OR = 6.18$; CI 1.4-29.61). None of the individuals analyzed was homozygous for the *CCR5*δ32 allele, it is consistent with previous reports in populations with Amerindian component. Our data suggest that some chemokine receptors gene polymorphisms, additionals to that reported, *CCR5* 59029, may be involved in a differential susceptibility to chagasic cardiomyopathy. Therefore, more studies are necessary to determine linkage disequilibrium between these polymorphisms and to establish haplotypes that affect surface CCR5 expression on different blood cell types, just as it has been reported for acquired immunedeficiency syndrome.

IM68 - TGF-b IN THE PATHOGENESIS OF GLOMERULONEPHRITIS IN EXPERIMENTAL VISCERAL LEISHMANIASIS.

Prianti, M.G.P *(Instituto de Medicina Tropical)*; Castro L. R, L.R.C *(Instituto de Medicina Tropical)*; Carvalho, M.D.T.C *(Instituto de Biologia Molecular do Paraná)*; Goto, H.G. *(Instituto de Medicina Tropical)*

Introduction: In visceral leishmaniasis (VL), renal involvement is very frequent and the pathogenesis is unclear yet. In previous studies using dogs, hamsteres and mouse with VL, we observed proliferative glomerulonephritis (GN). (Costa et al. Braz J Med Biol Res. 33:1455, 2000; Mathias et al., Braz J Med Biol Res. 34(4):539-43, 2001; Prianti et al., Braz J Med Biol Res. 40(6):819-23, 2007). The inflammatory infiltrated one consisting by mononuclear cells, mainly cells T CD4 (Costa et al. Braz J Med Biol Res. 33:1455, 2000). It is known that Cytokines is involved in renal disease, and TGF-b play an important role in the pathogenesis in glomerulonephritis Aim: To study the participation of TGF-b in pathogenesis in GN in VL, using Leishmania (L.) chagasi-infected mice. Methods: BALB/c mice were infected through intraperitoneal route with 2x107 purified Leishmania (L.) chagasi (MHOM/BR/72/strain 46) amastigotes. We evaluated by morphometry the expression of CD11+ and F4/80+ cells by immunohistochemistry and analyzed the expression of TGF-â by renal cells using ELISA method. The analysis was in different time periods. Results: We observed in infected animals CD11+ and F4/80+ cells in greater amount from 7 days PI, reaching a peak at 15 days PI. The analysis showed presence of TGF-b was significant in 7 days post infection. Conclusion: The data suggest important participation of CD11+ cell and F4/80+ macrophage infiltration in glomerulonephritis in murine visceral leishmaniasis, and the TGF-b contribute for this process. Supported by, LIM-38 (HC-FMUSP), CAPES, CNPq and FINEP

IM69 - Modulatory Role of CCL3/MIP-1α β-Chemokine in Experimental Chagas Disease

Wassita, M. T. *(Laboratório de Pesquisas em Autoimunidade e Imuno-Regulação - IOC, FIOCRUZ)*; Gibaldi, D. *(Laboratório de Pesquisas em Autoimunidade e Imuno-Regulação - IOC, FIOCRUZ)*; Lannes-Vieira, J. *(Laboratório de Pesquisas em Autoimunidade e Imuno-Regulação - IOC, FIOCRUZ)*

Chagas disease is caused by the intracellular protozoa *Trypanosoma cruzi*. The immune response triggered by the parasite and its antigens is crucial for parasite control, but also play a role in disease progression. Cytokines are proposed to act in both processes. Macrophages have important functions during infection control, producing Nitric Oxide (NO) and Tumor Necrosis Factor (TNF). On the development of disease IL-10 can be released leading to modulation of the immune response. Our group demonstrates the production of CC or β-chemokines by murine macrophages treated with GPI-anchored mucin from trypomastigote forms of *T. cruzi*. In fact, several studies show the production of CCL5/RANTES, CCL3/MIP-1α, CCL4/MIP-1β and CCL2/MCP-1 chemokines by *T. cruzi*-infected macrophages inducing control of parasite replication by a NO-dependent way. In this work we are evaluating the differential contribution of CCL3/MIP-1α on the production of cytokines (TNF and IL-10) and NO by *T. cruzi* infected-macrophages. The levels of NO, TNF-α and IL-10 were evaluated in the cultures of peritoneal macrophages obtained from CCL3-/- *T. cruzi* Colombian strain-infected mice. These cells produced higher levels of NO than observed in macrophages from background control infected mice. Our study is currently focused on the evaluation of the infection index and the mechanisms by which CCL3 absence up-regulates NO production.

IM70 - Control of lesions in TNFRp55-/- mice infected with *Leishamania major* may be partially achieved with stem cells or thalidomide.

Seixas, A. R. *(Instituto de Ciências Biológicas)*; Afonso, C. C *(Instituto de Ciências Biológicas)*; Arantes, M. E. *(Instituto de Ciências Biológicas)*; Vieira, Q. *(Instituto de Ciências Biológicas)*

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.

IM71 - Detection of IgG anti-*Toxoplasma gondii* antibody by Immunoenzymatic Assay and Indirect Immunofluorescence in domestic and stray dogs from Caraguatatuba

Aureliano, D.P. *(Instituto Adolfo Lutz - São Paulo)*; Gutierres, A. *(Instituto Adolfo Lutz - São Paulo)*; Kawarabayashi, M. *(Instituto Adolfo Lutz - São Paulo)*; Barbosa, J.A.R. *(Instituto Adolfo Lutz - São Paulo)*; Taniguchi, H.H. *(Instituto Adolfo Lutz - São Paulo)*; Tolezano, J. E. *(Instituto Adolfo Lutz - São Paulo)*; Hiramoto, R.M. *(Instituto Adolfo Lutz - São Paulo)*

Toxoplasma gondii is a feline coccidian that can infect harmblood animals and cause serious damages to health. The human and animals became infected by ingesting food and water contaminated with oocysts or raw meat with tissue cysts. Some studies have shown a high prevalence due a close relationship with the environment conditions and these diets in the domestic animals. These animals would be available as a sentinel of environment and human contamination. Thus, the present study has the aim to evaluate the *T. gondii* seroprevalence in domestic and stray dogs of Caraguatatuba, São Paulo state, Brazil. It was examinated 214 serum samples of domestic and stray dogs collected in 2002 and 2006. This evaluation was realized by detection of IgG anti-*T.gondii* antibody by serological tests ELISA and IFI. The seroprevalence of IgG anti-*T.gondii* antibody by ELISA in the period of 2002 was 58,5% and in the period of 2006 was 67,9% by ELISA and 67,1% by IFI. The high prevalence observed in these dogs may be caused by *T. gondii* oocysts environment contamination probably caused by presence of felines .However the differences observed in the seroprevelence rates between the year of 2002 and 2006 could be explained by the increase of cats population or the increase of consume of meat with tissue cysts. The serum samples of 2002 and 2006 were

analyzed by ELISA and IFI and both methods agree even so they have a difference of antigen type.

IM72 - Reliability between indirect or quantitative determination of specific IgG ELISA in the diagnosis of human toxoplasmosis.

Silva, I.J. *(Instituto de Medicina Tropical de São Paulo)*; Meireles, L.R. *(Instituto de Medicina Tropical de São Paulo)*; Pinto, S.P. *(Instituto de Medicina Tropical de São Paulo)*; Cardoso, R.P.A. *(Instituto de Medicina Tropical de São Paulo)*; de Andrade Jr., H.F. *(Instituto de Medicina Tropical de São Paulo e FMUSP)*

Toxoplasmosis, a world-wide zoonotic infection, is generally asymptomatic and benign in immunocompetent individuals, but it can be serious in immunocompromised patients particularly in individuals with acquired immunodeficiency syndrome and in children infected in utero. The parasitological diagnostic is of difficult execution and unavailable in most of the medical centers. Serological survey is the main diagnostic tool in this infection and several procedures have been developed and commercialized, as the indirect haemagglutination (IHA), indirect immunofluorescence assay (IFAT), and enzyme linked immunosorbent assay (ELISA), using several indirect quantitative approaches for IgG quantification, mostly using Units, impending reliable comparison between methods. To clarify those comparisons, we standardize a quantitative IgG ELISA assays, using internal human IgG standards, using several chromogens in an IFAT tested serum panel, determining its sensibility and reliability for mass IgG determination. Broad range chromogen ABTS is useful when low serum dilutions are used, but with low screening in weaker or border line reactors. TMB was the highest sensibility for negative serum but with low threshold for quantitative determination. The assays were reproducible and reliable intra and inter tests, allowing expression as μg specific IgG/ ml of serum. However, titers in IHA or IFAT were poor related with quantitative determination of IgG by ELISA. These data shows that are different mass levels of specific IgG those are responsible by each specific test reaction. These differences could be ascribed to the type of antigen preparation in each assay or IgG subclass characteristic for each type of assay. In conclusion, serology of toxoplasmosis will remain a matter of discussion, allowing several interpretation and external panels for testing in routine laboratories must be necessary. This work was supported by LIMHCFMUSP and FUNDAP

IM73 - Development of synthetic peptides for differential diagnosis of Visceral Leishmaniases

Baratta, J . A . B *(Instituto de Ciências Biológicas - UFMG)*; Lima, M . P . L *(Instituto de Ciências Biológicas - UFMG)*; Oliveira, D . M . O . *(Instituto de Ciências Biológicas - UFMG)*; Moreira, R . S . M . M . *(Instituto de Ciências Biológicas - UFMG)*; Fumagalli, M. A. C. F. *(Instituto de Ciências Biológicas - UFMG)*; Ribeiro, C. C. R. *(Instituto de Ciências Biológicas - UFMG)*; Alves, D. C. R. A. *(Instituto de Ciências Biológicas - UFMG)*; Campos, A. A. C. *(Instituto de Ciências Biológicas - UFMG)*; Coelho, E. A. F. C. *(Instituto de Ciências Biológicas - UFMG)*; Chávez-Olórtegui, C. C. O. *(Instituto de Ciências Biológicas - UFMG)*

Leishmaniases are parasitic diseases present worldwide, including Brazil. The diagnostic is difficulty by a lot of factors, principally because the similar symptoms with other common diseases, such as Schistossomosis. Another difficult is the low specificity of the serologic tests present in the market. Dogs are the main domestic reservation of the parasite Leishmania, and they can be considered an important infection source for the sandflies and consequently for men. The vaccine present currently in the market is able to induce high production of specific antibodies in a large percentage of immunized animals, which could be interpreted as infected ones. In this study, phage display techniques and spot synthesis are used to identify and qualify selected peptides obtained by dog´s purified antibodies (IgG class). These dogs are previously immunized with the commercial vaccine, and the main objective is selecting those peptides that react only with the immunized animals sample. Phage selection process carried out, and at this time, about 50 phages displaying peptides that could be used in the diagnosis were selected. After this selection, the most reactive phages with the immunized animals sample were identified to be used in the future in diagnostic kits. Support: FAPEMIG, CNPq, PRPq/UFMG

IM74 - A recombinant cysteine proteinase from *Leishmania (Leishmania) chagasi* used as a target for delayed type hypersensitivity assay and serodiagnosis of canine visceral leishmaniasis.

Pinheiro, P.H.C. *(Faculdade NOVAFAPI/FACIME-UESPI)*; Pinheiro, A.M.V.N. *(Faculdade NOVAFAPI)*; Costa, F.A.L. *(Universidade Federal do Piauí)*; Ferreira, J.H.L. *(Faculdade NOVAFAPI)*; Dias, S.S. *(Universidade Federal de São Paulo)*; Katz, S. *(Universidade Federal de São Paulo)*; Barbiéri, C.L. *(Universidade Federal de São Paulo)*

In a previous study we demonstrated that a recombinant cysteine proteinase from *Leishmania (L.) chagasi*, rLdccys1, is a suitable immunological marker for several stages of visceral leishmaniasis (VL) in humans and dogs. The involvement of this antigen in cellular immune responses was evaluated by *in vitro* proliferation of peripheral blood lymphocytes isolated from humans and dogs presenting several clinical forms of VL (Pinheiro *et al.*, Infect. Immun. 73: 3787-3789, 2005). In addition, we demonstrated that this recombinant cysteine proteinase presents high sensitivity and specificity for serodiagnosis of human visceral leishmaniasis by ELISA assay (Dias *et al.*, Am. J. Trop. Med. Hyg. 72: 126-132,

2005). The aim of the present work was to use the rLdccys1 antigen in delayed-type hypersensitivity (DTH) and ELISA assays. DTH responses were determined after intradermal injection of 12 μg rLdccys1 in the neck and the induration was measured at 0, 24, 48 and 72 h after injection. All dogs with subclinical form of VL (n=56) showed intradermal response to rLdccys1 manifested by induration with redness and swelling at the site of the antigen challenge. In these animals the diameter of indurations surpassed 10 mm and peaked at 48 h, whereas the symptomatic group (n=47) displayed no significant reactivity to the recombinant antigen. ELISA assays were performed with serum samples from *L. (L.) chagasi*-infected dogs living in Teresina, Piauí State, Brazil and preliminary data showed a sensitivity of 98.6% when rLdccys1 was used as antigen. The test specificity evaluated with serum samples from dogs with ehrlichiosis and babesiosis, frequent co-infections occurring in the endemic area under study, showed a specificity of 93.5% with the rLdccys1 antigen. Overall, these data showed that the *L. (L.) chagasi* recombinant cysteine proteinase is a suitable antigen for diagnosis of canine VL.

Supported by FAPESP, CAPES, Faculdade NOVAFAPI and FACIME/UESPI.

IM75 - Leishmania chagasi used in Phage Display and Spot Synthesis techniques in order to obtain sinthetic peptides for diagnostic of canine Visceral Leishmaniasis

Ribeiro, C. C. R. *(Instituto de Ciências Biológicas/Universidade Federal de Minas Gerais)*; Alves, D. C. R. A. *(Instituto de Ciências Biológicas/Universidade Federal de Minas Gerais)*; Baratta, J. A. B. *(Instituto de Ciências Biológicas/Universidade Federal de Minas Gerais)*; Lima, M. P. L. *(Instituto de Ciências Biológicas/Universidade Federal de Minas Gerais)*; Oliveira, D. M. O. *(Instituto de Ciências Biológicas/Universidade Federal de Minas Gerais)*; Moreira, R. S. M. M. *(Instituto de Ciências Biológicas/Universidade Federal de Minas Gerais)*; Campos, A. A. C. *(Instituto de Ciências Biológicas/Universidade Federal de Minas Gerais)*; Machado, C. M. T. M. *(Instituto de Ciências Biológicas/Universidade Federal de Minas Gerais)*; Coelho, E. A. F. C. *(Instituto de Ciências Biológicas/Universidade Federal de Minas Gerais)*; Chávez-Olórtegui, C. C. O. *(Instituto de Ciências Biológicas/Universidade Federal de Minas Gerais)*

Leishmaniasis is a progressive fatal infection considered a problem of World-wide Health. Of all the potential animal hosts transmitting parasite *Leishmania*, domestic dogs are the most important in harboring and transmitting the disease to humans, due to the close association between humans and dogs as pets. The difficult in the diagnosis of canine visceral leishmaniasis (CVL) is one of the principal difficulties for disease control. Because of the unspecific tests present on the market, health boosted dogs are being considered infected ones. Another problem is the high level of cross-reactivity in tests between *Leishmania* species and other parasites, such as *Trypanosoma cruzi* and *Schistosoma mansoni*. About these facts, this work focuses in the research for developing of a CVL immunodiagnostic kit. For this purpose, *Phage Display* technique was used for selecting peptides expressed in the phages surface selected using soluble extract (SLA) of *Leishmania chagasi* affinity. The phages carry exogenous random peptides were selected, purified and, now, they have been sequenciated and synthesized by *Spot Synthesis* technique and then, tested in serological tests (ELISA) to verify their sensibility and specificity in relationship to CVL sera.
Support: FAPEMIG, CNPq, PRPq/UFMG

IM76 - Utilization of synthetic peptides for serological diagnosis or canine Visceral Leishmaniases

Oliveira, D.M. *(Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais)*; Moreira, R.S.M. *(Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais)*; Machado, C.M.T. *(Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais)*; Ribeiro, C.C. *(Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais)*; Alves, D.C.R. *(Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais)*; Baratta, J.A. *(Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais)*; Lima, M.P. *(Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais)*; Campos, A.A. *(Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais)*; Coelho, E.A.F. *(Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais)*; Chávez-Olórtegui, C. *(Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais)*

Leishmaniasis are parasitic diseases that have a high incidence in the world. Dogs are an important domestic reservoir in Visceral Leishmaniasis (VL) cycle. Additionally, a high percentage of healthful dogs that reside in endemic areas of leishmaniasis or other diseases developing positive results in the diagnosis tests. At the sometime, animals which are immunized with the commercially available vaccine can also develop positive serology because of parasite antigens. The diagnosis of canine VL is difficult because of low specificity of used tests. So, the utilization of new and innovated techniques to produce more specific antigens that don't have reaction with another diseases and can discern between serum samples of vaccinated dogs and infected ones, is desirable. In this work, we used Phage Display and Spot Synthesis techniques to identify, characterize and produce synthetic peptides signing elaboration of a specific diagnosis test (ELISA) to canine VL. Preliminary results using serum samples of infected dogs with VL indicate that synthesize peptides were able to recognize just serum samples of dogs with active VL. Next time, will be used a bigger cross-section to search the sensibility and specificity comparing with another results of diagnostic tests, using Leishmania chagasi extract like con-

trol.
Support: FAPEMIG, CNPq, PRPq/UFMG

IM77 - Occurence of CD34$^+$ progenitor cells during the splenic ampliation in experimental rodent malaria

Hermida, F.P.M. *(Instituto de Ciências Biomédicas)*; Vieira, D.P. *(Instituto de Pesquisas Energéticas e Nucleares)*; Fernandes, E.R. *(Faculdade de Medicina da Universidade de São Paulo)*; de Andrade Jr., H.F. *(Instituto de Medicina tropical de São Paulo)*

Malaria is caused by *Plasmodium sp.*, which control by the host depends on the spleen. Crucial in malaria control, this organ is responsible for parasite clearing, which is achieved by a filtration network. The increase of parasitemia implies in amplification of this network to warranting the control of the infection. This amplification involves endothelial and myeloid progenitor cells, which presented the CD34 antigen in their surface. We studied the distribution and amount of CD34$^+$ cells in the spleen of mice infected with rodent malaria, to define the role of those cells in spleen amplification and infection control. Groups of C57Bl/6j mice were infected with 10^6 parasitized RBC of 2 strains of *Plasmodium chabaudi*, CR, self resolving, and AJ, lethal, and lethal strain of *Plasmodium berghei*, ANKA. Parasitemia was followed daily. Sequentially, the spleen was weighted and processed for histology and flow cytometry. Proportion of spleen pulps was determined by morphometry in usual histology. In the self controlling strain, the spleen structure was maintained during spleen amplification, not seen in lethal models. Hematopoiesis foci and germinal centers were observed in all models. The distribution of CD34$^+$ cells was increased in the red pulp in the 4^{th} day p.i., in all models. At the 8^{th} day p.i., CD34$^+$staining was faint in most cells of the red pulp unclear, suggesting a differentiation of red pulp infiltrating cells in committed cell lineages. By flow cytometry, free CD34$^+$ cells appear like a wave at the 4^{th} day p.i. in all models. *P. chabaudi* models presented the same level of those cells, which was larger in the *P. berghei* mice, despite absence of malaria control. In the present work, increase of spleen CD34$^+$ cells do not correlate with infection control. This work was supported by CAPES and LIMHCFMUSP.

IM78 - POSITIVE EFFECT OF APIRASE ACTIVITY IN *Leishmania* INFECTION

Marques-da-Silva, E. A. *(Universidade Federal de Ouro Preto)*; Figueiredo, A. B. *(Universidade Federal de Ouro Preto)*; Fietto, J. L. R. *(Universidade Federal de Viçosa)*; Afonso, L. C. C. *(Universidade Federal de Ouro Preto)*

Leishmaniasis is a parasitic disease with a variety of clinical forms, which are related to the *Leishmania* specie involved. In the murine model, *Leishmania (L.) amazonensis* causes chronic non-healing lesions in *Leishmania (V.) braziliensis* or *Leishmania (L.) major*-resistant mice strains. These parasites may express ecto-nucleotidases (NTPDases and 5'- nucleotidases), enzymes that convert extracellular ATP, a molecule with pro-inflammatory effects released by injured or pathogen-activated cells, to adenosine, an essential molecule to *Leishmania* metabolism with known immunomodulatory properties. Previous data from our laboratory have shown that *L. amazonensis* exhibits higher ability in hydrolyzing adenine nucleotides than their counterparts *L. braziliensis* or *L. major*, and adenosine treatment is able to induce larger lesions and higher parasitism in C57BL/6 mice infected with *L. braziliensis*. In the present study, we evaluated the presence of NTPDases in membrane preparations from these three *Leishmania* species by western blotting analysis, which revealed expression of these enzymes only in *L. amazonensis* preparations. In order to verify the effects of nucleotide hydrolysis inhibition in *Leishmania* infection, we used the P2 purinoreceptors antagonist and ecto-ATPase inhibitor suramin, at the moment of *L. amazonensis* inoculum in C57BL/6 footpad. Suramin induced decreased lesion size and parasitism, but did not alter IFN-γ production seven weeks after inoculum. In addition, we inoculated C57BL/6 mice with *L. braziliensis* in the presence of apirase and/or adenosine analogue NECA (5'-(N-ethyl-carboxamido)adenosine), in order to verify the conjugated effects of decreased ATP and increased adenosine in the development of infection. A transient increase in lesion size in NECA and apirase/NECA-treated animals was observed. Together, these results suggest that, such as adenosine, apyrase activity may favor *Leishmania* infection. This research is sponsored by: FAPEMIG, CNPq, PIBIC-CNPq, CAPES, PIP-UFOP

IM79 - Antigenic polymorphism and recognition of variable domains of merozoite surface protein 1 of *Plasmodium vivax* (PvMSP-1) by naturally acquired antibodies of subjects from Brazilian Western Amazonia

Bastos, M. S. *(Instituto de Medicina Tropical de São Paulo)*; da Silva-Nunes, M. *(Instituto de Ciências Biomédicas)*; Hoffmann, E. H. E *(Instituto de Ciências Biomédicas)*; Malafronte, R. S. *(Instituto de Medicina Tropical de São Paulo)*; Wunderlich, G. *(Instituto de Ciências Biomédicas)*; Moraes, S. L. *(Instituto de Medicina Tropical de São Paulo)*; Ferreira, M. U. *(Instituto de Ciências Biomédicas)*

Introduction: The merozoite surface protein 1 of *Plasmodium vivax* (PvMSP-1), a major target for malaria vaccine development, contains six highly polymorphic domains interspersed with conserved sequences. Although there is evidence that the sequence divergence in PvMSP-1 has been maintained over five million years by balanced selection exerted by host's acquired immunity, the variant-specificity of naturally acquired antibodies to PvMSP-1 remains little investigated. Objectives: To analyze the extent to which PvMSP-1 sequence diversity affects the development of an-

tibody responses to this major malaria-vaccine candidate antigen. Results: We show that 15 recombinant proteins corresponding to PvMSP-1 variants commonly found in local parasites were poorly recognized by 376 noninfected subjects aged 5-90 years exposed to malaria in rural Amazonia; less than onethird of them had detectable IgG antibodies to at least one variant of blocks 2, 6 and 10 that were expressed, although 54.3% recognized the invariant C-terminal domain PvMSP-119. Although the proportion of responders to PvMSP-1 variants increased substantially during subsequent acute *P. vivax* infections, the specificity of IgG antibodies did not necessarily match the PvMSP-1 variant(s) found in infecting parasites. Discussion e Conclusions: We discuss the relative contribution of antigenic polymorphism, poor immunogenicity, and original antigenic sin (the skew in the specificity of antibodies elicited by exposure to new antigenic variants due to preexisting variant-specific responses) to the observed patterns of antibody recognition of PvMSP-1. We suggest that antibody responses to the repertoire of variable domains of PvMSP-1 to which subjects are continuously exposed are only elicited after several repeated infections and may require frequent boosting, with clear implications for the development of PvMSP-1-based subunit vaccines. This study was supported by grants from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP).

IM80 - *Lutozomyia longipalpis* saliva-induced resistence to *Leishmania (V.) braziliensis* infection is dependent of $CD4^+CD25^+FOXP3^+IL\text{-}10^+$ inhibition and IFN-γ-producing $CD8^+$T cells activation in inoculation site

Carregaro, V. *(Faculdade de Medicina de Ribeirão Preto)*; Costa, DL *(Faculdade de Medicina de Ribeirão Preto)*; Brodyskin, CI *(Centro de Pesquisa Gonçalo Muniz, Fundação Oswaldo Cruz)*; Barral, A.M. *(Centro de Pesquisa Gonçalo Muniz, Fundação Oswaldo Cruz)*; Barral-Netto, M. *(Centro de Pesquisa Gonçalo Muniz, Fundação Oswaldo Cruz)*; Cunha, FQ *(Faculdade de Medicina de Ribeirão Preto)*; Silva, JS *(Faculdade de Medicina de Ribeirão Preto)*

Introduction and Objective: Although Phlebotomines saliva plays a crucial role in the establishment of *Leishmania* infection, the immunization of mice with salivary gland sonicated (SGS) of *Lutzomyia longipalpis* or *Phlebotomus papatasii* resulted in a protection against the parasite. Herein, we demonstrated that the pattern of susceptibility or resistance to *Leishmania(V.)braziliensis* infection depends on distinct cellular recruitment induced by differential inoculum of *L.longipalpis* SGE into mice ear. **Methods and Results:** BALB/c mice inoculated i.d. with SGS from *L.longipalpis* once (SGS-1X) or three times (SGS-3X) into ear were infected with $2x10^3$ *L.(V.)braziliensis* promastigotes. The ear lesion size, parasites burden, cytokines production and inflammatory infiltrated were analyzed at 30^th day post infection. SGS-3X inoculation reduced the recruitment of $CD4^+$T, $CD4^+CD25^+$, Macrophages and neutrophil induced by SGE-1X, whereas $CD8^+$T cells migration was increased. The lesion size and parasites numbers into ear were also reduced in these mice. SGE-3X-induced protective effect was correlated with the initial recruitment of $CD8^+$T cells and enhancement of IFN-γ release. Interestingly, IL-10 levels as well as $CD4^+CD25^+FOXP3^+$ cells recruitment induced by SGS-1X were abolished by SGE-3X inoculation. Confirming the involvement of $CD8^+$T cells and IFN-γ upon SGE-3X-protective activity, it failed to control the development of *Leishmania(V.)braziliensis* infection in depleted mice of IFN-γ as well as $CD8^+$T cells. Corroborating to this results, the adoptive transference of SGE-3X-isolated $CD8^+$T cells to either SGE-1X-inoculated or to IFN-γ-depleted SGE-3X mice reversed the SGE-1X-exacerbative effect on *L.(V.)braziliensis* infection. **Conclusion:** Altogether, the results suggest that the pattern of resistance induced by SGE-3X is dependent of IFN-γ-producing $CD8^+$Tcell activaction which inhibits the $CD4^+CD25^+FOXP3^+$ cells recruitment and IL-10 release into inflammation site and, consequently the development of Leishmaniasis. Finnancial Support: FAPESP and USP.

IM81 - Toll-like receptor (TLR) 9 and IFNγ dependent-priming enhances TLR responsiveness during acute malaria infection.

Franklin, B. S. *(Instituto René Rachou, FIOCRUZ, Belo Horizonte, MG, Brazil)* Rodrigues, J. O. *(Instituto René Rachou, FIOCRUZ, Belo Horizonte, MG, Brazil)*; Rodrigues, S. C. *(Departamento de Bioquímica e Imunologia, ICB, UFMG, Belo Horizonte, MG, Brazil)*; Ropert, C. *(Instituto René Rachou, FIOCRUZ, Belo Horizonte, MG, Brazil)*; Golenbock, D. *(Department of Medicine, University of Massachusetts Medical Scholl, Worcester)*; Gazzinelli, R. T. *(Departamento de Bioquímica e Imunologia, ICB, UFMG, Belo Horizonte, MG, Brazil)*

The acute infection with *Plasmodium* is characterized by systemic release of pro-inflammatory cytokine which are thought to mediate symptoms, such as: shivering, headache, chills, spiking fever, sweating, vasodilatation and hypoglycemia. Evidence exist that Toll-like Receptors (TLRs) are critical elements for induction of such pro-inflammatory cytokines. Nevertheless, the mechanisms underlying this burst in pro-inflammatory cytokine responses during malaria infection are not fully understood. Here we show that malaria infection primes the innate immune system leading to hyperresponsivenes to subsequent TLR stimulation. In both mouse (*Plasmodium chabaudi* AS) and human (*Plasmodium falciparum*) malaria models, increased production of pro-inflammatory cytokines was observed upon subsequent TLR stimulation. Interestingly, this phenomenon was restricted to the acute infection and correlated with up-regulation of TLR expression. Importantly, *P. chabaudi* mediated-priming to TLR ligands

was significantly diminished in TLR9$^{-/-}$ and MyD88$^{-/-}$ and was completely abolished in IFN$\gamma^{-/-}$ mice. Furthermore, up-regulation of TLR expression was significantly diminished in IFN$\gamma^{-/-}$ and MyD88$^{-/-}$ mice. Together, our results suggest that TLRs and IFNγ are critical elements in priming the immune system during acute infection with *P. chabaudi* in mice. Thus, TLR9 may be the critical TLR that initiates host responses to *Plasmodium* components, leading to IFNγ production and up-regulation of TLR expression which may contribute to the burst of pro-inflammatory cytokines observed during acute infection. **Financial support:** CNPq, FAPEMIG, NIH, WHO and Millennium Institute for Vaccine Technology and development.