Imunologia - Immunology

IM01 - IFN−γ−INDUCED REACTIVE OXYGEN SPECIES (ROS) AND IFN−γ−INDUCED P47 GTPASES ARE ASSOCIATED WITH RESISTANCE TO INFECTION WITH *LEISHMANIA MAJOR*

dos Santos, L.A.V.D.R. *(UFMG)*; Santiago, H.C. *(UFMG)*; Tafuri, W.L. *(UFMG)*; Vieira, L.Q. *(UFMG)*

Resistance to *Leishmania major* is mediated by IFN-γ-activated mechanisms, being nitric oxide (NO) production by the expression of the inducible nitric oxide synthase (iNOS) gene recognized as the most potent leishmanicidal mediator. Infected IFN-γ -/- and iNOS -/- mice develop uncontrolled lesions with high parasite burden. Strikingly, while the IFN-γ -/- animal displays high susceptibility to *L. major* infection, presenting 100% of death in the 11t̂h week post-infection, iNOS −/− mice remain alive up to 30 weeks post-infection and, in addition, developed smaller lesions than their IFN-γ −/− partners. Further, IFN-γ −/− animals consistently presented more severe liver and spleen visceralization when compared to small parasite burdens found in iNOS −/− and WT controls. Recently, p47GTPases were found to play relevant role in resistance to intracellular parasites and, in fact, tissues from infected iNOS −/− and WT animals expressed higher levels of LRG-47, IGTP and IRG-47 than those from IFN-γ −/− mice. Additional studies revealed that ROS production was exacerbated in iNOS −/− macrophages and strongly suppressed in IFN-γ −/− ones, suggesting a possible role of such mechanism in leishmania resistance in absence of NO. Together, these results implicate that additional IFN-γ−induced mechanisms besides NO production are required to effective resistance to *L.major* infection. Supported by: FAPEMIG

IM02 - CLINICAL EVALUATION OF TWO NEOTROPICAL LEISHMANIA-Leishmania (Leishmania) amazonensis AND Leishmania (Viannia) braziliensis IN VERVET MONKEYS (Cercopithecus aethiops)

Gomes P *(IEC)*; Garcez P *(IEC)*; Brigido M *(CNP/IEC)*; Muniz J *(CNP/IEC)* Shaw J *(ICB/USP)*

This study verified for the first time the susceptibility of Vervet monkeys (*Cercopithecus aethiops*) to *L.*(*L.*)*amazonensis* and *L.*(*V.*)*braziliensis*. Twelve laboratory bred Vervet monkeys were divided into two groups (3 male, 3 female). Each animal was inoculated intradermally with $2x$ 10^6 stationary phase promastigotes into the tail's shaved dorsal surface. Group 1 received *L.*(*L.*)*amazonensis*. When the lesions cured they were challenged with *L.*(*L.*)*amazonensis* and when these cured with *L.*(*V.*)*braziliensis*. Group 2 received one *L.*(*V.*)*braziliensis* inoculation. Each animal was examined weekly during the first two months then monthly until all lesions cured. Primary inoculations of both groups produced lesions at the inoculation site. Anti-Leishmania Ig-G antibody levels were significantly higher with homologous antigens. *L.*(*L.*)*amazonensis* lesions were nodular except one that ulcerated. *L.*(*V.*)*braziliensis* produced open ulcers. The clinical parameters that yielded statistically significant differences were duration of infection, incubation period and lesion size. The mean duration of *L.*(*L.*)*amazonensis* infections (100.83 days) was significantly less (p=0.0027) than that of *L.*(*V.*)*braziliensis* infections (280 days). Lesion diameters of group 1's primary *L.*(*L.*)*amazonensis* infections were significantly smaller (p=0.0423) than group 2's *L.*(*V.*)*braziliensis* infections. Up to 37 days there was no significant difference in the mean lesion diameter of group 1's primary and challenge *L.*(*L.*)*amazonensis* infections. After this (42 & 60 days) the lesions of the homologous challenge were significantly smaller (p=0.0451 & p=0.0059 respectively). The mean incubation period (24.75 days) of the *L.*(*L.*)*amazonensis* challenge was significantly shorter (p=0.0162) than that of the primary infection (46.83 days), but its duration (mean 38.67 days) was significantly less (p=.0044). The *L.*(*L.*)*amazonensis* challenge failed to produce lesions in two monkeys. Group 1's *L.*(*V.*)*braziliensis* lesions were significantly (p=0.0386) larger at 31 days but smaller (p=0.0409) at 102 days compared to group 2 infections. *L.*(*L.*)*amazonensis* infection apparently alters the clinical course of subsequent *L.*(*V.*)*braziliensis* infections. **Financial support: WHO, PCMAN/FNS, CAPES, FAPESP.**

IM03 - THE INVOLVEMENT OF NEUTROPHILS IN THE RESISTANCE TO *Leishmania* (*L.*) *amazonensis* INFECTION

Carmo, E. V. S. *(UNIFESP/EPM)*; Katz, S. *(UNIFESP/EPM)*; Mortara, R. A. *(UNIFESP/EPM)*; Barbiéri, C. L. *(UNIFESP/EPM)*

Neutrophils are key players of the innate immune system that provide a first line of defense against invading pathogens and recent evidences suggest that neutrophils are also implicated in immunoregulation. The involvement of neutrophils in *Leishmania* (*L.*) *major* infection in murine models has been reported (Ribeiro-Gomes et al., **J. Immunol.**, 2004, 172: 4454-4462; Chen et al., **Parasitol. Intern.** 2005, 54: 109-118). The present work focuses on participation of neutrophils from two mouse strains, BALB/c and C3H/HePas, during infection with *Leishmania* (*L.*) *amazonensis*. Peritoneal macrophages cocultured with inflammatory neutrophils are able to kill *L.* (*L.*) *amazonensis* amastigotes, and no significant difference in the leishmanicidal activity was observed between the two mouse strains tested. Results from a coculture assay by use of peritoneal macrophages and neutrophils maintained either at the same compartment or separated by a cell-impermeable culture insert (Transwell system, 0.4 mm) indicated that the leishmanicidal activity is due to a soluble factor. ELISA dosages in the supernatants from these cocultures showed that the soluble leishmanici-

dal factor is neither IFN$-\gamma$ nor TNF$-\alpha$. The histopathology of foot lesions from C3H/HePas mice showed a persistence of neutrophils three weeks after infection. In contrast, in the BALB/c foot lesions there was a predominance of macrophages harboring a large number of amastigotes and absence of neutrophils. These results were confirmed by transmission electron microscopy analysis of foot lesion ultrathin sections. These results indicate a role of neutrophils in the control of *Leishmania* (*L.*) *amazonensis* infection in a resistant host and open perspectives to study the leishmanicidal mechanisms performed by these cells. Supported by FAPESP

IM04 - HEPATIC GRANULOMA FORMATION IN CANINE VISCERAL LEISHMANIASIS: A HISTOPATHOLOGICAL, PARASITOLOGICAL AND IMMUNOLOGICAL RETROSPECTIVE STUDY

Tafuri Wg.L. *(UFMG)*; Oliveira P.S. *(UFMG)*; SantÁna, J.A.P. *(UFMG)*; Michalick, M.S.M. *(UFMG)*; Tafuri W.L. *(UFMG)*

Canine Visceral Leishmaniasis in the New World is caused by *Leishmania (Leishmania) chagasi* which is transmitted by the phlebotomine *Lutzomyia longipalpis*. *L. chagasi* parasites elicit granuloma formation in the livers of murine (Murray & Nathan, 1999) and canine host (Tafuri et al., 1996, 2001). This structure is a special cellular exudate assembly composed mainly by mononuclear cells and it consists of a core of fused, parasitized resident macrophages with an encircling mononuclear cell mantle containing blood monocytes and T cells (Murray, 1999). This work has carried out a qualitative and quantitative study of hepatic granulomas of naturally infected dogs with *L. chagasi*. So far, we have studied seventy five naturally infected animals divided in distinct clinical groups (asymptomatic, oligosymptomatic and symptomatic). Dogs were sacrificed with lethal dose of Thionembutal and T61. Quantitative histological (number and granulomas diameter) and parasitological (streptoavidin peroxidase method, Tafuri et al., 2004) analyses have been done under a computer-assisted image analysis system (Kontron Electronic/Zeiss). Immunohistochemical assays have been carried out to characterize macrophages antigens as CD11b/CD18 (CR3), CD11c/CD18 (CR4) and MAC387 (L1 or calprotectin molecule). Asymptomatic animals have shown a direct association between the granulomas formation to a lower parasitism tissue load. Moreover, these animals showed a higher positive cells expression of CR3 and MAC387 (Oliveira et al., 2004). In leishmaniasis, cellular immune responses are paramount in determining healing or resistance to disease (Kaye et al., 2004). Some authors have discussed the antimicrobial efficacy of the granuloma response, because it seems to be imprecise and depends upon host and pathogens determinants. (Wilson e Weinstock, 1996, Murray, 2001). It means, that granuloma formation does not necessarily guarantee antimicrobial function if the effector cell fails to become activated or is intrinsically deficient in a basic microbial mechanism. Thus, we are looking forward to continue these studies.

IM05 - A histopathological, parasitological and complement receptor type 3 (CR3) expression study in spleens of naturally infected dogs with *Leishmania (Leishmania) chagasi* .

Lima, W.G. *(UFMG)*; Cagliari, M.V. *(UFMG)*; Michalick, M.S.M. *(UFMG)*; Tafuri, W.G. *(UFMG)*; Tafuri, Wg.L. *(UFMG)*

In America, canine visceral leishmaniasis (CVL) is a chronic systemic disease caused by *Leishmania (Leishmania) chagasi*. The success of the parasite infection is dramatically dependent of this early interaction in the vertebrate host. The major event of these systems is the binding of serum complement opsonized promastigotes to macrophage receptors. Complement receptor type 3 (CR3) appears to make a quantitatively greater contribution to this adhesion than other receptors (Mosser and Rosenthal, 1983; Gonçalves, 2005). The aim this work is to evaluate the CR3 spleens tissue expression and its correlation to the histological picture and parasitism load. Asymptomatic, oligosymptomatic and symptomatic mongrel dogs were obtained from Sabara/MG (Belo Horizonte metropolitan area). They were sacrificed with lethal dose of Sodic Thiopental (33%) and T-61. Tissue touch preparations of spleens were positive for all animals and the parasite burden was expressed as Leishman-Donovan units (LDU). The LDU indices was determined by microscopic enumeration of *Leishmania* amastigotes divided by 1000 cell nuclei and the result was multiplied by the organ weight. Other spleen samples were collected, fixed in buffer formalin at 10%. Spleens fragments were dehydrated, cleared, embedded in paraffin for histopathological (H&E) and immunohistochemical analysis. The streptoavidin-peroxidase immunohistochemistry method was carried out to detect amastigotes forms of Leishmania in spleens paraffin sections. For CR3 expression we used the same immunocytochemical method over frozen spleens tissues sections. Immunolabeled amastigotes and CR3 were quantified by morphometrical analysis using the KS300 software. Our previous results have not been indicated any correlation between the parasitism and tissue alterations in all distinct clinical groups. However, CR3 tissue expression has been higher in symptomatic animals than the other groups. We are looking forward to determine the CR3 tissue expression quantification in other lymphoid organs as lymph nodes and skin. Supported by: FAPEMIG

IM06 - DELAYED-TYPE HYPERSENSITIVITY ELICITED BY A RECOMBINANT CYSTEINE PROTEINASE OF *LEISHMANIA (L.) CHAGASI.*

Pinheiro,P.H.C. *(NOVAFAPI)*; Dias, S.S. *(UNIFESP/EPM)*; Ferreira, J.H.L. *(UNIFESP/EPM)*; Katz, S. *(UNIFESP/EPM)*; Barbiéri, C.L. *(UNIFESP/EPM)*

In a previous study we demonstrated that a recombinant cysteine proteinase from *Leishmania (L.) chagasi*, rLdccys1, represents a suitable immunological marker for several stages of visceral leishmaniasis (VL) in humans and dogs. The implication of this antigen in cellular immune responses was evaluated. The IFN-γ, IL-4, and IL-10 secretion in the supernatants from human and dog lymphocyte cultures was also analysed (Pinheiro et al., Infect. Immun. 73: 3787-3789, 2005). In the present work, the delayed-type hypersensitivity (DTH) elicited by the rLdccys1 antigen was measured in naturally infected dogs presenting several clinical forms of VL living in Teresina, Piauí State, Brazil. The DTH responses were determined after intradermal injection of 60 μg rLdccys1 in the neck and the induration was measured at 0, 24, 48 and 72 h after injection. Animals intradermally injected with PBS (0.1 ml) in the right hind footpad served as controls. All asymptomatic dogs ($n = 5$) 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. In the oligosymptomatic group ($n = 5$) animals showed reactivity of 3 mm to rLdccys1, whereas the symptomatic group ($n = 5$) displayed any reactivity to the recombinant antigen. These data are in accordance with previous results that showed a predominance of Th1 profile in cellular responses elicited by rLdccys1 in patients and dogs with asymptomatic VL, a mixed response, Th1 and Th2, in those presenting the oligosymptomatic disease, and a Th2 profile in the symptomatic subjects. Overall, these results showed the feasibility to use the rLdccys1 antigen in DTH assays for evaluation of the effectiveness of this antigen in protection studies in endemic regions of canine VL that are currently in progress. Supported by FAPESP and NOVAFAPI.

IM07 - COMPARISON OF DIFFERENT ADJUVANTS IN IMMUNIZATION SCHEDULES OF BALB/C MICE AND HAMSTERS WITH rLDCCYS1, A RECOMBINANT CYSTEINE PROTEINASE FROM *LEISHMANIA (L.) CHAGASI.*

Ferreira, J. H. L. *(Unifesp)*; Fedeli, C. E. C. *(Unifesp)*; Dias, S. S. *(Unifesp)*; Ferreira, J.H.L. *(UNIFESP/EPM)*; Katz, S. *(Unifesp)*; Barbiéri, C. L. *(Unifesp)*

Previous studies from our laboratory demonstrated the implication of a 30 kDa cysteine proteinase from *Leishmania (L.) chagasi* (p30) in partially protective cellular immune responses against homologous infection in BALBc mice (Pinto et al., 2000, Int. J. Parasitol. 30 p.599 607). Expression of the gene encoding the p30 antigen from *L. (L.) chagasi*, *Ldccys1*, in pHis vector resulted in a recombinant protein of 47 kDa, rLdccys1. The aim of the present study was the characterization of the immune responses triggered after the immunization of BALBc mice and hamsters with rLdccys1 in combination to some adjuvants. In a first screening, *Propionibacterium acnes* and Bacille Calmette Guerin (BCG) were used as adjuvants in comparison to complete Freunds adjuvant (CFA). Animals were immunized with 25 μg of rLdccys1 plus either 200 μg *P. acnes* or 1x10^6 BCG. Animals received two doses of rLdccys1 plus each adjuvant with two weeks interval by either subcutaneous or intraperitoneal routes. Two weeks after immunization lymph node and spleen cells from animals immunized by the s.c. and i.p. routes, respectively, were isolated for use in lymphoproliferative assays in the presence of rLdccys1 and for lymphokine dosages. Lymphocytes from BALBc mice and hamsters immunized with the recombinant antigen plus either P. acnes or BCG showed similar stimulation indexes (4 to 8) in the presence of rLdccys1, independent on the immunization route used. Significant levels of IFN γ were secreted in the supernatants from the lymphocyte cultures, whereas IL 4 and IL 10 were not detected. The effect of CpG oligodeoxynucleotides (ODN) on the immunogenecity of rLdccys1 is now under investigation. Supported by FAPESP.

IM08 - Detection of *Toxoplasma gondii* specific antibodies in dogs from Mato Grosso do Sul, Brazil, by serologycal assays

Débora Picanço Aureliano *(IAL)*; Massami Kawarabayashi *(IAL)*; Helena Hilomi Taniguchi *(IAL)*; José Augusto de Raeffray Barbosa *(IAL)*; Suely Aparecida Correa Antonialli *(ESPMTS)*; Jose Eduardo Tolezano *(IAL)*; Roberto Mitsuyoshi Hiramoto *(IAL)*

Toxoplasmosis is generally asymptomatic in immunocompetent individuals, with occasional eye involvement, but causes devastating disease in immature and patients with deficiency in immune system. The infection occurs by ingestion of oocysts excreted in faeces of infected felines or by ingestion of raw and inadequately cooked meat containing cysts. The infection by *Toxoplasma gondii* was demonstrated in wild and domestic animals. Toxoplasmosis in dogs is usually asymptomatic and recently, the free-living stray animals are suggested like environment indicator of the contamination by *T. gondii*. In order to evaluate the titers and frequency of anti-*T. gondii* antibodies there were tested 154 blood samples from the stray dogs captured in the Campo Grande, Mato Grosso do Sul State by enzyme-linked immunosorbent assay (ELISA) and Indirect Immunofluorescence (IFAT). The sera-prevalence of *T. gondii* infection determined by a specific IgG ELISA was 55,20 % and 59,7 % by IFAT. The sera titers found ranged from 1:16 to $\geqq$ 1:4096, being 1:16

(33,69%), 1:64 (7,61%), 1:256 (10,87%), 1:1024 (16,30%), 1:2048 (11,96%) and ≧ 1:4096 (19,57%). Both techniques showed high percentage of contamination by *T. gondii* in stray dogs from Mato Grosso do Sul State, as other urban areas of Brazil. Those data suggest an indirect indicative of the high environment contamination and could be used as a tool to measure the risk of toxoplasmosis incidence in areas of cohabitation between human population and stray dogs.

IM09 - A PRELIMINAR STUDY OF IN VITRO BINDING ASSAYS OF CANINE MONOCYTES-DERIVED MACROPHAGES AND PROMASTIGOTES FORMS *Leishmania (Leishmania) chagasi*

Sampaio, W.M *(UFMG)* Arruda, F.C.S *(UFMG)*; Perlatto Moura, E *(UFMG)*; Ribeiro, R.R *(UFMG)*; Alves, C.F *(UFMG)*; Melo, M.N *(UFMG)*; Michalick, M.S.M *(UFMG)*; Tafuri, W.L. *(UFMG)*; Tafuri, Wg.L *(UFMG)*

Canine Visceral Leishmaniasis is a zoonosis and dogs represent the domestic reservoir of the disease. In America the etiological agent is *Leishmania (Leishmania) chagasi*. The success of the parasite infection is dramatically dependent of this early interaction in the vertebrate host. The major event of these systems is the binding of serum complement opsonized promastigotes to macrophage receptors for complement (Mosser & Rosenthal, 1983; Gonçalves, 2005). In this study, we have carried out a binding and a survival assays methods to study the interaction between *Leishmania chagasi* promastigotes and monocytes-macrophages derived from dogs with different clinical status of the disease. In a 24 well plate, monocytes obtained from peripherical blood were adjust to $3x10^6$ cells/mL per well. Promastigotes forms of *Leishmania* (stationary phase) were adjusted to $5x10^7$ cells/mL. The *Leishmania* binding assay was performed in a 24 well plate during 45-60 minutes at 35^oC over coverslips. We carried out assays in the presence of normal serum or in the presence of a final concentration of 5% of C5 deficient (serum from AKR/J mice) mouse serum. To obtain monocyte derived macrophages, the mononuclear cells were added to Teflon beakers for 5 days at 37^oC in 5%C0_2. Then, *Leishmania* are added to macrophages for a one hour binding interaction with or without complement serum deficient as well as done in the first experiment. Then, the number of infected macrophages was counted in an optical microscope, as well as the number of parasites per macrophages. Our preliminary results have demonstrated that the number of parasites bound to macrophages was dramatically increased in the serum dependent group (with C5 deficient serum mouse) in all experiments. We are looking forward to study these cells from dogs with a distinct clinical status of the disease. Supported by: FAPEMIG

IM10 - AMASTIGOTE SURFACE PROTEIN-2 OF Trypanosoma cruzi: EVALUATION OF THE POLYMORPHISM IN T. CRUZI I AND II STRAINS.

CLASER C. *(UNIFESP)*; BOSCARDIN SB *(UNIFESP)*; RODRIGUES MR *(UNIFESP)*

Vaccination studies in mice provided evidence that the Amastigote Surface Protein-2 (ASP-2) is an important target for protective immunity against Trypanosoma cruzi infection (Garg& Tarleton, 2002, Boscardin et al., 2003, Fralish& Tarleton, 2003, Vasconcelos et al., 2004, Araújo et al., 2005). Based on the remarkable protective properties of ASP-2, we thought it would be important to evaluate its polymorphism in amastigotes of different strains of T. cruzi. RNA purified from intra-cellular amastigotes of T. cruzi II strains (Sylvio X10/4, DM28c, Tulahuén, G and Colombian) as well as the hybrid strain CL-Brener was used as template for RT-PCR reaction in the presence of oligo-dt primers and primers specific for the ASP-2 gene. PCR products of $\tilde{2}$.1 Kb were cloned and subjected to enzymatic restriction analysis and sequencing. Amastigote cDNA of the different strains contained 1 to 4 groups of genes/pseudogenes. The predicted amino acid sequences of the genes isolated from CL-Brener or Tulahuén presented a high degree of identity to the previously described genes of ASP-2 from Y and Brazil strains. In contrast, ASP-2 genes from Sylvio X10/4 and G strains displayed a limited identity to the previously described genes. Interestingly, they were very similar between them. All cDNA clones isolated from DM28c strain showed a premature stop codon denoting the presence of a pseudogene. The sequence of the ASP-2 genes from amastigotes of the Colombian strain is currently being finished. Our study suggests that ASP-2 expressed by T. cruzi II strains (Brazil and Y) or a hybrid strain (CL-Brener) presented a high degree of identity. On the other hand, ASP-2 of T. cruzi I strains may be quite different or eventually, may not be expressed. The biological and immunological relevance of the different sequences of ASP-2 is currently being investigated. Support: FAPESP.

IM11 - Canine Visceral Leishmaniasis: a comparative study between the inflammatory process and tissue parasitism in cervical lymph nodes of dogs naturally infected with Leishmania (Leishmania) chagasi

Arruda, F.C.S. *(UFMG)*; Lima, W.G. *(UFMG)*; Michalick, M.S.M. *(UFMG)*; Tafuri, Wg.L. *(UFMG)*; Tafuri, W.L. *(UFMG)*

Canine visceral leishmaniasis (CVL) is a severe systemic disease caused by *Leishmania (Leishmania) chagasi*. Cervical lymph nodes appear to be more reactive then popliteal ones and others. In fact, Ciaramella et al. (1997) and Lima et al. (2004) have shown a gross pathological picture more prominent than that of the other nodes. The aim of this

work was to evaluate the histopathological picture of cervical lymph node in parallel to the parasitism tissue load. Thirty mongrel dogs, naturally infected with *Leishmania (L) chagasi*, from Sabara, MG (Belo Horizonte metropolitan area) were classified as asymptomatic, oligosymptomatic and symptomatic animals. All them were sacrificed with lethal dose of Sodic Thiopental (33%) and T61. Fragments of cervical lymph nodes were used to prepare tissue touch preparations (Giemsa-stained smears). The presence of amastigotes was detected in all animals by light microscopy using immersion oil (objective 100X). The parasite burden was expressed as Leishman Donovan units (LDU). Other fragments were collected and fixed in formalin 10%. The tissue sections were paraffined for histopathological (H&E) and immunohistochemical analysis. The streptoavidin-peroxidase immunohistochemistry method was carried out for tissue amastigotes detection (Tafuri et al., 2004). Immunolabeled amastigotes were quantified by morphometrical analysis using the KS300 software. All fragments tissue samples showed a chronic lymphadenitis. Despite the presence of diffuse chronic inflammation involving the capsule throughout the medullary zones, the hypertrophy and hyperplasia of cortical and medullary zones were a common feature. However, the essential architecture of the lymph nodes was preserved without atrophic or degenerative areas. These alterations explain the enlargement of the lymph nodes, whereas hyperemia and edema were irregularly present and thus cannot explain the enlargement of the nodes. Not positive or negative correlation could be found comparing the LDU data or the parasitism load by immunohistochemical analyzes among all tissue cervical nodes alterations. Support: FAPEMIG

IM12 - Histopathological and parasitological analysis of skin tissues biopsies from distinct anatomical areas of dogs naturally infected with *Leishmania (Leishmania) chagasi*.

Perlatto Moura, E. *(UFMG)*; Alves, C.F. *(UFMG)*; Ribeiro, R.R. *(UFMG)*; Tafuri, Wg.L. *(UFMG)*; Michalick, M.S.M. *(UFMG)*; Tafuri, W.L. *(UFMG)*

Canine visceral leishmaniasis is an endemic disease in Latin America caused by *Leishmania (Leishmania) chagasi* and transmitted to man and animals by infected blood sucking sand flies of the genus *Lutzomyia*. Dogs are considered the mainly domestic reservoir of disease because they present an intense cutaneous parasitism. The aim of this study was to evaluate the intensity of inflammatory process and compare it to the parasitism tissue. We believe that there is an essential ear anatomical area that presents higher parasitism then other anatomical regions. For diagnosis analysis serological tests were carried out by enzyme-linked immunosorbent assay (ELISA). Twelve animals were sacrificed with lethal dose of Sodic Thiopental and T61. During the necropsy two anatomical sites of ear (extremity and middle) and one fragment of nose of naturally infected dogs with Leishmania chagasi were collected. All tissues were fixed in formalin (10%) and they were paraffined for histopathological (H&E) and immunohistochemical analysis. The streptoavidin-peroxidase immunohistochemistry method was carried out for tissue amastigotes detection by optical microscopy (Tafuri et al., 2004). Our results have demonstrated a discrete to an intense chronic inflammatory reaction. Interestingly, the inflammatory process were more frequently observed in the extremity of the ears than the middle anatomical area (p 0,05). On the other hand, all nose fragments tissues showed the same histological picture and it was similar to the middle of the ear area. Moreover, the presence of parasites in ear extremity was higher than the other evaluated anatomical regions. A positive correlation between the tissue inflammation, parasitism and serological data was confirmed in both anatomical sites (p 0,05). The skin biopsies are an important tool for CVL diagnosis. Then, the ear extremity appears to be more appropriated anatomical area to execute the biopsies.

IM13 - Histopathological and immunocytochemical analysis of 717 canine ear skin biopsies of animals naturally infected with *Leishmania (Leishmania) chagasi* throughout May 2003 to June 2005.

Figueiredo, M.M. *(UFMG)*; Lima, W.G. *(UFMG)*; Amaral, F.N. *(UFMG)*; Amaral, M.E. *(UFMG)*; Perlatto Moura, E. *(UFMG)*; Sampaio, W.M. *(UFMG)*; Ribeiro, V.M. *(UFMG)*; Michalick, M.S.M. *(UFMG)*; Tafuri, W.L. *(UFMG)*; Tafuri, Wg.L. *(UFMG)*

Canine Visceral Leishmaniasis (CVL) is a zoonosis and a chronic systemic disease of the dog caused by a protozoan *Leishmania chagasi* in the New World. In this work we describe a histological and immunocytochemical retrospective study of canine skin ears biopsies. Moreover, we intended to analyze the histology (inflammation) and parasitism data in order to understand this variable. Ears skin biopsies of a 717 animals naturally infected with *Leishmania chagasi* were receipted by General Pathology Department (ICB-UFMG). All skin fragments were prepared for histopathological and immunocytochemical exams. For histological analysis paraffined skin sections were stained by Hematoxilin-Eosin (H&E) and they were analyzed by optic microscope in order to evaluate the chronic inflammatory reaction. For immunohistochemistry studies the streptoavidin peroxidase immunohistochemistry method was carried out for *Leishmania* detection in canine paraffined tissues (Tafuri et al., 2004). In fact, they were easily observed within inflammatory macrophages in skin biopsies. Our results have demonstrated 107 positive animals (14,9%). This positivism appears to be lowed throughout 2003 to 2005. All animals showed a general chronic inflammatory reaction picture whereas the mononuclear exudate was diffuse in the upper dermis and localized mainly in the deep dermis. The exudate was mainly composed by plasmocytes, macrophages and lymphocytes. A moderate discrete inflammatory reaction was mainly found independently of the months or years. There are a straight relation

between the intensity of the inflammatory reaction and the parasitism. In fact, the statistical analysis (Spearmen correlation test) confirmed that a higher parasitism increases the inflammation. However, this relation was not strong based on the media coefficient relation (r2= 0,3659). It means that the parasitism determines an inflammatory reaction, but not necessarily an intense inflammatory process. Also, we have concluded that is rare to find negative animals with an intense chronic inflammation or vice and versa. Supported by: FAPEMIG

IM14 - Delayed Human Neutrophil Apoptosis induced by *Leishmania amazonensis* metacyclics and Role of *Lutzomyia longipalpis* Salivary Gland Lysates.

Froment, GS *(UFRJ)*; Oliveira, SMP *(FIOCRUZ)*; Peçanha, LMT *(UFRJ)*; Saraiva, EM *(UFRJ)*

While taking a blood meal, sand flies inject *Leishmania* metacyclic promastigotes and salivate into a blood pool in the vertebrate host skin. The saliva contains different compounds with vasodilator and anti-cloting activities among others. Metacyclics regurgitated into the blood pool must survive host defense mechanisms such as complement activation and neutrophil phagocytosis and citotoxicity. Neutrophils constitute the most abundant cell in the blood and are short-lived cells. It has been shown that *Leishmania* can survive inside neutrophils, suggesting that this parasite can prolong the short-live of this cells. So, we were interested in analyze whether *Leishmania amazonensis* could prolong the lifetime of human neutrophils, as well as the role of *Lutzomyia longipalpis* salivary gland lysates (SGL) in this effect. We investigated the spontaneous neutrophil early apoptosis and the influence of the co-incubation with metacyclics and/or SGL. Neutrophils purified from blood bank donors were incubated overnight with *L.amazonensis* metacyclics at a 1:3 ratio in RPMI supplemented with 10% FCS at 37°C/5%CO_2. Excised salivary glands were lysed by ultrasound, sterile filtered and half gland added to the neutrophils and to neutrophils-metacyclics interaction medium. Apoptosis was evaluated by cytometry using Annexin V FITC staining and cell viability assessed by propidium iodide (PI). *L.amazonensis* metacyclics inhibited neutrophil spontaneous apoptosis by 45% (range 23 - 85%). Addition of SGL to this system inhibited 50% the neutrophils apoptosis. Neutrophils incubation with metacyclics plus SGL showed 26% less apoptosis than neutrophils incubated with metacyclics. Spontaneous neutrophil apoptosis was not affected by SGL. Interestingly, SGL also inhibited 40% the number of $PI+$ cells in relation to neutrophils incubated with metacyclics. Here we show for the first time that SGL can potenciate the delayed neutrophil apoptosis induced by *L. amazonensis* metacyclics. Supported by: PIBIC-UFRJ, CNPq.

IM15 - PROTECTION AGAINST VISCERAL LEISHMANIASIS AFTER INTRANASAL IMMUNIZATION WITH THE FML SAPONIN VACCINE

Oliveira-Freitas E *(UFRJ)*; Borja-Cabrera GP *(UFRJ)*; Santos FN *(UFRJ)*; Paraguai de Souza E *(UFRJ)*; Souza LOP *(UFRJ)*; Pinheiro RO *(UFRJ)*; Palatnik de Sousa CB *(UFRJ)*

Aiming the development of a needle-free vaccine, we compared the FML-vaccine administered either by the footpad subcutaneous(sc) or the intranasal(in) route(3 doses of 150ug FML + 100ug saponin R in saline in Balb/c females). Antibody response was significantly enhanced soon after vaccination and sustained after challenge with L. chagasi amastigotes. As expected, significant increases of serum anti-FML IgA antibodies were found only after in treatment. No vaccine induced IgM enhancement. Pronounced increases of anti-FML antibodies were found in IgG class and its subtypes for both vaccines. IgG1and IgG2b were similar for both vaccines while IgG2a was higher for the sc route and IgG for the in route. In the nasal wash, significant increases of anti-FML antibodies IgA ($p \leqq 0.0001$)(Abs $492nm = 0.719$) followed by minor IgG1, IgG2a and IgG2b titers were induced only by the in vaccine. DTH response to L.(L.) donovani antigen, monitored 24 and 48h after injection showed significant swells over the saline control($p \leqq 0.005$) for both vaccines, before and after challenge. Both the in and sc vaccines induced similar DTH reactions($p \geqq 0.05$). FACS analysis disclosed for the sc vaccine normal proportions of CD8 lymphocytes among spleen and lymph node cells while CD4 lymphocytes were increased only in the related poplyteal lymph node(48.43 ± 7.43). The in vaccine, on the other hand induced normal ratios of CD4 and CD8 in both spleen and cervical lymph nodes. Increased in vitro proliferation was disclosed by MTT assay for lymph node cells of the scvaccine and for spleen cells of both vaccines. A 96 % decrease in liver parasite counts was detected for both vaccines ($p \geqq 0.05$). The in route was then as protective as the sc route what makes possible the development of a needle free FML-vaccine. Support: CNPQ;RHAE-CNPQ,PRONEX/FAPERJ,FAPERJ, Brazil. Fort Dodge Animal Health, Brazil and USA.

IM16 - COMPARATIVE ADJUVANT POTENTIAL OF THE QS21 AND CP05 PURIFIED SAPONINS IN THE FML VACCINE AGAINST MURINE VISCERAL LEISHMANIASIS

Chame-Barreto F *(UFRJ)*; Oliveira-Freitas E *(UFRJ)*; Borja-Cabrera GP *(UFRJ)*; Santos FN *(UFRJ)*; Paraguai de Sousa E *(UFRJ)*; Souza LOP *(UFRJ)*; Nico D *(UFRJ)*; Silva BP *(NPPN/UFRJ)*; Parente JP *(NPPN/UFRJ)*; Palatnik de Sousa CB *(UFRJ)*

QS21 and CP05 are two purified highly potent saponins containing a complex normoterpen moiety attached to C-28. QS21 also contains an aldehyde esposed on C-23, analog to the B7 ligand on the APC cells. Both saponins showed to be potent adjuvants of FML antigen. We investigated the effect of 25,50 and 100ug of each saponin as adjuvant for 150ug of FML, on three weekly doses by the sc route. After vaccination, the DTH response to L.(L.) donovani antigen was significantly enhanced for all vaccinated groups over saline control ($p \leqq 0.05$). Most potent reactions were seen in the 100ug groups for ech saponin although differences between concentrations or adjuvants were not significant. Splenocyte in vitro proliferation against Leishmania lysate or NH36 recombinant antigen was enhanced only for the CP05 or QS2150ug groups. Splenocyte FACS analysis disclosed a dose-dependent enhancement for the CP05 treatment of CD4 and CD8 T cell lymphocytes and a for the QS21 treatment only for CD4 cells. A pronounced decrease in liver parasitic load was obtained using 50ug of CP05 or using 25ug of QS21. Our results indicate the strong effect of increased number of nomoterpen moieties of the CP05 saponnin on the CD8 lymphocyte increase addition of aldehydes through chemical treatment of theses saponins is under progress. Support: CNPQ; RHAE-CNPQ, PRONEX/FAPERJ, FAPERJ, Brazil. Fort Dodge Animal Health, Brazil and USA.

IM17 - HEPATIC FIBROSIS IN CANINE VISCERAL LEISHMANIASIS: A HISTOPATHOLOGICAL STUDY OF A 96 HEPATIC TISSUE SAMPLES

Amaral M.E. *(UFMG)*; Lima W.G. *(UFMG)*; Michalick M.S.M. *(UFMG)*; Tafuri Wg.L. *(UFMG)*; Tafuri W.L. *(UFMG)*

Canine visceral leishmaniasis is an endemic disease in Brazil and dogs play a central role in the transmission of the disease to human beings. In the New World is caused by *Leishmania Leishmania chagasi* and it is transmitted by the blood sucking flies *Lutzomyia longipalpis*. The aim of this study was to evaluate the parenchyma hepatic collagen deposition of dogs naturally infected with L chagasi. In human beings, the hepatic fibrosis is depicted in Indian Kala-azar (Rogers et al., 1908) and in Brazil (Bogliolo, 1956). Ninety six infected animals with positive serological exams to Leishmania were divided in four clinical groups: controls, asymptomatic, oligosymptomatic and symptomatic animals. The dogs were sacrificed with lethal dose of Thionembutal 33% (1,0mL/Kg) and T61. During necropsy liver fragments were collected and fixed in formalin 10 % for histopathological studies (HE). Special stainings as Gomori, Heidenhain, Silver and Picrosirius Red were carried out to characterize the fibrilopoesis. The fibrosis as described by Roger et al. (1908) and Bogliolo (1956) were observed only in three cases (3,1%). In fact, in these cases a diffuse reticular fibers deposition were observed and they were aligned in various directions with compact network formation whereas some fibers were thicker than others. Frequently these collagen fibers encircled small groups of hepatocytes or a single cell acquiring the aspect of the Nattan-Larrier (1918) "monocellular cirrhosis". A stronger yellow-red birefringence was observed indicating one collagen type fibers deposition by Picrosirius Red staining. Moreover, some intralobullar collagen fibers were slight green indicating collagen type 3. Other hepatic pathologies as intralobular granulomas, degenerative hepatocytes, hyperplasia and hypertrophy of kupffer cells congestion, capsule and portal inflammatory reaction and pigment deposition were analyzed in parallel. However, there were no statistical differences among the distinct animals clinical status. Supported by: FAPEMIG

IM18 - Pre-infection of C57BL/6 Interleukin 10-Deficient Mice with *Leishmania major* Leads to control of Lesion Progression Caused by *Leishmania amazonensis*

González-Lombana, C. Z. *(UFMG)*; Seixas-Rego, V. A. *(UFMG)*; Macedo, J. P. *(UFMG)*; Afonso, L. C. C. *(UFOP)*; Vieira, L. Q. *(UFMG)*

Infection of mice with *Leishmania spp.* that causes cutaneous leishmaniasis has led to an understanding of many immunological events required for a successful host response towards these parasites. Many studies have demonstrated that the host response is determined by the species or strains of *Leishmania* and also that this response may vary in the same mouse strain. Since C57BL/6 mice infected with *L. major* develop a Th1 response that leads to spontaneous resolution of lesions and knowing that the susceptibility of these mice to *L. amazonensis* results from an inability to mount these responses, we tested the hypothesis that the previous induction of a Th1 response elicited by *L. major* would modify the outcome of infection with *L. amazonensis*. To address this issue, C57BL/6 and IL10 -/-mice, which had been previously inoculated into the left hind footpad with 1 x 10^4 metacyclic promastigotes of *L. major*, were infected with 10^4 metacyclic promastigotes of *L. amazonensis* in the contralateral footpad and the course of infection was followed for 11 weeks. C57BL/6 and IL10 -/- mice previously inoculated with *L. major* were able to control the lesion progression induced by *L amazonensis* throughout the infection. In contrast, measurable lesions were observed between 7 to 8 weeks post-infection in both C57BL/6 and IL10 -/- mice in-

oculated with *L. amazonensis*, which continuously increased in size and showed no signs of recovery. Furthermore, the parasite burden in lesions was ∼1,4 $\log_{10}$ lower in the previously *L. major*-infected mice compared with control mice. On the other hand, cells from previously *L. major*-infected C57BL/6 and IL10 -/- mice produced significantly higher levels of IFN-γ compared with those of control mice. These data indicate that in C57BL/6 mice, pre-infection with *L. major* allowed the control of lesion progression caused by *L. amazonensis*, which occurs through an IL10-independent mechanism.Support:CNPq,FAPEMIG,CAPES.

IM19 - CD8$^+$ T Cells Are Not Required for Vaccine-Induced Immunity Against *Leishmania amazonensis* in IL12P40 −/− C57BL/6 Mice

Hernández S., M. X. *(UFMG)*; Golgher, D. *(UFMG)*; Afonso, L. C. C. *(UFOP)*; Tafuri, W. *(UFMG)*; Vieira, L. Q. *(UFMG)*

Adoptive or vaccine-induced protection against leishmaniasis is largely dependent on cell−mediated type 1 immune response and IL12-driven IFN-γ production. Surprisingly, previous data from our laboratory described the efficiency of vaccination against *L. amazonensis* infection and, additionally, IFN-γ production was found up−regulated in vaccinated IL12 p40 −/− mice. Although CD8$^+$ T cells play important role in *L. amazonensis* infection and are able to produce IFN-γ, its role in specific parasite vaccination is obscure. The aim was to evaluate the effects of CD8$^+$ T cells in protection against *L. amazonensis* in vaccinated mice. IL12p40 −/− mice were immunized with two inoculations in a seven-day interval regimen with killed *L. amazonenis* vaccine (Leishvacin) plus *Corynebacterium parvum* as adjuvant. Twenty-eight days later, the animals received a booster and seven days later were infected in the left footpad with *L. amazonensis*. In order to deplete CD8$^+$ T cells, one group of vaccinated animals was treated with anti-CD8$^+$ mAb YST169 or control antibody on days −6, −3, +4, +7 and once weekly after *L. amazonensis* infection. Infection was followed for 8 weeks. The vaccinated CD8$^+$-depleted group developed smaller lesions when compared to the non-depleted group. CD8 depletion did not affect tissue parasitism or antibody response against the parasite. In addition, CD8$^+$-depleted animals displayed milder inflammation and better tissue integrity. IFN-γ production in spleen and draining lymph node was impaired in anti-CD8$^+$ depleted group, suggesting a role of CD8$^+$ cells in production of this cytokine throughout infection in IL12-independent vaccination. IL4 was not detected in supernatants from lymph node or spleen cell cultures. Such results suggest that CD8$^+$ T cells play a minor role in *L. amazonensis* vaccination in IL12p40 −/− animals, contributing to augmented pathology. Although these cells produce some IFN-γ the in absence of IL12, they do not affect the parasite tissue load.

IM20 - Suppressive effect of hyperbaric oxygen on the development of *Leishmania amazonensis*-induced lesions in susceptible mice.

Arrais-Silva, W. W. *(UNICAMP)*; Pinto, E. F. *(UFRJ)*; Rossi-Bergmann, B. *(UFRJ)*; Giorgio, S. *(UNICAMP)*

Hyperbaric oxygen (HBO) therapy has been shown to increase both systemic and tissue oxygen levels and to assist as an adjuvant treatment for numerous soft-tissues infection. Recently, we demonstrated that HBO is toxic for *Leishmania amazonensis* promastigotes and amastigotes. In the present study we evaluated the effect of HBO therapy on various diseases parameters of mice infected with *L. amazonensis*. BALB/c mice exposed to HBO (100% O_2, 2.5 ATA, 1 h before, 2 h immediately after amastigotes inoculation and then treated daily for 20 days) showed significantly delay on the development of lesion and caused a reduction of parasite burden compared with HBO unexposed group. At the end of HBO treatment, histological analysis showed a cellular population infiltration consisting of inflammatory cells and macrophages less infected than that seen in lesions of HBO unexposed mice, accompanied by a significant increase in interferon gamma (IFN-g) and tumor necrosis factor (TNF-a) and reduction of interleukin 10 (IL-10), indicative of a skewed Th1-Type response. These findings demonstrated, for the first time, that HBO treatment can play a pathogen control role during leishmaniasis and favored the development of Th1-Type response during infection. Supported by FAPESP, CNPq, CAPES and FAEP.

IM21 - Expression of hypoxia-inducible factor-1α in the cutaneous lesions of BALB/c mice infected with *Leishmania amazonensis*.

Arrais-Silva, W. W. *(UNICAMP)*; Paffaro Jr, V. A. *(UNICAMP)*; Yamada, A. T. *(UNICAMP)*; Giorgio, S. *(UNICAMP)*

The hypoxia-inducible factor-1α (HIF-1α) is expressed in response to hypoxia and has been recently demonstrated in a variety of cells such as tumor cells and tumor-associated macrophages. Several characteristics of leishmanial lesions in humans and in animal models, such as microcirculation impairment, metabolic demand for leukocyte infiltration into infected tissue, parasite proliferation, and secondary bacterial infection, are strong indications of a hypoxic microenvironment in the lesions. We evaluated HIF-1α expression in the cutaneous lesions of BALB/c mice during *Leishmania amazonensis* infection. Immunohistochemical analyses of the lesions demonstrated, only in the later stages of infection when the lesion size is maximal and parasite burden is enormous and massive numbers of recruited macrophages and ulcers are observed, positive HIF-1α-infected cells throughout the lesions. HIF-1α is expressed mainly in the cytoplasm

and around parasites inside the parasitophorous vacuoles of macrophages. This is the first evidence that macrophages in the microenvironment of lesions caused by a parasite produce a hypoxia-inducible factor. Supported by FAPESP, CNPq, CAPES and FAEP.

IM22 - A model of intradermal inoculation of *Leishmania amazonensis* metacyclic promastigotes in C57BL/6 mice

Seixas-Rego, V. A. *(UFMG)*; González-Lombana, C. Z. *(UFMG)*; Macedo, J. P. *(UFMG)*; Afonso, L. C. C. *(UFOP)*; Vieira, L. Q. *(UFMG)*

Leishmania infections can cause a broad spectrum of clinical manifestations, depending on the parasites species, host immune status and inoculation dose. In natural condition, low numbers of metacyclic promastigotes are transmitted into the skin of a vertebrate host by sand fly vectors. Diverse murine models of cutaneous leishmaniasis are valuable for the study of the disease pathogenesis and vaccine development. Mostly, infections described in the literature are done by the subcutaneous route. Moreover, high doses of stationary phase promastigotes are used, which implies that a high number of non-infective parasites are inoculated together with the infective metacyclic forms. In this model, C57BL/6 mice develop a chronic lesion that is characterized by a delayed inflammatory response in comparison to *L. major*, lower IFN$-\gamma$ production and a relative but not efficient control of parasites. Only recently the ear dermis has been used to follow lesion development and immune response. This work describes a intradermal model of cutaneous leishmaniasis in C57BL/6 mice using 1x10$\hat{4}$ metacyclic promastigotes of *Leishmania amazonensis*. Dermal lesions were observed at 2 weeks post-infection in both *L. major* (as control) and *L. amazonensis*−infected mice. Although the lesion size of the *L. major*−infected C57BL/6 mice peaked at 4 weeks with tendency of healing, lesions of *L. amazonensis*−infected C57BL/6 mice peaked later, between 7 to 8 weeks, and showed no signs of recovery. On the other hand, the parasite load was 3.3 and 1.7 log_10 in *L. amazonensis* and *L. major* infected C57BL/6 mice, respectively. Hence, we show here that inoculation of metacyclic parasites intradermally cause a non−healing chronic lesions. This model is more similar to the natural infection and should be a tool to study the mechanisms of resistance to *L. amazonensis*.

IM23 - IL-18 involvement in immune response and pathology to *L. amazonensis*

Helton C. Santiago *(UFMG)*; Shaden Kamhawi *(NIH)*; Mayra X. Hernandez Sanabria *(UFMG)*; Allen Cheever *(NIH)*; David Sacks *(NIH)*; Dragana Jankovic *(NIH)*; Leda Q. Vieira *(UFMG)*

IL-18 is known to play an important role in Type 1 immune response differentiation, cooperating with IL-12 to trigger an optimal IFN-γ production. IL-18 has been shown to be important in host defense driving an efficient type 1 immune response and host defense against *L. major* infection. However, its role in *L. amazonensis* infection is completely unknown and has not been investigated. C57BL/6 IL-18 KO mice were injected in the ear with 1000 purified *L. amazonensis* metacyclics and the lesion followed for 16 weeks. Surprisingly, IL-18 KO animals were found to be more resistant displaying significant milder lesions throughout the infection and 10 time fold decrease in parasite burdens when compared to WT mice. In addition, real-time RT-PCR analysis determined that IL-18 KO animals expressed higher levels of IFN-γ, NOS2 and LRG-47 mRNAs than WT mice at 12 weeks post-infection, but not at 16 weeks post-infection. The IL-18 deficient animals also displayed smaller inflammatory areas and also lower macrophage numbers in the infiltrates. Such results suggest that IL-18 plays a deleterious role during *L. amazonensis* infection in the C57BL/6 background. In addition, our preliminary results show that BALB/c IL-18 KO mice also develop smaller lesions when compared to WT controls. Whether IL-18 plays a direct counter regulatory role of the immune response to L. amazonensis or whether it augments susceptibility by improving inflammation and macrophage recruitment to the infection site is still under investigation. Support: CNPq, CAPES, FAPEMIG

IM24 - Endogenously produced IL-10 and IFN-gamma regulate cellular activity of leukocytes from cutaneous leishmaniasis patients

Gaze, ST *(UFMG)*; Keesen TKS *(UFMG)*; Carvalho, L.P *(UFBA)*; Machado, P.R.L *(UFBA)*; Carvalho, E. *(UFBA)*; Dutra, WO *(UFMG)*; Gollob, KJ *(UFMG)*

Leishmania braziliensis can promote at least three clinical forms of disease with the cutaneous form accounting for more than 90% of related cases. Important studies have shown that cytokines produced during disease can influence the severity or the cure of leishmaniasis. Therefore, to better understand the role of cytokines such as IL-10, IFN-gamma and TGF-beta in cutaneous leishmaniasis we performed a study blocking the activity of these key immunoregulatory cytokines. Peripheral blood mononuclear cells, from 8 cutaneous leishmaniasis patients, were cultured with IL-10, IFN-gamma or TGF-beta blockers in the presence or absence of SLA. Cultures were then studied for the frequency of activated monocytes and lymphocytes, as well as for expression of immunoregulatory cytokines. Cultures with anti-IL-10 had more CD14 cells committed toward TNF-alpha expression, as well as, CD4 blast lymphocytes committed to IFN-gamma expression. In cultures with anti-IFN-gamma an increase in the frequency of CD4 T cells committed to IL-10 expression, and a decrease in CD4 and CD8 blast lymphocytes committed in the IFN-gamma expression was seen. Moreover, a decrease in HLA-DR expression by monocytes was also seen. Interestingly, in cultures with anti-TGF-beta a decreased expression of the early activation marker, CD69 was

seen in CD8 T cells, as well as a decrease in HLA-DR intensity by CD14+ monocytes. These results indicate that, both down modulatory (IL-10), and inflammatory (IFN-gamma) cytokines are actively involved in producing the overall immune profile seen in SLA specific responses from cutaneous leishmaniasis patients. e-mails: kjgollob@icb.ufmg.br and gaze@icb.ufmg.br Financial support: WHO/TDR, CAPES, CT-Infra-FINEP, PADCT/CNPq

IM25 - Role of nitric oxide (NO) and the cytokines TNF and IFN$-\gamma$ on the immunization of mice using Leishvacin against *Leishmania amazonensis* infection

Côrtes, D. F. *(UFMG)*; Maioli, T. U. *(UFMG)*; Carneiro, C. M. *(UFOP)*; Afonso, L. C. C. *(UFOP)*; Vieira, L. Q. *(UFMG)*

Control of cutaneous leishmaniasis (CL) is problematic due to the sylvatic nature of both vector and reservoirs, making the inseticide spraying and elimination of reservoirs specially difficult. Immunization of the population at risk appears to be the more cost effective prophylactic measure against CL. Scott *et al* (1987) showed that BALB/c mice could be protected against *L. major* infection after immunization with *L. major* antigen. In our study, we enquire if the same happens with C57BL/6 mice and which is the role of NO and the cytokines TNF and IFN$-\gamma$ on the immunization process. For that purpose, we vaccinated C57BL/6, TNFR p55 $-/-$, iNOS $-/-$ and IFN$-\gamma$ $-/-$ mice with two doses of Leishvacin, using *C. parvum* as an adjuvant, in a seven$-$day interval regimen. After 30 days, these animals received a booster with Leishvacin only and then, after seven days, they were infected in the footpad with *L. amazonensis*. The course of infection was accompanied weekly and the animals were sacrificed on the 8th and the 11th week post$-$infection. Parasite quantification and the footpad histology were performed, besides cytokine measurements in lymph node and spleen cell culture supernatants and *in vitro* assays for NO and reactive oxygen intermediates (ROIs) measurements. We observed that the immunization triggered an inflammatory response and IFN$-\gamma$ production, which were efficient in lesion size control of C57BL/6 and TNFR p55 $-/-$ mice. Surprisingly, the immunization did not increase NO and ROIs production, which explains the inefficacy observed in parasitism control. The results obtained in this study are, therefore, critical in the search of an efficient vaccine against leishmaniasis.Supported by: FAPEMIG

IM26 - Immunological evaluation of attenuated Leishmania amazonensis antigens

Guedes, HLM *(UFRJ)*; Pinheiro, RO *(UFRJ)*; Lopes, JRC *(UFRJ)*; Chaves, PS *(UFRJ)*; Gomes, DCO *(UFRJ)*; De Simone, SG *(FIOCRUZ)*; Bergmann, BR *(UFRJ)*

L. amazonensis is the main agent of the anergic diffuse cutaneous leishmaniasis. We showed previously that a crude promastigote lysate of virulent L. amazonensis-LaAg induces T-cell anergy in vitro and that intramuscular pre-vaccination with LaAg leads to exacerbated disease in BALB/c mice. Infection with live attenuated parasites has been used in some countries, but little is known about the immunomodulatory effect of killed attenuated parasites. In this work, we aimed first at comparing in vitro the capacity of attenuated L. amazonensis antigens-LaAg-At and LaAg to modulate T cell responses nitric oxide production by lymph node cells of 7-day infected mice. We found that LaAg-At but not LaAg activated cell proliferation, while the NO production by macrophages was similarly inhibited by both. In vivo, two intramuscular injections of 25ug of LaAg-At increased the resistance of BALB/c mice to subsequent infection with L. amazonensis-GFP. Protection was accompanied by a reduced production of TGF-beta and increased IFN-gamma and proliferation in the lesion-draining lymph nodes during in vitro antigen recall responses. In conclusion, the anergenic and disease-promoting effect of LaAg is associated with virulence factors of Leishmania that activate TGF-beta production.

IM27 - Efficacy of parenteral vaccination with different serine proteases fractions of Leishmania amazonesis

Guedes, HLM *(UFRJ)*; Pinheiro, RO *(UFRJ)*; Rezende-Neto, JM *(UFRJ)*; Silva-Lopez, RE *(FIOCRUZ)*; De Simone, SG *(FIOCRUZ)*; Rossi-Bergmann, B *(UFRJ)*

Leishmania amazonensis is one of the most important ethiologic agent of American tegumentary leishmaniasis. Proteases play crucial roles in host-parasite interaction. Recentely, our group purified and characterized serine proteases of Leishmania amazonensis. In the present work, we evaluate the protectiveness of intramuscular immunization with serine protease fractions in the highly susceptible BALB/c mice. Serine proteases were purified from aqueous orLaFsol, detergent-soluble or LaFi and extracellular or LaFex extracts of L. amazonensis using a single step with aprotinin-agarose chromatography . These fractions were first evaluated in vitro as to their capacity to modulate the proliferation and citokine production of fresh lesion-draining murine lymph node cells and in vivo as to its protective immunization. Balb/c mice injected with two doses of 25ug of serine proteases prior to infection with fluorescent L. Amazonensis. The course of infection was monitored by the lesion sizes and at the end of experiment - day 95, the parasite loads were assessed by the fluorescent intensity of the infected footpad lysates and the production of citokines and spontaneous proliferation were measured in the lesion-draining lymph node cells. We found that immunization with LaFsol and LaFex promted increased susceptibility to subsequent infection, similar to found previously with whole parasite antigen. Interistingly, LaFi induced protection as seen by the smaller lesion sizes and parasite loads in the infection site.

The protective effect of LaFi was associated with upregulated T-cell proliferative response and significant reduction TGF-beta and IL-10 in in the lesion-draining lymph node cells on day 95 of infection. These results indicate that LaFi is capable to protect mice against murine cutaneous leishmaniosis, and that its particulated nature may be impostant for its adjuvant-independent effectiveness. Possibly, its protective effect is associated with depressed IL-10 and TGF-beta production in fresh lesion-draining murine lymph node cells.

IM28 - IMMUNOREGULATION IN HUMAN CUTANEOUS LEISHMANIASIS: ROLE OF GLUTATHIONE

VIANNA, P *(UFMG)*; ANTONELLI, LRV *(UFMG)*; CARVALHO, EM *(UFBA)*; MACHADO, PRL *(UFBA)*; ALMEIDA, RP *(UFBA)*; DUTRA, WO *(UFMG)*; GOLLOB, KJ *(UFMG)*

The human cutaneous clinical form of leishmaniasis is characterized by the presence of skins lesions, and is caused mainly by Leishmania braziliensis. The control of the infection requires the induction of an immune response capable of activating macrophages to a microbicidal state, which depends on the production of nitric oxide and killing of the parasites within macrophages. Glutathione (GSH) is the major intracellular redox buffer and plays a role in protecting cells against oxidant damage modulating the expression of several genes. We have observed that the host response to L. major infection can be significantly improved by increasing in vivo glutathione levels in the murine model. We are interested in how GSH modulation could be employed to improve immune responses in human leishmaniasis. For this purpose we evaluated the human PBMC response to Leishmania infection and SLA (soluble leishmania antigen) stimulation in the presence of two glutathione modulating agents: N-acetyl-L-cystein (NAC), a GSH precursor, and diethyl-maleate (DEM), a GSH depleting agent. The effects of GSH modulation on monocyte-parasite interactions through CD11b were analyzed by flow cytometry, as well as other surface markers and cytokines (CD86, CD14, CD25, CD69, TNF-alpha and IL-10). Monocyte infection by Leishmania metacyclic promastigotes (L. braziliensis), was studied using parasites stained with CFSE (carboxyfluorescein diacetate, succinimidyl ester) and analyzed by flow cytometry. Reducing GSH levels in human monocytes led to an increased frequency of infected monocytes. Increasing intracellular GSH levels led to a lower frequency of infected monocytes and CD11b expression, accompanied by an increased expression of TNF-alpha and CD86. Our data indicate that GSH modulation might be a useful pathway to improve the host response against Leishmania infection. NAC appears to induce beneficial changes in the immune response and interaction of human monocytes with L. braziliensis. Support: CAPES, WHO/TDR, CNPq/PRONEX, FINEP-CT-Infra, FAPEMIG. e-mails: kjgollob@icb.ufmg.br and privanna@icb.ufmg.br

IM29 - Exposure of phosphatidylserine in murine macrophages infected with *Leishmania amazonensis*

DaMata, J. P. *(UFMG)*; Horta, M.F. *(UFMG)*

During the *Leishmania* life cycle, infected macrophages eventually rupture and release amastigotes, which are infective forms for neighboring cells. Being an amplifying step, the cells death may be a key point in the development of leishmaniasis. To evaluate cell death of *L. amazonensis*-infected BALB/c peritoneal macrophages, we have initially used the MTT and Trypan blue assays. We have observed a reduction in the viability of the infected macrophages, particularly after 24 and 48 hours of infection. Understanding the importance of apoptosis em several parasitic diseases, we have then investigated the type of death of *L. amazonensis*-infected macrophages, asking whether they died through apoptosis. Previous results have shown that DNA of *in vitro*-infected macrophages were fragmented, as shown by agarose gels and the TUNEL technique. In agarose gels, we have observed that, 24 hours after infection, *L. amazonensis*-infected cells presented a DNA fragmentation that appeared as a ladder pattern with fragment sizes multiples of 200 bp, typical of apoptotic cells. The TUNEL technique also allowed us to observe that, in 24 hours after infection, around 40% of the macrophages had their nuclei labeled. We now show that *L. amazonensis*-infected macrophages also expose phosphatidylserine, another important feature of apoptosis, as shown by annexin V-FITC staining followed by flow cytometry analysis. Together, our results indicate that programmed cell death occurs in macrophages infected with *L. amazonensis in vitro*.

IM30 - Increased activity of matrix metalloproteinase-9 in *Trypanosoma cruzi* and *Leishmania* infected human macrophages.

Pinho, R.T. *(FIOCRUZ)*; Seguins, W.S. *(FIOCRUZ)*; Côrtes, L.M.C. *(FIOCRUZ)*

Matrix metalloproteinases (MMPs) constitute a large family of Zn^{2+-} and Ca^{2+-} dependent endopeptidases, implicated in tissue remodeling and chronic inflammation. They possess broad and overlapping specificities and collectively have the capacity to degrade all the components of the extracellular matrix (ECM). MMPs also play key roles in activation of growth factors and chemokines, being produced by many cell types, including lymphocytes and granulocytes, but in particular by activated macrophages. Their synthesis and secretion appear to be important in a number of physiological processes, including the inflammatory response. In Chagas' disease and in leishmaniasis the macrophages are infected by *T. cruzi* or *Leishmania* respectively. Little is known about MMPs production during protozoan infections and the contribution they make to immunity versus pathology. In the present study we report the interaction be-

tween monocyte-derived human macrophages from normal donors with *T. cruzi* or with *Leishmania*, to verify the alteration of the MMPs in this system. We detected by SDS-PAGE-gelatin, an increase of MMP-9 (92kDa) production in culture supernatants of *T. cruzi* or *Leishmania* infected macrophages. The increase of MMP-9 production was detected at 24, 48 and 72 h post-infection and was also confirmed by immunoblotting analysis using anti-MMP-9 antibody. On the other hand MMP-9 production was down modulated when macrophages cultures were treated with interferon-gamma or IL-4 before infection.

IM31 - Immunomodulatory effect of glycoconjugates from *Leishmania (Leishmania) amazonensis* on macrophage function

Tanaka AK *(UNIFESP)*; Takahashi HK *(UNIFESP)*; Straus AH *(UNIFESP)*

The surface of *Leishmania* is constituted by a complex network of glycoconjugates, essential for their survival and development in the sandfly vector and in the mammalian host. The current study was focused on nitric oxide and tumor necrosis factor production by mouse peritoneal macrophages stimulated by *L. (L.) amazonensis* promastigote cell-surface glycolipid, the lipophosphoglycan (LPG), and the proteophosphoglycan (PPG), a mucin-like glycoprotein secreted by promastigotes in the culture supernatant (pPPG). *In vitro* experiments were performed with peritoneal macrophages subjected to different stimuli as purified pPPG and LPG, bacterial lipopolysaccharide and mouse recombinant interferon-gamma. It was observed that pPPG and LPG *per se* were not able to induce nitric oxide production but they synergize with interferon-gamma the macrophage production of nitric oxide in a dose dependent manner. Moreover, pPPG or LPG inhibit the macrophage secretion of tumor necrosis factor induced by lipopolysaccharide. These results reinforce that pPPG and LPG are important glycoconjugates on the survival of *L. (L.) amazonensis* parasites in the beginning of infection through their ability to interfere in the expression of cytokines and in the synthesis of nitric oxide by macrophages. On the other hand, amastigotes: i) are responsible for parasite persistence in the host; ii) express much lower concentration of LPG and; iii) usually present PPG with different carbohydrate structures, so, a particulary interesting question relays about the role of amastigote glycoconjugates. Thus, PPGs secreted by amastigote forms (aPPG) were purified and its carbohydrate composition analyzed by gas chromatography-mass spectrometry. It was observed that aPPG and pPPG present mannose:galactose:glucose at proportion of 0.5:1:4.5 and 0.8:1:1.4, respectively. This structural difference may reflect on the distinct roles of these glycoconjugates on macrophage function. The effect of aPPG and also amastigote-specific glycosphingolipids on cytokine induction are under investigation. *Supported by FAPESP and CNPq.*

IM32 - EFFECTS OF ATP METABOLISM IN *Leishmania* INFECTIVITY

Marques-da-Silva EA *(UFOP)*; Lima Jr DS *(UFOP)*; Oliveira JC *(UFOP)*; Fietto JLR *(UFOP)*; Afonso LCC *(UFOP)*

Leishmania parasites are devoid of the enzymatic machinery required for "de novo" synthesis of the purine nucleus. These parasites express enzymes that convert extracellular ATP to nucleosides that are then internalized. This study provides evidences that differences in *Leishmania* ability to convert immunostimulatory ATP to the immunomodulator product adenosine may explain differences in parasite infectivity. In order to verify this correlation, we determined the enzymatic activity of *Leishmania (L.) amazonensis* (PH8 strain), *Leishmania (V.) braziliensis* (M2903 strain) and *Leishmania (L.) major* (Friedlin strain) by measurement of nucleotide hydrolysis. Apyrasic and 5′−ectonucleotidasic activities were higher in the more virulent PH8 metacyclic promastigotes - obtained after two passages in culture (P2) after isolation from C57BL/6 mouse - than in the other two less virulent species. In order to verify the influence of adenosine in *Leishmania* infectivity, we administrated this nucleoside in different concentrations at the moment of inoculation of *Leishmania (L.) braziliensis* in C57BL/6 mice footpad (1,0 x 10^5 metacyclic/dose) and verified that adenosine concentrations ranging from 50 to 1000 μM slightly delayed the lesion healing. This delay in lesion healing does not seem to be dependent on IL-10 production - a cytokine induced by adenosine - since IL-10-deficient mice showed similar behavior when infected in the presence of 50 μM adenosine. In parallel experiments we evaluated the enzymatic activity present in salivary gland extracts of *Lutzomyia longipalpis*. Interestingly, these glands showed higher 5′-ectonucleotidasic activity and smaller apyrasic activity if compared with results obtained from metacyclic promastigotes, suggesting the existence of complementary effects of these components in the parasite establishment. Together, these results suggest that decreased ATP and increased adenosine concentrations improved by apyrase and 5′-ectonucleotidase activities can explain, at least in part, the differences observed in infectivity of different *Leishmania* species. **This research is sponsored by: FAPEMIG, PIBIC-CNPq, CAPES, PIP-UFOP.**

IM33 - Vaccination with p36(LACK) DNA vaccine induces IFN-gamma but it does not protect BALB/c mice against *Leishmania chagasi* in the presence of IL-10

Marques-da-Silva *(UFOP)*; Coelho EAF *(UFMG)*; Fernandes AP *(UFMG)*; Afonso LCC *(UFOP)*; Rezende SA *(UFOP)*

Visceral leishmaniasis is a progressive disease caused by *Leishmania chagasi* in South America. The acquisition of

immunity following infection suggests that vaccination is a feasible approach to protect from this disease. Since LACK antigen is of particular interest as a vaccine candidate because of the prominent role it plays in the pathogenesis of experimental *Leishmania major* infection, we evaluated the potential of a p36(LACK) DNA vaccine in protecting BALB/c mice challenged with *L. chagasi*. In this study, mice received intramuscular doses of LACK DNA vaccine. We evaluated the production of vaccine-induced cytokines and whether this immunization was able to reduce parasite load in liver and spleen. We detected a significant production of IFN-gamma by splenocytes from vaccinated mice in response to *L. chagasi* antigen and to rLACK protein. However, we did not observe a reduction in parasite load neither in liver nor in the spleen of vaccinated animals. In order to better understand the lack of protection observed, we also analyzed the IL-10 production by spleen cells prior and after infection. We observed an increase in IL-10 production by spleen cells obtained from mice prior to infection in pCI-neo-LACK vaccinated mice in response to rLACK protein when compared to spleen cells from mice inoculated with pCI-neo or PBS. Furthermore, we observed that IL-10 production by spleen cells from i.m. vaccinated and PBS inoculated mice that were challenged four weeks after booster was higher in response to particulate *Leishmania* antigen if compared to non stimulated cells. Thus, the present study shows that a LACK DNA vaccine was able to induce an increase in IFN-gamma production, but it did not protect BALB/c mice against *L. chagasi* in the presence of high levels of IL-10. Supported by: FAPEMIG, UFOP

IM34 - Intranasal immunization with LACK-DNA promotes systemic tecidual mRNA expression and protects BALB/c mice against *L. Chagasi* infection

Gomes D C O *(UFRJ)*; de Melo L D B *(UFRJ)*; Pinto E F *(UFRJ)*; Pacienza-Lima W *(UFRJ)*; Larraga V *(CIB)*; Lopes U G *(UFRJ)*; Rossi-Bergmann B *(UFRJ)*

LACK (Leishmania analogue of the receptor kinase C) is a conserved protein Leishmania species, that is associated with the immunopathogenes and susceptibility of BALB/c mice to infection with Leishmania. Recently we have demonstrated that intranasal immunization with a plasmid carring the LACK gene of Leishmania infantum (LACK-DNA) promotes protective immunity against Leishmania amazonensis. In the preent study, we sought to investigate 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 ug of LACK-DNA expressed LACK mRNA in the spleen, brain, cervical linfonode and poplite linfonode, 4 weeks later. As this storage, their cervical lymph node and spleencell were sensitized enought to produce incresead levels of both IFN-γ and IL-4 upon whole Leishmania chagasi (LcAg) or recombinant LACK (rLACK) restimulation in vitro.Eleveted levels of TNF-α were reduced and Leishmania-especific IgG were detected in the serum. Mice pre-vacinated with i.n. LACK-DNA and challange i.v. with L. chagasi 7 days after the second immunization dose displayad signifcant lower parasite loads in the liver and spleen at 1 month of infection. Their physical aparence was visibly healthier than non-vacinated animals. Pre-vaccinated infected animals produced higher amounts of IFN-γ and IL-4 but reduced levels of IL-10 during antigen recall response in the spleen as compared with spleen infected controls, reduced level of TNF-α accompanied by higher Ag specifc IgG were seen in the serum. Together, these data show that intranasal vaccination with LACK-DNA promotes systemic expression of the antigen that is accompanied by a strong productive immunity against infection with L. chagasi in mice. This is the frist report on the feasibility of using the pratical intranasal route for an effective vaccination against visceral leishmaniasis.

IM35 - A ROLE FOR P2X7 RECEPTOR IN LEISHMANIASIS.

Chaves, S. P. *(UFRJ)*; Torres-Santos, E. C. *(UFRJ)*; Persechinni, P. M. *(UFRJ)*; Rossi-Bergmann, B. *(UFRJ)*; Coutinho-Silva, R. *(UFRJ)*

P2X7 receptor (P2X7R) is a component of purinergic receptor family, wich are activated by extracellular nucleotides like ATP and UTP. Its activation leads to pore formation on the cell membrane, allowing the passage of molecules smaller than 900 Da. To evaluate the role of P2X7R in leishmanial infection, we followed the course of infection of P2X7R-competent (P2X7R +/+) and P2X7R-deficient (P2X7R -/-) C57Bl/6 mice with *Leishmania amazonensis*-GFP. After infection in the footpads, their lesion growth, parasite loads and cytokine production in the draining lymph nodes were evaluated. Although P2X7R -/- mice displayed significantly larger lesions, their parasite burden was lower than the P2X7R +/+ mice. Their capacity to produce IFN-γ was much higher than the P2X7R +/+ controls, compatible to their lower parasite burden. No difference in IL-10 production was observed. The intraperitoneal injection of *L. amazonensis*-GFP led 24 h later to a lower infection rate of the local macrophages in P2X7R -/- mice, as assessed by the fluorimetry of freshly adhered peritoneal cells. These preliminary results suggest that P2X7R is important in the establishment of leishmanial infection, and its absence seem to carry compensatory inflammatory mechanisms.

IM36 - THE ANTILEISHMANIAL ACTIVITY OF THE AQUEOUS EXTRACT OF A MONOCLONAL *KALANCHOE PINNATA* SPECIMEN

CRUZ, E. A. *(IBCCF)*; MUZITANO, M.F. *(NPPN)*; LAGE, C. L. S. *(IBCCF)*; COSTA, S.S. *(NPPN)*; ROSSI-BERGMANN, B. *(IBCCF)*

We have previously demonstrated the therapeutical efect of the oral treatment with the aqueous leaf extract of *Kalanchoe pinnata* (Kp, Crassulaceae) against murine and human cutaneous leishmaniasis [1,2]. In order to proceed in clinical studies with a more standardized product, we sought to generate a monoclonal specimen of the plant and to evaluate the effect of two different plant growth hormones on the antileishmanial activity of the leaf extract in vitro. Thus, a leaf sample was propagated in vitro using culture medium devoid of hormones or containing 2 mg/ml of either kinetin (KIN) or indol acetic acid (IAA). The leaf extracts of the developed plants were compared with an active extract from a wild sample. We observed that despite stimulating increased nitric oxide production by *Leishmania amazonensis* - infected mouse peritoneal macrophages, the extracts from either KIN- or IAA-stimulated specimens were equaly effective in preventing intracellular parasite growth as the wild and the medium-only grown plant extracts (66%, 57.8%, 70% and 67% respectively, tested at 100 μg/ml). We conclude that in vitro propagated *Kalanchoe pinnata* is a good source of active antileishmanial components as the wild plant, displaying strong potential for the production of standardized extracts to be used in further clinical trials. References: [1] Da-Silva, S.A.; Costa, S.S and Rossi-Bergmann, B. (1999). The anti-leishmanial effect of *Kalanchoe* is mediated by nitric oxide intermediates. *Parasitology* **118**:575-582. [2] Torres-Santos, E.C.; Da-Silva, S.A.G.; Santos, A.P.P.T.; Almeida, A.P.; Costa, S.S. and Rossi-Bergmann, B. (2003) Toxicological analysis and effectiveness of oral *Kalanchoe pinnata* on a human case of leishmaniasis. *Phytotherapy Research.* **17**:801-803.

IM37 - EFFICACY OF AN INTRANASALLY ADMINISTERED VACCINE CONTAINING FRACTIONS OF L. AMAZONENSIS ANTIGEN AGAINST CUTANEOUS LEISHMANIASIS.

Rayol, A *(UFRJ)*; Rossi-Bergmann, B *(UFRJ)*

We have previously demonstrated that intranasal vaccination of mice with whole antigen of Leishmania amazonensis (LaAg) protects mice against cutaneous leishmaniasis (Infect Immun, 2004, 72:4521). In this work we analyse the efficacy of the soluble and insoluble fractions of LaAg in protecting against cutaneous leishmaniasis. LaAg was fractioned in insoluble and soluble fractions by centrifugation (100 000 G). BALB/c mice received 2 doses (one week interval) of 20 mg of total antigen, insoluble and soluble fractions of LaAg, through instillation into the nasal cavities. One week later the animals were challenged in the right footpad with 2x105 fluorescent L. amazonensis parasites transfected with GFP. The lesions were accompanied by paquimetry during 10 weeks, when the animals were sacrificed. The cytokines produced by the cervical and lesion-draining popliteal lymph nodes were evaluated by ELISA. We demonstrate that the insoluble fraction was more effective in providing protection against cutaneous leishmaniasis.

IM38 - EFFICACY OF AN INTRANASALLY ADMINISTERED VACCINE CONTAINING L. donovani ANTIGEN AGAINST CUTANEOUS LEISHMANIASIS.

Rayol, A *(UFRJ)*; Gomes, DC *(UFRJ)*; Pinto, EF *(UFRJ)*; Rossi-Bergmann, B *(UFRJ)*

We have previously demonstrated that intranasal vaccination of mice with whole antigen of Leishmania amazonensis (LaAg) protects mice against cutaneous leishmaniasis (Infect Immun, 2004, 72:4521). Due to the observed similar effects of LaAg and L. donovani antigen (LdAg) on T cell anergy in vitro, in the present work we sought to evaluate the efficacy of LdAg as a nasal vaccine against L. amazonensis infection. LdAg was prepared as previously described for LaAg. BALB/c mice received 2 doses (one week interval) of 20 mg LdAg through instillation into the nasal cavities. Two weeks later the animals were challenged in the right footpad with 2x105 fluorescent L. amazonensis parasites transfected with GFP. The lesions were accompanied by paquimetry during 10 weeks, when the animals were sacrificed. The parasites in the infected feet were quantitated by the fluorescence intensity of the footpad tissue homogenate. The cytokines produced by the cervical and lesion-draining popliteal lymph nodes were evaluated by ELISA. In conclusion, LdAg seems to be effective in preventing murine cutaneous leishmaniasis when intranasally administered.

IM39 - IMMUNE RESPONSES INDUCED BY VACCINATION WITH A NUCLEOSIDE HYDROLASE (NH) EXPRESSING PLASMID AGAINST CHALLENGE INFECTION WITH *Leishmania amazonensis*

COSTA, MMS *(UFMG)*; ZANIN, FHC *(UFMG)*; COELHO, EAF *(UFMG)*; TAVARES, CAP *(UFMG)*; GAZZINELLI, RT *(UFMG)*; FERNANDES, AP *(UFMG)*

In the present study, the immune responses and the ability to induce protection, i.e., reduction on edema of infected footpad and on parasite loads were evaluated in mice immunized either with NH and A2 antigens or their association against *Leishmania (Leishmania) amazonensis* infection. The nucleoside-hidrolase (NH) protein, a 36 kDa protein, was identified in *L. (L.) donovani*, but is shared by var-

ious *Leishmania* species, including *L. (L.) amazonensis*. Immunization with NH was previously reported to induce partial reduction in edema of the infected footpad, against challenge infection against *L. (L.) mexicana*. A2 is an amastigote stage-specific antigen that is protective against *L. (L.) amazonensis* infection. BALB/c mice ($n = 8$, per group) were immunized intramuscularly with 2 doses of 100 μg of VR1012-NH or pCDNA- A2 plasmids, or with an association with these two plasmids, administered at 4 weeks intervals. The immune responses were investigated 30 days after, when levels of IFN- γ and IL-10 in splenocytes cultured and IgG1 and IgG2a antibodies were measured. Mice were then infected with $1x10^6$ stationary phase promastigotes of *L. (L.) amazonensis*. The disease's course was monitored weekly by measuring footpad thickness. After 9 weeks, mice were sacrificed and spleen, blood and infected tissue samples were collected for analysis. Parasite loads were determined in tissue fragments from the infected footpad by limiting dilution assay. Although increased levels of IFN-γ and decreased levels of IL-10 were observed in mice immunized with VR1012-NH or pCDNA3-A2 compared to control infected mice, protection, i.e, significant decreased in edema and parasite loads were observed only in A2 or A2/NH immunized animals. These results suggest that, under the conditions evaluated, NH was not protective against *L. (L.) amazonensis* challenge infection. Differences among immunization procedures and *Leishmania* virulence may explain these discrepancies among different studies.Supported by: FAPEMIG

IM40 - INTERFERENCE BY THE LACK ANTIGEN ON IMMUNE RESPONSES AND PROTECTION INDUCED BY DNA IMMUNIZATION WITH THE A2 ANTIGEN AGAINST *Leishmania (Leishmania) chagasi* INFECTION.

ZANIN, F.H.C. *(UFMG)*; COELHO, E.A.F. *(UFMG)*; SILVA, E.A.M *(UFOP)*; REZENDE, S.A. *(UFOP)*; TAVARES, C.A.P. *(UFMG)*; GAZZINELLI, R.T. *(UFMG)*; FERNANDES, A.P. *(UFMG)*

Antigenic interference on immune responses induced by a defined vaccine was previously described in a study involving the *Leishmania* LACK antigen.To investigate if this phenomena is restricted to LACK antigen and *L.(L.) amazonensis* infection we tested the association of LACK and A2 antigens in immunization experiments against *L. (L.) chagasi* infection. The A2 antigen is protective against *L. (L.) donovani* infection and it is cross-protective against *L. (L.) amazonensis* infection. BALB/c mice were immunized in the left hind footpad with 100 μg of each plasmid DNA (pCDNA3-A2, pCI-LACK) or with a mixture of 100 μg of each (pCDNA3-A2 plus pCI-LACK).Two vaccine doses were administered at 3 weeks intervals. Control mice received only PBS or pCI DNA. After 4 weeks, mice were infected with an intravenous injection containing $1x10^7$ late-log-phase promastigotes of *L. (L.) chagasi*. Immune responses (IFN-γ, IL-10 and IgG isotypes) were evaluated before and 30 days after infection, when parasite burdens at spleen and liver were also evaluated. Although high levels of IFN-γ were detected in culture supernatants of splenocytes from LACK or A2, association with LACK significantly abrogated the specific A2 IFN-γ production. In contrast, mice immunized with LACK produced, before infection, increased levels of IL-10 in response to total parasite antigens or recombinant LACK. This cytokine profile persisted after infection and thus, only the BALB/c mice immunized with A2 were protected against infection. Although further investigations are required, these data suggest that induction of IL-10 could be an important mechanism by which LACK inhibits immune responses against *L. (L.) chagasi* infection and that this antigen may act as a virulence factor for this species such as it is for *L. (L.) major*. Supported by: FAPEMIG

IM41 - Immuno modulation of *Leishmania (L.) amazonensis*curse infection in murine model.

VALVERDE. J.G *(Oswaldo Cruz)*; GONÇALVES DA COSTA, S.C *(Oswaldo Cruz)*

In attempt to evaluate the vaccine schedule against *Leishmania (L.) amazonensis*, two experimental murine models, with different immune response to BCG and *Leishmania* were used, the DBA/2 and C57BL/6 strains. Previous reports showed that the majority of inbred mice are susceptible to *Leishmania amazonensis* infection and one of them is C57BL/6. In contrast DBA/2 develop a persistent lesion that remain stationary with a small palpable nodule, without clearance of the infection. Previous reports with these strains, showed that BCG associated with microsomal fraction increase the infection in DBA/2, since they are non-responder to BCG. Otherwise this didn't occurred with C57BL/6, that is responder mice to this immunomodulator. The objective of this report is analyze the use of cyclophosphamide(Cy) associated to BCG and the Riboleish vaccine in immunization of C57BL/6 e DBA/2. Previous report showed a good correlation between Delayed-type-hypersensitivity (DTH) and clinic and experimental resistance to cutaneous*Leishmania*, thus the DTH were prime tested in all experimental groups.To compare DTH of vaccinated mice challenged with a viable dose of *L. amazonensis* that received the same riboleish antigen as eliciting dose, two inbred strain were employed. The experiments showed that a higher DTH was obtained in C57BL/6 mice which was associated with resistance expressed by the course time of the lesion. A significant protection was observed in riboleish immunized mice treated with both immunomodulators.C57BL/6 vaccinated pre-trated with BCG alone, however, were not protected. DTH induced by riboleish in DBA/2 mice was lower than that observed in C57BL/6, even in protocols employing both immunomodulators. The DBA/2 showed high mortality race in group that received Cy, what difficult the analysis of Kinetic lesion. The importance of this report is that we did not take into account if humans or animals were able to responder to BCG, and it has been used in humans vaccination.

IM42 - EFFECTIVE MUCOSAL VACCINATION AGAINST CUTANEOUS LEISHMANIASIS.

Pinto, E.F. *(UFRJ)*; Costa, B.L.S. *(UFRJ)*; Rayol, A. *(UFRJ)*; Larraga, V. *(CIB)*; Rossi-Bergmann, B. *(UFRJ)*

Parenteral vaccination with adjuvant-free antigens does not lead to protection against leishmaniasis and may in fact enhance the susceptibility against the disease. In this work we sought to investigate the feasibility of using the mucosa for the administration of disease-promoting leishmanial antigens as an alternative vaccination strategy to induce protection against cutaneous leishmaniasis. BALB/c and C57Bl/6 mice were orally or intranasally vaccinated with two doses of 100 ug or 10 ug, respectively, of total Leishmania amazonensis promastigote lysate (LaAg) prior to cutaneous infection. We found that both strains of mice developed increased resistance to infection. Oral immunization of BALB/c mice with LaAg led to decreased IL-10 and increased TGF-b production in the mesenteric lymph nodes. In the periphery, an increased production of IFN-g and a diminished Jones Mote - type cutaneous hypersensitivity reaction (TH2) were observed, compatible with a immune deviation to a TH1 response. gdTCR+ T cells seem to be an important component in antigenic sensitization of the gut mucosa since their depletion prior to and during oral immunization reverted protection. The nasal mucosa also proved to be an appropriate route for vaccination with LaAg. Attempts to induce protection in BALB/c mice with intranasal p36/LACK, a subunit component of LaAg that knowingly activates TH2 responses was unsuccessful. Interestingly, intranasal instillation of 30 ug of plasmid DNA codifying p36/LACK rendered the animals very resistant against infection with L. amazonensis. Parasite growth was effectively controlled, and at 5 months after challenge LACK-reactive cells in both the mucosal and in the lesion-draining lymph nodes were producing high levels of IFN-g. These results demonstrate for the first time the effectiveness of the mucosal routes particularly the nasal one for practical administration of adjuvant-free crude antigens or LACK-DNA for long-lived memory vaccination against cutaneous leishmaniasis.

IM43 - MODULATION OF MURINE MACROPHAGE EFFECTOR FUNCTION BY GLUTATHIONE

Bortolo V *(UFMG)*; Vianna P *(UFMG)*; Dutra WO *(UFMG)*; Gollob KJ *(UFMG)*

Leishmania are obligate intracellular protozoan parasites that infect host macrophages. The murine model of *L. major* infection has been used for investigation of the mechanisms controlling disease development. It is well documented that the control of the infection requires the induction of an immune response capable of activating macrophages to a microbicidal state, which depends mainly on the production of nitric oxide (NO) and killing of the parasites living within macrophages. The initial macrophage-parasite interaction is crucial for the establishment of host cell infection. The leukocyte integrin Mac-1 or CD11b is one important molecule for host cell invasion. We have recently observed that the host response to L. major infection can be significantly improved by increasing *in vivo* glutathione (GSH) levels. When *L. major* infected BALB/c mice are treated with N-acetyl-cystein (NAC), a GSH precursor, the histopathologic outcome of disease is greatly improved. Macrophages are the main effector cells controlling parasite replication, so we have investigated whether GSH modulation can increase the leishmanicidal activity of macrophages. To approach this question, murine macrophages were stimulated *in vitro* with IFN-γ and LPS in the presence of two glutathione modulating agents: NAC, a GSH precursor, and diethyl-maleate (DEM), a GSH depleting agent. The effects of GSH modulation on macrophage-parasite interaction through CD11b were analyzed by flow cytometry, the nitric oxide production by Griess reaction and the quantitative expression of cytokines (IL-10 and TNF-α) and iNOS by Real-Time RT-PCR. Our data indicate that the macrophage functions studied can be improved or impaired by GSH modulation. Modulation of macrophage function by GSH could be a useful pathway to improve the host response to *Leishmania* infection. **Support**: FAPEMIG, WHO/TDR, CNPq/PRONEX, and FINEP-CT-Infra. E-mails: vitor_bortolo@yahoo.com.br, privianna@icb.ufmg.br, waldutra@icb.ufmg.br and kjgollob@mono.icb.ufmg.br

IM44 - The identification of two novel saliva proteins of *Lutzomyia longipalpis* by antibodies of dogs with canine visceral leishmaniasis (CVL)

Bahia D *(CPqRR)*; Gontijo NF *(UFMG)*; Léon IR *(IOC)*; Perales J *(IOC)*; Campos LA *(CPqRR)*; Oliveira G *(CPqRR)*; Corrêa-Oliveira R *(CPqRR)*; Reis AB *(UFOP)*

Lutzomyia longipalpis saliva contains an extremely potent vasodilator, known as maxadilan, a 7 kDa protein which produces a long-lasting erythema at the bite site. In addition to antihaemostatic properties, sand fly saliva has immunomodulatory activities and evidence has been found that *Leishmania* parasites use this activity to facilitate their establishment in the vertebrate host. Several approaches have been taken towards the development of vaccines and therapy to treat dogs with CVL, such as the use of *L. braziliensis* and saliva antigens in the composition of anti-CVL-vaccine. The aim of present work is to identify saliva antigens of *Lu. longipalpis* which are recognised by antibodies of dogs with CVL (symptomatic and asymptomatic). Saliva soluble antigens were separated in SDS-PAGE-gel, stained with Coomassie and electro-transferred onto a nitrocellulose membrane. Western blot experiments were conducted by incubating nitrocellulose sheets with dog serum, followed by detection of anti-saliva reactive antibodies with anti-dog IgG/alkalyne phosphatase. The reactivity was developed by adding NBT-

BCIP. Six protein bands have been identified in the saliva of *Lu. longipalpis* (84, 62, 47, 30, 20, 17kDa). Two proteins were detected by western and may have potential use as vaccine targets. The protein bands were excised from the gel, transferred to 0.5mL tubes, cut into smaller pieces and treated with tripsin. The peptides were sequenced by chromatography coupled to ESIION TRAP mass spectrometry. The proteins which were recognised by dog antibodies have molecular weights of 28.6 and 47.3kDa. Expasy bioinformatics tools were used to detect signal peptides in both proteins by using the *Sigcleave* software. DNA cloning and characterisation of the recombinant DNA-derived from *Lu. longipalpis* proteins will be employed in future experiments. Taken together these results provide promising information which encourages experiments towards the development of multicomponent vaccination. Supported by: FAPEMIG,CNPq,PAPESIIIb/FIOCRUZ-RJ.

IM45 - IL-4 and IL-13 as susceptibility factors to the infection by Leishmania (L.) amazonensis in C57BL/6 and BALB/c mice

Felizardo, T.C. *(ICB/USP)*; Borges, N. B. *(ICB/USP)*; Elsas, M. I. *(ICB/USP)*; Abrahamsohn, I. A. *(ICB/USP)*; Lima, G. M. C. A. *(UFGo, ICB/USP)*

The factors that determine the susceptibility of C57BL/6 mice to infection by *L.amazonensis* are not completely understood. We verified the influence of the cytokines IL-4 and IL-13 on the course of infection of C57BL/6 and BALB/c mice inoculated with 10,000 *L.amazonensis* metacyclic forms into the footpad or into the dermis of the dorsum. IL-4 deprived (KO) C57BL/6 mice developed a small and persistent footpad lesion but did not develop a visible lesion at the dorsal site in spite of the local presence of parasites and systemic parasite dissemination. The parasitism (LDA) was lower in mice inoculated in the dorsum. IL-10 and IFN-gama levels were higher in popliteal LN cell-culture supernatants in footpad-infected mice. Although IL-4-KO-C57BL/6 mice controlled the infection better than C57BL/6 mice, those mice did not cure the infection and treatment with mAb anti-IL-10 receptor did not alter the parasitic load on the 30th week. We extended the study to IL-4-alpha-Receptor-KO-BALB/c mice to analyze the influence of IL-13 in the infection. Footpad and dorsal skin lesions in IL-4-alpha-R-KO BALB/c mice were smaller than those observed in IL-4 KO-BALB/c and resembled those observed in IL-4-KO-C57BL/6 mice. A major difference, however, relates to dissemination: there was no systemic parasite dissemination from either the footpad or dorsal inoculation sites in IL-4-alpha-R-KO-BALB/c mice. Furthermore, by the 15th week, the mice inoculated in the dorsum contained parasites only in the inoculation site, in comparison with the presence of parasites both in the footpad and in its draining Ln. The results indicate that both IL-13 and IL-4 contribute to susceptibility of BALB/c mice to L.amazonensis while IL-10 does not act as susceptibility factor in long-term-infected C57BL/6 mice. However, the absence of signaling by IL-4/IL-13 was not enough to completely eradicate the infection in BALB/c mice at the analyzed times. Supported by FAPESP . e-mail: tcfeliz@usp.br

IM46 - Evaluation of infection by *Leishmania (L.) amazonensis* in C57BL/6 mice inoculated in the footpad or in the dorsal skin

Felizardo, T. C. *(ICB/USP)*; Borges, N. B. *(ICB/USP)*; Abrahamsohn, I. A. *(ICB/USP)*; Lima, G. M. C. A. *(UFGo e ICB/USP)*

The murine model is widely used to study infection by *Leishmania*. Despite modifications in the dose and parasite forms used for inoculation, the footpad has been used as preferential site of inoculation. We compared the infection by L. amazonensis in C57BL/6 mice infected in the dorsal skin or footpad using 10 thousand metacyclic promastigotes. The kinetics of infection, parasitic load, cytokine and histology were verified. The dorsal lesions were ulcerative and expanding while the footpad lesions were nodular and self-contained. The parasite numbers in the dorsal lesion and in the draining superficial inguinal LNs were lower than in footpad lesions and draining popliteal LN on the 3rd, 10th and 24th weeks after inoculation. Although no difference was found in the frequency of IFN-gamma producing cells between inguinal and popliteal LNs, IFN-gamma levels detected in the supernatants of cell cultures of inguinal LNs draining the dorsal infection site were lower and not altered by anti-IL-10 addition to the cultures, whereas in the footpad-draining popliteal LN cultures, the treatment with anti-IL-10 enhanced IFN-gamma secretion on the 10th week of infection. The frequencies of Gr1+ (10th week) and CD45R/B220+ cells (24th week) were higher in the popliteal and inguinal LN respectively. Histopathology of dorsal and footpad lesions showed cellular infiltrates in the dermis in the absence of lesion (3rd week) and staining for IL-10, IL-12, IFN-gamma and SMAD-2 on the 10th week after infection in both tissues. Our results show that the development of lesion, parasitic load, dissemination and cellular responses to *L. amazonensis* in mice can be markedly influenced by route of infection. Supported by FAPESP. e-mail: tcfeliz@usp.br

IM47 - EFFECT OF INSULIN LIKE GROWTH FACTOR (IGF)-I ON PHOSPHATIDYLSERINE EXPOSITION ON *Leishmania Amazonensis*

Vendrame, CMV *(IMT-USP, ICB-USP)*; Carvalho, MDT *(IICB-USP)* Goto, H *(IMT-USP, FMUSP)*

IGF-I are polypeptides stimulating proliferation and differentiation of different cells. We reported previously that IGF-I induce proliferation of *Leishmania* in vitro and exacerbates the lesions in mouse cutaneous leishmaniasis (Goto et al.PNAS 95: 13211, 1998). Recently, de Freitas et al (Current Biology, 11: 1870, 2001) showed that phosphatidylser-

ine (PS) exposition and its recognition by a specific receptor (PSR) on macrophages were implicated in the infectivity of amastigotes of *Leishmania*, showing that unicellular organisms use apoptotic features as evasion mechanism, for the establishment and/or maintenance of infection, and they called it apoptotic mimicry. In this study, we searched the effect of IGF-I on PS exposition directly on *Leishmania* and their effect on parasitism in *L. amazonensis*-infected macrophages in vitro using lesion derived-amastigotes or stationary phase promastigotes (10^6parasites/mL) that were pre-incubated for 5 min with IGF-I (50 ng/mL or 100 ng/mL) or the stimuli maintained in the culture. PS exposition was evaluated after 24 h by binding of FITC conjugated anexin V by flow cytrometry. Effect on parasitism was also evaluated in BALB/c mouse peritoneal macrophages ($5x10^5$/well) that were infected either with *Leishmania (L.) amazonensis* amastigotes or promastigotes (*Leishmania*:macrophage = 2:1). Either macrophages or *Leishmania* were pre-incubated for 5 min with IGF-I (50 ng/mL) or maintained in the culture, or maintained without IGF-I (control). IGF-I induced an increase in macrophage parasitism with amastigotes and promastigotes. IGF-I did not alter the phosphatidylserine exposition on promastigotes, however it induced an increase of PS exposition on lesion derived-amastigotes. Anexin V binding was positive in 4.78% in the control, 11.59% when amastigotes were pre-incubated with IGF-I (50ng/ml) or 18.45% when maintained in the culture; it was dose dependent. The data suggest that IGF-I induces a phenomenon similar to apoptotic mimicry on lesion-derived amastigotes that may favor intracellular parasite growth. Supported by: FAPESP, CNPq, FINEP, LIM-38 (HC-FMUSP).

IM48 - EFFECT OF INSULIN LIKE GROWTH FACTOR (IGF)-I ON *LEISHMANIA*-MACROPHAGE INTERACTION IN VITRO

Vendrame, CMV *(IMT-USP e ICB-USP)*; Carvalho, MDT *(ICB-USP)*; Manuli, ER *(IMT-USP)*; Rios, FJO *(ICB-USP)* Goto, H *(IMT-USP e FMUSP)*

IGF-I induces proliferation and differentiation of different cells. It is one of the first factors encountered by *Leishmania* promastigotes when injected into the skin, and subsequently inside macrophages. We reported previously that IGF-I induces proliferation of *Leishmania* in vitro and exacerbates the lesions in mouse cutaneous leishmaniasis (Goto et al. PNAS 95: 13211, 1998). In this study, we searched the effect of IGF-I on leishmanicidal mechanisms and cytokine production in *Leishmania*-infected macrophages in vitro. BALB/c mouse peritoneal macrophages were infected with *Leishmania (L.) amazonensis* amastigotes or stationary phase promastigotes. Either macrophages or *Leishmania* were pre-incubated for 5 min with rIGF-I (50 ng/mL) or maintained in the culture. IGF-I induced increase in macrophage parasitism. It did not alter de H_2O_2 production, but the nitric oxide (NO) level was decreased when IGF-I was present: while in the control was 10.3+/-2.5, when macrophages were pre-incubated with IGF-I was 5.8+/-0.9, when *Leishmania* was preincubated with IGF-I, 3.3+/-1.8, and when maintained in the culture 6.2+/-1.5. We observed decreased expression of iNOS in macrophages, but increased expression and activity of arginase in the presence of IGF-I in macrophages and parasites. IGF-I did not alter the cytokine production by *Leishmania* amastigote-infected macrophages but, it altered in promastigote-infected macrophages. While in *Leishmania*-infected macrophages (control) we observed following levels (median pg/mL), TNF-α = 53, TGF-β = 295, IFN-γ = 187, in the presence of IGF-I, we observed TNF-α = 275, TGF-β = 1049, IFN-γ = 84 (data from IGF-I pre-incubated macrophages). The data suggest an effect of IGF-I favoring intracellular parasite growth acting on NO and polyamine production through the effect on enzymes on L-arginine metabolic pathway, and modulation of cytokine production. Supported by: FAPESP, CNPq, FINEP, LIM-38 (HC-FMUSP).

IM49 - INTERPLAY BETWEEN PARASITE CYSTEINE PROTEASES AND THE HOST KININ SYSTEM MODULATES VASCULAR LEAKAGE AND MACROPHAGE INFECTION BY PROMASTIGOTES OF THE *Leishmania donovani* complex

Svensjo, E. *(IBCCF)*; Barbosa, C.V. *(IBCCF)*; Batista, P.R. *(IBCCF)*; Brodskyn, C.I. *(LIP)*; Lima, A.P.C.A. *(IBCCF)*; Schmitz, V. *(IBCCF)*; Saraiva, E. *(UFRJ)*; Silva, R. *(LIP)*; Muller-Esterl, W. *(FU)* Scharfstein, J. *(IBCCF)*

Kinins, the vasoactive peptides proteolytically liberated from kininogens, were recently characterized as alert signals for innate immunity. In this study we demonstrate that *Leishmania donovani* and *L. chagasi*, i.e., etiological agents of visceral leishmaniasis (VL), activate the kinin system *in vivo*, *ex-vivo* and *in vitro*. Intravital microscopy in the hamster cheek pouch showed that topically applied promastigotes induced macromolecular leakage (FITC-dextran) in post-capillary venules. Peaking at 15 min, the parasite-induced leakage was enhanced by captopril (Cap), an inhibitor of angiotensin-converting enzyme (ACE), a kinin-degrading metallopeptidase. The enhanced microvascular response was cancelled by HOE-140, an antagonist of the B2 bradykinin receptor (B2R), or by pretreatment of promastigotes with the irreversible cysteine proteinase inhibitor N-methylpiperazine-urea-Phe-homoPhe-vinylsulfonebenzene (N-Pip-hF-VSPh). In agreement with the abovementioned data, the promastigotes induced edema in the paw of Cap-treated J129 mice, but not in Cap-B2R-/- mice. Interplay between kinin-releasing parasite proteases, kininogens, and kinin-degrading peptidases (i.e., ACE) modulated parasite uptake and progression of macrophage infectivity in cell cultures. Our study demonstrates that activation of the kinin system by *L.chagasi* and *L. donovani* modulates inflammation and upregulate innate macrophage responses. Additional studies are required to determine if changes in

kinin system homeostasis may modulate innate and/or adaptive immunity in the more complex settings of sand-fly transmitted leishmania infection. Supported by CNPq, MCT, FAPERJ, VW Foundation, Wellcome Trust

IM50 - KININOGEN HIGHJACKING BY L. CHAGASI PROMASTIGOTES: A POTENTIAL PATHWAY FOR TARGETED ACTIVATION OF BRADYKININ RECEPTORS OF MACROPHAGES

Oliveira, D.L. *(IBCCF)*; Schmitz, V. *(IBCCF)*; Barbosa, C.V. *(IBCCF)*; Lima, A.P.C. *(IBCCF)*; Müller-Esterl, W. *(UFMS)*; Scharfstein, J. *(IBCCF)*

Pathogenic trypanosomatids express high contents of papain-like cysteine proteases (CP), considered important virulence and/or survival factors in parasitic diseases. Analysis of substrate specificity indicated that the major CP from *Trypanosoma cruzi* (cruzipain) is a tissue kallikrein-like kininogenase (Del Nery et al., 1997), i.e., this enzyme liberates kinins from high/low molecular weight kininogens (HK/LK). Kinins are pro-inflammatory peptide that signal cells via two distinct GPCRs, B_1R and B_2R. Driven by cruzipain, parasite-evoked activation of these GPCRs induce vigorous $[Ca^{+}2]i$, thereby potentiating invasion of cardiomyocytes and endothelial cells (Scharfstein et al., 2000). Moreover, trypomastigotes evoke edema in mice by sequentially activating B_2R and B_1R (Todorov et al., 2003). Clues from these studies led us to investigate the role of kinins as hormones that induce innate immunity. Proof of principle came from studies showing that kinins induce maturation of dendritic cells, converting them into full-fledge inducers of adaptive immunity (Aliberti et al., 2003). After showing that these principles apply to the context of T. cruzi infection, we turned our attention to Visceral leishmaniasis because members of L. donovani complex share with T. cruzi the ability to activate the Kinin system through CP, inducing microvascular leakage and modulating macrophage infection via the CP/kinin/B2R pathway (Svensjo et al., in press). In the accompanying abstract, we showed evidence that B2R signaling of macrophages is a pathway leading to increased uptake of promastigotes. Here we asked if promastigotes may retrieve the kinin-parental proteins (HK/LK) from serum, perhaps simulating effects associated with bleeding, a common manifestation of sand-fly transmitted leishmania infection. Our data show that L. chagasi can stably bind HK on their cell surfaces. We are currently testing the hypothesis that parasite sequestering of HK/LK may allow for long-distance transport and targeted release of short-lived kinin hormones on susceptible macrophages. Funded by CNPq, FAPERJ, Wellcome Trust

IM51 - IMMUNE RESPONSE OF DOGS IMMUNIZED WITH Leishmania chagasi AMASTIGOTE RECOMBINANT ANTIGENS IN COMBINATION WITH A PLASMID ENCODING RECOMBINANT CANINE IL-12.

Santos, POM *(UFBA)*; Pereira, AM *(FIOCRUZ)*; Fraga, RE *(UFBA)*; Santos, LR *(UFBA)*; Alcântara, AC *(FIOCRUZ)*; Rodrigues, MS *(FIOCRUZ)*; Teixeira, MCA *(FIOCRUZ)*; dos-Santos, WLC *(FIOCRUZ)*; Pontes-de-Carvalho *(FIOCRUZ)* Oliveira, GGS *(FIOCRUZ)*

Introduction and Objectives: Zoonotic visceral leishmaniasis is an endemic disease in the Mediterranean basin, Asia and South America. Dogs are considered the major reservoir of the causative agent, Leishmania chagasi. In principle, an effective canine vaccine could contribute to the control of the disease in both humans beings and dogs. The protective immune response against canine visceral leishmaniasis seems to be a Th1-type cellular response. IL-12 is a potent immunomodulator, capable of inducing Th1-type immune responses to co-administered antigens. The immunogenicity for dogs of two L. chagasi amastigote recombinant antigens, in combination with recombinant canine single-chain IL-12 encoding plasmid, is described herein, Methods and Results: Two recombinant L. chagasi antigens (Lc9 and Lc13) were selected from a cDNA library, using pool of sera of dogs naturally infected with L. chagasi and with Leishmania specific DTH. The Lc9 and Lc13 proteins used for immunization were produced in E. coli. Canine IL-12 was constructed as a single-chain in pcDNA3.1zeo plasmid (pIL-12) and was shown to be biologically active. Groups of dogs were injected with three doses, in three-week intervals, of: i) saline/saponin (3 dogs); ii) antigens/saponin (4 dogs) or iii) antigens/800 ug of pIL-12/saponin (3 dogs). Dogs injected with antigens in saponin, with or without pIL-12, developed specific humoral immune responses, but fail to show specific lymphoproliferative response and interferon gamma in in vitro assays. Conclusion: Immunization with Lc9 and Lc13, associated with 800 ug of pIL-12 or not, in saponin, induced a humoral immune responses but failed to induce a Th1 immune response. The lack of cellular immune response in the animals injected with pIL-12 may be due to the administration of an insufficient amount of the plasmid.

IM52 - Anti-*Leishmania amazonensis* antibodies detected by flow cytometry as a tool for diagnosis of American Tegumentar Leishmaniasis

Pissinate, J F *(NDI - UFES)*; Merlo, B B *(NDI-UFES)*; Martins-Filho, O A *(CPqRR-FIOCRUZ)*; Dietze, R *(NDI-UFES)*; Lemos, E M *(NDI-UFES)*

The diagnosis of American tegumentar leishmaniasis (ATL) usually requires the combination of immunological and para-

sitological tests. In practice, the most common complementary exams employed to diagnose the disease are the Montenegro skin test and the direct investigation of parasites in biopsy specimens. Despite its high sensibility, the skin test is not able to distinguish between previous and current infection. Many efforts have been made in order to develop more sensible and less invasive tests to assist in the ATL diagnosis. The goal of this study was to evaluate the efficiency of anti-*L. amazonensis* antibodies detected by flow cytometry on ATL diagnosis. Sera were obtained from 60 patients with cutaneous and 10 patients with mucosal leishmaniasis. For cross-reactivity studies we evaluated sera from 50 patients with different diseases and 34 sera from healthy individuals. Samples of diluted sera were incubated with fixed *L. amazonensis* promastigotes and then with FITC conjugated anti-human IgG. The results were expressed as percentage of positive fluorescent parasites (PPFP) and the samples were considered positive when the $PPFP > 25\%$. The best sensibility and specificity were obtained using serum dilution at 1:8.000, which were 97% and 71%, respectively. The highest frequency of cross-reactivity was observed for sera from Chagas disease and visceral leishmaniasis patients. As an effort to increase the specificity, we are testing the IgG subclasses reactivity and *L. braziliensis* promastigotes as antigens.

IM53 - PARTICIPATION OF MACROPHAGES, T CELLS AND IMMUNOGLOBULIN IN GLOMERULONEPHRITIS IN EXPERIMENTAL VISCERAL LEISHMANIASIS

Prianti, M.G. *(IMT)*; Saldanha, L.C.B. *(FMUSP)*; Goto, H *(FMUSP)*

Introduction: Glomerulonephritis (GN) is present in visceral leishmaniasis (VL), but the pathogenesis is not fully known yet. In previous studies in dogs with VL, we observed predominance of proliferative patterns of GN. We showed the presence of CD4+ T cells in GN in canine VL (Costa et al. Braz J Med Biol Res. 33:1455, 2000) and IgG in the renal lesions in hamster VL, (Mathias et al., Braz J Med Biol Res 34:539, 2001). **Aim:** To study the pathogenesis, in this work we extended the analysis of the renal lesions in mouse VL. We have studied in kidney the presence of CD4+ and CD8+ T cells, F4/80+ cells (macrophages) as well as of IgG deposits and cellular proliferation. . **Methods**: BALB/c mice were infected either through intravenous or intraperitoneal route with $2x10^7$ purified *Leishmania (L.) chagasi* (MHOM/BR/72/strain 46) amastigotes. We analyzed by morphometry the number of total cells in different time periods, mesangial proliferation using silver staining, and detected IgG, CD4+ and CD8+ T cells and F4/80 antigen by immunihistochemistry. **Results:** We observed focal glomerular hipercellularity due to mesangial proliferation from 7 through 30 days post-infection. Moderate IgG deposits were observed from 7 days post infection that decreased in intensity from 15 through 30 days post infection. CD4+ T, CD8+ T F4/80+ cells were also present in glomeruli, peaking at 15 days PI. **Conclusion:**The results suggest that IgG participates in early phase of infection, and CD4+ and CD8+ T and F4/80+ cells participate concomitant and/or subsequently in the pathogenesis of glomerulonephritis in murine visceral leishmaniasis. Supported by, CAPES& LIM-38 (HC-FMUSP).

IM54 - Performance of Anti-*Leishmania chagasi* Antibodies Detected by Flow Cytometry for Monitoring Treatment Efficacy in Human Visceral Leishmaniasis

Gomes, I.T. *(CPqRR-FIOCRUZ)*; Carvalho, S.G. *(UNIMONTES)*; Martins-Filho, O. A. *(CPqRR-FIOCRUZ)*; Dietze, R. *(NDI)*; Lemos, E. M. *(NDI)*

The search for active disease markers and their validation for post-therapeutic cure criteria have been investigated. However, serological cure criteria still remain inconclusive for visceral leishmaniasis (VL). To date, the criteria adopted for establishing the efficacy of VL treatment depends on the clinical improvement of patients and negative result in parasitological methods 12 months after treatment. The aim of the present work was to assess the flow cytometry performance in the study of anti-*L. chagasi* antibodies after treatment in order to find a laboratory indicator of treatment efficacy. In this study, the profile of IgG and its subclasses were analyzed by flow cytometry using sera from 21 patients with positive parasitological examination for VL, collected prior to and after treatment. Samples of diluted sera were incubated with fixed promastigote and then with FITC conjugated anti-human IgG or IgG subclasses. The results were expressed as percentage of positive fluorescent parasites (PPFP) for each individual sample, establishing $\leq$ 50% as the cutoff between treated and non-treated patients. According to our data, all patients evaluated 12 months after treatment showed PPFP $\leq$ 50% for IgG. In early cure assessment, a decrease in antibody levels was detected in 71% of patients analyzed 6 months after treatment. Nevertheless, 2 months after treatment the PPFP values decreased in less than 50% of the patients. Concerning IgG subclasses, reactivity was found only for IgG1 and IgG3, and IgG3 showed greater performance in therapeutic monitoring. PPFP levels dropped in 90.5% and 71.24% of patients after 6 and 2 months of treatment, respectively. Our results suggest the potentiality of flow cytometry in studying anti-*L. chagasi* antibodies and monitoring VL therapeutic efficacy.

IM55 - Course of *Leishmania (L.) amazonensis* infection in deprived mice of IL-12 or of IFN-γ The Molecule CD28

Toma,LS *(ICB-USP)*; Lima,GMCA *(ICB-USP)*

It is accepted that IL-12 and IFN-γ are required for the leishmanicidal effect. Studies indicate that co-stimulatory signals is necessary to T cell proliferation and cytokine response. The purpose of this work is to investigate the infection by *L.amazonensis* in C57BL/6 mice deprived of co-stimulatory molecule CD28 knockout (KO), IL12 KO and IFN- γ KO as compared with the wild type (WT) controls. The mice were inoculated into the footpad with 10^5 or 10^6 stationary phase promastigotes and 10^3 metacyclic purified. The kinetics, parasitemia and cytokine production were monitored. We observed that CD28 KO mice developed controled and smaller lesion in comparison to the other groups, independent of inoculum dose of parasites. The number of parasites in the footpad and popliteal draining LN was lower in CD28 KO mice inoculated with stationary phase promastigotes. When the mice were inoculated with metacyclic forms no difference was noted in the popliteal LN parasitic load in CD28 KO and WT mice, but in the footpad the parasite number was lower in the CD28 KO mice. The IL-10 production was significantly lower in the supernatant cells culture of draining LN from CD28 KO mice. Similar levels of IL-12 and IFN- γ were detected between CD28 KO mice and WT mice. IFN-γ KO and IL-12 KO mice were shown to be highly susceptible to the infection with severe and metastic lesions and higher parasitemia. Taken together, these data suggested that molecule CD28 play a role in susceptibility to *L.amazonensis* infection and the presence of IL-12 and IFN-γ are essential to resistance. Supported by CAPES e-mail: lstoma@usp.br

IM56 - Characterization of immunological compartment and cytokines involved in host resistance to experimental infection with natural recombinant (type I/III) strains of Toxoplasma gondii

RESENDE, M.G. *(CPqRR)* FUX, B. *(CPqRR)*; GAZZINELLI, R. T. *(CPqRR)*; VITOR, R. W. A. *(UFMG)*; ROGRIGUES, S. C. O. *(UFMG)*; MELLO, M. N. *(UFMG)*

Toxoplasma gondii is an obligate intracellular coccidian, belonging to the phylum Apicomplexa. The parasite can be found within many different species of mammals and birds (DUBEY& BEATTIE, 1988). In mice, the various strains of the parasite differ enormously in their virulence and disease presentation (HOWE& SIBLEY 1995). Some studies show that the structure of *T. gondii* population is clonal, being that most strains fall into one of the three categories denominated Type I, Type II and Type III lineages. The Type I lineage was shown to exclusively contain those strains that are highly virulent, whereas Type II and Type III strains display lower virulence in mice (SIBLEY et al.,1992). We analyzed eight genetic markers and the biological behavior of two different recombinants strains of Toxoplasma gondii, D8 and G2 (I/III) in various lineages of mice in compares with ME49 (II) and P-Br (I/III). As previously shown for the ME-49 strain to confirm the importance of cytokines in host resistance to these strains, we used the IFN-γ-, IL-12 - and iNOS - mice. All the strains presented low virulence in the acute phase of infection and were cystogenics during the chronic infection shown like type I/III. The C57BL/KsJ congenic strain containing MHC haplotype "d" was more resistant than the parental strains (C57BL/6), CB10H2 congenic strain containing MHC haplotype "b" were more susceptible than the parental strain (BALB/c) when infected with the ME-49, but not with the P-Br, D8 and G2 strain. These findings are relevant to understanding the complex immunologic mechanisms that protect against *T. gondii* infection.

IM57 - Analysis of the humoral response in BALB/c mice immunized with 255 Gy irradiated *T. gondii* tachyzoites and challenged with cysts of ME49 strain

Costa-Silva, T. A *(IAL)*; Galisteo, A. J. Jr *(IPEN)*; Meira, C. S. *(IAL)*; Pereira-Chioccola, V. L. *(IAL)*; Andrade Jr, H. F. *(IMT)*; Hiramoto, R. M. *(IAL)*

Toxoplasma gondii infection is usually asymptomatic in immunocompetents hosts, with occasional eye involvement. Toxoplasmosis can cause severe disease in fetus of acutely-infected pregnant woman, immunocompromised (AIDS) and therapeutically immune suppressed patients, as cancer or transplant recipients. At the moment, no effective vaccines against *T. gondii* are available for humans, and vaccines for the veterinary have been showing low efficiency. Susceptible C57BL/6j mice immunized with irradiated tachyzoites have been demonstrated immune response similar to the chronically infected mice, with significant decrease of the cysts in brain. In this work groups of resistant BALB/c mice were immunized with three sequential i.p. doses of 255 Gy RH strain-irradiated tachyzoites ($1x10^7$) using an uniform source of ^{60}Co -rays in a γCell^{TM}. The mice groups were challenged after 18 days from the last dose with 10 cysts of Me49 strain by oral route. Blood samples from the tail vessel were collected weekly in standardized filter paper, stored in freezer and the IgG antibody detected by Enzyme-linked immunosorbent assay (ELISA). Specific antibody response showed an increase in IgG levels after the immunization with irradiated tachyzoites. After challenging with Me49 strain cysts, immunized mice presented high levels of IgG antibodies as compared with control group and no detectable cysts in brains were observed by light microscopy. These results were different from immunized C57Bl/6j mice, where a decrease in number of cysts was found. These results show that the immunization can induce an increase of the humoral response and can prevent cysts forming in BALB/c mice.

IM58 - Nitric oxide inhibition caused by *Toxoplasma gondii* infection in activated macrophages depends on the parasite genotype.

Oliveira FC *(U E Norte Fluminense)*; Maciel RC *(U E Norte Fluminense)*; Seabra SH *(UENF)*; de Souza *(UFRJ)* DaMatta RA *(U E Norte Fluminense)*

Toxoplasma gondii has a clonal population structure with limited genetic diversity. Three different strains types (I, II, III) exist with different levels of virulence toward mice. While type I is fatal, type II and III are controlled resulting in chronic infections in mice. We have demonstrated that *T. gondii* from the type I genotype (RH strain) regulates nitric oxide (NO) production after infection of activated macrophages. Here we determine if a type II genotype *T. gondii* (ME-49) can also control NO production. For that, tachyzoites of ME-49 were obtained from the supernatant of infected Vero cells and RH from peritoneal washes of infected mice. Mice peritoneal macrophages were seeded over coverslips, cultured in Dulbecco's Modified Eagle's Medium with 5% fetal bovine serum and activated with interferon-gamma and lipopolysaccharide. Macrophages were infected with a 10 to 1 tachyzoite macrophage ratio and cells collected after 2 and 24h. Supernatants after 24h of infection were assayed for NO by the Griess reagent. Parasite infectivity and multiplication were counted under a light microscope. After 2h no differences between both strains were detected in the percentage of macrophages with parasite, although ME-49 presented a higher mean number of interiorized parasites. After 24h, RH persisted in activated macrophages, and ME-49 was controlled presenting a lower mean number of interiorized parasites and percentage of macrophages with parasites. RH reduced NO production in macrophages, however ME-49 did not. These results strongly suggest that the RH virulence is related to its capacity to control NO production and persist in activated macrophages. Supported by: FAPERJ, CNPq.

IM59 - CROSS-RECOGNITION OF *PLASMODIUM VIVAX* VARIANT ANTIGENS (VIR) BY SERA FROM MOUSE IMMUNIZED WITH RECOMBINANT PROTEINS.

Oliveira TR *(USP)*; Becerra-Fernandez C *(USP)*; del Portillo HA *(USP)* Soares IS *(USP)*

The variant antigens of *Plasmodium vivax* (VIR) are expressed on the surface of infected erythrocytes and encoded by members of the multicopy vir gene family. In spite of the sequence variation, these antigens may constitute an important target for vaccine development if we were able to induce antibody to cross-reactive epitopes. Toward that goal, seven recombinant proteins corresponding to four vir subfamilies (A, B, C, and E) were produced in *Escherichia coli* as glutathione S-transferase (GST) fusion proteins. BALB/c mice were immunized subcutaneously twice with 5 μg of each recombinant protein emulsified in Complete Freund's adjuvant. The recombinant proteins MSP1-19 and soluble GST were used as control. IgG antibodies titers against each recombinant protein were detected by ELISA. After the second immunization with each recombinant protein, sera from mice of the different groups displayed high antibody titers against the homologous antigen. In addition, we obtained cross-recognition of heterologous antigens encoded by the different vir sub-families. Our results suggest that immunizations with a single recombinant protein may elicit specific, as well as cross-reactive antibodies against VIR proteins. Supported by FAPESP and CNPq

IM60 - MODULATION OF THE IMMUNE RESPONSE IN MALARIA-INFECTED MICE AND ITS EFFECTS ON EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS (EAE)

TALAISYS, R.L. *(Unicamp)*; FARIAS, A.S. *(Unicamp)*; PEREIRA, C.G. *(Unicamp)*; CARVALHO, B.O. *(Unicamp)*; GUARALDO, A.M. *(Unicamp)*; SANTOS, L.M.B. *(Unicamp)*; COSTA, F.T.M. *(Unicamp)*

Malaria afflicts 300-500 million people per year mainly in Third World countries. However, although infected individuals develop the clinical symptoms of the disease, the innate immune response can control replication of the parasite blood forms via the production of cytokines (IFNγ, TNFα, IL-12 and IL-8), mainly by NK, γ δ, CD4 T cells. The balance of cytokine levels and the timing of their production control the parasite growth and the development of severe forms of the disease, e.g. cerebral malaria. Recent studies in malaria-endemic areas in Africa have shown that children repeatedly exposed to the *Plasmodium* parasite do not respond to allergens as much as European children. In this study, we verified whether modulation of the immune system in malaria-infected mice can interfere with the evolution of autoimmune disorders such as experimental autoimmune encephalomyelitis (EAE). EAE is used an animal model for human multiple sclerosis (MS), an inflammatory demyelinating autoimmune disease of the central nervous system that is characterized by the activation of IFNγ and TNF-α producing T cells; however, $\gamma\delta$ T cells appear to reduce the clinical symptoms of EAE. In order to evaluate whether *Plasmodium* infection could modulate the immune system and thus impairing or aggravating the EAE, C57BL/6 mice were infected with 10e6 *P. chabaudi chabaudi* AS-parasitized erythrocytes (PE) before or concomitantly with the peak of EAE clinical symptoms. In preliminary experiments, we observed that the concomitant induction of EAE in malaria-infected mice reduced the clinical symptoms of EAE, and that this reduction was negatively correlated with the levels of parasitemia. In contrast, when EAE was induced after malaria infection, an increase in the symptoms of EAE was observed. These data demonstrate that the immune response against malaria par-

asites can modulate, positively or negatively, autoimmune disorders; and open perspectives to evaluate the immunological and parasitological mechanisms involved.

IM61 - Cellular and humoral immune responses to P. vivax Merozoite Surface Protein 9 (PvMSP9) in naturally exposed individuals from Rondônia State-Brazil

Lima-Junior, JC *(FIOCRUZ)* Rodrigues-Alves, B *(FIOCRUZ)*; Tran, TM *(EVRC)*; Meyer,V-S E *(EVRC)*; Singh, B *(EVRC)*; De-Simone, SG *(IOC/FIOCRUZ)*; Santos, F *(FUNASA)*; Barnwell, JW *(NCID)*; Galinski, MR *(EUSM)*; Oliveira-Ferreira, J *(FIOCRUZ)*

Merozoite Surface Protein-9 of *Plasmodium vivax* has orthologs in several malaria parasite species. PvMSP-9 consists of 979 amino acids and possesses a signal peptide, a cluster of four cysteines within the N-terminus region, and two species-specific blocks of tandem repeats at the C-terminus. We have demonstrated that *P.vivax* monoclonal antibody and polyclonal antiserum against native *P.cynomolgi* MSP-9 inhibit erythrocyte invasion of *P.vivax* merozoites in vitro and that PvMSP-9 is immunogenic in mice. In this study we evaluate the antibody and T cell reactivity to PvMSP-9 in individuals naturally exposed to malaria. In a cross-sectional study carried out in Porto Velho, Rondônia state, cells and sera from individuals living in Ribeirinha(N=188), a riverine native community along the Madeira river and Colina, a transmigrant community living in a rural area close to Porto Velho(N=122), were assessed for IFN-gamma and IL-4 cellular response by ELISPOT using PvMSP-9 synthetic peptides and for IgG antibody responses to PvMSP-9 recombinant proteins by ELISA. Our preliminary data show that individuals naturally exposed to P. vivax infections presented a cellular immune response to 6 of the 11 PvMSP-9 derived peptides. However the cytokine profiles were different between the two communities. In Ribeirinha, the frequency of positives individuals was similar for IFN-gamma and IL-4 and most immunogenic peptides were MSP9A(22.8%) and MSP9H(22.4%) for IFN-gamma and MSP9L(24.4%) and MSP9J(18%) for IL-4. In Colina, the transmigrant population, the frequency of positive individuals was mainly for IFN-gamma and the most immunogenic peptides were MSP9L(29.8%), MSP9E and MSP9H(29.2%), for IL-4 the higher frequency was observed in MSP9L(9.5%). Studies are in progress to evaluate the antibody response in these communities, and the association between cellular and humoral immune responses may provide information on the characteristics of acquired immunity to this *P.vivax* antigen and its potential as a vaccine candidate.

IM62 - Activation of TLR2 by alpha-galactose-enriched vesicles shed by trypomastigotes of *Trypanosoma cruzi*

ACT Torrecilhas *(USP)*; ES Nakayasu *(UTEP)*; LL Nohara *(USP)*; JF Jacysyn *(USP)*; C Ropert *(CPqRR-FIOCRUZ)*; RT Gazzinelli *(UFMG)*; W Colli *(USP)*; MJM Alves *(USP)*; IC Almeida *(UTEP)*

Infective trypomastigote forms of *Trypanosoma cruzi*, the causative agent of American trypanosomiasis, spontaneously shed into the culture medium vesicles of about 20-80 nm that resemble mammalian cell-derived exosomes. Gel-filtration and immunoaffinity chromatography with immobilized anti-alpha-galactosyl antibodies (anti-alpha-Gal Abs) has been used here to fractionate these vesicles into three major populations (P1, P2, and P3). P2 showed to be highly glycosylated, abundantly expressing alpha-Gal epitopes, and therefore able to bind strongly to immobilized anti-alpha-Gal Abs. These alpha-Gal containing vesicles (alpha GalV) strongly induced proinflammatory cytokines (IL-12, TNF-alpha) and NO in C3H/HeJ murine macrophages (MOs), via a Toll-like receptor 2 (TLR2)-dependent signaling pathway. The alpha GalV also induced TLR2 activation in CHO/CD14 TLR2-transfected cells. Macrophage invasion by trypomastigotes was considerably increased by prior incubation with alpha GalV. This phenomenon was reverted by prior vesicle treatment with alpha-galactosidase, indicating that alpha-Gal residues are somehow involved in the invasion process. It can be concluded that vesicles released by trypomastigotes interact with host cells inducing TLR2 activation, and drastically increasing macrophage invasion by the parasite. Thus, it can be proposed that alpha GalV represent novel parasite virulence factor. On-going proteomic analysis of these vesicles may shed some light on their chemical composition and biogenesis. Supported by FAPESP and CNPq

IM63 - Mice deficient in LRG-47 display enhanced susceptibility to *Trypanosoma cruzi* infection associated with defective hematopoiesis and intracellular control of parasite growth

Helton C. Santiago *(UFMG)*; Carl G. Feng *(NIH)*; Andre Bafica *(NIH)*; Ester Roffe *(UFMG)*; Rosa M. Arantes *(UFMG)*; Allen Cheever *(NIH)*; Gregory Taylor *(DUMC)*; Leda Q. Vieria *(UFMG)*; Julio Aliberti *(DUMC)*; Ricardo T. Gazzinelli *(UFMG)*; Alan Sher *(NIH)*

IFN-γ is known to be required for host control of intracellular *Trypanosoma cruzi* infection in mice, although the basis of its protective function is poorly understood. LRG-47 is an interferon inducible p47GTPase that has been shown to regulate host resistance to intracellular pathogens. To investigate the possible role of LRG-47 in IFN-γ-dependent control of *T. cruzi* infection, LRG-47 knock-out (KO) and wild-type (WT) mice were infected with the Y strain of this parasite and host responses analyzed. When assayed at day 12 following parasite inoculation, LRG-47 KO, in contrast to IFN-γ KO mice, controlled early parasitemia almost as effectively as WT animals. However, the infected LRG-47 KO mice displayed a rebound in parasite growth at day 15 and all succumbed to the infection by day 19. Further analysis indicated that LRG-47 deficient mice exhibit unimpaired pro-inflammatory responses throughout the infection. Instead, reactivated disease in the KO animals was associated with severe splenic and thymic atrophy, anemia and thrombocytopenia not observed in their WT counterparts. In addition, in vitro studies revealed that IFN-γ-stimulated LRG-47 KO macrophages display defective intracellular killing of amastigotes despite normal expression of TNF and NOS2 and that both NOS2 and LRG-47 are required for optimum IFN-γ-dependent restriction of parasite growth. Together, these data establish that LRG-47 can influence pathogen control by simultaneously regulating macrophage-microbicidal activity and hematopoietic function.

IM64 - Kinetics of immuglobulin (IgG) and isotypes (IgG 1 and IgG2) in dogs infected with benznidazole-susceptible, partially resistant and resistant *Trypanosoma cruzi* strains

Caldas, I.S. *(UFOP)*; Crepalde, G.P. *(UFOP)*; Diniz,L.F. *(UFOP)*; Guedes, P.M.M. *(UFMG)*; Veloso,V.M. *(UFOP)*; Lana, M. *(UFOP)*; Tafuri, W.L. *(UFMG)*; Bahia,M.T. *(UFOP)*; Bahia,M.T. *(UFOP)*

This study have been designed to evaluate the kinetic of Immunoglobulin G (IgG) and isotypes (IgG1 and IgG2) and their correlation with the cure after benznidazole treatment using dogs as experimental model. The animals were inoculated With Y, Colombian, VL-10, ABC or Berenice-78 *T. cruzi* strains and treated with 7 mg/Benznidazol/kg. Blood samples were collected from a regular interval of 15-30 days for antibodies quantification. For therapeutic evaluation were used parasitological (hemoculture) and serological (ELISA and LMCo) assays. Animals infected with Berenice-78, Y and ABC strains presented 100%, 100% and 75% of cure, respectively. Animals infected with the Colombian and VL-10 strains did not respond to benznidazole-treatment. IgG and IgG2 antibodies were detected 15 days after inoculation in sera of infected control groups, and all animals displayed high levels of antibodies. Diverse pattern of IgG1 were observed in animals inoculated with different strains, being: (1) high levels in VL-10 infected animals (absorbance peak-ap: 0.461 ± 0.139nm) and Colombian (ap: 0.383 ± 0.124nm); (2) absent or in low levels (ap: 0.307 ± 0.112nm) in Berenice-78; (3) similar to negative controls (ap: 0.136 ± 0.072nm) in ABC (ap: 0.209 ± 0.025nm) and Y infected animals (ap: 0.181 ± 0.037nm). After treatment IgG and IgG2 antibodies levels in Colombian and VL-10 infected animals were similar in treated and untreated dogs, nevertheless, during treatment was observe reduction in IgG and IgG2 levels only in VL-10 infected animals. On the other hand, before and during the treatment was not observed difference among the IgG1 levels in cured and not cured animals. However, after the treatment IgG1 to be detected among not-cured animals infected with VL-10 and Colombian strains, and not detected in not-cured animals infected with the ABC strain. Our results indicate that IgG1 production during the *T. cruzi* infection of dogs is related to the parasite strain and with failure therapeutic. Supported by: UFOP, CNPq and FAPEMIG.

IM65 - Different IgG1 profile in *Beagle* dogs infected with distinct *Trypanosoma cruzi* strains

Guedes, P.M.M. *(UFMG)*; Veloso, V.M. *(UFMG)*; Afonso, L.C.C. *(UFOP)*; Caldas, I.S. *(UFOP)*; Tafuri, W.L. *(UFMG)*; Lana, M. *(UFOP)*; Chiari, E. *(UFMG)*; Galvão, L.M.C. *(UFMG)*; Bahia, M.T. *(UFOP)*

This research has been designed to evaluate the kinetic of Immunoglobulin G (IgG) and isotypes (IgG1 and IgG2) production and their correlation with the proliferation of peripheral blood mononuclear cells (PBMC) using *Beagle* dogs as experimental model. Animals were inoculated by intraperitoneal route with 4 x 10^3 blood trypomastigotes (Tryp)/kg of Y, ABC or Berenice-78 *T. cruzi* strains. The animals parasitemia was examined daily. Blood samples were collected in a regular interval of 15 and 60 days (acute and chronic phases) for antibodies quantification and lymphocyte proliferation. Animals inoculated with ABC strain showed higher parasitemia than Y or Berenice-78 strain infected animals, with peak of parasitemia at 20,600, 10,000 and 5,000 tryp/0.1ml of blood, respectively. The same pattern of trypomastigote-induced lymphocyte proliferative response was observed in all infected animals. The anti- *T. cruzi* lymphocyte proliferative response was higher in dogs infected with Y strain (Stimulation index peak/SIP: $18,08 \pm 6,13$), intermediate with ABC (SIP: $11,44 \pm 2,73$), and slow stimulation index (SIP: $9,16 \pm 2,74$) was observed in Be-78 in-

fected animals. The IgG and IgG2 antibodies were detected 15 days post-inoculation, and all animals showed a similar level of these antibodies. However, higher levels of IgG1 antibodies were detected in animals infected with Berenice-78 strain (absorbance peak: 0.419 ± 0.139nm). In animals infected with ABC strain, IgG1 antibodies were absent or detected in low levels (absorbance peak: 0.187 ± 0.046nm), and with Y strain IgG1 level (absorbance peak: 0.103 ± 0.026nm) was similar to those observed in negative controls (mean absorbance: 0.074 ± 0.014nm). Our results indicate that the production of IgG1 isotype during the *T. cruzi* infection of *Beagle* dogs is correlated to the parasite strains and IgG1 levels are inversely correlated with the trypomastigote-induced PBMC proliferation. Supported by: PRONEX, CNPQ, FAPEMIG, UFOP and UFMG

IM66 - Anatomophatologic alterations of linfatic organs and cardiac muscle in *Beagle* dogs infected with Y and Berenice-78 strains of *Trypanosoma cruzi*

Veloso, V.M. *(UFOP)*; Guedes, P.M.M. *(UFMG)*; Caldas, I.S. *(UFOP)*; Souza, S.M. *(UFOP)*; Tafuri, W.L. *(UFMG)*; Lana, M. *(UFOP)*; Carneiro, C.M. *(UFOP)*; Chiari, E. *(UFMG)*; Bahia, M.T. *(UFOP)*; Galvão, L.M.C. *(UFMG)*

The aim of this study is to evaluate the presence of cardiomegaly, splenomegaly and lymphadenopathy and its correlation with cardiac inflammation in *Beagle* dogs infected with *Trypanosoma cruzi*. Twenty-four *Beagle* dogs were inoculated with 4 x 10^3 trypomastigotes/kg of the Y or Berenice-78 *T. cruzi* strains. Animals were euthanasied 30 days and two years after inoculation for anatomophatological analysis. The organ weight (spleen, lymphnode,heart) were determined and related with animal weight and macroscopic alteration. For histopathological analysis a tissue fragment was collected from the right atrium and analyzed in a semi-quantitative way. The parasitemia was detected 11-20 and 14-30 days after infection in animals inoculated with Y and Berenice-78 strains, respectively. Y infected animals showed higher parasitemia (peak: 10.000tryp/0.1ml) than Berenice-78 (peak: 5.000 tryp/0.1ml). All infected animals presented splenomegaly and cervical lymphadenopathy in acute phase. However, only 50% (Y) and 25% (Berenice-78) of infected animals showed cardiomegaly. During chronic phase only 25% of Berenice-78 infected animals showed lymphadenopathy, and all infected with Y and Be-78 strains remained showing splenomegaly, but less pronounced than observed in acute phase. Cardiomegaly was observed in 75% and 66% of the animals infected with Y or Berenice-78, respectively. The histopathological analysis showed severe myocarditis in all infected dogs during the acute phase. In chronic phase the myocarditis was moderate in animals infected with Berenice-78 or Y strain, however, Y infected dogs showed more fibrosis than those infected with Berenice-78. Previous studies showed moderate and absent myocarditis in dogs infected with 2 x 10^3 trypomastigotes/kg of the Y or Berenice-78 strains, respectively. Our results showed different patterns of alterations in *Beagle* dogs infected with Berenice-78 and Y strains, and indicate the correlation between the histopathological and macroscopic cardiac alterations. Data reforce the *Beagle* dog as a model for studies in Chagas disease. Supported by: PRONEX,CNPQ,FAPEMIG,UFOP and UFMG.

IM67 - MECHANISM OF LECTIN COMPLEMENT PATHWAY ACTIVATION BY *T. cruzi*

Cestari, IS *(IOC-FIOCRUZ)*; Evans-Osses, I *(IOC-FIOCRUZ)*; Ramirez, MI *(IOC-FIOCRUZ)*

Trypanosoma cruzi parasite has a digenetic life cycle alternating between insects and vertebrates. *T.cruzi* needs to evade host innate immunity to establish infection. The first vertebrate defense mechanism is the complement system, which is composed by proteins activated in a cascade culminating with parasite lyses. The complement system may be activated by Classical Pathway (CP), immunoglobulin binding to parasite surface; Lectin Pathway (LP), Mannan-Binding Lectin binding to carbohydrate surface or Alternative Pathway (AP), C3b binding to proteins surface. Previous studies supported the concept that trypanosomatids lysis is mediated by AP according to the absence of natural antibodies with trypanolytic activities in Normal Human Serum (NHS) (Nogueira, N et all_1975, Rimoldi, MT et all_1989). Although reports suggest that AP is responsible for *T.cruzi* lyses, our experiments with NHS_50% and EGTA-treated_NHS_50% (inhibits classical/lectin pathway) showed that 4% of epimastigote *T.cruzi* Y strain survived after the three pathways activation at 10 minutes-37^{o}C, however 50% survived after AP activation. The LP and CP share the same route, being necessary to analyze their activation separately. NHS pre-incubation parasites at 4^{o}C (Lectin activation) followed by EGTA-treated_NHS_50% (inhibiting classical pathway) kinetics at 37^{o}C allowed compare synergic effect of LP and AP to AP alone. In 10 minutes 5% of parasites survived LP and AP synergic effect, however 20% survived AP activation. Incubating NHS_50% with increasing mannose concentration 0,1mM-10mM inhibited *T.cruzi* LP activation in a dose-dependent manner from 26%-54% at 10 minutes-37^{o}C. These indicated that (i) LP is responsible for quick complement activation while AP acts slowly, (ii) LP probably shares a route with AP accelerating its activation and (iii) mannose inhibits LP suggesting that *T.cruzi* LP activation is mediated by mannose rich molecules. We are investigating molecules that activate LP in *T.cruzi*, as well as the LP resistance mechanism during differentiation from non-infective to metacyclic parasites. Support_Fiocruz/CNPq

IM68 - Activity of matrix metalloproteinase-9 and metalloproteinase-2 in *Trypanosoma cruzi* infected murine cells.

Pinho, R.T. *(FIOCRUZ)*; Seguins, W.S. *(FIOCRUZ)*; Côrtez, L.M.C *(FIOCRUZ)*

Extracellular matrix (ECM) plays a central role in maintaining the structural integrity of primitive multicelular organisms, as well as that of highly complex mammals. Matrix metalloproteinases (MMPs) family endopeptidases is composed of at least 23 members that collectively are capable of cleaving most major macromolecules of the ECM. MMP-9 is mainly produced by inflammatory cells, such as monocytes/macrophages, neutrophiles and eosinophiles, whereas MMP-2 is synthesized constitutively by mesenchymal cells such as fibroblasts, macrophages, endothelial and epithelial cells. MMP-2 is also produced by C2C12 myogenic murine cell line. The MMP-2 activation is concomitant with the regeneration of new myofibres (Kherif et al., Developmental Biology, 1999). In this study, we investigated the production and secretion of MMP-9 and MMP-2 in murine J774 macrophage and C2C12 myogenic *T. cruzi* infected cell cultures. The J774 and C2C12 infected and non infected culture supernatants and cells were collected at 24, 48 and 72 h and enzyme production was analyzed by SDS PAGE-gelatin. We detected in J774 cells an increase in the MMP-9 48 and 72h post-infection. For C2C12 *T. cruzi* infected cells it was observed a decreased of MMP-2 enzyme (molecular mass at 66, 60 and 55 kDa; Kherif et al., Developmental Biology, 1999) at 48 and 72 h.

IM69 - FLOW CYTOMETRY ANTI-FIXED EPIMASTIGOTES ANTIBODIES ASSAY (FC-AFEA) AS A NON-CONVECIONAL SEROLOGICAL ALTERNATIVE TO MONITOR CURE AFTER ETIOLOGIC TREATMENT OF CHRONIC CHAGAS DISEASE.

WENDLING, A.P.B. *(UFMG)*; VITELLI-AVELAR, D.M *(CPqRR)*; MARTINS-FILHO, O.A. *(CPqRR)*; ELOI-SANTOS, S.M. *(UFMG)*

We have performed FC-AFEA, described by Cordeiro et al (2001), in a group of 60 chronic chagasic patients, classified in: NOT TREATED (NT, n=19), TREATED NOT CURED (TNC, n=17) and TREATED AND CURED (TC, n=24) patients. FC-AFEA was performed using serial dilution (1:128 to 1:16384) and data were expressed as percentage of positive fluorescent parasites (PPFP). Cut-offs to differentiate IgG reactivity were determined using the ‘‘Receiver Operating Curve“that discriminated the following values: NEGATIVE = PPFP$\leq$20%; LOW POSITIVE = 20%<PPFP$\leq$60%; and HIGH POSITIVE = PPFP>60%. Considering these PPFP values, we have first used 1:256 sera dilution. All NT and TNC presented HIGH POSITIVE IgG reactivity, consistently with their clinical status. Nevertheless, when we analyzed TC group we found only 2 patients (8%) presenting NEGATIVE PPFP results, whereas 92% of TC showed POSITIVE PPFP values (PPFP>20%), with 33% of them showing HIGH POSITIVE IgG reactivity (PPFP>60%), not compatible with their clinical status. To solve this query we have searched for alternative sera dilutions to discriminate better cured and not cured patients. Using 1:2048 sera dilution, all NT and TNC samples showed HIGH POSITIVE PPFP values; and all TC samples showed NEGATIVE or LOW POSITIVE PPFP values (PPFP$\leq$60%), consistent with their clinical status. Thus, FC-AFEA performed at 1:2048 sera dilution was able to precisely discriminate the clinical status of the chagasic patients after etiological treatment and suggested the use of FC-AFEA to monitor cure after etiological treatment, considering this rule: *if 1:2048 is used as the reference dilution, a HIGH POSITIVE PPFP result observed after etiological treatment gives a precise conclusion of therapeutic failure. On the other hand, a NEGATIVE or LOW POSITIVE PPFP result (PPFP$\leq$60%) strongly suggested successful treatment efficacy after etiological therapeutics.* Finncial Support: CPqRR/FIOCRUZ, CNPq, CAPES, WHO.

IM70 - FLOW CYTOMETRY ANTI-FIXED EPIMASTIGOTES ANTIBODIES ASSAY (FC-AFEA) PRESENTS LESS CROSS-REACTIVITY WITH MAJOR TROPICAL ENDEMIC DISEASES IN BRAZIL

WENDLING, A.P.B. *(UFMG)*; VITELLI-AVELAR, D.M. *(FIOCRUZ)*; DIAS, J.C.P. *(FIOCRUZ)*; MARTINS-FILHO, O.A. *(FIOCRUZ)*; ELOI-SANTOS, S.M. *(UFMG)*

We have compared FC-AFEA reactivity, described by Cordeiro et al (2001), in a group of 28 chagasic patients (CH), 26 patients with classic Kalazar (LV), 20 patients with American Cutaneous Leishmaniasis (LT), 20 toxoplasmosis patients (TX), 20 malaria patients (MA), 21 Schistosoma mansoni infected individuals (ESQ) and 12 non-infected controls (NI). FC-AFEA was performed using serial dilution (1:128 to 1:16384) and data were expressed as percentage of positive fluorescent parasites (PPFP). Cut-offs to differentiate IgG reactivity by FC-AFEA were determined using the “Receiver Operating Curve“ that discriminated the following values: NEGATIVE = PPFP$\leq$20%; LOW POSITIVE =20%<PPFP$\leq$60%; and HIGH POSITIVE = PPFP>60%. Using 1:256 sera dilution, our data demonstrated that 86% of CH showed HIGH POSITIVE PPFP values and all NI presented LOW POSITIVE or NEGATIVE PPFP values. Considering the patients with different endemic diseases, 52% of LV, 5% of LT, 15% of TX, 17% of MA and 10% of ESQ also presented HIGH POSITIVE PPFP results. In an attempt to minimize this unspecific reactivity, we have searched for alternative sera dilutions. The titration curve indicated 1:1024 sera dilution and PPFP=60% as a promising experimental condition. In this way, 82% of CH was HIGH POSI-

TIVE while 52% of LV and 5% of ESQ still presented HIGH POSITIVE PPFP values. We found that FC-AFEA presented a sensitivity of 91.2% and specificity of 82%, representing a better performance in comparison to conventional methodology. We propose FC-AFEA as a diagnostic tool for Chagas disease since a differential IgG FC-AFEA could be observed at sera dilution 1:1024 where 82% of CH and 9.8% of individuals bearing other relevant parasitic disease showed high positive PPFP values. Thus, these data allowed the identification of 1:1024 as the most promising sera dilution to be used with diagnosis purposes. Financial Support: CPqRR/FIOCRUZ, CNPq, CAPES, WHO.

IM71 - TRIGGERING OF TOLL-LIKE RECEPTORS 2 (TLR2) AND 4 (TLR4) BY *Trypanosoma cruzi* INFECTION OF MACROPHAGES.

Giselle Cavalcanti *(UFRJ)*; Weberton K. Pires *(UFRJ)*; Thais Souto-Padron *(UFRJ)*; Marise Nunes *(FIOCRUZ)* Maria Bellio *(UFRJ)*

Toll-like receptors (TLR) recognize pathogen-associated molecular patterns (PAMPs), initiate the innate immune response against microorganisms and modulate the acquired response to pathogens. Recently, it has been demonstrated that MyD88 KO mice, which lack this adaptor molecule involved in TLR signaling, are more susceptible to the infection with *T. cruzi*. *Trypanosoma* derived GPI anchors activate macrophages in a TLR2-dependent manner, although *tlr2* gene deletion had no major impact on parasitemia nor on mortality. On the other hand, we have recently demonstrated that the lack of expression of a functional TLR4 causes higher parasitemia and accelerated mortality to *T. cruzi* infection. Nonetheless, the mechanisms by which this occurs were not determined yet. Therefore, the aim of the present work was to analyze the role of TLR4 and TLR2 in the innate response to*T. cruzi in vitro*. For this, host cell invasion by Y strain *T. cruzi*, as well as parasite survival and release, were evaluated in cultures of peritoneal macrophages obtained from the C3H/HeN (wt) and C3H/HeJ (TLR4 P712H), as well as C57BL/6 (wt) and TLR2 KO strains of mouse, respectively. Moreover, the production of hydroxyl radicals (OH.) and the generation of nitric oxide (NO) were investigated by the use of the ion chelator desferrioxamine (DFO) and of the nitric oxide synthase (NOS) inhibitor L-NMMA, respectively. We concluded that both TLR2 and TLR4 signaling pathways are triggered in macrophages during the infection with *T. cruzi*, and the consequent generation of ROS and RNI plays a significant role in the control of the *in vitro* infection.

IM72 - ACTIVATION OF BRADYKININ B_2 RECEPTORS BY *Trypanosoma cruzi* TRYPOMASTIGOTES MODULATES CXC-CHEMOKINE PRODUCTION BY MACROPHAGES

Schmitz, V. *(IBCCF)*; Oliveira, D. L. *(IBCCF)*; Scharfstein, J. *(IBCCF)*

Plasma leakage through post capillary venules is a common response to tissue injury provoked by physical trauma, noxious stimuli or host exposure to pathogens. Reduction of endothelial barrier function occurs when injured tissue cells, such as mast cells, nociceptive neurons, macrophages and other cell types release chemokines and/or other pre-formed vascular permeability inducing factors. As plasma proteins extravasate to peripheral tissues, inflammation is amplified as result of activation of proteolytic cascades, eg. complement, fibrinolytic and kinin system. In previous studies, we found evidences that tissue culture trypomastigotes (TCT, Dm28) initiate inflammation in subcutaneous tissues by activating TLR2 via a developmentally regulated PAMP (tGPI-mucin). Analysis of the dynamics of the inflammatory process by intravital microscopy revealed that neutrophil-evoked plasma leakage promotes the accumulation of kininogens (i.e. cruzipain substrate) into peripheral tissues. Here we studied the impact of parasite-induced activation of the kinin system in macrophage function, based on the assumption that chemokine release may contribute to the escalation of the inflammatory response. Interactions of thioglycollate-induced inflammatory macrophages (TG-MO) with TCT or EPI (controls) were performed at various host:parasite ratios in complete medium. The CXC-chemokines MIP-2 and KC were used as read outs for macrophage activation. Our data show that Angiotensin converting enzyme (ACE) and cruzipain play opposite roles in the regulation of MIP-2 secretion induced by TCT, respectively acting as down-modulators and up regulators of macrophage function via the kinin/B_2R pathway. These results suggest that levels of CXC chemokines produced by macrophages is, at least to some extent, coupled to the levels of kinins liberated in infected tissues. Funded by CNPq, FAPERJ, Wellcome Trust

IM73 - BRADYKININ ADJUVANT CONVERTS EPIMASTIGOTE EXTRACTS INTO PROTECTIVE IMMUNOGENS BY DRIVING EFFECTOR-MEMORY T CELL DIFFERENTIATION VIA IL-12 INSTRUCTIVE PATHWAY

Monteiro A.C.S. *(IBCCF)*; Schmitz, V. *(IBCCF)*; Oliveira, D.L. *(IBCCF)*; Castro, C.F. *(IBCCF)*; Arruda, L.B. *(UFRJ)*; Bonomo, A. *(UFRJ)*; Rodrigues Jr., J.M. *(USP)*; Silva, C.L. *(USP)* Scharfstein, J. *(IBCCF)*

Recently, we demonstrated that bradykinin (BK) is a "danger" signal that convert immature dendritic cells into induc-

ers of type 1 immunity (Aliberti *et al.*, 2003).The maturation responses elicited by BK are transduced by a constitutively expressed GPCR, the bradykinin B_2 receptor (B_2R). More recently, we demonstrated that *T. cruzi* trypomastigotes (Dm28c), a kinin releasing pathogen, activate immature DC via the kinin/B_2R pathway, rather than via TLR2, TLR4 or other MyD88-dependent pathways. Studies *in vivo* demonstrated that type 1 immune responses are directly correlated with levels of IL-12 production by CD11c DC in draining lymph nodes, the latter being in turn linked to extent of kinins released in the primary sites of infection (Monteiro *et al.*, submitted). Here we explored the potential use of BK as an adjuvant that stimulates type 1 immunity against intracellular pathogens. We choose to use epimastigotes extracts (Epi-Ag) as the immunogen, because (i) it does not induce protective immunity by conventional vaccination protocols (ii) it lacks potent pro-inflammatory PAMPS. BALB/c male mice were immunized with Epi-Ag combined to BK, pre-treated or not with ACE inhibitor. After boosters, mice were challenged and IFN-*gamma* production by Ag stimulated spleen cells were determined. Our results revealed that mice immunized with BK/Epi-Ag were protected from lethal challenge. This process was associated to an increased production of type 1 cytokines by CD4+ and CD8+ T cells accompanied by a higher Ag specific IgG2a serum levels. The benefits of BK adjuvanticity were nullified in animals pretreated with HOE-140, the antagonist of the B_2R. Adoptive transfer of spleen-derived T cells, isolated from the optimally immunized mice, into naive recipient male Balb/c, conferred resistance to lethal infection, suggesting that effector/memory T cells are generated and expanded by vaccine containing the BK adjuvant. Supported by CNPq, MCT, FAPERJ, VW Foundation and REDE TB (Projeto Milênio).

IM74 - GENDER AND GENETIC BACKGROUND DIFFERENTIALLY MODULATE KININ-DRIVEN LINKAGE OF INFLAMMATION TO INNATE AND TYPE-1 ADAPTIVE IMMUNITY IN MICE INFECTED BY *Trypanosoma cruzi.*

Granato AP *(IBCCF)*; Monteiro AC *(IBCCF)*; Schmitz V *(IBCCF)*; Oliveira DL *(IBCCF)*; Arruda LB *(IMPPG)*; Andrade DS *(IBCCF)*; Pesquero JB *(UNIFESP)*; Scharfstein J *(IBCCF)*

We have recently developed a subcutaneous model of *T. cruzi* infection (Dm28c) whereby tissue culture trypomastigotes convert immature dendritic cells (DC) into full-fledge inducers of type 1 immunity (CD4 and CD8) *in vivo* by triggering the constitutively expressed bradykinin receptor (B_2R). Driven by kinin "danger"signals liberated from kininogens through the proteolytic activity of cruzipain, the activated DC upregulate IL-12 and co-stimulatory CD40/C86 molecules. After migrating to popliteal lymph nodes (LN), the mature DC prime naive T cells, thereby linking innate to adaptive immunity in our *T. cruzi* infection model (Monteiro *et al.*, submited). Angiotensin converting enzyme (ACE/kininase II), a metallopeptidase that degrades the bradykinin/lysyl-bradykin (BK/LBK) plays an essential role in this process, attenuating the pro-inflammatory and Th1-instructive activity of BK/LBK. Here we report preliminary data showing that LN T cells isolated from infected females of the J129 (wt) mouse strain upregulate IFN-γ production, the type 1 response being cancelled by HOE-140 (B_2R antagonist) or absent in B_2R -/- mice. Gender has no influence in the kinin/B_2R -driven responsiveness of BALB/c. Experiments with ACE inhibitors show that J129 males, like BALB/c, do not spontaneously mount a vigorous type 1 response, due to the overriding influence of the ACE-dependent pathway of kinin degradation. Our study suggests that sex/genetic differences in ACE activity, by determing extent of kinin generation in in primary sites of *T. cruzi* infection, modulate the linkage between inate and adaptive immunity. Our findings may help to clarify the molecular basis of gender influence in susceptibility to Chagas disease (Basquiera *et al.*, 2003; de Souza *et al.*, 2001; Barretto AC *et al.*, 1993; Pereira JB *et al.*,1992). Funded by CNPq, FAPERJ, Wellcome Trust

IM75 - Evaluation of Fe employing X-Ray Fluorescence Methodology (XRF) in mice skin during acute phase of experimental infection with *Trypanosoma cruzi*

Estevam, M. *(UEL)*; Borges, C.L *(UEL)*; Pinge-Filho, P *(UEL)*; Appoloni, C.R *(UEL)*

Recent technological improvements allow the method of in vivo XRF to provide useful sensibility for diagnostics or monitoring in biomedical applications. One potential application involves monitoring of Fe in human skin with hereditary blood disorder as beta-thalassaemia and another ailments that request invasive methods for diagnostic but they are high undesirable. In addition, many systemic infections provoke a host hypoferremic response that reduces the level of Fe in the in the plasma transferring iron pool and thus limits the availability of extracellular Fe. *Trypanosoma cruzi* is protozoan parasite causing widespread human disease in Latin America, known as Chagas' disease. C57BL/6 Mice (resistant to infection) when infected with *T. cruzi* had a biphasic hypoferremic response. Treatment of those mice with exogenous Fe enhanced the mortality rate of *T. cruzi* infection, whereas depletion of iron was protective. This work investigates the use of a Si PIN-diode detector and a 238 Pu source (13 and 17KeV; 13%; 95.2mCi; 86.v) for measurement of Fe skin levels from susceptible Swiss mice infected with *T. cruzi* (Strain Y). XRF spectra were analyzed using a set of AXIL-WinQXAS programs elaborated and disseminated by the IAEA. The correlation coefficient of the calibration model (sensitivity curve) was 0.97. Measurements on skin mice phantoms containing concentrations of Fe in the range from 10 to 150 parts per million (ppm), indicate that we are able to detect Fe at levels of the order of 8 ppm, using monitoring periods of 100 seconds and skin entrance dose less than 6 mSv. Preliminary measurements on skin from susceptible

infected mice suggest that the pathogenicity of the *T. cruzi* correlated with its growth rate and with the amount of Fe available by XRF. So, the employed methodology allows the measurement of the pretended skin Fe concentration during experimental Chagas' disease.

IM76 - Association between the IL10 -1082 G/A and CTLA4 +49 A/G gene polymorphisms and the occurrence of the indeterminate clinical form of Chagas disease.

Costa, G.C. *(UFMG)*; Moreira, P.R. *(UFMG)*; Rocha, M.O.C. *(UFMG)*; Gollob, K.J. *(UFMG)*; Dutra, W.O. *(UFMG)*

Chagas disease evolves from an acute into a chronic phase, where patients can be classified according to clinical manifestations and signs. Most individuals are asymptomatic, classified as indeterminate, and approximately 30% of patients develop conductive (non-dilated) and/or contractile (dilated) heart dysfunctions, classified as cardiac patients. Our group, seeking to understand the immunological mechanisms involved in the pathogenesis of Chagas disease, showed that peripheral blood mononuclear cells (PBMC) from indeterminate patients display an immunomodulatory profile, characterized by production of IL10 and expression of CTLA4, as compared with PBMC from non-chagasic individuals. This profile can be a consequence of the disease, with multiple factors influencing the establishment of this response, or a result of genetic polymorphisms. Functional studies performed by others demonstrated that the genotype, -1082 GG of the polymorphism -1082 G/A located in the promoter region of the IL10 gene, is associated with high production of IL10. Other studies showed that the genotype, +49 AA of the polymorphism +49 A/G located in the first exon of the CTLA4 gene, is associated with higher surface expression of this receptor. The aim of this study was to study possible associations between these gene polymorphisms and clinical forms of human Chagas disease. The analyses were carried out using the RFLP method in patients with distinct forms of Chagas disease (indeterminate, non-dilated or dilated cardiopathy). Although our analysis did not show any association of individual polymorphisms with specific clinical forms of Chagas disease, the combined genotype made up of allele G of -1082 polymorphism for IL10 (G+), along with allele A of +49 polymorphism for CTLA4 (A+), was significantly higher amongst indeterminate patients. These results show that the occurrence of these polymorphisms are associated with the immunomodulatory profile seen in indeterminate patients, suggesting a protective role for these genotypes in human Chagas disease.

Quimioterapia - Chemotherapy

QT01 - The antiestrogen tamoxifen inhibits L. (L.) amazonensis growth - Perspectives in experimental leishmaniasis

Miguel DC *(USP)*; Uliana SRB *(USP)*

Leishmania (L.) amazonensis is the most important causative agent of diffuse cutaneous leishmaniasis in Brazil. The drugs currently used in the treatment of cutaneous leishmaniasis include pentavalent antimonials, amphotericin B and pentamidine. These drugs are administered by parenteral routes, require long courses of treatment and present high toxicity and costs. Tamoxifen (TMX) is widely used in the treatment and in the prevention of breast cancer due to its activity as an estrogen receptor modulator (Jordan VC, Nat Rev Drug Discov, 2003). However, it has become increasingly apparent that many biological activities of TMX are independent from the estrogen receptor machinery, as modulation of calmodulin, caspases and kinases, interference in ceramide metabolism and inhibition of the acidification of intracellular organelles. We have previously demonstrated that TMX inhibits the growth of promastigotes (IC_50: 11,1 μM) and amastigotes (IC_50: 13,3 μM) of *L. (L.) amazonensis*. The activity of TMX was further tested against *L. (L.) amazonensis*intracellular amastigotes. Infection rates on resident peritoneal macrophages infected in vitro decreased by 93% when cells were treated with 10 μM TMX. We also tested the activity of TMX on *L. (L.) amazonensis*infected BALB/c mice. Topic administration of the drug (10/25 mg/kg/d for 15/7 days) resulted in partial decrease in lesion sizes after treatment. Groups of infected mice were also treated topically with emulsions containing TMX and 2% limonene in ethanol. This monoterpene was chosen because of its properties in enhancing the percutaneous permeation of TMX (El-Kattan AF, Int J Pharm, 2001). However, we did not observe significant differences in the reduction in lesion sizes in comparison with the group treated only TMX. These data may provide clues to establish TMX as an alternative antileishmanial compound. Supported by FAPESP.

QT02 - ANTILEISHMANIAL ACTIVITY OF LIMONENE

Arruda, DC *(USP)*; Katzin, AM *(USP)*; Uliana, SRB *(USP)*

Limonene is a monoterpene found in a variety of fruits, vegetables and herbs. This terpene, a product of the isoprenoid pathway in plants, is widely used as food flavouring or additive in cosmetics. Several studies have shown that limonene exhibits chemopreventive and chemotherapeutic activity in rodent cancer (CROWELL et al., 1994; LU et al., 2004). Limonene has been shown to possess antibacterial and antifungal activities (AL-BURTAMANI et al., 2005). This terpene also inhibits *Plasmodium falciparum*

growth *in vitro* by interference in the biosynthesis of dolichol and ubiquinone and in protein isoprenylation (MOURA et al., 2001; RODRIGUES GOULART et al, 2004). This study shows that limonene inhibits the growth of promastigotes and amastigotes of *Leishmania*. Promastigotas and amastigotas of *L. amazonensis* were killed by limonene with IC50 values of 252,85±4,96 μM and 147,3±4,67 μM, respectively. Limonene also inhibited the growth of promastigotes of *L. braziliensis* and *L. chagasi* with IC50 values of 185,85±19,1 μM and 201,30±17,6 μM, respectively. The terpene inhibited the proliferation of intracellular amastigotes by *in vitro*. To investigate whether limonene/s mechanism of action includes interference in the isoprenoids pathway, *L. amazonensis* promastigotes were treated with 85 μM limonene and labelled with [14C]-acetic acid, [2-14C]-mevalonate, [3H]-farnesyl pyrophosphate (FPP) or [14C]-leucine. The analysis of the hexane extract of these parasites by HPLC or HPTLC showed inhibition of dolichol, ergosterol and ubiquinone biosynthesis after labelling with [14C]-acetic acid but not with [2-14C]-mevalonate, [3H]-FPP and [14C]-leucine. These results indicate that limonene is probably an inhibitor of hydroxymethylglutaryl-CoA reductase, which is the enzyme responsible for the synthesis of mevalonate from hydroxymethylglutaryl-CoA (HMG-CoA).

QT03 - Anti-*Leishmania amazonensis* Activity of 8,10,18-Trihydroxy-2,6-dolabelladiene (TRIOL) obtained by reduction of an analogous compound isolated from the brown alga *Dictyota pfaffii*

Soares, D. C. *(UFRJ)*; Santos, S. R. *(UFRJ)*; Teixeira, V. L. *(UFF)*; Saraiva, E. M. *(UFRJ)*

Leishmaniasis, a disease that affects 12 million people worldwide, is found in five continents and is endemic in the tropical and sub-tropical regions. Recently, these numbers are increasing due to the co-infection with HIV-1. Pentavalent antimonials, still the first choice treatment for this infection, present several side effects and parasite resistance is been reported. All that stimulates the search for new antileishmanial agents. Dolabellane diterpenes are presents in several organisms such mollusk, fungi, moss, plants and especially in brown algae of the order Dictyotales found in Atol das Rocas, Northeast of Brazil. This compound presented interesting biological activities such as antifungal, antimalarial and finally inhibitory activity of the reverse trascriptase enzyme of HIV-1. In our studies we investigated the activity of TRIOL obtained by chemical reduction of the 10,18-Diacetoxy-8hydroxy-2,6-dolabelladiene, isolated from the brown alga *Dictyota pfaffii* in intracellular amastigotes of *Leishmania amazonensis*, as well as its citotoxicity for macrophages. TRIOL showed an inhibitory effect of 53, 34 and 25% of the amastigotes survival in the concentrations of 50, 10 and 1μM, respectively. This effect was independent of nitric oxide (NO) production by the macrophages, since TRIOL is unable of induce NO in treated macrophages stimulated or not with IFN-γ and LPS. We also investigated the possible toxic effect of this compound for the host macrophages by Trypan dye exclusion and XTT assays. Both tests showed that TRIOL at 50μM was not toxic. Our results point TRIOL as a promising compound for further studies aiming the development of an anti-leishmania therapy. Supported by: CAPES, CNPq and Faperj.

QT04 - Activity of Perillyl Alcohol, Perillyl aldehyde and Perillic Acid against Leishmania major

Niño de Guzmán JRA *(ICB II)*; Uliana SRB *(ICB II)*

The chemotherapy of leishmaniasis is far from satisfactory and represents a considerable constraint for the disease control. The first choice pentavalent antimonials as well as the second choice drugs amphotericin B and pentamidine are highly toxic and resistance to the antimonial compounds is spreading. Perillyl alcohol (POH), perillyl aldehyde (PCO) and perillic acid (PCOOH) are derived from limonene, a monoterpene synthesized by plants through the isoprenoid biosynthesis pathway. Limoneno is oxidized by limonene monooxygenase to POH and by alcohol dehydrogenase and aldehyde dehydrogenase to PCO and PCOOH.
Limonene and its metabolic derivatives have been evaluated in the therapy against cancer in various preclinical models, due to their action as inhibitors of the posttranslational isoprenylation of small G proteins and induction of apoptosis.
We have investigated the activity of POH, PCO and PCOOH against promastigotes of *L. major* and the citotoxicity of these drugs against J774Aî macrophages and human fibroblasts HFF. Cell viability was evaluated by cleavage of MTT. The sensitivity of *L. major* promastigotes to these compounds was not uniform. IC50 for POH were calculated as 730 and 310 μM, respectively after 24h and 48h of incubation. However, POH was toxic to macrophages with a CC50 of 260 μM. *L. major* promastigotes were killed by PCO with an IC50 of 37 μM after 24h but PCO was also toxic to macrophages with a CC50 of 50 μM. Human fibroblasts were less sensitive to PCO with a CC50 of 330 μM. The IC50 of PCOOH against *L. major* promastigotes after 24h was 630 μM while the CC50 for J774Aî macrophages was 1660 μM.
In conclusion, the metabolites of limonene have antileishmanial activity but their citotoxicity for cells as macrophages represent a possible limitation. The activity of PCO deserves more investigation, given the greater tolerance of fibroblasts against this drug.

QT05 - A new potent Leishmanicidal activity of copper complexes coordinated with polypyridinics ligands

Edgar Marchán *(IIBCA-UDO)*; Antonio Gómez *(IIBCA-UDO)*; Zulay Simoni *(IIBCA-UDO)*; Oscar Corona *(IVIC)*; Maribel Navarro *(IVIC)*

Leishmaniasis is a severe world health public problem nowadays without effective control although multidisciplinarities efforts carried out to eradicate it. Based on our previous knowledge developed in relation to the design of copper DNA intercalators with leishmanicidal activity (J.Biol.Inorg.Chem, 2003., 8:401-408 and J.Inorg.Biochem, 2003.,97:364-369) in the present study we describe the biological activity of new polypyrydinics copper complexes. *Leishmania (L) mexicana* (NR strain) promastigotes maintained in Schneiders drosophila medium supplemented with 5% heat inactivated foetal calf serum at 26^o C were treated with $[Cu(Phe_3P)_2$phenanthroline$]NO_3$ and $[Cu(Phe_3P)_2$biquinoline$]NO_3$ dissolved in DMSO. The complexes were added when the cultures reached 10^7 parasites/mL in the exponential phase of growth and the inhibition kinetics was monitored during 72 h by direct counting in a Neubauer chamber, and viability was measured by trypan blue exclusion. The copper complexes showed a potent dose dependent antiproliferative effect on *L (L) mexicana* promastigotes. $[Cu(Phe_3P)_2$phenanthroline$]NO_3$ after 21 h added induced leishmanicidal activity at concentration as low as 5 nM (LD_{23}) whilst $[Cu(Phe_3P)_2$biquinoline$]NO_3$ requires scarcely 10 nM to reach the same lethal dose. Direct observation at sublethal concentrations (IC_{40} =1 nM and IC_{60} = 5 nM during 72 h, respectively) showed that both drugs caused parasites loss motility, granulations and considerably swelling previous to cell lysis. In summary our findings showed a couple of drugs with a potent leishmanicidal activity *in vitro* which could be associated with the polypyridinics structure coordinated to the transition metal and must be evaluated in terms of the possible strong interactions between planar ligands and parasite DNA. Therefore constituting a new promising drugs to be analyze *in vivo* in the permanent search of alternative chemotherapeutics agents to combat the Leishmaniasis

QT06 - Aqueous extract of *Cariocar braziliense* Camb inhibits arginase from *Leishmania (L.) amazonensis*

Hoff C *(CEULP/ULBRA)*; Ribeiro ECB *(CEULP/ULBRA)*; Floeter-Winter LM *(IB-USP)* Silva ER *(CEULP/ULBRA)*

Arginase is a metalloenzyme that play a pivotal role in polyamine precursor metabolism in *Leishmania*. We have explored the plants from Cerrado in search of selective inhibitors for *Leishmania* arginase. The leaves of *Cariocar braziliense* had been dehydrated (60^oC) to produce a constant dry mass. The dry leaves were powdered and a 10% (w/v) mixture in water was boiled during 15 minutes for extract production. Liver extract, buffered in Tris 100 mM/EDTA 1 mM, pH 7.0 was used to measure the activity of arginase from rat. Recombinant arginase of *L. (L.) amazonensis* was prepared from cultures of *E. coli* transformed with the plasmid plarg. The bacterial cells were broken by freeze-thaw in buffer MOPS 100mM pH 7,2. The fraction soluble was utilized to purify recombinant arginase in column HisTrap and DEAE FF Sepharose. The inhibition test were carried out using 100 mM of L-arginine pH 9,6 containing 20% (v/v) of the plant extract in the enzyme reaction. The activity of arginase from rat (liver type) and *Leishmania* showed 53% and 87%, respectively, of decrease in the presence of the plant extract. A 64-time dilution of the plant extract has inhibited in 10% and 63% the arginases from rat and *Leishmania*, respectively. The enzymatic kinetic showed that the inhibition of arginase of rat is competitive while that the inhibition of arginase of *Leishmania* is not competitive. The isolation, from *Cariocar braziliense*, of the molecule responsible for the arginase inhibition was initiated through chromatography in silica gel. These studies can contribute to description of a new active drug against *Leishmania*. Supported by COPPEX-CEULP/ULBRA, FAPESP and CNPq.

QT07 - ANTILEISHMANIAL ACTIVITY OF PLANTS USED IN BRAZILIAN FOLK MEDICINE

Fernanda G Braga *(UFJF)*; Magnum O Matos *(UFJF)*; Maria LM Bouzada *(UFJF)*; Rodrigo L Fabri *(UFJF)*; Elia Scio *(UFJF)*; Elaine S Coimbra *(UFJF)*

Leishmaniasis, caused by organisms of the *Leishmania* genus, is one of the major infectious diseases affecting the poorest regions of the world. The search for more effective drugs against *Leishmania* became extremely necessary. Today, chemotherapy is limited to pentavalent antimonials, drugs that are toxic and difficult to administrate because of their long-term treatment and high cost. Brazil is well known for the exuberance and variety of its tropical plants. Cultural habits and expensiveness of the pharmaceutical drugs have caused the use of folk medicinal plants. Many plants are used in Brazil in the form of crude extracts, infusions or plasters to treat common infections without any scientific evidence of efficacy. Considering this, we decided to check the *in vitro* effect of methanolic extracts of *Polygonum hydropiperoides*, *Stachytarpheta cayennensis*, *Syzygium jambolanum*, *Solanum nigrum*, *Pothomorphe umbellata* and *Bixa orellana*, currently used in the folk medicine in our region. Theses extracts were assayed against *L. amazonensis* promastigotes. Each concentration was screened in triplicate and it was performed in flat-bottomed 96-weel plastic tissue-culture plates. Promastigotes forms from logarithmic phase culture were suspended to yield 2 millions of cells/ml. The viability of promastigotes was checked using the tetrazolium-dye (MTT) colorimetric method. The results are expressed as the concentrations inhibiting parasite growth by 50% (IC_50) after

three days incubation period. Among the select plants, *S. nigrum* and *P. umbellata* had a very significant activity against *L. amazonensis* promastigotes with IC_50 of 40 μg/ml and 60 μg/ml, respectively. The others extracts presented IC_50 values higher than 500 μg/ml. In preliminary studies with *L. chagasi* promastigote forms, in the same conditions above described, *Solanum nigrum* did not demonstrated inhibitory effect against this specie. These results highlights the interest of medicinal plants. Others extracts are being investigated in our laboratory against different forms of *L. amazonensis* and *L. chagasi*.

QT08 - SYNTHESIS AND ANTILEISHMANIAL ACTIVITIES OF PURINE DERIVATIVES

Fernanda G Braga *(UFJF)*; Magnum O Matos *(UFJF)*; Marcos J V Prado *(UFJF)*; Mariza D Cancio *(UFJF)*; Adilson D da Silva *(UFJF)*; Elaine S Coimbra *(UFJF)*

Infections due to protozoa of the genus *Leishmania* are a major worldwide health problem, with high endemicity in developing countries, affecting millions of people and causing severe socio-economical losses. The drugs of choice for the treatment of leishmaniasis are pentavalent antimonials, but toxic side effects, limited efficacy to control parasite proliferation and drug resistance are frequently encountered. In order to find new drugs against leishmaniasis we decided to check the *in vitro* effect of ethyl(6-mercaptopurine)acetate, 1-cloro-3-(6-mercaptopurine)-propane, 1-desoxi-1-(6-mercaptopurine)-beta-D-fructopiranose, 2-amino-6-oxy-8-azapurine, 6-mercaptopurine riboside and tionicotinamida, after theirs synthesis. All compounds were assayed against *L. amazonensis* promastigotes. Each concentration was screened in triplicate and it was performed in flat-bottomed 96-well plastic tissue-culture plates. Promastigote forms from logarithmic phase culture were suspended to yield 2 millions of cells/ml. The viability of promastigotes was checked using the tetrazolium-dye (MTT) colorimetric method. The results are expressed as the concentrations inhibiting parasite growth by 50% (IC_50) after three days incubation period. Among the selected compounds, 2-amino-6-oxy-8-azapurine and 1-cloro-3-(6-mercaptopurine)-propane had very significant activity against *L. amazonensis* promastigotes with IC_50 of 12 μM and 50 μM, respectively. The others compounds presented IC_50 values higher than 500 μM. Further experiments are being carried out in order to investigated cytotoxicity against mammalian cells, besides analysis with amastigote forms of *L. amazonensis* and promastigote forms of *L. chagasi* for a better study of this new approach for the chemotherapy of leishmaniasis. Supported by FAPEMIG.

QT09 - The effect of essential oils and purified fractions from *Cymbopogon citratus* and *Ocimum basilicum* in *Leishmania* species

Cortez, TS *(UFRJ)*; Oliveira, RG *(UFRJ)*; Fortunato, CM *(UFRJ)*; Rodrigues, IA *(UFRJ)*; Alviano, DS *(UFRJ)*; Alviano, CS *(UFRJ)*; Rosa, MSS *(UFRJ)*

Parasites of the genus *Leishmania* are transmitted by the bite of sandflies and infect cells of the mononuclear phagocyte lineage of their vertebrate hosts. Depending on both virulence factors of the parasite itself and on the immune response established by the host, a spectrum of diseases known as leishmaniasis can appear, which can be cutaneous and/or visceral. The number of drugs available for the treatment of human and animal trypanosomiasis, amoebiasis, leishmaniasis, and malaria are limited. Considering the side effects and the resistance that pathogenic protozoan builds against these drugs, more attention should be given to the extracts and biologically active compounds which are isolated from plant species commonly used in herbal medicine. In the present study, we reported the effect of the essential oil from brazilian medicinal plants, investigating their effects on *L. amazonensis* and *Leishmania major* parasites.The parasites and/or the peritoneal mouse macrophages were treated with different concentrations of *Cymbopogon citratus* and *Ocimum basilicum* essential oils and their purified compounds. The essential oil from *C. citratus*, 200μg/mL, and the essential oil from *O. basilicum*, 350μg/mL, were able to eliminate 100% of parasites in 90 minutes of incubation on both *Leishmania* species.The treatment with the purified fractions citral, from *C. citratus*, linalool and eugenol,from *O. basilicum*, presented more intensive action where low concentrations were needed. Tests with mice peritoneal macrophages were also evaluated. Neither the essential oils and their purified fractions showed toxicity to housed cell. These results point new perspectives by the use of this plant to cutaneous leishmaniose treatment. Financial support: CNPq, FUJB, FINEP, and PRONEX

QT10 - PAMAM DENDRIMERS AS CARRIERS OF ANTILEISHMANIAL DRUGS

Falcão, C.A.B. *(IBCCF)*; Furtado, V.L.R. *(IQ)*; Rossi-Bergmann, B. *(IBCCF)*

Dendrimers are branched and versatile quasi-spherical polymers with unique molecular weights and diameters in the 2- to 10-nm size range. They are constructed by radial growth that produces concentric shells (generations). Poly (amidoamine) (PAMAM) dendrimers are the first complete dendrimer family to be synthesized, characterized and commercialized. In this work, we describe for the first time, the use of a PAMAM dendrimer systems to enhance the efficacy of antileishmanial drugs, using the pentavalent antimonial Pentostan as a drug prototype. PAMAM dendrimers of generations G3.5 (COOH), G4 (NH_2) and G4 (OH) were

conjugated with different numbers of Pentostan molecules. To test the antiamastigote activity of the different conjugates, resident macrophages of BALB/c mice were infected with fluorescent (GFP-transfected) *Leishmania amazonensis*. The conjugates were added in triplicates and the cultures were incubated for a further 72h. The cells were transferred to a black microplate for fluorescence quantitation using a plate fluorimeter. At the end of the culture period, the supernatants were collected for evaluation of LDH concentration as an indicator of cell lysis and toxicity. We found that conjugation with the different generations of PAMAM dendrimers enhanced the Pentostan activity. At 2μg/ml the inhibitory activity of free Pentostan was 20% of controls without drugs. After conjugation with G3.5 (COOH), G4 (OH) and G4 (NH_2) dendrimers, the inhibitory activity of Pentostan was enhanced to 41%, 63 % and 77%, respectively. Although the G4 (NH_2) generation seemed more effective in potentiating the antileishmanial activity of Pentostan, it also produced unselective toxicity as measured by increased LDH release. Conjugation of Pentostan with the 3 different generations of PAMAM dendrimers enhanced the antileishmanial activity. In all, this study demonstrates that G4 (OH) PAMAM dendrimer may be a useful tool for controlled release of antileishmanial drugs.

QT11 - *In vivo* evaluation of plant products inhibition on *Leishmania major* cells in association with computational tools

Costa, F. C. *(USP - IFSC)*; Nicoluci, R. P. *(USP - IFSC)*; Andricopulo, A. D. *(USP - IFSC)*; Rocha, W. C. *(UFSCar - DQ)*; Vieira, P.C. *(UFSCar - DQ)*; Thiemann, O. H. *(USP - IFSC)*

According to the World Heath Organization (WHO, 1998), Leishmaniasis affect 88 countries resulting in 12 million people infected. At present new drugs are necessary for the treatment of leishmaniasis since a lack of safe and effective chemotherapyes in worsening the world scenario of the disease. Our purpose is to explore the purine salvage pathway as a target for the development of new drugs that may come as an alternative treatment for leishmaniasis as a consequence that the *Leishmania* parasites are purine auxotrophs. We used recombinant adenine phosporibosyl transferase (APRT) from *Leishmania* to evaluate the inhibitory capacity of several compounds from natural or comercial origin. The screening allowed the identification of 4 compounds that were tested against *Leishmania* and as a control on human epithelial cells in culture. These inhibitors were employed as templates for the computational searches and a new set of commercially available compounds was identified. To further test the novel compounds, we have established and compared the Neubawer chamber counting method and the colorimetric method using MTT as a substrate to establish a High-Throughput alternative for the *in vivo* screening of compounds. The results obtained and comparison of the inhibitory activity are presented and discussed. **Supported by: FAPESP (CEPID)**

QT12 - THERAPEUTIC ACTION OF EXTRACELLULAR NUCLEOTIDES ON CUTANEOUS LEISHMANIASIS.

Chaves, S. P. *(UFRJ)*; Torres-Santos, E. C. *(UFRJ)*; Persechinni, P. M. *(UFRJ)*; Coutinho-Silva, R. *(UFRJ)*; Rossi-Bergmann, B. *(UFRJ)*

P2 receptors (P2R), a family of receptors activated by extracellular nucleotides, are highly expressed on macrophages, and their activation has been described in intracellular infections such as caused by *Mycobacteria tuberculosis* and *Chlamydia trachomatis*. In this work we evaluate whether P2R could be used as an antileishmanial target for exogenous nucleotides. Thus, BALB/c mice infected with *L. amazonensis*-GFP for 7 days were treated twice a week during three weeks with local injections with 20 μl of 50mM ATP (an agonist of P2XR and P2YR); 1 mM oxidized ATP (oATP, an antagonist of P2X7R); 1 mM oATP plus 50 mM ATP; or 5 mM UTP (an agonist of P2YR). We observed that treatment with either ATP, UTP and oATP significantly reduced the parasite loads by 20, 80 and 1600 times, respectively, in relation to PBS, as measured by the limitant dilution of the infected tissue lysate. *In vitro*, peritoneal macrophages were infected, and after 48 h at 37 °C they were treated with ATP (0.5 mM), ADP (0.1 mM), Adenosine (0.1 mM), UTP (0.3 mM), UDP (0.1 mM) or oATP (0.1 mM) in PBS during 30 min. Although UTP and UDP treatment stimulated nitric oxide production during the subsequent 48h, the intracellular parasite growth was inhibited only in ATP-treated cells (36% of inhibition). These results suggest that P2 receptors are potential targets to the antileishmanial effect of some nucleotides, and that the immunological environment of the lesion may be important for this effect.

QT13 - ER-119884 and E5700, two novel squalene synthase inhibitors as chemotherapeutic agents against *Leishmania amazonensis*

Rodrigues, JCF *(UFRJ)*; Urbina, J. A. *(IVIC)*; de Souza, W. *(UFRJ)*

Parasites of the *Leishmania* genus require for viability and growth the de novo synthesis of sterols such as episterol and 5-dehydroepisterol as they are not able to use only sterol found in their mammalian hosts, cholesterol. Squalene synthase catalyzes the first committed step in the sterol biosynthesis and has been investigated as a potential target in the treatment of human hypercholesterolemia. Leishmaniasis affects millions of people around the world and is associated with significant levels of morbidity and mortality in endemic countries. The chemotherapeutic approaches currently employed are very unsatisfactory and there is an urgent need for safer and more efficacious anti-*Leishmania* agents. In this work we investigated the effect of two novel squalene synthase

inhibitors, ER-119884 and E5700, on *L. amazonensis*. The more active compound against the extracellular promastigote form was ER-119884, with an IC50 of 1.4 nM, while for E5700 the corresponding value was 11.2 nM. Against intracellular amastigotes, grown in cultured macrophages ER-119884 at 50 nM caused total growth arrest after 48h and loss of cell viability after 72h. Differential interference contrast microscopy revealed the presence of promastigotes with several flagella and altered morphology, when cultivated in presence of only 1 nM of both compounds for 24h.The main ultrastructural change observed was the appearance of large vacuoles, possible characteristic of autophagic process, many of them in close association with the mitochondrion, which were observed after the exposure of promastigotes to 5 nM ER-119884 for 24h. Alterations were also found in the mitochondrion-kinetoplast complex, in plasma membrane, flagellar membrane and flagellar pocket, in the Golgi complex and nuclei, as well an increase of lipid inclusions. We conclude that ER119884 and E5700 are promising lead compounds for the development new chemotherapeutic agents for the specific treatment of Leishmaniasis. **Financial Support**: FAPERJ, CNPq, Pronex and European Commission

QT14 - Preliminary studies with different squalene synthase inhibitors, an important enzyme of the sterol biosynthesis, on *Leishmania amazonensis*

Rufino, C. E. *(UFRJ)*; Rodrigues, J. C. F. *(UFRJ)*; Gilbert, I. H. *(WSPA)*; de Souza, W. *(UFRJ)*

Leishmania parasites cause Leishmaniasis, a disease that affects millions of people around the world and comprise three clinical forms: visceral, cutaneous and mucocutaneous. The main and unsatisfactory chemotherapy used is based in pentavalent antimonials as pentostan and glucantime. Sterol biosynthesis is an important target to develop new chemotherapeutical approaches because these parasites require an amount of endogenous sterol for growth and viability, as ergosterol. Squalene synthase catalyzes the first committed step in sterol biosynthesis. In this work, we investigated the effect of eighteen squalene synthase inhibitors on promastigote forms of Leishmania amazonensis, as a preliminary study for tests on intracellular amastigotes and *in vivo* infections. WSP 1267 presented the best IC50 in the concentration range of 40 nanomolar. Other compounds were less efficient presenting an IC50 higher than 1.0 micromolar, whereas no inhibition was observed for seven compounds. Growth curve of WSP 1267 showed a total growth arrest and cell lysis with only 1.0 micromolar and 24h. In addition, in presence of 0.5 micromolar the parasites died after 96h. Differential interference contrast microscopy revealed the presence of parasites with several flagella and completely altered in its cell morphology, when cultivated in presence of 0.1 to1.0 micromolar. Transmission electron microscopy showed dramatic changes in the ultrastructure of the treated parasites, such as an intense mitochondrial swelling where the matrix became less electron dense, the presence of many autophagic structures, some of them in close association with the mitochondrion and flagellar pocket, an increase in lipid inclusions, the appearance of blebs in the plasma membrane and abnormal chromatin condensation. We conclude that WSP 1267 is able to inhibit the promastigotes growth, causing dramatic changes in their morphology, becoming a potential candidate for tests in intracellular amastigotes and *in vivo* infections. **Financial support**: CNPq, Pronex, FAPERJ and European Commission.

QT15 - COMPARATIVE STUDY OF THE EFFICACY OF FORMULATIONS CONTAINING FLUCONAZOLE OR PAROMOMYCIN FOR TOPICAL TREATMENT OF INFECTIONS BY *Leishmania major*

MUSSI, S.V. *(UFMG)*; FERNANDES, A.P. *(UFMG)*; FERREIRA, L.A.M. *(UFMG)*

Emphasis has been given lately to the development of alternative therapeutical approaches for cutaneous leishmaniasis, including the identification of formulations for topical treatment. In the present study the activity of two hydrophilic formulations was evaluated in animals experimentally infected by Leishmania major: a hydrophilic gel (PAHG) containing paromomycin (10%), an aminoglycoside antibiotic and a cream (FLUC) containing fluconazole (1%), a bis-triazole antimycotic. After development of ulcerated lesions, 15 BALB/c mice infected with Leishmania major were divided into 3 groups of 5 animals each: 1) PAHG group: lesions were covered with 50 μL of 10% PAHG, twice a day, for 12 days; 2) FLUC group: lesions were covered with 50 μL of 1% FLUC, twice a day, for 12 days and 3) placebo group: treated with the cream without fluconazole. During and after treatment, the size of lesions was determined weekly using a caliper. Further evaluations included the occurrence of relapses, nodules and metastasis in other sites on the skin of animals through careful observation of paws and tails. Animals were followed for an additional period of 70 days after the end of treatment. The topical treatment activity of PAHG was higher than that observed for FLUC treatment. The PAHG formulation was effective in promoting healing of ulcers in all animals, 28 days after the beginning of treatment, while none of the animals were cured by the FLUC treatment. These results suggest that the PAHG formulation could be suitable for clinical studies and may represent an alternative novel formulation for topical treatment of cutaneous leishmaniasis.

QT16 - SELECTIVE ANTILEISHMANIAL ACTIVITY OF A NEW SYNTHETIC NAPHTHOQUINONE

Pacienza-Lima, W. *(UFRJ)*; Chaves, S.P. *(UFRJ)*; Costa, P.R. *(UFRJ)*; Silva, A.J. *(USS)*; Rossi-Bergmann, B *(UFRJ)*; Torres-Santos, E.C. *(UFRJ/USS)*

Naphthoquinones are a class of natural products with a wide spectrum of biological activities, including antileishmanial, antioxidative and antitumoral. In this work, we demonstrate the antileishmanial activity of a new synthetic naphthopterocarpanquinone (LQB-17). Promastigotes of *Leishmania amazonensis* were cultivated with LQB-17 in several concentrations in 199 medium for 72h. LQB-17 presented strong antipromastigote activity, inhibiting the parasite growth by more than 95% at 10 μM. Antiamastigote activity was evaluated by incubating infected peritoneal macrophages with the drug for 48h. We found that LQB-17 was inhibited the amastigote growth as seen by the decreased percentage of infected macrophages and decreased numbers of amastigotes per total macrophages by 90% at 10 μM. No significant toxicity to macrophages was observed until 20 μM, as monitored by lactate dehydrogenase release. This molecule did not affect the nitric oxide production by infected macrophages up to the concentration of 40 μM. Altogether, these results show for the first time the selective antileishmanial activity of LQB-17 and indicate that this compound acts directly on the parasites, once that it did not modulate the NO production by macrophages.

QT17 - EFFECTS OF NATURAL PRODUCTS ON *Leishmania amazonensis*

Alves, E.S *(CPqGM-FIOCRUZ)*; Lanfredi-Rangel, A *(CPqGM-FIOCRUZ)*; Lima, M.E.F. *(UFRRJ)*; Vannier-Santos, M.A. *(CPqGM-FIOCRUZ)*

Leishmania are protozoan parasites responsible for a several diseases collectively called as leishmaniasis, which comprise a significant health problem in many regions of the world. Current treatment is based on a limited number of chemotherapeutic agents which are rapidly becoming ineffective, and are characterized by high toxicity and cost. Therefore, new leishmanicidal drugs are required and natural products of the Brazilian flora comprise a valuable source of new antimicrobial agents and were poorly studied on protozoa. Here, we have tested effects of curcumin isolated from *Curcuma longa* and piperine isolated from *Piper nigrum* on the growth of promastigotes of *Leishmania amazonensis*. Every 24 hours, samples were collected for counting at Neubauer chamber. At early stationary phase (5^{th} day), curcumine and piperine at 50μM inhibited, respectively, 98,7% and 90% of the promastigote growth. The ultrastructural analysis of curcumine and piperine treated parasites is ongoing. These data indicate that the action mechanisms of these compounds should be elucidated in detail and these further studies may provide new therapeutical tool for leishmaniasis treatment. Acknowledgments: This work was supported by FIOCRUZ, CNPq, PROCAD/CAPES and FAPESB

QT18 - BRAZILIAN AMPHIBIAN VENOMS AS NEW TOOLS FOR ANTIPROTOZOAL AND ANTIFUNGAL COMPOUNDS: AN IN VITRO APPROACH

Tempone, A.G *(IAL)*; Jared, C *(IBU)*; Antoniazzi, M.M *(IBU)*; Hiramoto, R.M *(IAL)* Motoie, G *(IAL)*; Oliveira, F.P *(IAL)*; Melhem, MSC *(IAL)*

Amphibian skin secretions are known as a rich source of biologically active molecules, most of which are alkaloids, biogenic amines, and peptides, including compounds that performance defense mechanisms against microorganisms. In this work, we have studied the in vitro activity of four brazilian anuran venoms, Bufo paracnemis, Bufo ictericus, Phyllomedusa distincta and Phrynohyas venulosa, against Leishmania L. chagasi, Toxoplasma gondii (RH strain) and two fungal strains, the azole-resistant Candida krusei and the azole-susceptible Candida parapsilosis isolates. Our data showed that only B. paracnemis venom presented antileishmanial activity against promastigotes, with an EC50 of 248.8 ug/mL. The crude venom activity against infected macrophages showed toxicity for mammalian cells, impeding an accurate light microscopy analysis. Thus, fractionation of crude venom was the choice for the better study of active compounds. FPLC-molecular exclusion chromatography of B. paracnemis yielded 5 peaks, with most active compounds concentrated in peaks 4 and 5, resulting in low molecular weight compounds. The incubation of the four crude venoms with tachyzoites of T. gondii showed only a strong inhibitory effect of P. distincta, without affecting the morphology of macrophages. The light microscopy analysis showed 100% treatment at 25 ug/mL of previously tachyzoites-infected LLC-MK2 cells. All amphibian crude venoms were moderate effective against both Candida species at 500 ug/mL, but P. distincta crude venom inhibited the growth of the C. parapsilosis by 100% at the same concentration. B. ictericus also inhibited the growth of the azole-resistant C. krusei by 35% at the same concentration. Amphibian venoms have been shown promising data in the search for new lead compounds. Further isolation of the active compounds could represent an interesting and useful tool for the development of new drugs for Leishmaniasis, Toxoplasmosis and mycosis. These data was supported by Instituto Adolfo Lutz and Instituto Butantan.

QT19 - MOLLUSK SECRETIONS FOR DRUG DISCOVERY STUDIES: THE ANTILEISHMANIAL ACTIVITY OF THE AFRICAN GIANT SNAIL, ACHATINA FULICA

Motoie. G *(IAL)*; Rocco, S.C2 *(IAL)*; Pinto, P.L.S *(IAL)*; Tempone, A.G *(IAL)*

Leishmaniasis remains to afflict the poorest population in developing countries, and despite of the increased number of cases, no considerable effort has been made for new drug development. The lack of efficient and less toxic drugs against Leishmaniasis and the mainstay of Gaspar Vianna's antimonial for clinical therapy for almost one century, shows the imperative necessity of new lead compounds. The African giant snail (Achatina fulica Ferussac), is one of the largest land snail species in the world, now found abundantly in tropical and subtropical regions. Some important biological activities derived from its skin secretions, as antibacterial and antiangiogenic activity in tumor cells have been described. In this work, we described the effective antileishmanial activity of the crude skin secretion of A. fulica and studied the mammalian citotoxicity and its possible mode of action against promastigotes. The crude secretion showed an Effective Concentration 50% (EC50) of 98.37 ug/mL against L.(L) chagasi. Through enzymatic assays, we have found L-amino acid oxidase (LAO) activity in crude secretion, and revealed that the hydrogen peroxide produced by LAO is one of the compounds responsible for the antileishmanial effect. The use of catalase for H2O2 scavenging in Leishmania cultures incubated with crude secretion abolished 54% of parasite death. To study the effect of crude secretion on promastigotes membrane, the permeability assay using ethidium bromide was performed. The fluorescent microscopy images suggest that the killing activity is other than pore-forming activity. Despite of the moderate toxicity for LLC-MK2 mammalian cells (EC50 83.25 ug/mL), these promising data provided the valuable information that other compounds than LAO could be involved in the antiparasitic effect. If adequately studied these data could serve as an useful tool for the development of new drugs for Leishmaniasis. This work was supported by grants from Instituto Adolfo Lutz.

QT20 - QUANTITATIVE EVALUATION OF THE POTENTIAL AS GIARDICIDE OF ANALOGOUS OF METRONIDAZOLE

Busatti, H. G. N. O. *(UFMG)*; Vieira, A. E. D. *(UFMG)*; Viana, J. C. *(UFMG)*; Alves, R. J. *(UFMG)*; Gomes, M. A. *(UFMG)*

Giardia lamblia is world-wide distributed and is responsible for diarrhea, malabsorption and weight loss. In underdeveloped countries, the illness presents considerable importance for public health, causing physical and mental depauperation in many children. *Giardia* isolated from human beings proceeding from different regions of the world present differences in sensitivity to metronidazole. It was observed a significant number of refractory patients to the treatment. In this way, the search of new drugs, more effective against the parasite, becomes necessary. This work has objective to produce and to investigate the potential as giardicide of analogous of metronidazole produced by chemical modifications in their structure. Two of the studied analogous mesilate metronidazole (MTZMs) and iodine metronidazole (MTZI) had their ability to interfere in the viability and vitality of trophozoite evaluated. It was determined DE_50 the dose that inhibits 50% of the growth and the minimum inhibitory concentration (MIC) of metronidazole and the analogous MTZMs and MTZI. The trophozoite of *G. lamblia*, strain Portland (ATCC 30888), had been cultivated in the TYI-S-33 medium at 37_*o*C. Trophozoites, in logarithmic phase of growth, had been distributed in cell culture plates for association with derivatives. The plates containing the trophozoites had been incubated in CO_2 at 37_*o*C. Metronidazole and its derivatives had been associated to the *Giardia*, in increasing concentrations, to determine DE_50 and MIC. For quantification, a colorimetric method was used. Metronidazole presented DE_50 = 1.96 μM and MIC = 34.10 μM, MTZMs DE_50 = 0,69 μM and MIC = 10,32 μM and MTZI DE_50 = 0.40 μM and MIC = 6,69 μM. When compared to metronidazole, the derivatives presented high potential as giardicide. Supported by: CNPq busatti@gmail.com

QT21 - EFFECTS OF THE VEGETAL EXTRACT OF Mentha x piperita LIN. (LAMIACEAE) ON THE MORPHOLOGY, MULTIPLICATION AND ADHESION OF Giardia lamblia TROPHOZOITES

Flávia Vidal *(UERJ)*; Juliana Vidal *(UERJ)*; Ana Paula Gadelha *(UERJ)*; Carlos Lopes *(UERJ)*; Marsen Coelho *(UERJ)*; Luiz Henrique Monteiro Leal *(UERJ)*

INTRODUCTION: *Giardia lamblia* is the causative agent of giardiasis. In order to find a more natural treatment, we analyzed the antigiardial effects of different extracts and fractions from *Mentha x piperita* MATERIAL AND METHODS: Parasites were cultivated in TYI-S-33 medium at 37C. For the growth assays, parasites were incubated with different concentrations of the compounds for 2, 4, 6, 24 and 48h. The final number of cells were counted using a Neubauer chamber. The same protocol was used for the video-microscopy assays. After the experiment, an aliquot was removed and observed at the light microscope. For the electron microscopy experiments, the parasites were processed and visualized in a Zeiss EM 906 (TEM). For the cell adhesion analysis, trophozoites were grown on coverslips with different concentrations of the compound. After incubation, the attached parasites were fixed and a specific number of cells was determined by counting using the KS400 software. RESULTS: All extracts from Mentha x piperita, with exception of the infusion, reduced the growth of *G. lamblia* trophozoites. The methanolic extract presented the highest inhibitory activity. Fractions

derived from the methanolic extract were also tested. The dichloromethane fraction, showed the greatest activity, after 48 hours with IC 50 % value of 0,75 μ g/ml. The residual fraction showed weak activity against the trophozoites. By video-microscopy, holes and protusions were observed, and some cells apparently lose their osmoregulative ability, acquiring a round aspect. TEM analysis showed trophozoites displaying holes on the cytoplasm and lamellar body-like structures. The adhesion assays demonstrated great activity of the dichloromethane fraction, with an inhibition of almost 100 % after 48h of treatment.

QT22 - EFFECTS OF DIETHYLDITHIOCARBAMATE IN TROPHOZOITES OF *Giardia lamblia*

Santana-Anjos,K.G. *(CPqGM - FIOCRUZ/BA)*; Lanfredi-Rangel, A. *(CPqGM - FIOCRUZ/BA)*; Oliveira,M.F. *(UFRJ)*; Vannier-Santos, M.A. *(CPqGM - FIOCRUZ/BA)*

The microaerophilic protozoan *Giardia lamblia* inhabits the upper small intestine mucosa of vertebrate hosts, where it is exposed to different concentrations of oxygen. Despite the fermentative metabolism, *Giardia* trophozoites consume O_2 and produce oxygen free radicals and therefore mechanism for detoxification are required. Devoid of glutathione, *Giardia* express high concentrations of cystein-rich proteins (CRP, also known as variable surface protein or VSP), as an antioxidant defense. This mechanism involves redox cycling for maintenance of a reduced intracellular environment and avoidance of the oxidative stress. In this regard, substances that interfere in the antioxidant response of this protozoan could comprise a powerful chemotherapeutic strategy for *Giardia lamblia* infection. Here, we analyzed the effects of DETC, a superoxide dismutase (SOD) inhibitor, on parasite proliferation, cell architecture, lipid peroxidation and thiol expression. DETC inhibited parasite proliferation and induced lipid peroxidation associated with ultrastructural alteration. Since this protozoan is devoid of SOD, here present data indicate SOD-independ DETC effects. Thiol groups detection with the fluorescent probe ortophthaldialdehyde (OPA). Cells treated with 0.2 mM DETC displayed washed out cytoplasm. Some of them, presented interrupted cytokinesis as demonstrated by the presence of three ventral disks in a only cell. The peripheral vesicles also had an increased volume, presumably caused by homophilic fusion. Taken together these data indicate that DETC enhance the oxidative stress in Giardia trophozoites by reacting with thiol groups. This work was supported by FIOCRUZ, CNPq, PROCAD/CAPES and FAPESB.

QT23 - HYPERBARIC OXYGEN THERAPY AMELIORATES MURINE CEREBRAL MALARIA CLINICAL OUTCOMES

BLANCO, Y.C. *(Unicamp)*; LOPES, S.C.P. *(Unicamp)*; ARRAIS-SILVA, W.W. *(Unicamp)*; TALAISYS, R.L. *(Unicamp)*; GIORGIO, S. *(Unicamp)*; COSTA, F.T.M. *(Unicamp)*

Cerebral malaria (CM) causes approximately 2 million deaths per year, mainly in African children. Sequestration in the brain of parasitized erythrocytes (PE) and leukocytes is thought to be responsible for this pathology. CM is characterized by blood flow blockage, oxygen supply impairing, and modulation of endothelial cell receptors and host immune response. It is believed that PE sequestration avoids parasite clearance in the spleen. PE and leukocyte are often described within the brain from patients who died of CM; however ethical considerations limit investigations of therapies capable to diminished or prevent CM clinical outcomes. In a mouse model of CM with *Plasmodium berghei* ANKA histological studies have shown that PE and leukocytes sequestered in brain capillaries. Hyperbaric oxygenation (HBO) therapy increases systemic oxygen levels and has been successfully used against bacterial and fungal infections. HBO also assists as adjuvant to diabetics and tissue-graft surgeries. Recently, studies have shown that HBO reduces proliferation of *Leishmania amazonensis* by altering macrophage susceptibility to infection. Herein, we investigated the in vivo effects of HBO therapy on murine CM manifestation. We have observed that *P. berghei* ANKA-infected mice exposed daily to HBO (100% *O*_2, 3 ATA, for 1H) display higher levels of survival in comparison to the non-exposed animals. Moreover, HBO therapy significantly interferes on CM clinical outcomes. *P. berghei* ANKA-infected mice under HBO treatment presented less pronounced alterations on temperature, weight, hematocrite and parasitemia. These data demonstrate a protective effect of HBO therapy on murine cerebral malaria, suggesting modulation of host immune response, alteration on parasite proliferation and/or reduction of PE cytoadhesion in the brain.

QT24 - Inhibition of the proteolytic activity of the proteasome by terpenes in cultures of the *Plasmodium falciparum.*

Rodrigues Goulart H *(ICBII-USP)*; Balanco J M F *(ICBII-USP)*; Peres V J *(ICBII-USP)*; Kimura E A *(ICBII-USP)*; Katzin A M *(ICBII-USP)*

The ubiquitin-proteasome pathway is the principal cellular mechanism for controlled protein degradation. The existence of a *Plasmodium* proteasome has been shown directly by cloning of the 20S proteasome *beta*-subunit gene. During the schizogony the erythrocytic stages the parasite undergoes radical morphological changes and many rounds of replication, events that likely require proteasome activity. Studies reported in our laboratory have demonstrated that terpenes

(farnesol, limonene, linalool or nerolidol) inhibit the development of the intraerythrocytic stages of parasite *in vitro*, probably by interfering in the elongation of the isoprenic chain attached to coenzyme Q, dolichols, and inhibiting the isoprenylation of proteins in *P. falciparum*. Preliminary results have demonstrated that terpenes or lactacystin might be affecting the cellular proteasomal function, and thereby induce cell death in cultures of the *P. falciparum*. We measured chymotrypsin protease activity of the proteasome and observed that the activity of this enzyme was inhibited by terpenes. How terpenes disturb the cellular proteasome function is not clear. Either the proteasome function is inhibited directly by blocking the 20S proteasome core cavity, or indirectly through the generation of oxidative stress. Oxidative stress is known to inhibit the proteasome function, and terpenes probably could be inducing the oxidative stress by interfering in the elongation of the isoprenic chain attached to coenzyme Q in *P. falciparum*. Our result favors this hypothesis, because mitochondrial abnormalities could lead to generation of oxidative stress inside the cell. Additional events as the decrease of the mitochondrial membrane potential have been observed when cultures of the *P. falciparum* were stained with DiOC6 and treated with lactacystin or terpenes. This suggests that inhibition of the proteolytic activity of the proteasome in *P. falciparum* could be acting as a starter of a cascade of events similar to apoptosis and could therefore be a promising therapeutic target. Supported by FAPESP.

QT25 - Comparison between two methods to test new antimalarials against *Plasmodium falciparum* in vitro, the traditional culture with hipoxantine incorporation.

Freitas, IO *(CPqRR)*; Soares RPP *(CPqRR)*; Santana E *(UFAL)*; Benetti MRN *(UFRGS)*; Krettli AU *(CPqRR)*

Malaria treatment has been hindered by the limitations in the drug arsenal, so that the search of new antimalarials is needed to find alternative drugs. Our aim is to compare two in vitro methods to evaluate antimalarial activity of extracts and molecules of medicinal plants that could be useful against clone W2 of *Plasmodium falciparum* chloroquine-resistant : (i) the traditional test, where parasites are exposed to drugs for three consecutive days with daily medium changes and in the fourth day, blood smears stained with Giemsa are examined by optical microscopy; (ii) the method based on the incorporation of hypoxanthine, where parasites are exposed to drugs for only 24 hours, enabling the determination of drug-response curves and IC_{50} values (half maximum inhibitory response). Molecules or fractions of two medicinal plants were tested: *Cecropia* sp (CAT) and *Symphyopappus* sp (SHY). The SHY extract, CAT molecule and chloroquine showed lower IC_{50} in the traditional tests, as compared to the hypoxanthine test, respectively: (1) SHY 4,9 and 17,8 ug/ml; (2) AT 5,87 and 11,4 ug/ml; (3) chloroquine 32,3 and 51,2 ng/ml. In spite of both methods being accurate, the traditional test showed IC_{50} values up to 4-fold lower a data attributed to the longer contact drug-parasites. The hypoxantine method could be responsible for the loss of drugs with borderline activity. Tests with a higher number of samples are under progress with *P. falciparum* clones chloroquine-resistant and/or chloroquine-sensitive.

QT26 - Antimalarial activity in vitro of 4-(pyrazolyl)-chloroquine analogues against Plasmodium falciparum chloroquine resistant parasites

Cunico, W. *(Fiocruz)* Soares, R.P.P. *(CPqRR)*; Cechinel, C.A. *(UFSM)*; Bonacorso,H.G. *(UFSM)*; Martins,M.A.P. *(UFSM)*; Zanatta,N. *(UFSM)*; de Souza,M.V. *(fIOCRUZ)*; Krettli, A.U. *(CPqRR)*

Malaria remains one of the most important diseases of man affecting mainly the population living in tropical and subtropical areas. Chloroquine (CQ) and other quinoline have been frontline drugs of malaria chemotherapy for much of the past 40 years. Since resistance of *P. falciparum* to such drugs is increasing, the search of new antimalarials is need. The antimalarial activity of CQ-pyrazole analogues synthesized from 1,1,1-trifluor-4-methoxy-3-alken-2-ones and 4-hydrazino-7-chloroquinoline was tested in vitro against a CQ-resistant *P. falciparum* clone. Parasite growth in the presence of these drugs was measured in comparison to controls with no drugs in the [^{3}H]-hypoxanthine incorporation assay. Briefly, trophozoite stages in sorbitol-synchronized blood were cultured at 2% parasitaemia and 2.5% hematocrit, in the presence of the test molecules diluted in RPMI medium without hypoxanthine; a CQ control was used as a reference antimalarial drug in each test. Inhibition of parasite growth was evaluated through the levels of [^{3}H]-hypoxanthine incorporation by the parasites in control and test samples, done in triplicates, then plotted to generate dose-response curves. The half-maximal inhibitory response (IC_{50}) compared with parasite growth in the drug-free controls was estimated using a software program (Microcal, Origin Software, Inc., Northampton, MA, USA). All assays were performed in triplicate. All but one of the eight dihidropyrazolyl chloroquine derivatives tested showed a significant activity in vitro being a promising new class of antimalarials. The 4-(pyrazolyl)-7-chloroquinolines tested were mostly inactive thus, the aromatic functionality of the pyrazole ring is critical. These analogues will be tested in vivo in mice to evaluate the potential of this group.

QT27 - Two Novel Squalene Synthase inhibitors arrest *Toxoplasma gondii* tachyzoites proliferation

Erica S. Martins-Duarte *(UFRJ)*; Julio A. Urbina *(IVIC)*; Wanderley de Souza *(UFRJ)*; Rossiane C. Vommaro *(UFRJ)*

Currently the most frequently used treatment for human toxoplasmosis consists is combination of antifolates, such as pyrimethamine and sulfadiazine, which act synergistically and are effective in the acute phase of disease. Although their efficacy is well known, this regime is associated with severe adverse effects that may lead to the discontinuation of the therapy, emphasizing the need for safer drugs. In the present work the antiproliferative effect of ER119884 and E5700, two novel inhibitors of squalene synthase (SQS), a key enzyme of the sterol biosynthesis pathway, were tested in *Toxoplasma gondii* infected LLCMK2 epithelial cells alone or in association with 24,25(R,S)epiminolanosterol (EIL), a known inhibitor of Δ24(25)sterol methyl transferase in fungi and protozoa. Both compounds had potent, dose-dependent, anti *T. gondii* activity, with IC50 values of 0.61μM and 0.23μM for ER119884 and E5700 after 24h of interaction, respectively and 0.44μM and 0.19μM after 48h. The association of 0.1μM ER119884 with EIL after 48h was capable to reduce the IC50 of this drug from 0.36μM to 0.18μM with a FIC of 0.72, demonstrating that the combination of these drugs had an additive effect. On the other hand, the association of 0.02μM E5700 reduced the IC50 of EIL from 0.36μM to 0.13μM with a FIC of 0.46, demonstrating that the combination of these drugs was synergistic. In order to elucidate the possible cellular target of these compounds, treated cells were processed for transmission electron microscopy. Tachyzoites incubated with 3μM of ER119884 or E5700 presented disrupted mitochondrial cristae and general swelling of this organelle. The abnormal formation of endoplasmatic reticulum surrounding cytoplasm and organelles portions strongly suggests the presence of autophagy events. This work was supported by CNPq, CAPES, Pronex-Faperj and European Commission

QT28 - Dendrimers as nanoagents against Toxoplasmosis.

Prieto MJ *(UNQ)* MJ Morilla *(UNQ)*; EL Romero *(UNQ)*

Dendrimers are nanoscopic devices that could be used for controlled release of drugs conveniently loaded to their structure in order to achieve favourable pharmacokinetic biodistribution, and intracellular delivery. In this work we present the protocols designed to load sulfadiazine (SDZ) to PAMAM G 4 and G 4,5 dendrimers, their quantitative and qualitative structural characterisation as well as followed their uptake, intracellular transit and citotoxicity on two cell lines. The results showed that both PAMAM G4 and G4,5 could be used for complexing and increasing the aqueous solubility of the hydrophobic SDZ up to a maximum of 35 molecules SDZ/dendrimer molecule, which resulted in a SDZ aqueous solubility in the order of 35 x dendrimer solubility. By minidyalisis through 3 Kda benzoilated membranes, it was followed the structural stability of the complexes that were stable both in buffer and plasma at 1/500 fold dilution along 24 h. The citotoxicity measured by MTT/formazan on Vero cells (endocytosis) and J774 (phagocytosis) of G4,5 complexes was lower than for G 4 at least at 30 mM dendrimers and 1 mM SDZ. Having shown that structurally stable complexes dendrimers-SDZ can be captured without toxicity by potential host cells, these results allow to propose the complexes G4,5-SDZ as suitable candidates for massive and selective delivery of the antitoxoplasmic drug SDZ to infected cells.

QT29 - NEW NAPHTHOQUINONES ACTIVE AGAINST *Trypanosoma cruzi*

Costa, E. M. *(FIOCRUZ)*; Menna-Barreto, R. F. S. *(FIOCRUZ)*; Salles, R. *(UFRJ)*; Pinto, M. C. F. R. *(UFRJ)*; Pinto, A. V. *(UFRJ)*; De Castro, S. L. *(FIOCRUZ)*

Naphthoquinones isolated from *Tabebuia* and their synthetic heterocyclic derivatives are the subject of our screening of new compounds with trypanocidal activity. Among more than 60 compounds, up to now, we identified three naphthoimidazoles obtained from beta-lapachone as the most active compounds against bloodstream trypomastigotes. In this work, from C-allyl lawsone 1 and nor-lapachol 2 new naphthoquinones were synthesized, their structures established and their activity against *T. cruzi* evaluated. From 1, 3 and 4 were obtained by electrophylic addition of iodine to the lateral double bond followed by cyclization to a furan ring, and 5 by the acid-catalyzed reaction of ring formation by dissolution in sulfuric acid. From 2, 6 was synthesized by addition of bromine followed by aniline. For assays with trypomastigotes, the values of IC50 for 1d oscillated among 157.5 to 641.2 microM, showing that the iodinated derivative 3 was about 2.2 times more active than the original naphthoquinone 1. More striking was the increase of 6.4 times in trypanocidal activity was observed when comparing the aminated derivative with the quinine 2. The effect of 3, 4 and 5 was also assayed on proliferation of epimastigotes, being observed that this form of the parasite is much more susceptible to the naphthoquinones than trypomastigotes, with values of IC50 for 1 to 4d values between 2.6 and 24.9 microM. For the first time in our studies we observed trypanocidal activity of furanic napthoquinones. We will investigate the mode of action of these naphthoquinones against *T. cruzi*. The trypanocidal activity of the new naphthoquinones stimulates extended studies with other pathogenic trypanosomatids as well as the synthesis of new analogues with redox properties, reinforcing the strategy of a rational approach in of the development of drugs active against Chagas disease.

QT30 - Effect of a Brazilian green propolis extract against Trypanosoma cruzi

Salomão K. *(FIOCRUZ)*; Barbosa HS. *(FIOCRUZ)*; De Castro SL. *(FIOCRUZ)*

Propolis possesses a variety of biological activities and, during the last decades an increasing number of studies about the chemical composition, biological activity and therapeutic uses of propolis have been published (De Castro, Ann Rev Biol Sci 3: 49, 2001). The composition of this resin is variable and complex depending on the botanic sources where the bees collected vegetal exudates (Bankova et al. Apidologie 31: 3, 2000). In a study with a Brazilian green propolis, the chemical composition of its ethanol extract (Et-Bra) was determined, showing this extract activity against trypomastigotes of T. cruzi, and several fungi and bacteria species of medical importance (Salomão et al. Lett Appl Microbiol 38: 87, 2004). In the present work we analysed the effect of Et-Bra on the infection of peritoneal macrophages, the ultrastructure of epimastigotes and trypomastigotes of T. cruzi and on treated parasites and labelled with acridine orange and rhodamine to detection of acidic compartments and mitochondrial membrane potential fluorescence of epimastigotes by flow cytometry and fluorescence microscopy. The inhibition of macrophage infection was dose- and time dependent in the range of 15 to 30 microg/ml. The ultrastructure showed that trypomastigotes treated with Et-Bra (30 to 60 microg/ml/1 day) displayed "blebs"of the body and flagellum membrane. Epimastigotes treated with 50 to 400 microg/ml/1 day displayed damages on the mitochondria, reservosomes, Golgi complex and, in higher drug concentrations, alterations in their morphology. The treatment of parasites decreased rhodamine and acridine orange fluorescence, indicating interference at the potential of the mitochondrial membrane and on the functional activity of the reservosomes, compromising your cellular viability revealed by flow cytometry. The results obtained with Et-Bra point out to the involvement of the amyrins, besides that of phenolic derivatives, on the biological activity of propolis.

QT31 - Evaluation of nitrosyl complexes able to delivery nitric oxide on therapeutic of Chagas disease

Osakabe A.L. *(FMRP - USP)*; Silva J.J.N. *(USP)*; Silva J.S. *(FMRP - USP)*; Franco D.W. *(FMRP - USP)*

Acute infection with *Trypanosoma cruzi* is characterized by immunosuppression mediated by T cells and macrophages. Nitric oxide (NO) production during the initial phase of acute infection might participate in the clearance of parasites by macrophages, whereas its overproduction during the late phase of acute infection would account for the immunosuppression. NO-donors block *Trypanosoma*, *Plasmodium* and *Leishmania* life cycle by inactivating parasite enzymes, e.g., cysteine proteinases. In the present study we synthesized two nitrosyl complexes, trans-$[Ru(NO)(NH_3)_4(isn)](BF_4)_3$ and trans-$[Ru(NO)(NH_3)_4(imN)](BF_4)_3$ (ISN and IMN respectively) and evaluated the potential trypanocidal using *in vivo* assay. In this assay we used five swiss mice per group, female, infected with 1000 Y strain trypomastigote forms of *T. cruzi*, treated with 10 and 100 nmol of each compound. The treatment started a day before infection and continued until 15^{th} post infection. The parasitemia was performed in alternate days by Brener method and the period of survival was observed. Our results showed that the parasitemia of mice treated with the two compounds, in the concentration of 100 nmol/mice/day, was lower than the controls (treated with PBS). Also the mortality was lower in the groups of treated mice. An interesting result was found in ISN group once two mice survived and they showed the same initial parasitemia than the others three mice of that group, but on 11^{th} day the two survival mice acquired the lowest parasitemia indicating that the parasite must be controlled early during the infection for survival, as showed in this experimental model. We concluded that the best dose in our experimental treatment was 100 nmol/mice/day of ISN since in this assay some mice infected survived if compared with control.

QT32 - TRYPANOCIDAL ACTIVITY OF *Piper* SPECIES (PIPERACEAE)

Ambrósio, D. L. *(UNESP)* Passerini, G. D. *(UNESP)*; Lopes, A. A. *(UNESP)*; Batista Jr, J. M. *(UNESP)*; Kato, M. J. *(USP)*; Bolzani, V. S. *(UNESP)*; Furlan, M. *(UNESP)*; Cicarelli, R. M. B. *(UNESP)*

American trypanosomiasis is caused by the hemoflagellate protozoan *Trypanosoma cruzi* that can be transmitted by triatomine bugs, congenital route or through blood transfusion. This is a serious parasitic disease that occurs in Latin America, with considerable social and economic impacts. Millions of people (estimated at 16-18 million in the decade of the 1990s) have been infected, and more than 25% of the population is at risk of being contaminated in Central and South America. Two drugs, nifurtimox and benznidazole, are available for the treatment of infected people but are poorly tolerated in the acute phase and inefficient in the chronic phase of the disease. In view of these considerations, there is the need to search for new efficient chemopreventive and chemotherapeutic agents. Natural products mainly of plant origin have been studied as a source of new drugs against *T. cruzi*. The objective of this project was the investigation of trypanocidal activity of a crude extracts and fractions from leaves of *Piper aduncum* (1), *Piper crassinervium* (2) and *Piper gaudichaudianum* (3)(PIPERACEAE). The crude extracts and hexanic, ethyl acetate and hydroalchoolic fractions were evaluated against *Trypanosoma cruzi* epimastigote forms (Y strain). The *in vitro* assay was carried out using 105 epimastigote forms/ well in the microtiter plates containing LIT medium. In each well, was used a serial dilution of the extracts or fractions. The efficacy of the drugs was measured using counting in the Neubauer's chamber. The IC 50 found for 1, 2, 3 were 112.4; 98.2; 210,5 and the hexanic, ethyl acetate and hydroalchoolic fractions

were 220,6; 101,5; 83.70 for *P. aduncum*; 90.3; 118.4; 200.1; for *P. crassinervium* and 46.07; 315.5; 473.2 μg/mL for *P. gaudichaudianum*, respectively. These results showed that the crude extracts of Piper species have significative *in vitro* activity. Supported by Fapesp and Capes.

QT33 - ACTIVITY OF *Eugenia jambolana* Lam. DERIVATIVES AGAINST *TRYPANOSOMA CRUZI*: POTENCIAL USEFUL SUBSTANCE ON CHAGAS DISEASE CHEMOTHERAPY

Gibaldi, D. *(UFRJ)*; Ramos, M. F. S. *(UFRJ)*; Siani, A. C. *(UFRJ)*; Henriques, M. G. M. O. *(UFRJ)*

The parasite *Trypanosoma cruzi* is the causative agent of Chagas' disease, an endemic illness in Latin America. Current treatment of human patients shows chemotherapeutic failure and unpleasant side effects. Moreover, the increased resistance of the parasite to reference drugs is one of major problems for the successful of the treatment. Medicinal herbs and their products have been used in folk medicine throughout centuries, and several essential oils and their constituents have been found to demonstrate anti-microbial activity. Recently we demonstrated the trypanocidal activity of *Eugenia jambolana* essential oil against trypomastigotes forms of *T. cruzi* only, on release of trypomastigotes forms of parasite by infected macrophage, and development of amastigotes inside these cells. As demonstrated, the toxicity against parasites was independent of cell activation. In the present work we show the trypanocidal effect of different constituents of *E. jambolana* essential oil. Chemical profile of this oil shows several derivatives, terpens as majotity. Two substances, Terpinolen and Citral demonstrated potential trypanocidal activity on non-cytotoxic concentrations to peritoneal macrophages. When tested against trypomastigotes forms in vitro, the substances showed a dose depended activity in 24h of incubation (Terpinolen ED50 1.1μg/mL; Citral 2.1μg/mL). On release of trypomastigotes and growth of amastigotes in co-culture with macrophages, the essential oil derivatives also demonstrate effect. Studies of the activity of *E. jambolana* essential oil derivatives are currently been carried out to evaluate the effects of these substances on experimental Chagas' disease.

QT34 - Effects and mechanisms of action of miltefosine against *Trypanosoma cruzi*: studies *in vitro* and *in vivo*.

Saraiva, V.B. *(UFRJ)*; Pinhão, F. L. *(UFRJ)*; Wengert, M. *(UFRJ)*; Gome-Quintana, E. *(UFRJ)*; Mendonca-Previato, L. *(UFRJ)*; Previato, J.O. *(UFRJ)*; Pirmez, C *(UFRJ)*; Caruso-Neves,C *(UFRJ)*; Heise, N *(UFRJ)*

(1)Laboratório de Glicobiologia (1)Laboratório de Bioquímica Renal (2)Laboratório de Imunopatologia. (victors@biof.ufrj.br) Miltefosine, an anti-tumoral synthetic agent now available for oral treatment of visceral leishmaniasis in humans, also presents toxicity against the causative agent of Chagas' disease *Trypanosoma cruzi*. In tumor cells and *Leishmania* miltefosine interferes with cellular signaling through inhibition of protein kinase C. In a previous study using a cell-free system, we have observed that miltefosine was able to inhibit the ouabain-insensitive Na^+-ATPase activity in *T. cruzi*. Since in the renal proximal tubule the Na^+-ATPase activity can be stimulated through the activation of PKC, we decided to evaluate if there is a linkage between the inhibitory effects of miltefosine and these two intracellular signaling pathway components. The *T. cruzi* Na^+-ATPase activity in the presence of calphostin (PKC inhibitor), was not altered but, PMA (PKC activator) was able to inhibit this activity. Miltefosine inhibited the PKC activity in a dose-dependent manner and, as observed for mammalian cells, PMA and calfostine activated and inhibited respectively PKC activity. These results suggested an interference of miltefosine in the cellular signaling through inhibition of PKC but, different from mammalian cells, the *T. cruzi* Na^+-ATPase activity is not dependent on activation through PKC. Studies *in vivo* comparing the oral treatment of *T. cruzi* (Y-strain) infected Swiss mice with miltefosine and benznidazol showed 100% survival for both drugs. The animals treated with miltefosine showed a significant reduction of the parasites detected in blood when compared to the untreated animals. Nevertheless, no parasites were detected in the blood of animals treated with benznidazol. After 15 days of infection the animals were sacrificed, and histopathological studies showed a decrease in the inflamation area of the heart and liver and absence of amastigote nests in both groups treated. Supported by: CAPES, CNPq and FAPERJ.

QT35 - EFFECT OF L-LEUCINE METHYL ESTER ON *Trypanosoma cruzi* AMASTIGOTES AND TRYPOMASTIGOTES

Adade C. *(FIOCRUZ)*; Soares M. J. *(FIOCRUZ)*; DeCastro S. L. *(FIOCRUZ)*

Some amastigotes of *Leishmania* species present large lysosome-like organelles, the megasomes, which contain large amounts of cysteine proteinase. It has been shown that these amastigotes can be killed by amino acid esters such as L-leucine methyl ester (Leu-OMe), by a mechanism that involves hydrolysis of this compound in megasomes by cysteine proteinases, leading to amino acid accumulation in the organelles followed by osmotic lysis. Due to the biochemical and morphological similarities between reservosomes and megasomes, in a previous work we have analyzed the action of Leu-OMe on *T. cruzi* epimastigotes and could observe that this compound was effective against this evolutive form. The present work was designed to verify the action of Leu-OMe on the life forms of *T. cruzi* present in the vertebrate host. Bloodstream trypomastigotes were obtained from infected albino Swiss mice and then incubated in RPMI medium supple-

mented with 10% fetal bovine serum, at 37^o C, with different concentrations (0.5 to 8 mM) of Leu-OMe for 24 hours. Effect of this compound on parasite lysis was evaluated by counting with a Neubauer chamber. Mice peritoneal macrophages were collected, plated at 3x10^5 cells/well and infected with bloodstream trypomastigotes at a 10:1 parasites:host cell ratio. After three hours of interaction, the culture was washed and incubated with 0.125-1 mM of the drug. The percent of infected macrophages and number of amastigotes per cell were daily evaluated up to 72 hours post-infection. The ED-50 for trypomastigote lysis was estimated as about 1.75 mM. Treatment with Leu-OMe inhibited the macrophage infection in about 87-98% after 72 hours of incubation. The number of intracellular parasites decreased in 82-92% after the same time of incubation. Our data demonstrate that Leu-OMe was effective against the vertebrate forms of *T. cruzi*. Further studies are underway to investigate the possible intracellular targets of the drug.

QT36 - EVALUATION OF THE MICROBIOCIDAL *in vitro* ACTIVITY OF ARJUNOLIC ACID AGAINST *Trypanosoma cruzi*

Souza-Neta, L.C. *(GESNAT-IQ-UFBA)*; Menezes, D. *(CPqGM-FIOCRUZ)*; Guaré Cruz, F. *(GESNAT-IQ-UFBA)*; Martins, D. *(GESNAT-IQ-UFBA)*; Vannier-Santos, M.A. *(CPqGM-FIOCRUZ)*

Trypanosoma cruzi is the ethiological agent of the Chagas disease or American trypanosomiasis, which affects about 16-18 million people in South and Central Americas, where circa 40 million remain at risk [1]. Benznidazole is the drug of choice in Brazil against acute and early chronic phases of Chagas disease. Although arjunolic acid was not effective against *Leishmania amazonensis* [2], we decided to test it upon the *T. cruzi*. Arjunolic acid was isolated in species *Myrcia guianensis* and *M. rotundifolia* (Myrtaceae) which were collected in the Lagoa do Abaeté (Bahia). We used *Trypanosoma cruzi* (Y strain) epimastigote forms incubated or not with 100-500μM arjunolic acid. Parasites were maintained in LIT medium with 10% FBS at 26ºC. It reduced in vitro proliferation with an apparent IC_50 of 171μM. We used ultrastructural analysis in order to approach the arjunolic acid mode action upon T. cruzi. Parasites were fixed in 2.5% glutaraldehyde and 4% paraformaldehyde in 0.1M sodium cacodylate buffer pH 7.2 and prepared for transmission electron microscopy. We observed remarkable alteration of the cell architecture. The parasite plasma membranes were often finely corrugated and the subpelicullar microtubules disorganized. The overall cell compartmentation appeared disordered and altered basal body numbers were observed. Taken together, these data indicate that arjunolic acid affects *T. cruzi* cell membrane and membrane-cytoskeleton connection, impairing parasite survival and proliferation in vitro. Supported by: FIOCRUZ, CNPq, CAPES and FAPESB. [1]- World Health Organization. 1995. Chagas' disease: important advances in elimination of transmission in four countries in Latin America. WHO Press no. 183. [2]- Torres-Santos et al. Phytomedicine. 2004; 11(2-3): 114-20.