XXX ANNUAL MEETING ON BASIC RESEARCH IN CHAGAS DISEASE - XIX MEETING OF THE BRAZILIAN SOCIETY OF PROTOZOOLOGY - HOTEL GLÓRIA, CAXAMBU, MG, BRASIL - 10-12 NOVEMBER 2003. **Rev. Inst. Med. trop. S. Paulo, 45**(Suppl. 13), November, 2003.

IM1 - INFLAMMATORY MEDIATORS AND MAST CELLS IN HUMAN LOCALIZED CUTANEOUS LEISHMANIASIS LESIONS.

Miglio-Junger, J.[1]; Pirmez, C.[1]; Oliveira-Neto, M.P.[3]; Oliveira, M.P.[1,2].

[1]Department of Biochemistry and Molecular Biology, Oswaldo Cruz Institute, FIOCRUZ; [2]School of Medicine, Estácio de Sá University; [3]Evandro Chagas Research Institute/FIOCRUZ. E-mail: marciapo@ioc.fiocruz.br

In localized cutaneous leishmaniasis (LCL), the lesions are described as a chronic granulomatous inflammatory reaction composed of macrophages, T lymphocytes, plasma cells and mast cells. Despite of the T cell and type 1 cytokines are essential for the infection control, other cell types and inflammatory mediators may be important in this process. The aim of this study is to evaluate the expression of inflammatory mediators and mast cells subpopulations in human LCL lesions. The inflammatory infiltrate of LCL lesions caused by *Leishmania (Viannia) braziliensis* was analyzed by immunostaining using monoclonal antibodies that recognize inflammatory mediators (histamine, leukotriene B4/ LTB4 and prostaglandin F2a/PGF2a), mast cell proteases (tryptase and chymase) and iNOs. A total of 16 frozen biopsies obtained from localized cutaneous lesions were studied. The patients, all of them living in endemic areas of Rio de Janeiro, were classified in two groups: 9 with a period of evolution up to three months (early lesions) and 7 patients with more than three months of illness duration (late lesions). The number of cells expressing histamine was significantly elevated in early lesions, when compared to those with more chronic illness duration. Expression of PGF2a was found in both groups, with a slightly elevated amount of positive cells in early lesions. However, the number of LTB4 positive cells tended to increase in late lesions. These results indicate that histamine and lipid mediators could be involved not only in the initial stages of the inflammatory infiltrate development, but also in mechanisms that contribute to the progression of lesions. Both clinical groups presented similar patterns of iNOs expression. In patients with early lesions, the amount of tryptase+ cells was significantly higher than the chymase+ cells. However, in late lesions chymase+ cells were found in greater quantity. These results suggest that changes in mast cells phenotype could be involved in the immunopathogenesis of cutaneous lesions.

Financial Support: PAPES/FIOCRUZ; CNPq

IM2 - MODULATION OF EXPERIMENTAL *LEISHMANIA (L.) MAJOR*-INFECTION IN MICE BY NORMAL POLYSPECIFIC IMMUNOGLOBULIN INTRAVENOUS THERAPY

Nogueira, A.[1], Olivieri, B.P.[2], Montalvao, F.[1], Modesto, H.[1], Kaveri, S.[3], Amaral, V.F.[1], Araújo-Jorge, T.C.[2] & Vasconcellos, R..[1]

[1] Departamento de Imunobiologia, Instituto de Biologia, UFF, [2]Laboratório de Biologia Celular, DUBC, IOC/FIOCRUZ, Brazil and [3]Institut de Cordeliers, INSERM U430, France. E-mail: ritavasc@vm.uff.br

Natural antibodies (NAb) are normally present in the sera of healthy individuals in the absence of a specific antigenic stimulation. Given the knowledge on the important role of NAbs, it is possible to associate deviations of these repertoires to an effective response towards protozoa infections. Indeed, our recent published date showed a nice correlation between NAb repertoire and resistance of mice to experimental *Trypanosoma cruzi* infection. At the present work we associate a skewed autoantibody repertoire to resistance or susceptibility to *Leishmania (L.) major* infection. Using a semi-quantitative immunoblot technique, we demonstrated that BALB/c mice (susceptible) presented an increased IgG autoreactive repertoire late after infection. In contrast, at the same time after infection, C57BL/6 mice (resistant) displayed an increased IgM autorreactive repertoire. We are now directly testing the role of NAb in the modulation of *L. major* infection. For such aim, BALB/c mice will be infected with *L. (L.) major*, submited or not to Intravenous immunoglobulin (IVIg) therapy. IVIg is a pool of normal polyspecific immunoglobulins obtained from 10000 people, representing a normal spectrum of serum IgG reactivities. IVIg has been successfully used on the treatment of autoimmune diseases and in chronic inflammatory processes At our work, we are using the following IVIg therapeutic schemes: (1) Infection and no treatment; (2) IVIg 3 weeks before infection; (3) IVIg 1 week post-infection. (4) IVIg 6 weeks post-infection. These experiments are in course. Infection parameters are being evaluated and serum samples being collected to further analysis of the autoantibody reactive repertoire. Mechanisms involved in the possible protection given by IVIg will be addressed on our studies.

IM3 - EFFECT OF PASSIVE TRANSFER OF ANTI-MSP3 AND ANTI-GLURP ANTIBODIES ON *PLASMODIUM FALCIPARUM* GROWTH *IN VITRO* AND *IN VIVO*

Bianco-Jr, C.[1], Carvalho, L.J.M.[1], Alves, F. A.[2], Luna, T.G.S.[1], Oliveira, S. G.[3], Theisen, M.[4], Muniz, J.A P.C.[2], Daniel-Ribeiro, C.T.[1]

[1]Laboratory of Malaria Research, Department of Immunology, Instituto Oswaldo Cruz/FIOCRUZ, Rio de Janeiro; [2]National Primate Center, [3]Evandro Chagas Institute; Funasa, Belem, Brazil; [4]Statens Seruminstitut, Copenhagen, Denmark. e-*mail*: cesare@ioc.fiocruz.br

Introduction: The Merozoite Surface Protein-3 (MSP3) and the Glutamate Rich Protein (GLURP) of *Plasmodium falciparum* were selected as candidate antigens based on epidemiological data and also because of their strong effect in antibody-dependent cellular inhibition (ADCI) assay of *P. falciparum* growth. We have tested 18 different antigen-αdjuvant formulations containing recombinant proteins or peptides derived from these two proteins in *Saimiri* and *Aotus* monkeys, the WHO-recommended primate models for malaria vaccine trials. Most formulations were immunogenic and in some cases able to induce a partial anti-parasite immunity, as verified in challenge experiments with *P. falciparum*. However, it is important to know whether antibodies raised by immunization with recombinant antigens have similar effects to those elicited against the native proteins by infection. In the present work, we aim to study the protective effect of antibodies raised against the native MSP3 and GLURP proteins through passive transfer experiments in *Saimiri* and *Aotu*s monkeys, as well as their effect in ADCI assays. Methods: *Saimiri sciureus* and *Aotus infulatus* monkeys were repeatedly infected (at least four times) with blood stages of the *P. falciparum* FUP or FVO strains, respectively, until becoming refractory to further infections. Sera was repeatedly collected and pooled until obtained a volume of nearly 100ml for each species. This pooled malaria-immune serum will be precipitated with Ammonium Sulphate, dialyzed and passed through Sephadex columns to obtain purified total IgG. The IgG will then be passed through CNBr-Sepharose coupled to a recombinant hybrid MSP3-GLURP protein and properly eluted, allowing the acquisition of purified anti-MSP3 and anti-GLURP antibodies. After extensive dialysis and concentration adjustment, the antibody preparation will be tested in ADCI assays and also passively transferred to naïve monkeys, which will then be challenged with 50,000 *P. falciparum*-parasitized erythrocytes. Parasitemia will be daily evaluated and monkeys treated with Choloroquine or Mefloquine if necessary. Results: we have performed the repeated infections and obtained the pooled immune serum. The antibody purification step is ongoing.

Supported by PDTIS/FIOCRUZ and FAPERJ

XXX ANNUAL MEETING ON BASIC RESEARCH IN CHAGAS DISEASE - XIX MEETING OF THE BRAZILIAN SOCIETY OF PROTOZOOLOGY - HOTEL GLÓRIA, CAXAMBU, MG, BRASIL - 10-12 NOVEMBER 2003. **Rev. Inst. Med. trop. S. Paulo, 45**(Suppl. 13), November, 2003.

IM4 - SOME OBSERVATIONS ON THE SUSCEPTIBILITY OF *CEBUS APELLA* (PRIMATES: CEBIDAE) TO THE EXPERIMENTAL INFECTION BY *LEISHMANIA (L.) CHAGASI*.

Carneiro, L.A.; Tabosa, K.F.; Brígido, M.C.O.; Campos, M.B.; Lainson, R. & Silveira, F.T.

Instituto Evandro Chagas (SVS), Belém, Pará, Brasil.

Introduction: The monkey *Cebus apella* has a very large geographical distribution in South America, and in north Brazil this species has been associated with the silvatic cycle of *Leishmania (V.) shawi*, a dermotropic parasite causing cutaneous leishmaniasis of man (Lainson et al.,1988). This monkey has been successfully used as a model for studying cutaneous leishmaniasis (Lainson & Shaw, 1977; Silveira et. al., 1989, 1990, 1997) and, for this reason, we are at present investigating its susceptibility to experimental infection with *Leishmania (L.) chagasi.* Objectives: To determine the susceptibility of *Cebus apella* to experimental infection with *Leishmania (L.) chagasi* and the animal's usefullness as a model for Americans visceral leishmaniasis. Materials & Methods: 10 specimens of *Cebus apella* were used - 4 males and 6 females 8 werw adults and 2 juveniles. All were born and raised in captivity. *Protocol*: 6 monkeys (3 that had previously been used to study the animal's susceptibility to *L. (V.)* shaw, and 3 that had no previous contact with *Leishmania*) were inoculated intradermaly into the base of the tail with 2 x 10^6 promastigotes from stacionary cultures in Difco B45 culture medium. Four others, all having had no previous contact with *Leishmania*, were inoculated with 3 x 10^7 amastigotes from infected hasmters by two routes: two by intravenous injection and two by intraperitoneal inoculation. *Evaluation of infections*: clinical examination, IgG humeral response (IFAT) and a search for amastigotes in Giemsa-stained bone-marrow smears were made monthly. Results: In animals inoculated with promastigotes we have till now found no signs or symptoms of clinical infection 16 month post inoculation: neither have we been able to detect parasites in the bone- marrow or demonstrate IgG antibody against *L. (L.) chagasi*. Among the animals injected with amastigotes, the monkeys inoculated by the intravenous route showed parasites in bone- marrow smears one month later. The two inoculated by the intraperitoneal route have till now shown no parasites in the bone-marrow at one month p.i.. Other indications of infection have not been observed till now, doubtless due to the short period of incubation. Conclusion: It is as yet too early to say if *Cebus apella* can serve as a satisfactory model for American visceral leishmaniasis, but the present results are considered encouraging.

IM5 - OUTCOME OF *LEISHMANIA (V.) BRAZILIENSIS* INFECTION IN THE EAR DERMIS OF MICE

de Oliveira, CI[1], Oliveira, F[1], Novais, FO[1], Barral, A[1,2] and Brodskyn, C[1,2]

[1] Centro de Pesquisas Gonçalo Muniz, FIOCRUZ, Salvador, BA, Brasil; [2] Universidade Federal da Bahia, Salvador, BA, Brasil

Extensive work with *Leishmania major* has revealed that Th1 T cells and associated cytokines IFN-γ and TNF-α mediate healing while Th2 cells and associated cytokine IL-4 mediate susceptibility. However, little work has been done in *L. (V.) braziliensis*, probably due to the fact that an experimental model is not readily available. Recent reports have shown that parasite inoculation in the ear dermis closely resembles the natural infection, leading to important findings concerning the pathogenesis of disease. We investigated the course of infection with *L. (V.) braziliensis* (MHOM/BR/01/BA788) by injection of 10^5 parasites in the ear dermis of BALB/c mice. Parasite burden was assessed weekly and by day 35 post infection, parasites achieved a 1000-fold expansion. Thereafter, parasites were gradually destroyed so that beyond day 63 they could not be detected at the inoculation site. Histopathological evaluation revealed an intense inflammatory infiltrate at the peak of lesion development (day 35 post infection) composed mainly by infected macrophages and granulocytes and scarce lymphocytes. Accordingly, RT-PCR failed to detect IFN-γ at the inoculation site. On the other hand, TNF-α expression was detected at days 14, 35 and 49 post infection. Concerning the draining lymph nodes, parasites were detected from day 14 to day 125 post infection, although at lower levels. In terms of cytokine production, RT-PCR showed the presence of IFN-γ and TNF-α throughout the infection period. Regarding chemokine expression, we observed, by RT-PCR, the presence of MCP-1, MIP 1a, MIP 1b and RANTES in the draining lymph nodes. Presently, we are evaluating the chemokine expression at the inoculation site by immunohistochemistry. Similar to recently published data, inoculation of *Leishmania* parasites in the ear dermis resembles the natural infection. Our results confirm that BALB/c mice cure an infection with *L. (v.) braziliensis* due to the development of a parasite-specific Th1 response. To our knowledge, this is the first report showing that the activation of the Th1 pathway also occurs after inoculation of *L. (v.) braziliensis* in the ear dermis.

Supported by: FAPESB, CNPq

IM6 - SERA FROM CHRONIC CHAGASIC PATIENTS WITH MUSCARINIC ACTIVITY PROLONGS QT INTERVAL IN ISOLATED RABBIT HEARTS.

Medei, E*, Hernandez, Q. C*, Costa, P.C.S*, Linhares, VS*, Pedrosa, R.C#, Masuda, M.O*, Campos de Carvalho, A.C*, Nascimento, J.H.M*.

Laboratório de Eletrofisiologia Cardíaca, Instituto de Biofísica, Carlos Chagas Filho* e Hospital Universitário Clementino Fraga Filho#,Universidade Federal do Rio de Janeiro. RJ-Brasil.

Introduction: Chronic chagasic patients (CChP) with cardiac disease present several arrhythmias that could evolute in sudden death. QT interval parameters are potential prognostic markers of arrhythmogenicity risk, cardiovascular mortality and have been evaluated in chagasic patients (Circulation. 2003, 108(3): 305-12). We previously showed that sera from CChP induced alterations in cardiac electrogenesis and impair atrioventricular conduction in isolated hearts (Circ 1997, 96(6): 2031-7). These effects could be explained by β-adrenergic and muscarinic receptor activation. The aim of our study was analyze the acute effect of CChP sera, previously characterized as having muscarinic activity, on QT interval in isolated rabbit hearts.

Methods and Results: Rabbits (both gender) were killed by cervical dislocation and hearts were immediately cannulated through the aorta, and perfused by modified Langendorff technique with Tyrode solution (in mmol/L; NaCl 137, glucose 9, $NaHCO_3$ 18, KCl 2.7, NaH_2PO_4 1.8, $MgCl_2$ 0.5, $CaCl_2$ 2.7, bubbled with carbogenic mixture 5%CO_2/ 95% O_2). The experimental protocol consisted of three 20 minutes perfusion period (control, serum and washout). In the second period serum from CChP (n=16) and normal blood donors (NBD, n=10) was diluted 1:100 (vol.:vol.) in control solution. The QT interval was measured in all periods (10 representative beats from each period. The QT interval measured in presence of CChP serum (255 ± 6,2; mean ± SEM) was significantly different of control (241,3 ± 6,2; $p<0,01$) and washout periods (233,8 ± 6,5; $p<0,001$). NBD sera had not effect on QT interval; control (271,1±12,1), serum (280±13,3), and washout (277,8±13,1).

Conclusion: Our result suggest that sera from CChP with muscarinic like activity were able to prolong QT interval. This effect can contribute for the genesis of some arrhythmias present in CChP.

Support: CNPq, PRONEX-MCT, FAPERJ.

XXX ANNUAL MEETING ON BASIC RESEARCH IN CHAGAS DISEASE - XIX MEETING OF THE BRAZILIAN SOCIETY OF PROTOZOOLOGY - HOTEL GLÓRIA, CAXAMBU, MG, BRASIL - 10-12 NOVEMBER 2003. **Rev. Inst. Med. trop. S. Paulo, 45**(Suppl. 13), November, 2003.

IM7 - IGF-I AFFECTS DIFFERENTLY THE LEISHMANICIDAL REACTIVE OXYGEN- AND NYTROGEN INTERMEDIATE-DEPENDENT MECHANISMS

[1]VENDRAME, C.M.V.; [1,2] CARVALHO, M.D.T.; [1] GOTO, H.

1 Lab. Soroepidemiologia e Imunobiologia IMTSP e 2 Lab. de Imunofisiopatologia ICB-IV, USP

Insulin-like growth factors are polypeptides stimulating proliferation and differentiation of a wide variety of cell types, are present in the blood, in many tissues and particularly in macrophages. It is one of the first factors encountered by the *Leishmania* promastigotes as soon as they are injected into the skin, and subsequently after internalization by macrophages. We have previously reported that insulin-like growth factor-I induces proliferation of *Leishmania* promastigotes and amastigotes in vitro and exacerbates the lesion development in cutaneous leishmaniasis in mice (Goto et al. Proc.Natl.Acad.Sci.95:13211,1998). Since leishmanicidal effect of murine macrophages is mediated by reactive oxygen and nitrogen intermediates, effect of IGF-I on exacerbation of lesion development might be related to these leishmanicidal mechanisms. We have previously seen that NO production is decreased in macrophage infected with *Leishmania (L.) amazonensis* upon IGF-I stimulation (Rev.Inst.Med.Trop.S.P Suppl.12 (IM12) 2002). In this study, we searched the effect of IGF-I or IGF-II on induction of H_2O_2 production in *L. (L.) amazonensis*-infected macrophages. BALB/c mouse peritoneal macrophages (2 x 10^5/ well) were infected with stationary phase *Leishmania (L.) amazonensis* (WHOM/BR/00-LTB-0016) promastigotes (*Leishmania*:macrophage=2:1) at 33ºC for 3 hours. Either macrophages or *Leishmania* were pre-incubated for 5 minutes with rIGF-I or rIGF-II (50 ng/ml) or maintained in the culture system, or maintained without IGFs (control). Cultures were set up in sextuplicates and we evaluated the reactive oxygen species produced by macrophages on supernatant by H_2O_2 assay using horse radish peroxidase-dependent oxidation of phenol red. We present here data from one representative experiment from four. H_2O_2 level (nmol/mL) in the control was 5.1±0.9 (mean + standard deviation), with preincubation with IGF-I of macrophages was 4.1±1.0, of *Leishmania* was 7.0±0.7 and when IGF-I was maintained in culture, 4.4±0.6. H_2O_2 level (nmol/mL) with preincubation with IGF-II of macrophages was 4.5±0.9, of *Leishmania*, 6.9±1.2 and when maintained in the system 4.3±0.4. Contrary to the observed decrease of NO production by macrophages in the presence of IGF-I, neither IGF-I nor IGF-II leads to a significant decrease of H_2O_2 production by macrophages.

Supported by: FAPESP, CNPq and LIM-38 (HC-FMUSP).

IM8 - EFFECT OF INSULIN-LIKE GROWTH FACTOR (IGF) II ON THE LESION DEVELOPMENT IN EXPERIMENTAL CUTANEOUS LEISHMANIASIS

[1]VENDRAME,C.M.V.; [1]MANULI, E.R.; [1,2]CARVALHO, M.D.T.; [1]GOTO, H.

1 Lab. Soroepidemiologia e Imunobiologia IMTSP e 2 Lab Imunofisiopatologia ICB-IV, USP

We have previously reported that a inflammation related- and constitutively present growth factor, insulin-like growth factor-I, induces a direct proliferative response on *Leishmania* promastigotes and amastigotes in vitro and exacerbates the lesion development in cutaneous leishmaniasis in mice (Goto et al. – Proc. Natl. Acad. Sci. 95: 13211, 1998). We had not verified any direct effect of IGF-II on *Leishmania* promastigotes and amastigotes therefore the study was not procceeded with IGF-II. However, since IGF-II is a factor with effect on a wide variety of cell types, particularly in macrophages, its effects on host macrophage-parasite interaction is likely. Therefore, here we studied the effect of IGF-II on lesion development in cutaneous leishmaniasis in mice. BALB/c mice were injected in the hind footpad with 10^7 stationary phase *Leishmania (L.) amazonensis* (WHOM/BR/00-LTB-0016) promastigotes, preincubated for 5 min with or without rIGF-II (50 ng/ml). The contralateral footpad of each animal was injected with sterile PBS as a control. The progression of the lesion was evaluated measuring foot thickness with a dial caliper at 14, 28, 42 days post-infection (PI). Lesion size was calculated subctrating from the thickness of the infected foot, the thickness of the contralateral non-infected footpad. The size of the lesion in control *Leishmania*-infected mice was 0.06±0.06 mm (mean + standard error) at 14, 0.92±0.51 at 28, and 2.74±0.71 at 42 days PI while, surprisingly in IGF-II pre-incubated *Leishmania*-infected mice, 0.02± 0.03 at 14, 0.23 ±0.15 at 28, and 1.70± 0.46 at 42 days PI. We evaluated in parallel the in vitro NO production in the supernatant of BALB/c mice peritoneal macrophages infected with *Leishmania*, pre-incubated for 5 min with or without rIGF-II (50 ng/ml). Cultures were set up in sextuplicates and we evaluated the nitrite [that reflects the nitric oxide (NO) production] in the supernatant by Griess method. We have done 3 similar experiments and we present here data from a representative experiment. NO levels (mM) in the promastigote-infected macrophages was in the control without IGF-II 4.5±2.3 (mean ± standard deviation) and 8.0±0.5 with promastigotes preincubated with rIGF-II.

Our results show that the mice injected with *Leishmania* pre-incubated with IGF-II developed a smaller lesion from day 28 PI. Observation of increased NO production by macrophages infected with IGF-II- preincubated *Leishmania* suggests that IGF-II have opposite in vivo and vitro effect compared with IGF-I.

Supported by: FAPESP, CNPq and LIM-38 (HC-FMUSP).

IM9 - EFFECTS OF GLYCOINOSITOLPHOSPHOLIPIDS (GIPL), EXTRACTED FROM *T. CRUZI* IN THE MODULATION OF THE ADAPTIVE IMMUNITY.

Lemos, R.*; Peixoto, J. R.*, Lintomen, L.*; Previato, J.O.[#]; Mendonça-Previato, L[#]; Elsas, P. P. X.*; Gaspar Elsas, M.I.[+]; Bellio, M*.

* IMPPG-UFRJ, [#]IBCCF-UFRJ, +Fiocruz

Molecules from the surface of *Trypanosoma cruzi* parasite have been described as playing a role in parasite adhesion, infectivity and also in the inflammatory response due to parasite infection. This initial response, determined by receptors present in phagocytes and APCs, may be very important in guiding the adaptive immunity generated against the parasite. Glycoinositolphospholipid (GIPL) belongs to the GPI family of anchors and is one of the most abundant surface molecule in epimastigotes forms of *T.cruzi* (eGIPL). Evidence suggests the occurrence of GIPLs also in infective trypomastigote and intracellular amastigote forms of *T. cruzi*. Recent work developed in our laboratory has described that eGIPL can induce a proinflammatory response in a Toll like receptor 4 (TLR4)-dependent manner. In the present study we demonstrate that the treatment with eGIPL can enhance the Th2 response in C57BL/10 and BALB/c mice immunized with OVA. Mice injected with eGIPL 24 h prior to the second OVA/alum immunization developed an increase in bone marrow eosinopoiesis, as revealed by bone marrow cultures in the presence of rIL-5. Moreover, an augmentation in the serum levels of OVA-specific IgE is also observed in the eGIPL-treated mice.

Supported by: FAPERJ, CNPq, CAPES and PRONEX-MCT

IM10 - LEISHMANIASIS: A DYNAMICAL SYSTEM APPROACH.

De Almeida, M.C*. & Moreira, H.N.[#]

*Departamento de Patologia e Núcleo de Medicina Tropical. UnB. DF. Brazil.

XXX ANNUAL MEETING ON BASIC RESEARCH IN CHAGAS DISEASE - XIX MEETING OF THE BRAZILIAN SOCIETY OF PROTOZOOLOGY - HOTEL GLÓRIA, CAXAMBU, MG, BRASIL - 10-12 NOVEMBER 2003. **Rev. Inst. Med. trop. S. Paulo, 45**(Suppl. 13), November, 2003.

[#]Departamento de Matemática. UnB. DF. Brazil

The TH1/TH2 paradigm has been largely used in the interpretation of several diseases, particularly in leishmaniasis. But, so far we are aware, there is not a mathematical description of this model related to leishmaniasis. We have extended and modified a previous published set of equations (Bergmann et al 2002. *Bull Math Biol* 63, 425-446) in order to adapt it to leishmanial disease particularities. The main modifications were: 1) the assumption of a logistic parasite growth curve instead of an exponential one, 2) the assumption of the TH2 arm of the response having a positive action on parasite growth. The set of three simultaneous differential equations describing the TH1 arm, TH2 arm and parasite growth were analysed for conditions of existence and stability of the solutions.

Stability solutions were obtained for the following situations and its possible clinical correlations: 1) TH2 and parasite extinction [TH1 cure], 2) TH1 extinction, TH2 and parasite coexistence [stable TH2 infection], 3) TH2 extinction, TH1 and parasite coexistence [stable TH1 infection] and 4) TH1, TH2 and parasite coexistence [stable TH1/TH2 infection]. Geometrically the situations 1) and 2) were characterised as stable nodes, and situations 3) and 4) as stable spiral focus. In the last situation, a phase plane analysis showed an oscillatory behaviour of TH1, TH2 and parasite in relation to time. Some predictions of the model agree with experimental data: TH1 cure, stable TH1 infection and stable TH1/TH2 infection. Host "death" (TH1 and TH2 extinction with parasite survival), TH2 cure (TH1 and parasite extinction) and TH1/TH2 cure were predicted as possible states (equilibrium points), but they were not attractors (stable conditions) in the model. Less obvious results were: 1) stable TH2 infection, 2) after infection the necessity of parasite presence for stable TH1/TH2 coexistence. The behaviour of the parasite growth is an important element for the stability conditions. For example, in stable TH1/TH2 infection one of the sufficient conditions was expressed by a relation in which inhibitory effects of host carrying capacity predominates over stimulating TH2 action on parasite growth, on the other hand a TH1 destructive action on the parasite, higher than parasite intrinsic growth rate, was important in determining the stability of TH1 cure. The stable TH2 infection is dependent on a decreasing TH1 response and on inhibitory effects of host carrying capacity that predominates over stimulating TH2 action on parasite growth. The system is intrinsically unstable for TH2 cure

IM11 - EFFECT OF ORAL TREATMENT WITH THE ENDOTHELIN RECEPTOR ANTAGONIST BSF-461314 IN THE MYOCARDITIS DURING MURINE EXPERIMENTAL *T. CRUZI* INFECTION

Roffê E[1], Souza ALS[1], Barcelos LS[1], Alves RO[3], Machado PP[1], Camargos EP[2], Romanha AJ[3] & Teixeira MM[1]

[1]Lab. Imunofarmacologia, Departamento de Bioquímica e Imunologia, ICB, UFMG; [2] Lab. Parasitologia Celular e Molecular, Centro de Pesquisas René Rachou, Fiocruz/MG; [3]Lab. Neurobiologia, Departamento de Morfologia, ICB, UFMG.

Endothelins have been shown to participate in leukocyte activation in several models of inflammation and are suggested to participate in pathological and functional damages to the cardiac endothelium during *T. cruzi* infection. In an attempt to investigate the role of endothelins in the acute and chronic *T. cruzi*-driven myocarditis, we infected C57BL/6 mice with 5000 tripomastigotes of the Y *T. cruzi* strain. Mice were treated with BSF-461314 (30 mg/Kg/day, *per os*), an endothelin ET-A receptor antagonist, or PBS, starting on day 1 post-infection (p.i.) until sacrifice on day 10, 15 or 20 p.i. Parasitemia, myocardial histopathology (inflammation and infection scores) and cardiac cytokines measurement by ELISA were assessed at these days. The parasitemic curve in BSF group did not show significant difference when compared with the control group, although inflammation was much more intense in the BSF than PBS group 10 days after infection. Moreover, a higher mortality was found in the BSF group (30% compared to 10%). In regard to cytokine production, the analysis revealed that *T. cruzi* infection induces increased levels of IL-10 and IL-4 (both on day 10 day p.i.) and MCP-1 and TNF-α (throughout the whole experimental period). Treatment with BSF increased IL-10 levels, what could be correlated with more intense myocarditis found in these animals. In conclusion, BSF treatment does not have a strong effect in *T. cruzi*-infected mice. Future *in vitro* studies will assess the role of endothelins in macrophage activation and *T. cruzi* killing.

Financial support: CAPES, FAPEMIG.

IM12 - EFFECT OF POLY(I:C), AN INTERFERON INDUCER, UPON PARASITE BURDEN, SUPEROXIDE AND NITRIC OXIDE PRODUCTION IN *LEISHMANIA*-INFECTED HUMAN AND MURINE MACROPHAGES

Santana G, Khouri AR, Cuvillier A, Barral-Netto M and Van Weyenbergh J

LIMI, CPqGM-FIOCRUZ, Salvador-BA

We previously demonstrated an IFN-beta-induced, NO-independent increase in parasite burden in human macrophages infected with *Leishmania (L.) amazonensis in vitro.* In murine macrophages, however, IFN-alpha/beta has been shown to induce iNOS *in vitro* and *in vivo*. To further explore the molecular mechanism of macrophage activation/desactivation by type I IFN *in vitro*, we investigated the effect of poly(I:C), an interferon inducer mimicking viral double stranded RNA, upon parasite burden, superoxide and NO production in both human (monocyte-derived) and murine (bone marrow-derived) macrophages. We found that poly(I:C) was able to induce NO (measured as nitrite) production in both human and murine macrophages, which could be reverted by the addition of an iNOS inhibitor (L-NMMA) or a neutralizing anti-IFN-alpha/beta antibody. Surprisingly, poly(I:C) did not significantly reduce intracellular *Leishmania* amastigotes in human or murine macrophages, indicating that NO production by itself might not be sufficient for a leishmanicidal effect. In contrast, an inhibitor of superoxide dismutase strongly decreased parasite burden in both human and murine macrophages, argueing for a significant participation of superoxide in parasite clearance. A strong superoxide production, as measured by a hydroxylamine-Griess assay, was indeed observed in human macrophages, either spontaneously or after triggering with PMA and poly(I:C). In uninfected as well as *Leishmania*-infected murine macrophages, a low spontaneous superoxide production was observed, which could be modestly triggered by PMA and poly(I:C), but not by LPS. In conclusion, superoxide and nitric oxide production appear to be reciprocally regulated in human and murine macrophages. Endogeneous IFN-alpha/beta production upon poly(I:C) treatment is able to induce NO in both models, without a significant leishmanicidal effect, which could be easily obtained through inhibition of superoxide desmutase.

FINANCIAL SUPPORT: FIOCRUZ

IM13 - PARTICIPATION OF IMMUNOGLOBULINS AND CELLS IN THE PATHOGENESIS OF GLOMERULONEPHRITIS IN VISCERAL LEISHMANIASIS.

M.G. Prianti, M. Yokoo, F.A.L. Costa, W. C. Santos, H. Goto

Laboratório de Soroepidemiologa e Imunobiologia (LIM 38), Inst. Medicina Tropical de São Paulo – USP

Renal involvement in visceral leishmaniasis (VL) is very frequent, but the

XXX ANNUAL MEETING ON BASIC RESEARCH IN CHAGAS DISEASE - XIX MEETING OF THE BRAZILIAN SOCIETY OF PROTOZOOLOGY - HOTEL GLÓRIA, CAXAMBU, MG, BRASIL - 10-12 NOVEMBER 2003. **Rev. Inst. Med. trop. S. Paulo, 45**(Suppl. 13), November, 2003.

pathogenesis is still unclear. Deposition of immune complexes has been considered the major mechanism of glomerulonephritis (GN) in VL. However, in previous studies in dogs and hamsters with VL, we detected deposit of IgG in glomeruli (Mathias et al., Braz. J. Med. Biol. Res. 2001), but also the presence of $CD4^+$ and $CD8^+$ T cells (Costa et al., Braz. J. Med. Biol. Res. 2000) in the renal lesions. To further study the pathogenesis, we started the characterization of the renal lesions in BALB/c and C57/BL6 strains of mouse infected intraperitoneally with $2x10^7$ purified *Leishmania (L.) chagasi* (MHOM/BR/72/strain 46) amastigotes. We observed focal glomerular hipercellularity, and interstitial focal inflammatory infiltrate that decreased in intensity from four to eight weeks. IgG deposits were detected in glomeruli and its intensity also decreased from four to eight weeks. Since in mouse VL develops a glomerular lesion that is similar to that observed in human, dog and hamster, we procceeded the study of participation of immunoglobulins and cells in the pathogeneisis of renal lesions in VL in BALB/c mice. Naive mice were injected either with *L. (L.) chagasi*-infected hamster serum and/or with splenic cells from *L. (L.) chagasi* antigen immunized BALB/c mice (N = 3 – 4/group). Control mice received serum and cells from naive animals (N = 3/group). Forty eight hours after transfer, the recipient animals were sacrificed and the kidney taken for analysis. We quantified by morphometry the number of cells/glomerulus, analyzing 50 glomeruli/animal. The median number of cells/glomerulus in control animals was 47.0, in recipient of immune splenic cells and serum, 65.6, of infected hamster serum, 60.2, of immune splenic cells, 65.0. The results suggest the participation of both immune serum and cells in the induction of glomerular hipercellularity in VL.

Supported by: CAPES, CNPq, LIM-38 (HC-FMUSP).

IM14 - TGF - B AND T CELL APOPTOSIS AS IMMUNOSUPPRESSIVE MECHANISM OF *LEISHMANIA AMAZONENSIS* ANTIGEN

Pinheiro RO[1], Lopes JRC[2], Camargo, NM[1], Fentanes RF[1] and Rossi-Bergmann B[1]

1– Instituto de Biofísica Carlos Chagas Filho, UFRJ, Rio de Janeiro, Brazil. 2 – Instituto de Microbiologia Professor Paulo de Góes, UFRJ, Rio de Janeiro, Brazil.

Diffuse cutaneous leishmaniasis is a disease normally associated with *Leishmania (L.) amazonensis* and anergy to parasite antigens. We have systematically observed that whole *L. (L.) amazonensis* antigens (LaAg) as used in Leishvacin™, a vaccine currently under clinical trials in Brazil, strongly inhibits specific and mitogenic murine T cell responses. Here we show *in vitro* that TGF-β plays a key role in the anergic mechanism of LaAg since addition of anti-TGF-β to lymph node cells from 7 day-infected BALB/c mice restores their capacity to respond to the antigen with increased proliferation. *In vivo*, we found that i.m. immunization of BALB/c, but not C57Bl/6, with 2 doses of LaAg (25mg/dose) with a 7-day interval increases the production of TGF-β and IL-10 while decreasing TNF-α. The vaccination in BALB/c but not in C57Bl/6 induced an increase in the numbers of $CD4^+$ apoptotic cells in the lymph nodes. Moreover, when animals were infected with $2x10^6$ fluorescent *L. (L.) amazonensis*-GFP seven days after the second vaccine dose, BALB/c but not C57Bl/6 mice developed increased lesion growth and significantly higher parasite loads, as compared with non-vaccinated controls. Contrary to LaAg, vaccination with *L. (V.) braziliensis* Ag was protective. Administration of anti-TGF-β (100mg/dose) during LaAg vaccination promoted a milder infection in BALB/c mice and reduced the parasite loads to levels similar to non-vaccinated controls. Interestingly, the pattern of IFN-γ production was not altered by anti-TGF-b treatment, despite the increased IL-12, TNF-α and NO production. These results, although carried out in rodents, warns that such vaccine may produce a disease-aggravating effect in more susceptible individuals by an apoptotic mechanism possibly mediated by TGF-β.

Supported by: FAPERJ, CNPq, CAPES

IM15 - IL-4 IS NOT A SUSCEPTIBILITY FACTOR IN MICE INFECTED WITH *LEISHMANIA (L.) AMAZONENSIS* BY THE DORSAL SKIN ROUTE

Felizardo, T.C.[1], Nunes, A.R.[1], Abrahamsohn, I.A.[1] and Lima, G.M.C.A.[1]

[1] Instituto de Ciências Biomédicas, USP, São Paulo, Brasil.

We report on the influence of IL-4 on the susceptibility and lesion caused by *L. amazonensis* infection in mice infected in the dorsal skin in comparison to footpad infection. Although most experimental studies in cutaneous leishmaniasis used the paw sole as infection site, mammals or man are usually infected in other areas where the skin is much thinner and not under constant friction. Ten million *L. amazonensis* (Josefa strain) stationary phase promastigotes were injected in the footpad or in the dorsum of susceptible BALB/c or resistant C57BL/6 mice and in their respective IL-4-deficient (IL-4 KO) strains. The kinetics of infection, parasite load, cytokine and serum antibody levels were verified. The parasite counts in the draining lymph nodes of the dorsal injection site were similar in IL-4 KO and in wild-type BALB/c mice. Interestingly, no lesion was observed in the dorsum of C57BL/6 IL-4 KO mice, in spite of detectable parasites in the draining lymph nodes. Production of IL-12p40 and IFN-γ by draining lymph nodes from C57BL/6 IL-4 KO mice inoculated in the footpad was elevated. However, when the inoculation site was the dorsal skin, no significant differences were noted in cytokine production levels by lymph node cells. The serum levels of *Leishmania*-specific IgG2a and total IgE were high in the IL-4 KO and in wild-type BALB/c mice, respectively. In conclusion, in our experiments, IL-4 was neither sufficient nor necessary for susceptibility to *L. (L.) amazonensis*, but the route of infection is an important determinant factor that influences the outcome of infection.

Supported by: FAPESP

IM16 - CELLULAR IMMUNE RESPONSE TO *LEISHMANIA (V.) BRAZILIENSIS* AND SUPERANTIGENS IN HUMAN AMERICAN TEGUMENTARY LEISHMANIASIS

[3]Cylleno, J.L.; [1,3]Oliveira, M.P.; Este, [1]M.G.M; [2]Mattos, M.; [2]Oliveira-Neto, M.P.; [4]Afonso, L.C.C.;[1]Pirmez, C.

1.Departamento de Bioquímica e Biologia Molecular, Instituto Oswaldo Cruz / FIOCRUZ; 2. Instituto de Pesquisa Evandro Chagas /FIOCRUZ; 3. Escola de Medicina, Universidade Estácio de Sá; 4. Laboratório de Imunoparasitologia, ICEB, Universidade Federal de Ouro Preto

Cellular mediated immune response is essential for the control of human American tegumentary leishmaniasis (ATL). The inflammatory infiltrate is composed of a high number of memory and activated cells, but only a very small percentage is *Leishmania*-specific reactive T cells. Clinical observations have shown that the development of secondary infection by *Staphylococcus aureus* in cutaneous and mucosal lesions is common. Since staphylococcal toxins can induce a massive human T cell stimulation and cytokine production, the aim of this study was to analyze the "in vitro" T cell immune response induced by *Leishmania* and staphylococcal toxins in human ATL. A total of 49 patients with active cutaneous (LCL) or mucosal (ML) disease were studied. Lymphocyte proliferative response (LPR) assays were performed using peripheral blood mononuclear cell cultures stimulated with *L. (V.) braziliensis* total antigens (Lb Ag), mitogen (concanavalin-A) and superantigens derived from *S. aureus* (enterotoxin A/SEA and enterotoxins B/SEB). Supernatants obtained from T cell cultures were tested for IFN-γ and TNF-α production using ELISA assays, and the results were expressed as pg/ml. The LPR was positive for both leishmanial and bacterial stimulus (median SI: Lb Ag=4,0; SEA=1,6 and SEB=5,8). The levels of IFN-γ were elevated when compared to non-stimulated

XXX ANNUAL MEETING ON BASIC RESEARCH IN CHAGAS DISEASE - XIX MEETING OF THE BRAZILIAN SOCIETY OF PROTOZOOLOGY - HOTEL GLÓRIA, CAXAMBU, MG, BRASIL - 10-12 NOVEMBER 2003. **Rev. Inst. Med. trop. S. Paulo, 45**(Suppl. 13), November, 2003.

cultures, particularly in PBMC stimulated with Lb Ag and SEB (median: Lb Ag=1855,2; SEA=449,8 and SEB=3710). TNF-α production was detected only in cultures stimulated with SEB (median=43,4). Superantigens are able to induce T cell proliferation and cytokine release. Therefore, the development of bacterial co-infection during active disease could lead to the recruitment of non-*Leishmania* specific cells and the increase of type 1 cytokines in the inflammatory infiltrate, suggesting a role for these bacterial superantigens in the immunopathogenesis of human tegumentary leishmaniasis.

Supported by: CNPq & PAPES-Fiocruz

IM17 - RT-PCR DETECTION OF CXCL12 CHEMOKINE IN SPLEEN DURING EXPERIMENTAL *PLASMODIUM CHABAUDI* AJ STRAIN INFECTION IN C57BL/6J MICE.

Garnica MR and Andrade Jr HF.

Lab. Protozoologia – Instituto de Medicina Tropical de São Paulo – FMUSP. Av.Dr.E.C.Aguiar, 470 – 05403-000. São Paulo SP Brazil e-mail: margoth@usp.br

The mechanisms of parasitemia clearance in malaria, one of the major parasitic disease in the world, are not well understood, but in the intact host is dependent of the spleen, the site for killing of the intraerythrocytic parasite. During malaria infection, there are a clear increase in spleen volume and cellularity. Recently, we studied the CXCL12 chemokine production in spleen during rodent malaria, both by *P.berghei* and *P.chabaudi* CR strain infected mice, showing that this chemokine presented a specific pattern of synthesis, that induces homing and activation of immune cells, especially those produced by the spleen organizer reticular cell. We studied the production of chemokine, by mRNA detection by RT-PCR, The supplementation of this chemokine in lethal model results in an attempt to the control of parasitemia. *P.berghei* malaria is frequently related to immunopathological disease, as cerebral malaria and exacerbated TNFa production, but *P.chabaudi* AJ malaria, equally lethal, presented less immune response and death due anemia. We decided to study the production of SDF-1a and related chemokines, looking for new aspects of those chemokines in the spleen function in malaria.

We infected groups of three animals that were sacrificed on a CO_2 chamber, with careful dissection of spleen, with 1/3 of the spleen immediately placed in three volumes of Trizol ™ and conserved at –70ºC until mRNA extraction. These procedures were repeated at the 4th, 6th, 7th, 8th and 11th days after infection. Parasitemia was determined daily by tail blood smears. The extracted RNA was submitted to RT-PCR with oligo-dT for cDNA synthesis at the first round, with specific primers in the second round PCR. We detect the specific product of each chemokine in TBE-PAGE and silver staining, with semi quantitative comparison with mRNA β-actin bands using Imaje J. SDF-1a mRNA is present only before the 4th day after infection, when the parasitemia is detected in the blood, but disappeared in the 11th when the infection results in death of almost all mice. Interestingly, the constitutive SDF-1b mRNA was undetected during the evolution of the experimental malaria. These data suggests that the production of SDF-1b is greatly affected during this malaria, aside to the fact that there are no organized production of SDF-1a in this lethal malaria infection by *P.chabaudi* AJ. Those data also suggests that the isoforms of those chemokines could be involved in dichotomic effects in the malaria, both related to immune or hematological response to the disease.

Supported by: FAPESP (00/10863-6) and LIMHCFMUSP-49.

IM18 - TUMOR NECROSIS FACTOR RECEPTOR II(TNFR II) EXON 6 POLYMORPHISM IN AMERICAN TEGUMENTAR LEISHMANIASIS

Silva, F.M.D[1]., Oliveira-Neto, M.P[2]., Mattos, M[2]., Maretti-Mira, A.C[1]., Santos, A.R[3]., Pirmez, C[1].

[1]Department of Biochemistry and Molecular Biology, [2]Evandro Chagas Hospital, [3]Department of Hanseniase, Fundação Oswaldo Cruz, Rio de Janeiro, Brazil.

Background: The pathogenesis of infection has been associated with a pro and anti-inflammatory cytokine profile, which can be directly influenced by individual genetic inheritance. In this regard, it has been shown that susceptibility and resistance to intracellular pathogens is often associated with an allelic polymorphism of cytokine-coding genes and theirs receptors, resulting in a differential clinical expression among individuals presenting the same pathology.

American tegumentar leishmaniasis (ATL) is an endemic disease mainly caused by *L. (Viannia) braziliensis* in Brazil. The disease is expressed either by a self-healing localized cutaneous ulcer (LCL) or a destructive inflammation of the oro-nasal mucosae (ML). In both forms there is a mixture of Th1 and Th2 cytokines. However, Th1 cytokines predominate in LCL forms, in contrast to the MCL form, where the expression of IL-4 is four-fold higher than in LCL. In addition, it has been previously demonstrated the association between TNF promoter polymorphisms and ML.

Aims: The aim of the present work was to evaluate the possible association of a single nucleotide polimorphism at the nucleotide 196 within the exon 6 of the gene coding for TNF-α receptor and the severity of ATL.

Patients and Methods: Patients were selected within a particular clinical presentation (30 with LCL and 30 with ML). This group was subdivided according to response to therapy: half presented a good response to Glucantime and in 15, achievement of cure demanded either an additional course of treatment or patients presented reactivation of the lesion within a period of 6 months after the first course of Glucantime. Controls consisted of 28 samples from blood donors, and 32 individuals with no present or past history of cutaneous or mucosal lesion, but with a positive Montenegro's skin test. Genomic DNA was extracted from biopsy of the lesions or peripheral blood, after clearance by the Fiocruz Ethical Committee.

Samples were genotyped by PCR-RFLP using TNF receptor II specific primers. The amplified products were then digested using *Nla*III restriction enzyme.

Results: The allele frequency analysis suggests an association between the presence of the mutant 196G allele and patients presenting a bad therapeutic response. No polymorphism association was observed when LCL was compared to ML patients or controls. These results suggest that this particular polymorphism could have a role in the pathogenesis of the disease, either by hampering the resolution process or by exacerbating the inflammatory reaction. Complementary research with a larger number of samples and their functional analysis of the immune response are necessary to confirm this hypothesis.

Supported by CNPq and PAPES/Fiocruz

IM19 - FUNCTIONAL AND MORPHOLOGICAL EVALUATION OF PERITONEAL MAST CELLS DURING EXPERIMENTAL ACUTE INFECTION WITH *TRYPANOSOMA CRUZI* IN RATS

Azevedo AS[1, 2,] Melo RCN[2], Oliveira MP[1], Ferraz WP[2], Barreto EO[3], Parreira GG[4], Pirmez C[1]

[1]Laboratory of Immunopathology, Department of Biochemistry and Molecular Biology, IOC-FIOCRUZ-RJ, Brazil; [2]Laboratory of Cellular Biology, Department of Biology, Federal University of Juiz de Fora, Juiz de Fora, MG, Brazil;

XXX ANNUAL MEETING ON BASIC RESEARCH IN CHAGAS DISEASE - XIX MEETING OF THE BRAZILIAN SOCIETY OF PROTOZOOLOGY - HOTEL GLÓRIA, CAXAMBU, MG, BRASIL - 10-12 NOVEMBER 2003. **Rev. Inst. Med. trop. S. Paulo, 45**(Suppl. 13), November, 2003.

[3]Laboratory of Inflammation, Department of Physiology and Pharmacodynamics, IOC-FIOCRUZ-RJ, Brazil; [4]Department of Morphology, Federal University of Minas Gerais, Belo Horizonte, Brazil.

Mast cells are multifunctional cells capable of secreting a wide variety of mediators. Following activation, these cells express mediators such as histamine, serotonine, leukotrienes and prostanoids, as well as proteases and many cytokines and chemokines, all essential to the genesis of an inflammatory response. During *Trypanosoma cruzi* infection, it has been suggested that mast cells could contribute to the control of the parasite by recognizing and killing IgG-opsonized trypomastigotes and through secretion of mediators. Increased numbers of mast cells have been demonstrated in chagasic patients with chronic disease and in experimental models. In the present work, the involvement of mast cells was studied *in vivo* and *in vitro* during the acute experimental infection with *Trypanosoma cruzi* in rats. Female Holtzman rats infected with Y strain of *T. cruzi* (300.000 trypomastigotes, i.p.) were sacrificed in different time points of infection (24h, 48h, 12 and 20 days) for quantification of mast cell numbers and histamine levels in the peritoneum and histopathological analysis of the mesenterium. In parallel, mast cells were co-cultured with bloodstream trypomastigotes and the released and intracellular histamine levels were quantified. Compared to non-infected controls, the acute *T. cruzi* infection did not induce an increase of mast cells neither in peritoneum nor in mesenterium. However, degranulated mast cells, a high number of milk spots and a diffuse mononuclear inflammatory process were observed in the mesenterium, particularly at day 12 of infection, corresponding to the peak of the parasitemia. The *in vivo* intracellular histamine levels measured in the peritoneum was not different from the controls, but when mast cells were co-cultured with trypomastigotes, the intracellular histamine decreased after 30 minutes. Our data suggest that experimental infection with *T. cruzi* induce mast cell degranulation during the acute phase in rats and may participate in pathogenesis mechanisms during acute Chagas disease.

Supported by IOC/FIOCRUZ/RJ and CNPq.

IM20 - CELLULAR IMMUNE RESPONSE IN SHEEP AND CATTLE EXPERIMENTALLY INFECTED WITH *TOXOPLASMA GONDII*

Meireles, L. R.[1], Hiramoto, R. M.[1], Terentowicz, H. C. K.[1], Birgel Jr., E.[2], Galisteo Jr., A. J.[1], Andrade Jr., H. F[1].

1- ICB & IMTSP/USP, 2 - Veterinary Medicine School, USP. Contacts: E-mail lrmeirel@usp.br

Toxoplasma gondii is a protozoan parasite that can infect all warm-blooded animals. Sheep and cattle show different susceptibilities to *T. gondii* infection. Primary infection in pregnant sheep can result in abortion or the birth of weak lambs but they are then protected against further challenge by the development of an effective immunity. Cattle on the other hand, can be readily infected, but abortion or perinatal mortality has not been recorded. The evidence suggests that cattle develop a more effective immune response to *T. gondii* infection than sheep. Seronegative calves and lambs were maintained at the Veterinary Hospital/ USP in an environment with minimal risk of *Toxoplasma* infection. Two animals of each species were inoculated subcutaneously (s.c.) with 5 x 10^2 ME 49 *T. gondii* cysts, two other immunized (s.c.) with 10^7 irradiated tachyzoites (3 monthly doses), while one remained as control. Animals that received irradiated tachyzoites were challenged with 5 x 10^2 ME 49 cysts at 150th day. Serology was performed biweekly and cellular immunity evaluated in Ficoll-Hypaque purified lymphocytes, by PHA or antigen proliferation assays, by ^{3}H thymidine uptake. Infected animals, either cattle or lamb, presented a clear elevation in specific antibody serum levels, with usual antigen maturation pattern. Immunized animals, from both species, presented lower antibody titers, but with similar maturation profile. In proliferation assays, there is a clear antigen induced lymphocyte proliferation after the infection, in both species, despite a lower PHA response during infection, that remain for at least 6 months. After immunization, the lymphocyte response was lower than those driven by infection, but was also clearly seen after 6 months and challenge. PCR studies in organs of infected and immunized and challenged animals are in course. Those data show that the infection of ruminants with *T. gondii* resulted in intense immune activation, both humoral and cellular, a fact that could be also induced at lesser extent by immunization with irradiated tachyzoites.

This work was supported by LIMHCFMUSP and CAPES.

IM21 - EVALUATION OF LYMPHOPROLIFERATIVE RESPONSES IN HUMAN VISCERAL LEISHMANIASIS INDUCED BY A RECOMBINANT ANTIGEN FROM *LEISHMANIA (L.) CHAGASI* AMASTIGOTES.

Pinheiro, P.H.C., Dias, S.S., Katz, S., Eulálio, K.D. & Barbiéri, C.L.

Disciplina de Parasitologia, Escola Paulista de Medicina – Universidade Federal de São Paulo, São Paulo, S.P., SP, Brasil barbiericl@ecb.epm.br

A straight correlation between the IFN-γ production and resistance to visceralizing *Leishmania* species has been demonstrated in murine model. In humans IFN-γ production and T cell proliferation have been implicated in the control of *L. (L.) chagasi* infection, whereas IL-10 production has been correlated with pathology in *L. (L.) donovani* infections (Carvalho et al., 1992, J. Infect. Dis. 165:535-540; Ghalib et al., 1993, J. Clin. Invest. 92:324-329). In our laboratory an antigen of apparent molecular mass of 30 kDa (p30) was identified in *Leishmania (L.) chagasi* amastigotes and showed to induce lymphoproliferative responses mediated by $CD4^+$ Th1 and a partial protection against challenge with *L. (L.) chagasi* in BALB/c mice (Pinto et al., 2000, Int. J. Parasitol. 30:599-607). The present work evaluates lymphoproliferative responses induced by a recombinant form of *L. (L.) chagasi* p30 in people living in Teresina, the capital of the state of Piauí, Brasil, where a significant number of visceral leishmaniasis (VL) cases has been reported. The recombinant antigen (R30) was used in cultures of lymphocytes purified from human peripheral blood by Ficoll-Hypaque density gradient centrifugation. The recombinant antigen was produced by expression of the gene *Ldccys1* obtained by PCR amplification using genomic DNA from *L. (L.) chagasi* amastigotes and primers corresponding to the ORF of *L. (L.) chagasi Ldccys1* gene previously described (Omara-Opyene and Gedamu, 1997, Mol. Biochem. Parasitol. 90:247-267). A fragment of 1.3 kb was obtained and cloning of this fragment in pHis vector resulted in a recombinant protein of 47 kDa (R30).

The proliferation assays were carried out in lymphocyte cultures of fourteen patients hospitalized at the Infectious Disease Hospital, Teresina, which had a diagnosis of VL confirmed by identification of amastigotes in bone marrow tissue and enzyme-linked immunosorbent assay (ELISA) to detect antibodies to *L. (L.) chagasi*. All patients also presented VL clinical symptoms: fever, splenomegaly, anaemia, cough and wasting. Four patients treated and cured, as well as two uninfected controls were also included in the present study.

Peripheral lymphocytes from thirteen symptomatic patients were stimulated by R30 presenting stimulation indexes (SI) ranging from 4.0 to 16.0. All cured individuals presented SI to R30 ranging from 1.6 to 13.0, whereas normal subjects did not respond to stimulation by the antigen. The evaluation of lymphoproliferative responses to R30 is currently extended to a higher number of patients, and analysis of limphokine profile in the supernatants of lymphocyte cultures from these patients in the presence of R30 is also in progress. Our preliminary results indicate that R30 is recognized and induces T cell proliferation in both symptomatic and cured subjects, opening perspectives to use this antigen in protective immune schedules against *L. (L.) chagasi*.

Supported by CAPES, FAPESP and NOVAFAPI

XXX ANNUAL MEETING ON BASIC RESEARCH IN CHAGAS DISEASE - XIX MEETING OF THE BRAZILIAN SOCIETY OF PROTOZOOLOGY - HOTEL GLÓRIA, CAXAMBU, MG, BRASIL - 10-12 NOVEMBER 2003. **Rev. Inst. Med. trop. S. Paulo, 45**(Suppl. 13), November, 2003.

IM22 - INVOLVEMENT OF NITRIC OXIDE (NO) AND TNF-A IN THE ANEMIA AND IN THE OXIDATIVE STRESS IN ERYTHROCYTES FROM MICE SUSCEPTIBLE AND RESISTANT TO *TRYPANOSOMA CRUZI* INFECTION

Aparecida Donizette Malvezi[a], Rubens Cecchini[a], Fausto de Souza[a], Carlos Eduardo Tadokoro[b], Luiz Vicente Rizzo[b], Phileno Pinge Filho[a]

[a]Departamento de Ciências Patológicas, Centro de Ciências Biológicas, Universidade estadual de Londrina, CEP:86051-970. Paraná, Brasil; [b]Departamento de Imunologia, Instituto de Ciências Biomédicas IV,Universidade de São Paulo.

Trypanosoma cruzi (Y strain)-infected susceptible mice (Swiss and C57BL/6 $iNOS^{-/-}$) and resistant mice (C57BL/6) showed difference in parasitemia levels and survival rates. *T. cruzi* infection is associated with anemia and leukopenia only for C57BL/6 mice. The data obtained indicate that lethality in acute *T. cruzi* infection not necessarily is associated with low number of blood cells. Treatment of both strain with aminoguanidine (AG, 50mg/Kg) and the use of $iNOS^{-/-}$ mice, revealed that the anemia in mice is not reverted in the absence of NO. In addition, we showed that *in vivo* blockade of TNF-α provoked an dramatic increase in the percentage of reticulocytes in C57BL/6 mice infected, but didn't modify the measured values for hemoglobin content (Hb), hematocrit (HMT), and erythrocyte count (RBC) of those animals. Suggesting that the TNF-α produced during *T. cruzi* infection inhibits the erythropoiesis. The evaluation of the oxidative stress after induction by *t*-butyl hydroperoxide (*t*-BHT) revealed that the oxyghemoglobin oxidation was found to be higher in Swiss mice infected with *T. cruzi*. Treatment with AG protected completely against OxyHb oxidation. In the mice C57BL/6 strain, a strong iNOS responsive, the OxyHb oxidation rate increased from 21.6 to 31.9. Treatment with AG was able to reduce to 24.4. The rate of OxyHb oxidation in the mice C57BL/6 $iNOS^{-/-}$ increased from 28.0 to 37.4. Anti-TNF-α treatment did not affect the OxyHb oxidation in mice C57BL/6 infected. The oxygen uptake in Swiss mice erythrocyte was significantly increased after 14 days of infection ($p<0,05$) and reduced below the control levels for AG treatment ($p<0,001$). The C57BL/6 strain showed a significant decrease in oxygen uptake after 14 days of infection. However the AG treatment induced an additional significant decrease in this parameter ($p<0,01$). $iNOS^{-/-}$ mice showed a very significant reduction in the oxygen uptake ($p< 0,01$). Treatment with anti-TNF-α did not revel any difference related to 14 days infected C57BL/6 mice ($p>0,05$). In the mice Swiss, there were not any variations in $T^{ind}$for all groups studied ($p>0,05$). However, for the C57BL/6 strain a significant reduction ($p<0,05$) was observed after 14 days of infection with complete recuperation of the control levels after treatment with AG ($p<0,01$). Also, a significant decrease in T^{ind} was seen for the C57BL/6 $iNOS^{-/-}$ after 14 days of infection ($p< 0,001$) in comparison with normal $iNOS^{-/-}$). The treatment with anti-TNF-α also protected against the reduction of antioxidant capacity of erythrocyte on day 14 pos infection ($p<0,001$).

IM23 - PROFILE OF PLASMATIC CYTOKINES AND AUTOANTIBODIES IN THE MALARIA ASSOCIATED ANEMIA IN INDIVIDUALS FROM THE BRAZILIAN AMAZON

Fernandes, A.A.M.*; Ventura, A.M.R.S.**; Carvalho, L.J.M.*; Zanini, G.M.*; Banic, D.M.*; Ferreira-da-Cruz, M.F.*; Souza, J.M.**& Daniel-Ribeiro, C.T.*

*Laboratory of Malaria Research, Department of Immunology, Instituto Oswaldo Cruz/Fiocruz, Rio de Janeiro; **Evandro Chagas Institute, Funasa, Belém - Brazil. e-mail: *fernandes@ioc.fiocruz.br*

In Brazil, the malaria associated anemia is recorded in all age groups including adults, differently from the observed in hyperendemic areas where malarial anemia is more frequently observed in children below two years old. The mechanisms of malarial anemia induction are poorly understood, but the participation of cytokines and autoantibodies (AAb) has been considered. In the present work we evaluated the profile of the cytokines TNF-α, IFN-γ, IL-10, IL-12, MIF, the MCP-1 chemokine and the presence of anti-erythrocyte (aERY-AAb) and anti-phospholipids (aPHO-AAb) autoantibodies in the anemia associated to *P. vivax* (Pv) and *P. falciparum* (Pf) infections. Blood samples from 117 (77 Pv, 38 Pf and 2 Pv/Pf) patients with acute malaria assisted at the Instituto Evandro Chagas/IEC/Belém and at the Hospital Municipal de Paragominas, Pará State, as well as a control group (38 individuals with no history of malaria) were assayed for hematological and biochemical parameters. The plasma concentrations of cytokines, chemokine and the presence of autoantibodies were measured by ELISA. Twenty patients with acute malaria were anemic (13 Pv and 7 Pf), being 16 with mild (Hb 10 to 12g/dl), three with moderate (Hb 7 to < 10g/dl) and one with severe (Hb < 7g/dl) anemia. Surprisingly, the seven anemic patients infected by Pf presented mild anemia, whereas those presenting moderate to severe anemia were infected with Pv. A significant increase in the levels of TNF-α, IFN-γ, IL-10, IL-12 and MCP-1 was observed in patients with malaria as compared to the control group, whereas the levels of MIF did not change. There was a positive correlation between the levels of TNF-α, IL-10 and MCP-1 with the parasitemia degree but not with the presence of anemia. The plasmatic levels of IL-12 were significantly higher among patients with different degrees of anemia than in those with malaria without anemia. The presence of aERYAAb and aPHOAAb were significantly more frequent in patients with malaria than in the control group, but there were no correlations between the presence of these AAb and the parasitemia degree or the presence or intensity of the anemia. The analysis of new samples recently collected from patients with malaria at Paragominas is being performed to complete this study.

Supported by CAPES, Fiocruz and CNPq

IM24 - CENTRAL NERVOUS SYSTEM INVOLVEMENT IN EXPERIMENTAL *LEISHMANIA (L.) AMAZONENSIS* INFECTION

Abreu-Silva, A. L.[1/2]; Calabrese, K.S.[2];Tedesco, R.C.[3]; Mortara, R.A.[4], and Gonçalves da Costa, S.C.[2] Depto de Patologia, Universidade Estadual do Maranhão, Maranhão, Brasil.

1. Depto de Protozoologia, Lab. de Imunomodulação, IOC/FIOCRUZ; 2. Departamento de Morfologia, Universidade Federal de São Paulo, Escola Paulista de Medicina, São Paulo; 3. Departamento de Microbiologia, Imunologia e Parasitologia, Universidade Federal de São Paulo, Escola Paulista de Medicina, São Paulo, Brasil.

The main goal of this paper is to describe pathological alterations of the central nervous system observed in experimental tegumentar leishmaniasis. BALB/c and Swiss mice were subcutaneously infected with 10^4 *L. (L.) amazonensis* amastigotes. Animals were sacrificed and the whole brain was removed for histological and immunocytochemical studies. Histological examination revealed that 66,6% of infected mice presented a discrete hyperemia and inflammatory infiltrate in the meninges, composed by mononuclear cells and neutrophils with no detectable parasites. However, parasitized macrophages were detected in the cerebral parenchyma, as well as mast cells, lymphocytes and polymorphonuclear cells. Necrosis in cerebral parenchyma was also observed. Confocal fluorescence microscopy showed that $CD8^+$ T lymphocytes are the major component of the inflammatory infiltrate in CNS. Besides these cells, $CD4^+$, CD11b and dendritic cells are present, in small numbers, in the inflammatory processes of the CNS. In conclusion, *L. amazonensis* is able to cross blood-brain barrier and cause significant pathological changes in the CNS.

XXX ANNUAL MEETING ON BASIC RESEARCH IN CHAGAS DISEASE - XIX MEETING OF THE BRAZILIAN SOCIETY OF PROTOZOOLOGY - HOTEL GLÓRIA, CAXAMBU, MG, BRASIL - 10-12 NOVEMBER 2003. **Rev. Inst. Med. trop. S. Paulo, 45**(Suppl. 13), November, 2003.

IM25 - MIP 1A AND RANTES IN HUMAN LOCALIZED CUTANEOUS LEISHMANIASIS

[1]Corrêa Coelho PA; [2]Oliveira MP; [2]Pirmez C; [1]Nunes Jr E, [1]Da-Cruz AM.

1. Laboratório de Imunidade Celular e Humoral, Instituto Oswaldo Cruz/Fiocruz; 2. Departamento de Bioquímica e Biologia Molecular/Instituto Oswaldo Cruz/ Fiocruz. e-mail: alda@ioc.fiocruz.br

Background: Chemokines are cytokines with chemotatic activity with important role in the selective recruitment of leucocytes into inflammatory areas. Also, these mediators are responsible for the activation of various cells in inflammatory sites. MIP 1a (macrophage inflammatory protein 1a) and RANTES (regulated upon activation normal T cell expressed and secreted), both classified as C-C chemokines, are attractants for lymphocytes, monocytes and eosinophils. These chemokines are produced not only by non-inflammatory resident cells, such as epithelial cells, fibroblasts, keratinocytes and endothelial cells, but also by monocytes and lymphocytes. Previous studies demonstrated that the predominance of Th1 cytokines over Th2 cytokines is correlated to the resistance against *Leishmania* infection. Although, previous works reported the association of RANTES and MIP1a with Th1 response, the role of chemokines in human leishmaniasis still remains to be elucidated.

Objectives: To determine the production of MIP and RANTES in mononuclear cells cultures and their expression "in situ".

Material and Methods: Twenty-three patients with active localized cutaneous leishmaniasis (LCL), all of them living in endemic areas of Rio de Janeiro, were studied. Cultures of mononuclear cells obtained from blood (PBMC) and biopsies (LMC) were stimulated with *Leishmania (V.) braziliensis* promastigote antigens (Ag-Lb). The supernatants were tested for MIP1a (n=16 cases) and RANTES (n=5 cases) using ELISA assays. Immunohistochemistry assays were performed using monoclonal antibodies on frozen sections (n=6 cases).

Results: We observed a significant decrease of MIP1a production in Ag-Lg stimulated LMC cultures (19,8 ± 22,7 ng/ml), when compared to the controls (52,3 ± 40,2 ng/ml). In PBMC, similar levels of MIP1a were observed in both Lb-Ag stimulated (43 ± 43,16 ng/ml) and control cultures (42,6 ± 32,96 ng/ml). RANTES production was detected in PBMC (1665 ± 134 g/ml) and LMC cultures (1028 ± 257 pg/ml). The expression of MIP1a was detected only in three cases and positive focus were mainly localized in the upper dermis. RANTES staining was present in all LCL samples examined. In this case, the majority of chemokine producing cells were basal epidermis keratinocytes, but we also observed few scattered positive cells in the inflammatory infiltrate.

Conclusion: LCL lesions have been considered as a self-healing condition. Our findings indicate that *Leishmania* infection could be correlated with the inhibition of MIP1a production. Futhermore, the synthesis of RANTES, but not MIP1a, might play a role in immmunoregulatory mechanisms associated with the outcome of disease.

Supported by PAPES/Fiocruz.

IM26 - IMPORTANCE OF FAS/FAS-L ENGAGEMENT IN THE CARDIAC INFLAMMATORY INFILTRATION INDUCED BY *TRYPANOSOMA CRUZI* INFECTION

Oliveira, G.M.; Meuser-Batista, M.; Araújo-Jorge, T. C.; Henriques-Pons, A..

Departamento de Ultra Estrutura e Biologia Celular; Laboratório de Biologia Celular; Instituto Oswaldo Cruz. Av. Brasil 4365 Manguinhos, 21045-900, Rio de Janeiro / RJ, Brazil.

Trypanosoma cruzi is the etiologic agent of Chagas' disease and affects 16-18 million people in Latin America. The infection is characterized by an acute phase with trypomastigote forms circulating in the blood and also intracellular proliferating amastigote forms in different tissues. It is also observed considerable inflammatory infiltration throughout the cardiac tissue leading to severe cardiac alterations and congestive heart failure. This is possibly the result of multiple aggressive mechanisms to the tissue, determined by different cytotoxic molecules, cellular populations and physiological alterations of the organ. One of the most important cytotoxic pathways employed to induce cellular death is based in the engagement of Fas/Fas-L molecules and the goal of this project is to evaluate whether the Fas-based cytotoxic pathway plays a role in the development of *T. cruzi*-induced myocarditis. Our results indicate that the lack of Fas-L (glg/gld mice) disfavor the cellular inflammatory infiltration, in accordance with previous results using Coxsackievirus (1), and the advance of cardiac fibrosis, although the mortality rate is comparable to Balb/c infected mice. The histopathological analysis of cardiac samples taken from gld/gld mice infected with *T. cruzi* Y strain revealed numerous parasite nests distributed throughout the tissue but scarcely associated to inflammatory infiltration. In contrast to Balb/c infected mice, which showed fewer parasites but intense inflammatory infiltration. Enzimatic evaluation of cardiomyocytes destruction based in creatin kinase (CK) activity showed higher cellular death in Balb/c mice. Besides, we found no marked regenerative anemia, but AST and ALT dosage revealed discrete hepatic injury in both mice but with normal levels of urea in the acute phase. Flow cytometry analysis of cardiac inflammatory cells harvested from *in vivo* infected mice showed predominance of $CD8^+$ T cells in Balb/c and gld/gld mice on the 15^{th} day post infection and remarkably more than 90% of the cells were $D32^+$ (FcgRII) T cells. The labeling of intracellular citokines revealed a Th1 pattern, with high levels of IL-2 and IFN-γ in both mice, but also IL-10 in gld/gld mice. We found equivalent levels of perforin, bcl-2 and iNOS in both mice. In this work we also analyzed cardiomyocytes from adult infected mice by flow cytometer and observed the production of IFN-γ and TNF-α but also high levels of iNOS and bcl-2 after infection. These results indicate the importance of Fas/Fas-L pathway in the cardiac inflammatory infiltration triggered by *T. cruzi* infection and demonstrate the adaptation of the cardiac tissue facing the infection.

Refernce

(1) Seko Y., Kayagaki N., Seino K-I., Yagita H., Okumura K., Nagai R. Role of Fas/Fas-L pathway in the activation of infiltrating cells in murine acute myocarditis caused by coxsakievirus B3. J. Am. Coll. Cambridge 2002; 39:1399-1403.

Supported by: CNPq, FAPERJ e CAPES.

IM27 - COURSE OF INFECTION AND IMMUNE RESPONSE OF C57BL/6 MICE CHALLENGED WITH STATIONARY PHASE OR METACYCLIC *LEISHMANIA* PROMASTIGOTES

Maioli, T.U.[1], Castro, J.M.A.[1], Carneiro, C.M.[2], Teixeira, M.M.[3] and Afonso, L.C.C.[1]

[1]Laboratório de Imunoparasitologia – DECBI / NUPEB, [2]Depto. Análises Clínicas, Escola de Farmácia, Universidade Federal de Ouro Preto, [3]Depto. Bioquímica e Imunologia, ICB, Universidade Federal de Minas Gerais

Natural infection by *Leishmania* parasites is initiated by the inoculation of a few infectious metacyclic parasites into the host skin. Most of the laboratory studies however use stationary phase promastigotes that are a mixture of infective and non-infective promastigotes. In this work we, initially, compared the course of infection and immune response in animals infected with either total stationary phase (total) or purified metacyclic promastigotes of *L. (L.) amazonensis* (PH8 strain). C57BL/6 mice were inoculated with 2 x 10^5 total stationary phase promastigotes or with 1 x 10^5 metacyclic promastigotes. Curiously, lesion size in animals infected with total promastigotes was smaller than in those infected with metacyclic promastigotes, even though tissue parasitism was similar between the two groups, which suggested an increased inflammatory response at the site

XXX ANNUAL MEETING ON BASIC RESEARCH IN CHAGAS DISEASE - XIX MEETING OF THE BRAZILIAN SOCIETY OF PROTOZOOLOGY - HOTEL GLÓRIA, CAXAMBU, MG, BRASIL - 10-12 NOVEMBER 2003. **Rev. Inst. Med. trop. S. Paulo, 45**(Suppl. 13), November, 2003.

of infection. This was confirmed by histological analysis of the lesions at two weeks after infection, even though no differences were noted thereafter. Measurement of IFN-γ and IL-4 production by lymph node and spleen cells did not show any difference between the two groups. In order to evaluate the inflammatory potential of these two parasite forms, thioglycollate elicited peritoneal macrophages were infected in vitro with total or metacyclic promastigotes and TNF-α production measured after 6 hours. Surprisingly, our results show that metacyclic promastigotes from *L. (L.) amazonensis* induced higher levels of TNF-α production than total stationary phase promastigotes. In summary, our results show that while the presence of non-metacyclic promastigotes in the inoculum affect the inflammatory response to the parasite *in vivo*, this observation cannot be explained by the ability of these parasites to induce TNF-α production *in vitro*.

Financial Support: CAPES-DS, UFOP

IM28 - PAFR -/- MICE PRESENTS DELAYED IFN-γ PRODUCTION AND AUGMENTED SUSCEPTIBILITY TO *LEISHMANIA (L.) AMAZONENSIS*

Helton da Costa Santiago, Maíra Farias B. Pires, Wagner Luíz Tafuri, Mauro Martins Teixeira, Leda Quercia Vieira

Departamento de Bioquímica e Imunologia e Departamento de Patologia Geral, ICB< UFMG.

Leishmania (L.) amazonensis is an intracellular parasite and the causative agent of cutaneous leishmaniasis in New World. Although some IFN-γ is produced in response to infection in the C57BL/6 mouse strain, these mice are unable to resolve infection completely. The lipid-derived mediator, PAF, has been associated with induction of NO, migration of neutrophils and killing of intracellular *L. (L.) amazonensis in vitro* and *in vivo*. To evaluate the involvement of PAF and its receptor (PAFR) in resistance to *L. (L.) amazonensis*, PAFR deficient mice (PAFR -/-) and its wild type C57BL/6 control (WT) were infected with $1x10^6$ stationary forms of *L. (L.) amazonensis* in hind footpads. The infection was followed for 11 weeks. PAFR KO mice were more susceptible to infection and developed larger and progressive lesions (6 mm at 11th week). In contrast, WT mice developed a lesion that stabilized at around 2 mm. Qualitative histopathological analysis revealed a more severe lesion in PAFR -/- with an intense and extensive necrosis. There did not appear to be a difference in the inflammatory infiltrate. Parasitism was higher in PAFR -/- (around 10 fold increase per mg of tissue) when compared to WT. Interestingly, the concentration of IFN-γ, IL-4 and TNF-α as accessed by ELISA at the lesion site at 4th and at 11th and were similar in both groups. In cultures of lymph node and spleen, the level of IFN-γ was smaller in PAFR -/- mice at 4th week of infection when compared to WT. On the other hand, at 11th week, the IFN-γ level is similar in both groups. IL-4 was not detected in lymph node or spleen cell cultures. Moreover, production of the chemokine MCP-1 was similar in both groups, whereas the concentration of RANTES was different in both time points analyzed. There was a strikingly lower concentration of RANTES in the lesion site of PAFR -/- mice. Recent data have shown the importance of immunoglobulins in the susceptibility to *L. (L.) amazonensis*. IFN-γ, RANTES and PAF can modulate the activation of B cells. In order to investigate the involvement of immunoglobulins in the present model, anti-*L. (L.) amazonensis* antibodies in serum of infected animals were accessed by ELISA and our results shown that PAFR -/- presented a higher titer of anti-*L. (L.) amazonensis* antibodies and an impressive up regulation of IgG1 secretion. Our results point to an important role of PAFR in resistance to *L. (L.) amazonensis,* promoting early activation of IFN-γ production. Moreover, RANTES may also be involved in the resistance to this parasite.

Support: CAPES

IM29 - INFLUENCE OF NORMAL MICROBIOTA ON SOME ASPECTS OF IMMUNE RESPONSE DURING EXPERIMENTAL INFECTION WITH *TRYPANOSOMA CRUZI* IN MICE

Duarte R.[1], Silva A.M.[1], Vieira L.Q.[2], Afonso, L.C.C.[3] and Nicoli J.R.[1]

[1]Departamento de Microbiologia and [2]Departamento de Bioquímica-Imunologia, ICB – Universidade Federal de Minas Gerais, Belo Horizonte; [3]Departamento de Ciências Biológicas – ICEB/NUPEB, Universidade Federal de Ouro Preto, Ouro Preto, MG, Brazil.

To study the influence of normal associated microbiota on systemic immunological responses during experimental Chagas' disease, germ-free and conventional NIH Swiss mice were infected with Y strain of *Trypanosoma cruzi*. Conventional mice showed a slightly higher survival than the germ-free ones as well as, seven days after the infection, a tendency to a lower parasitemia. Additionally, higher IFN-γ, TNF-α and NO productions ($P < 0.05$) by spleen cell cultures and higher blood levels of specific immunoglobulins of IgG2a isotype ($P < 0.05$) were observed in conventional animals when compared to their germ-free counterparts. On the other hand, germ-free mice showed higher production of IL-10 by spleen cell cultures ($P < 0.05$). In conclusion, the presence of the normal microbiota induces a more efficient Th1 immune response during an experimental infection with *T. cruzi* in mice.

Financial Support: CNPq and FAPEMIG

IM30 - IMPLICATION OF TRANSFORMING GROWTH FACTOR B IN CHAGAS' DISEASE MYOCARDIOPATHY

Mariana C. Waghabi[1], Alejandro M. Hasslocher-Moreno[2], Sérgio S. Xavier[2], Maria de Lourdes Higuchi[3], Michelle Keramidas[4], Sabine Bailly[4], Jean-Jacques Feige[4] and Tania C. Araújo-Jorge[1]

[1]Laboratory of Cell Biology, Department of Ultra structure and Cell Biology, Institute Oswaldo Cruz, [2]Department of Clinical Research, Hospital Evandro Chagas, Fundação Oswaldo Cruz, Rio de Janeiro, Brazil; [3]Service of Pathology, Heart Institute, University of São Paulo, São Paulo, Brazil and [4]INSERM EMI 0105, Department of Molecular and Structural Biology, Commissariat à l'Energie Atomique, Grenoble.

Cardiac dysfunction with progressive fibrosis is a prominent feature of chronic Chagas disease. Transforming growth factor beta (TGF beta) is a strong inductor of extracellular matrix synthesis and accumulation. To test if TGF beta could play a role in the pathogenesis of chagasic myocardiopathy, we measured TGF beta levels by ELISA in patients at different stages: asymptomatic indeterminate (IND), cardiac with ECG alterations but no or slight heart dysfunction (Card 1), and cardiac with ECG and echocardiographic alterations indicating moderate or severe heart dysfunction (Card 2). The three groups had significantly higher circulating levels of TGF beta than non-chagasic persons, and 27% of Card 1 patients had higher TGF beta levels than IND patients. We detected an important immune staining for fibronectin and for phosphorylated-Smad 2, a TGF beta transcription factor that reflects activation of the TGF beta signaling pathway, observed respectively in the extracellular matrix and in the nuclei of cardiomyocites from chagasic cardiac patients heart fragments. The higher levels of latent TGF beta observed in patients with chagasic myocardiopathy, together with intracellular activation of the TGF beta pathway and tissue fibrosis, suggest that TGF beta plays an important role in chagasic pathology. TGF beta may then represent a new target for both preventive and curative treatments of Chagas disease.

Grant support: Inserm-Fiocruz research program, PAPES/FIOCRUZ, IOC, CNPq, FAPERJ, CEA and INSERM.

XXX ANNUAL MEETING ON BASIC RESEARCH IN CHAGAS DISEASE - XIX MEETING OF THE BRAZILIAN SOCIETY OF PROTOZOOLOGY - HOTEL GLÓRIA, CAXAMBU, MG, BRASIL - 10-12 NOVEMBER 2003. **Rev. Inst. Med. trop. S. Paulo, 45**(Suppl. 13), November, 2003.

IM31 - DIFFERENTIAL PHENOTYPIC AND FUNCTIONAL CHARACTERISTICS OF CD28- AND CD28+ T CELLS BETWEEN CARDIAC AND INDETERMINATE CHAGASIC PATIENTS

Menezes, C.A.S.[1], Rocha, M.O.C.[2], Souza, P.E.A.[1], Chaves, A.C.L.[3], Gollob, K.J.[4], Dutra, W.O.[1]

[1]Department of Morphology, ICB, UFMG, [2]Medical School, CTR-DIP and Graduate Programme in Tropical Medicine, UFMG [3]Rene Rachou Research Center, FIOCRUZ, [4]Department of Biochemistry, ICB, UFMG.

Chronic human Chagas' disease presents as one of several different clinical manifestations ranging from an asymptomatic to a severe cardiac clinical form. The involvement of the host's immune response in the development and maintenance of the chagasic pathology has been demonstrated by several groups. We have shown that activated $CD4^+$ and $CD8^+$ T cells lacking CD28 expression are increased in the peripheral blood of chagasic patients. Since $CD28^-$ cells may not need co-stimulation to exert their effector functions, we hypothesize that $CD28^-$ cells are important in cardiomyocyte damage. Thus, our study focuses on the characterization of T cells with differential expression of CD28, to gain a better understanding of the biology of these important cell populations. We evaluated the expression of Vb-TCR regions 2, 3.1, 5, 8 and 17, as well as the expression of IFN-γ, TNF-α, IL-4 and IL-10 by $CD28^+$ and $CD28^-$ cell subpopulations from polarized indeterminate and cardiac chagasic patients using flow cytometry. The results were compared to those obtained from non-chagasic individuals. Analysis of TCR-Vb usage showed similar frequencies of all regions analyzed when we compared between $CD4^+CD28^-$ and $CD4^+CD28^+$ cells from chagasic patients and non-chagasic individuals. However, $CD8^+CD28^-$ cells from chagasic patients but not from non-chagasic individuals, displayed a reduced frequency of the analyzed Vbs when compared with the $CD8^+CD28^+$ subpopulation. This suggests that $CD8^+CD28^-$ cells from chagasic patients could display preferential expression of other Vbs due to a dominant expansion of these cells in response to an unknown disease related antigen. Evaluating the expression of key immunoregulatory cytokines by circulating mononuclear cells from chagasic patients, we detected a higher frequency of IFN-γ, TNF-α and IL-4 producing cells in the cardiac than in the indeterminate patients, possibly reflecting an inflammatory reaction in the first group. The levels of IL-10 were similar between groups. We also performed correlation analysis between the frequency of cytokines and the frequency of $CD4^+$ or $CD8^+$ T cells with differential expression of CD28. Interestingly, we observed a positive correlation between $CD4^+CD28^-$ T cells and IL-10 expression in indeterminate and cardiac patients. This result suggests that these cells may carry out an important modulatory role in the immune responses of indeterminates and cardiac patients.

Supported by PADCT/CNPq and WHO. E-mail: waldutra@mono.icb.ufmg.br and cristianemenezes@hotmail.com

IM32 - PHENOTYPIC AND FUNCTIONAL CHARACTERISTICS OF T CELLS FROM INDETERMINATE AND CARDIAC CHAGASIC PATIENTS AFTER EXPOSURE TO *T. CRUZI*-INFECTED MONOCYTES

Souza P.E.A.[1], Coelho J.S. [1], Rocha M.O.C. [2], Menezes C.A.S. [1], Rocha-Vieira E [3],· Chaves A.C.L. [4], Gollob K.J. [3], Dutra W.O. [1]

[1]Department of Morphology, ICB, UFMG, [2]Medical School, CTR-DIP and Graduate Programme in Tropical Medicine, UFMG [3]Department of Biochemistry, ICB, UFMG, [4]Rene Rachou Research Center, FIOCRUZ.

Infection with the protozoa *Trypanosoma cruzi* causes Chagas' disease, an inflammatory illness that affects approximately 18 million people in Latin America. 20-30% of the infected individuals develop specific cardiomiopathy whereas the great majority of the patients remain in the indeterminate clinical form. Several studies have demonstrated that host's immune response is critical in controlling parasitemia but also in leading to tissue pathology. Interaction between *T. cruzi* and monocytes is certainly an important event in regulating cellular reactivity in Chagas' disease since it may affect T cell response during disease. The aim of this work was to study the effects that infection or expose to parasite antigen to monocytes from indeterminate and cardiac patients would have in T-cells from the patients. We purified adherent cells from peripheral blood mononuclear cells from chagasic and non-chagasic individuals and submitted them to parasite infection or exposure to parasite antigen and added T-cells to the culture. After a period of approximately 18 hours we evaluated the expression of co-stimulatory and adhesion molecules, activation markers and immunoregulatory cytokines, using flow cytometry. Our results showed that: (1) T cells from indeterminate patients showed high levels of expression of CTLA-4, mainly in $CD8^+$ T cells, suggesting an important role for this molecule in controlling cellular responses, specially cytotoxic function; (2) cardiac patients showed a reduced expression of CD86, important ligand for CTLA-4, suggesting a decreased immunomodulatory ability through this pathway; (3) lymphocytes from cardiac but not indeterminate patients showed high IFN-γ expression, as compared to non-chagasic individuals, establishing a direct correlation between this inflammatory cytokine and the severe cardiac clinical form; (4) lymphocytes from cardiac patients showed a high expression of IL-4, possibly important for the control of inflammatory response, critical for the long-lasting nature of the disease. These data show important immunological differences between T-cells from indeterminate and cardiac patients upon contact with the parasite and/or its antigens, offering new information concerning the cellular reactivity in human Chagas' disease.

Financial support: WHO, CNPq/PADCT, PRONEX

IM33 - STIMULATION BY FOOD PROTEINS AFFECTS CYTOKINE PROFILE AND RESISTANCE TO *L. (L.) MAJOR* INFECTION IN C57BL/6 MICE.

Amaral, J.F.[1]; Menezes, J.S.[2]; Vieira, L.Q.[1] and Faria, A.M.C [1].

[1] Departamento de Bioquímica e Imunologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais and [2] Departamento de Imunologia, Universidade de São Paulo, Brazil.

The majority of contacts with foreign antigenic materials occurs in the gut mucosa, and are represented by food proteins and the autochthonous microbiota. Approximately 30 kg of food proteins reaches the human intestine per year and 130±190 g of these proteins are absorbed daily in the gut (Brandtzaeg, 1998). In addition, the number of bacteria colonizing the human large intestine can reach 10^{12} microorganisms/g of stool (Macfarlance *et al*, 1997). The presence of a large gut-associated lymphoid tissue (GALT) has been usually attributed to stimulation by bacterial antigens because this tissue is drastically reduced in germ-free animals. Local production of secretory IgA (sIgA), as well as serum levels of IgA and IgG, but not IgM, are also reduced in these animals (Bos *et al*, 1988). Recently, we described an experimental model in mice that was designed to study the immunological effects of food proteins. These mice were fed a diet where the intact dietary proteins were replaced by equivalent amounts of amino acids (Aa) from weaning up to adulthood and investigated its effects on the development of the immune system. Adult animals that were reared on this balanced protein-free diet (Aa-mice) have a poorly developed GALT resembling suckling mice. Levels of secretory IgA and circulating IgG and IgA are also reduced in Aa-mice, whereas IgM levels are normal. *In vitro* cytokine production by cells from several lymphoid organs shows a predominant Th2 profile with a high concentration of IL-10 and IL-4, and a low concentration of IFN-γ (Menezes *et al*, 2003). These parameters also resemble the immunological patterns observed

XXX ANNUAL MEETING ON BASIC RESEARCH IN CHAGAS DISEASE - XIX MEETING OF THE BRAZILIAN SOCIETY OF PROTOZOOLOGY - HOTEL GLÓRIA, CAXAMBU, MG, BRASIL - 10-12 NOVEMBER 2003. **Rev. Inst. Med. trop. S. Paulo, 45**(Suppl. 13), November, 2003.

in pre-weaned mice suggesting food protein stimulation is required for the full maturation of the immune system. In the present study, we evaluated the effects of Aa diet in C57BL/6 mice infected with *L. (L.) major*. Adult C57BL/6 mice treated with Aa diet show an increase in footpad lesions as compared to control casein-diet treated mice. After 4 weeks of infection, they resemble susceptible BALB/c mice suggesting that a poor IFN-gamma mature immune response to the parasite is triggered. Indeed, analysis of *in vitro* cytokine production in popliteal lymph node cells of Aa-mice show a reduction in IFN-gamma production and an increase in IL-4 production. Our results suggest that the impairment in immunological maturation observed in C57BL/6 Aa- mice alters their cytokine profile and their resistance to *L. (L.) major* infection.

Supported: CAPES, CNPq and FAPEMIG

IM34 - POLIMORPHONUCLEAR NEUTROPHILS AS A SOURCE OF CYTOKINES IN REGULATING OF THE IMMUNE RESPONSE IN HUMAN VISCERAL LEISHMANIASIS

Peruhype-Magalhães, V [1,2]; Martins-Filho, OA [3]; Rabello ALT[4]; Baratta-Masini, A[1,5]; Teixeira-Carvalho, A[1,5]; Carvalho, SFG [6]; Silva L de A[7]; Prata A[7;] Corrêa-Oliveira, R [1].

[1]Laboratório de Imunologia Celular e Molecular, CPqRR, Belo Horizonte, Brasil; [2]Instituto Oswaldo Cruz, FIOCRUZ, Rio de Janeiro, Brasil; [3]Laboratório de Doença de Chagas, CPqRR, Belo Horizonte, Brasil; [4]Laboratório de Pesquisas Clínicas, CPqRR, Belo Horizonte, Brasil; [5]Instituto de Ciências Biológicas, UFMG, Belo Horizonte, Brasil; [6]Unimontes, Montes Claros, Brasil; [7]Faculdade de Medicina do Triângulo Mineiro, Uberaba, Brasil

The clinical forms of visceral leishmaniasis (VL) are critically influenced by the host immune response where the cure or disease progression has been related to the predominance of a type 1 or a type 2 immune response of T cells, respectively. However, the role of neutrophils as source of cytokines during early infection and on the modulation of the adaptative immune response, has not yet been reported. In this context, we have evaluated, at a single cell level, after a short-term *in vitro* stimulation, the cytokine patterns of neutrophils from adults and children with active LV form (ACT), asymptomatic LV form (AS), cured LV individuals (CR) and non-infected individuals (NI), living in endemic area. Our data demonstrate a decrease on the absolute number of TNF-α + cells in NI and an increased on the expression of IL-4 in NI and ACT, both, in adults and children. Additionally, our results demonstrate an increase on the absolute number of IFN-γ^+ and IL-4 $^+$ cells in AS and CR, both, in adults and children. The cytokine pattern of neutrophils, suggest that NI, would be susceptible to the infection and could also explain the disease progression in ACT. Furthermore, the cytokine pattern observed for AS and CR, suggest that in spite of what was observed, a tendency for a type 1 profile, these individuals modulate the response for secrete IL-4, perhaps to control the disease without allowing the host to inflame. Taken together, these findings suggested that the neutrophils could function as immunoregulatory cells by releasing cytokines, that could influence the outcome of the innate immune response and of the subsequent T cell-dependent immune response during early stages of LV.

Supported by: FAPEMIG, FIOCRUZ and CPqRR

IM35 - CYTOKINE EXPRESSION IN NEUTROPHILS ARE IMPORTANT IN DIRECTING THE ADAPTATIVE IMMUNE RESPONSE IN INDIVIDUALS SUSCEPTIBLE AND RESISTANCE TO *LEISHMANIA (V.) BRAZILIENSIS* INFECTION.

Baratta-Masini A [1,2], Martins-Filho OA [3], Mayrink W [4] and Corrêa-Oliveira R [1].

1 Laboratório de Imunologia Celular e Molecular - CPqRR - FIOCRUZ, Belo Horizonte, Brasil. 2 Departamento de Bioquímica e Imunologia - ICB-UFMG, Belo Horizonte, Brasil. 3 Laboratório de Doença de Chagas - CPqRR - FIOCRUZ, Belo Horizonte, Brasil. 4 Departamento de Parasitologia - ICB-UFMG, Belo Horizonte, Brasil

Neutrophils are the first cells recruited when the peripheral tissue is disrupted, and recent studies have demonstrated that neutrophils, once exposed to inflammatory signals, synthesize several cytokines. In this context, we evaluated, at a single cell level, the cytokine patterns of neutrophils, after a short antigen-specific stimulation *in vitro*. In this study, we evaluated four groups of individuals, including patients with active localized cutaneous leishmaniasis (LCL), subjects cured and treated with conventional chemotherapy (Q) and immunochemotherapy (IQ) and an endemic control group (NI). The results are expressed as number of positive cells/mm3 of peripheral blood. Our data demonstrate an increased in the levels of IFN-gamma, TNF-alpha and IL-12$^+$ neutrophils in LCL in comparison to the other groups. And increased level of IFN-gamma and TNF-alpha + neutrophils in IQ and Q in comparison to NI, and a decreased level of type 2 cytokine (IL-10 and IL-4) in cured patients (IQ and Q) in comparison to infected group (LCL) and control group (NI). Our data show that LCL individuals present a type 0 response, with the presence of type 1 cytokines and type 2cytokines$^+$ neutrophlis. Cured individuals present a predominance of type1$^+$ cytokine pattern by neutrophils, usually associated with activation of type 1$^+$ T cells and resistance to infection. Taken together, these findings suggested that neutrophils could function as immunoregulatory cells by expressing cytokines, that could influence the outcome of the innate response and of the subsequent T cell-dependent immune response.

Supported by: FAPEMIG, CNPq and FIOCRUZ – CPqRR.

IM36 - CONCENTRATION OF MACROPHAGES, TIA-1$^+$ CYTOTOXIC LYPHOCYTES AND CD57$^+$ NATURAL KILLER CELLS CORRELATE WITH THE DEVELOPMENT OF CHAGASIC MEGAESOPHAGUS.

da Silveira, A.B.M.[1], Lemos, E.M.[2], Arantes, R.M.E. [3], Linhares, D.C.[1], Correa-Oliveira, R.[4], Adad, S.J.[4], Reis, D.d'A[1].

Department of Morphology[1], ICB, UFMG, Institute of Infectious Diseases, UFES[2], Department of Pathology[3], ICB, UFMG, FIOCRUZ[4], Belo Horizonte; Department of Pathology, FMTM, Uberaba, Brazil[5].

Chagasic megaesophagus is characterised by lumenal enlargement and wall thickening. The organ may have different degrees of involvement, from a slight motor disturbance, only detectable by manometric and scintilographic studies, to large dilations that characterise the more advanced forms of chagasic mega syndrome. Histological studies demonstrated that patients with megaesophagus present lesions of the myenteric plexus, associated with inflammatory infiltrates. Despite being very well described in terms of anatomo-pathological findings, very little information is currently available regarding the phenotype of inflammatory cells and their possible role in the development of megaesophagus. We quantified CD57$^+$ *Natural Killer* cells, TIA-1$^+$ cytotoxic lymphocytes and CD68$^+$ macrophages in esophagus of patients bearing severe megaesophagus and also in chagasic patients without megaesophagus. Patients without mega were classified in two groups, as having high or low number of neurons. Both groups with decreased counting of neurons presented *Natural Killer* cells, as well as cytotoxic lymphocytes in the esophagus, whilst increased numbers of both cellular populations were observed in patients bearing megaesophagus. The levels of CD68$^+$ macrophages in the esophageal *muscularis propria* and plexus regions of chagasic patients were also increased in chagasic patients when compared to non-infected controls. Morphometric analysis of sections labelled with anti-CD68 or anti-PGP9.5 showed that, the higher levels of

XXX ANNUAL MEETING ON BASIC RESEARCH IN CHAGAS DISEASE - XIX MEETING OF THE BRAZILIAN SOCIETY OF PROTOZOOLOGY - HOTEL GLÓRIA, CAXAMBU, MG, BRASIL - 10-12 NOVEMBER 2003. **Rev. Inst. Med. trop. S. Paulo, 45**(Suppl. 13), November, 2003.

macrophages, the lower density of PGP 9.5+ nerve endings. Those data together point to the participation of cytotoxic lymphocytes, *Natural Killer* cells and macrophages in neuronal lesions occurring in the chronic phase of chagasic megaesophagus.

Financial support: CAPES, FAPEMIG, and WHO.

IM37 - COMPARATIVE STUDY: PRESENCE OF PARASITE KDNA, INFLAMMATION AND COUNTING OF NEURONS IN CHAGASIC PATIENTS WITH AND WITHOUT MEGAESOPHAGUS.

da Silveira, A.B.M.[1], Vago, A.R.[1], Arantes, R.M.E.[2], Adad, S.J.[3], Correa-Oliveira, R.[4], Reis, D.d'A.[1]

Department of Morphology[1] and Department of Pathology[2], ICB, UFMG, Belo Horizonte; Department of Pathology, FMTM, Uberaba[3], and FIOCRUZ, Belo Horizonte, Brazil[4].

The pathogenic mechanisms in chagasic megaesophagus are are not entirely understood. Neuronal lesions have been considered the hallmark of this syndrome, and they are frequently observed in association with ganglionitis, periganglionitis and fibrosis. It has been suggested that chronic lesions are consequence of parasite-related mechanisms, as well as immune-mediated cytotoxic damage. In this study we analysed the inflammatory process, the counting of neurons and the presence of kDNA parasite in the esophagus of patients with and without megaesophagus. The presence of kDNA parasite was demonstrated in esophagus of patients with digestive disease, as we had previously published. However, sixty percent of the chagasic patients without megaesophagus analysed in this study also presented parasite DNA in the organ. When analysed for neuronal number, this group could be classified in two, as having low or high counting of neurons. Patients without megaesophagus presenting high number of neurons did not show any inflammatory process, but two of them had parasite kDNA in the organ. Interestingly, all patients without megaesophagus presenting low number of neurons had parasite kDNA and also light inflammatory process. These data strongly suggest that chronic lesions in chagasic megaesophagus might be consequence of immune-mediated mechanisms, that last until the chronic phase of infection, and are dependent on the persistence of parasite in the tissue.

Financial support: CAPES and FAPEMIG.

IM38 - ROLE OF TNF-α RECEPTOR 1 (TNFRP55) IN INFLAMMATORY RESPONSES TO *LEISHMANIA (L.) MAJOR*

Oliveira, C. F.[1], Nogueira, T. S.[1], Santiago, H. C.[1], Santiago, L.[1], Ferraz, F. O.[1], Tafuri, W.[2], Arantes, R.[2], Gazzinelli, R.T.[1], Vieira, L.Q.[1]

[1]Department of Biochemistry and Immunology, [2] Department of General Pathology, ICB, UFMG – CP 486, 30161-970, BH, MG

Tumor necrosis factor (TNF-α) has an essential role in the activation of infected macrophages to kill *Leishmania major* after activation with IFN-γ. Although TNF-α has two receptors on the surface of most cells, the introduction of nitric oxide (NO) by TNF-α was believed to be mediated by the receptor 1 (TNFRp55). Mice in which this receptor was deleted by homologous recombination (TNFRp55-/-) resolved parasitism in the footpad when infected with *L. (L.) major,* but more slowly than C57BL/6 wildstrain. More interestingly, even after the levels of parasites at the site of infection were undetectable, TNFRp55-/- did not resolve lesions, and an intense inflammatory infiltrate was present after 25 weeks of infection. The aim of this word is to investigate the reason for the permanence of the cellular infiltrate in lesions from TNFRp55-/- mice infected with *L. (L.) major*. Thus, we determined the expression of chemokines by RT-PCR at the site of infection in C57BL/6 and TNFRp55-/- mice. RANTES and MCP-5 expression was upregulated in C57BL/6 wildtype and TNFRp55-/- mice. However, levels of these chemokines were downregulated at 11 weeks of infection in C57BL/6 mice, while there was still a high level of expression of both chemokines in lesions from TNFRp55-/- mice at this time point. In order to investigate the role of the TNFRp55 on the delayed-type hypersensitivity (DTH) in response to formalin-treated *L. (L.) major*, we injected 10^7 dead parasites in the right footpad of mice infected for 2, 4, 6 and 11 weeks in the left footpad. The size of the response was measured 3, 6, 24, 48 and 72 hours. Surprisingly, TNFRp55-/- mice presented no detectable DTH response and C57BL/6 presented a positive DTH from the 4th week of infection. We determined the expression of chemokines by RT-PCR in the right footpad injected with 10^7 dead parasites after 6 weeks of infection. C57BL/6 wildtype express higher levels of RANTES than TNFRp55-/- mice. We also studied the response of C57BL/6 and TNFRp55-/- mice to carrageenan. We injected carrageenan solution in the right footpad and the lesion was measured 3, 6, 12, 24, 48 and 72 hours. C57BL/6 wildtype present larger footpads at 6 hours after injection than TNFRp55-/- mice, however, TNFRp55-/- mice seemed not to resolve the cellular infiltrate 96 hours after injection. Thus, we conclude that the TNFRp55 plays a role in the resolution of a primary cellular infiltrate, maybe by mediating the downregulation of chemokine expression. As to the recall response, TNFRp55 seems to mediate the migration of cells to the site of a secondary antigenic exposure.

Support: CNPq

IM39 - LEVELS OF NITRIC OXIDE IN THE CANINE VISCERAL LEISHMANIASIS

MORADOR[1] C. C., PEROBA[1], S.C.G.; MALAQUIAS[1] L. C. C., TOSTES[1] T..L L., SILVEIRA[1] K. V., REIS[2] A.B.

1- Núcleo de Pesquisa em Imunologia - Universidade Vale do Rio Doce; 2- Laboratório de Análises Clínicas - Universidade Federal de Ouro Preto

The canine visceral leishmaniasis (CVL) is an illness of chronic course in which the dog evolved from an unapparent state of the infection (asymptomatic). These do not present clinical evident signals of infection, low levels of IgG and low tecidual parasitic load. These animals can evolve to serious symptomatic picture. Objectives: To quantify the sera levels of nitric oxide and correlate to the tecidual parasitic density (LDU) in dogs with different clinical forms of the CVL. Materials and Methods: It had been used sera from dogs infected and not infected with *Leishmania (L.) chagasi*, carrying different clinical forms of the infection. The dogs were clinically classified and grouped in: Not Infected (NI=20), Asymptomatic (AS=12), Oligosymptomatic (OS=12) and Symptomatic (SY=16). These dogs are proceeding from the Zoonotic Center Control from Belo Horizonte (CCZ/PBH). The dogs of groups AS, OS and SY presented positive serologic test in at least two of three serologic tests (RIFI, ELISA, ELISA-rK39). The quantification of nitric oxide was carried out indirect through the measure of nitrite and nitrate using the Griess method and the results expressed in ìM. Resulted and conclusions: A gradual fall in the levels of NO was observed with severe clinical form of the CVL. The animals from group AS had presented higher levels of NO in relation to NI and SY group. These data seem to point out an association of high levels of NO on asymptomatic dogs and minor LDU in the spleen, skin and popliteal lymph node. Support: UNIVALE

IM40 - IFN-γ AND IL-10 ARE PRODUCED BY NATURALLY INFECTED DOGS WITH VISCERAL LEISHMANIASIS.

Lima, V.M.F., Dossi A.C.S., Neto, JLJR

XXX ANNUAL MEETING ON BASIC RESEARCH IN CHAGAS DISEASE - XIX MEETING OF THE BRAZILIAN SOCIETY OF PROTOZOOLOGY - HOTEL GLÓRIA, CAXAMBU, MG, BRASIL - 10-12 NOVEMBER 2003. **Rev. Inst. Med. trop. S. Paulo, 45**(Suppl. 13), November, 2003.

Department of Clinic, Surgery and Animal Reproduction, Veterinary Medicine - UNESP, Araçatuba, Sao Paulo - Brazil

Zoonotic visceral leishmaniasis, caused by both *Leishmania (L.) infantum* and *L. (L.) chagasi* represent 20% of human visceral leishmaniasis in the world and its incidence is growing in urban and periurban areas of the tropics. In Brazil, visceral leishmaniasis (VL) is caused by *Leishmania (Leishmania) chagasi*. Dogs constitute the main domestic reservoir of these parasites transmitted to humans by phlebotomine sandflies. Most of the infected animals are susceptible and develop active disease, which is characterized by high anti-Leishmania antibody titers and depressed lymphoproliferative abilities. The immune response Th1 is correlated with resistance to the pathogen, but the high level of antibodies suggests that the immune response Th2 is associated with active disease. In this study, naturally infected dogs with clinical signs of the disease and positive ELISA test in sera were analyzed. After intravenous injection of 25mg/kg of thiopental (Thionembutal 12.5%) to each animal, 30 ml of blood was collected, lymphocytes were isolated with ficoll-paque-PLUS and RNA was isolated. Interferon-gamma and interleukin–10 profiles were detected by RT-PCR using specific primers. In all animals analized (20 / 20) interferon-gamma expression was detected, but only few animals (5 / 14) showed interleukin–10. The predominance of interferon-gamma production associated with active disease suggests that other cytokines rather than interleukin-10 must be produced to collaborate in the immune response Th2 observed in animals with active disease. The cytokines IL-4 and TGF-beta are being investigated.

Supported by FAPESP

IM41 - PHENOTIPIC PROFILE OF PERIPHERAL BLOOD LEUKOCYTES AND SPLENOCYTES IN CANINE VISCERAL LEISHMANIASIS

Giunchetti, R.C[2]; Corrêa-Oliveira, R.[2]; Mayrink, W.[1]; Carvalho, G.M.[4]; Genaro, O[1]; Martins-Filho, O.A[3] and Reis, A.B[1,5]

1- Laboratório de Leishmanioses ICB-UFMG, 2-Laboratório de Imunologia Celular e Molecular CPqRR, 3- Laboratório de Doença de Chagas CPqRR-FIOCRUZ. 4- Laboratório de Hematologia Clinica EF/UFMG. 5-Laboratório de Parasitologia e Histopatologia NUPEB-UFOP, alexreis@cpqrr.fiocruz.br

Canine Visceral Leishmaniasis (CVL) can manifest itself in a broad clinical spectrum ranging from asymptomatic to patent hepatosplenic disease. However, the role of different cell populations on the development and/or maintenance of CVL are still unclear. Considering that CVL causes a systemic impairment, it becomes relevant to study other cell compartment like the spleen – the major lymphoid organ interfacing the systemic circulation – where monocyte/macrophage homing and also antigenic stimulation can occur during CVL. We evaluated the immune status of 40 CVL dogs naturally infected with *L. (L.) chagasi* as well 20 healthy controls. The dogs were classified as asymptomatic (n=12), oligosymptomatic (n=12) and symptomatic dogs (n=16) based on their clinical and laboratory records. The phenotypic profile of both, peripheral blood leukocytes and splenocytes was analyzed by flow cytometry. Spleen biopsies were used to access the parasitism ("Leishman Donovan Units" – LDU). Our data demonstrated that symptomatic dogs presented decreased levels of circulating T-cells, including both $CD4^+$ and $CD8^+$ subpopulations, with a lower CD4/CD8 ratio in comparison to the control group. On the other hand, asymptomatic dogs presented high absolute number of $CD4^+$ and $CD8^+$ T-cells when compared to the other groups. Dogs with clinical diseases, including oligosymptomatic and symptomatic, presented a decreased number of $CD21^+$ cells and a progressive reduction of $CD14^+$ cells linked with the severity of the disease. Phenotypic analysis of splenocytes demonstrated a progressive increase in T-cell, mainly $CD8^+$, in all infected dogs in comparison to the control group, which lead to a decreased CD4/CD8 ratio in CVL dogs. A decreased percentage of B-cells, similarly observed in the PB, were observed with the development of patent disease. LDU was higher in the symptomatic animals in comparison to the other infected dogs. Our data pointed out that the maintenance of a stable chronic infection by *L. (L.) chagasi* in dogs involves an increase in T cell subpopulation, mainly $CD8^+$ cells, in both PB and spleen, re-emphasizing that this cell population may be evolved in the mechanisms of protective immunity in CVL. Dogs with symptomatic disease, presenting a reduced potential an antigen presenting cells - evidenced by the lower number of circulating monocytes and B-cells as well as a decreased percentage of B-splenocytes - showed lower results in the *in vitro* proliferate response and were more prone to develop patent disease.

This work was supported by CNPq, FAPEMIG, FBB, UFMG, FIOCRUZ (CPqRR).

IM42 - *TRYPANOSOMA CRUZI* CARRYING A TARGETED DELETION OF A TC52 PROTEIN-ENCODING ALLELE ELICITS ATTENUATED CHAGAS' DISEASE IN MICE

Garzón, E[1]., Nacife, V.P[3]., Monte-Alegre, A[1]., Cordeiro-da-Silva, A[2]., Guilvard E[1]., Bosseno, M.F[1]., Meirelles, M.N.L[3]., Guevara, A.G[4]., Borges, M[1,2]., Brenière S.F[1]., and Ouaissi, A[1].

[1] IRD-UR 008 "Pathogénie des Trypanosomatides", Centre IRD de Montpellier, 911 Avenue Agropolis, BP 5045, 34032 Montpellier, France.[2] Department of Biochemistry, Faculty of Pharmacy and Institute of Molecular and Cellular Biology, University of Porto, Portugal.[3] Department of Ultrastructure and Cellular Biology, Instituto Oswaldo Cruz, FIOCRUZ, Av. Brasil 4365, 21045-900, Rio de Janeiro, Brasil.[4] Laboratory of Clinical Investigations, Vozandes Community Services, Hospital Vozandes, Quito, Ecuador. Email: garzoni@mpl.ird.fr

The intracellular protozoan parasite *Trypanosoma cruzi* is the etiological agent of Chagas' disease. We have previously characterized a *T. cruzi* virulence factor named Tc52 sharing structural and functional properties with the thioredoxin and glutaredoxin protein family. Single mutant parasite clones ($Tc52^{+/-}$) exhibiting low virulence *in vitro* and *in vivo* were obtained by targeted *tc*52 gene replacement. In this report, we have extended our study to analyze the immune response and the disease phenotype in $Tc52^{+/-}$- infected BALB/c mice, during the acute and chronic phases of the disease. Significantly lower parasitemia were found in $Tc52^{+/-}$-infected mice, as compared to wild-type parasite (WT)-infected ones. However, the expansion of all classes of lymphocytes and macrophages was similar for both clones. Furthermore, except for IgG2b levels which were higher in the case of WT-infected mice, all classes of Ig presented no significant difference for WT and $Tc52^{+/-}$-infected animals. Interestingly, a lack of suppression of IL-2 production and of T-cell proliferation inhibition was observed in the case of spleen cells from $Tc52^{+/-}$-infected mice. Finally, the pattern of inflammation process was different and characterized as diffused in the case of $Tc52^{+/-}$-infected mice, or presenting numerous foci in the case of WT-infected mice. Localization of the Tc52 protein in tissue sections and infected heart cell primary cultures by immunofluorescence and immunogold labeling, respectively, revealed the presence of Tc52 at the amastigote surface and associated to aggregates within host cell vesicles. Taken together, these results reinforce the notion of Tc52 being a virulence factor playing a role in the phenotype of the immune response associated to the infection and on the course of the disease.

These investigations received financial support from Institut de Recherche pour le Développement (IRD), Institut National de la Santé et de la Recherche Médiacle (INSERM) and "Coopération Scientifique et Technologique Luso-Française: Project N°706 C3". FIOCRUZ and FAPERJ.

XXX ANNUAL MEETING ON BASIC RESEARCH IN CHAGAS DISEASE - XIX MEETING OF THE BRAZILIAN SOCIETY OF PROTOZOOLOGY - HOTEL GLÓRIA, CAXAMBU, MG, BRASIL - 10-12 NOVEMBER 2003. **Rev. Inst. Med. trop. S. Paulo, 45**(Suppl. 13), November, 2003.

IM43 - T CELL INDEPENDENT IMMUNITY DURING EARLY STAGES OF HUMAN INFECTION WITH *TRYPANOSOMA CRUZI*

Sathler-Avelar, R.1; Lemos, E.M2.; Reis, D.D.A.3; Medrano-Mercado, N.4; Araújo-Jorge, T.4; Correa-Oliveira, R.5; Eloi-Santos-S.1,6; Favato, D.1; & Martins-Filho, O.A.1*

1. Laboratório de Doença de Chagas – Centro de Pesquisas René Rachou – FIOCRUZ– Brazil; 2. Núcleo de Doenças Infecciosas – Universidade Federal do Espírito Santo – Brazil; 3. Departamento de Morfologia – Universidade Federal de Minas Gerais – Brazil; 4. Laboratório de Biologia Celular – Instituto Oswaldo Cruz – FIOCRUZ – Brazil; 5. Laboratório de Imunologia Celular e Molecular – Centro de Pesquisas René Rachou – Fundação Oswaldo Cruz – Brazil; 6. Departamento de Propedêutica Complementar – Faculdade de Medicina-UFMG – Brazil

In this study, a cross-sectional flow cytometric analysis of peripheral blood mononuclear cells (PBMC) was performed to evaluate the immunological status of *T. cruzi*-infected children, aiming to make known the immune response triggered during the early stages of Chagas disease. Fourty-two infected children were classified into four groups, based on their serological features, including early-acute phase (EA), late-acute phase (LA), recent chronic phase (RC) and not infected (NI). Our results demonstrated that three well-characterized major immunological changes could be identified according to the proposed phases of disease. EA was accompanied by expansion of conventional B-cells and up-regulation of CD54 molecule on monocytes, associated with neither monocyte activation phenotypes nor changes of NK or T-cell compartments. LA was characterized by a selective expansion of a distinct lineage of NK cells ($CD16^+CD56^-$) besides a persistent high frequency of B-cells. RC was still accompanied by a high levels of B-cells, leading to a reduced T/B ratio, mainly triggered by the expansion of B1 cells subset, besides a delayed expansion of HLA-DR bright+ monocytes. These findings reinforce the hypothesis that T. cruzi-derived antigens are able to activate NK-cells, before the development of T-cell-mediated immunity. Moreover, our data supported, previous remarks of increased levels of B1-lymphocytes during chronic human Chagas disease and evidenced that this event is already present in initial stages of chronic infection. We discussed that T-cell-mediated immunity during early stages of *T. cruzi* infection may stand for a phenomenon restricted to the cardiac and lymph node compartments, not detectable on the peripheral blood.

IM44 - *IN VIVO* NEUTRALIZATION OF *PLASMODIUM GALLINACEUM* SPOROZOITE INFECTIVITY USING SERA OF *P. FALCIPARUM* PATIENTS

Alves-Brito, C. F.[1], Matoso, L. F.[1], Mansur, S. B. E.[1], Rocha, E. M. M.[2] and Krettli, A.U.[1]

1.Laboratório de Malária, Centro de Pesquisas René Rachou / FIOCRUZ, Belo Horizonte/MG; 2.Departamento de Patologia, Centro de Ciências Biológicas, Universidade Federal de Alagoas, Maceió/Al.

Plasmodium falciparum (Pf) is the most prevalent species causing malaria worldwide. Practical difficulties, such as, high human infectivity and absence of experimental vectors in Brazil, hampers its use in research. Our group has been using *P. gallinaceum* (Pg), non-infectious to humans, as an alternative model to study malaria, particularly in the identification of protein as marker of acquired immunity in human Pf. We show that Pg sporozoites, react by indirect immunofluorescence (IIF) with sera from subjects exposed to Pf; sera from *P. vivax* subjects or normal sera were negative. Highest scores of positive sera (73-90%) with Pg sporozoites were in subjects denying malaria in the last 5 years or more. These sera also recognized several Pg proteins by immunoblot analysis mainly the circumsporozoite protein (CSP). In this study, we analyze the ability of crossreactive antibodies present in sera of immune Pf patients to neutralize the infectivity of Pg sporozoites. Fresh-isolated sporozoites were incubated with sera from Pf patients who described more than 10 malaria acute episodes. Two immune sera were able to partly neutralize the Pg sporozoite infectivity to chicks, its natural host, in relation to non-immune sera. Monoclonal antibodies anti-PgCSP which totally abrogate the Pg sporozoite infectivity in vivo and in vitro, recognized mainly the C-terminal of the CSP (CSC). Studies with human sera are now undertaken in an attempt to verify whether such CSC protein identifies protective antibodies in ELISA.

Supported by FAPEMIG and CNPq.

IM45 - MAPPING CD4 T-CELL EPITOPES WITHIN THE AMASTIGOTE SURFACE PROTEIN-2 OF *TRYPANOSOMA CRUZI*.

Araújo AFS, Boscardin SB, Tzelepis, F and Rodrigues MM

Departamento de Microbiologia, Imunologia e Parasitologia, Universidade Federal de São Paulo-Escola Paulista de Medicina, Rua Botucatu 862 6A, São Paulo, SP, Brazil, 04023-062. E-mail: mrodrigues@ecb.epm.br

Recently, independent groups studied the immunogenic properties of plasmids containing genes encoding the Amastigote Surface Protein-2 (ASP-2). Genetic immunization with *asp-2* gene generated immune responses mediated by antibodies, $CD4^+$ and $CD8^+$ T cells. Most relevant, DNA-vaccinated mice displayed remarkable protective immunity, surviving lethal infection with *T. cruzi* (Garg & Tarleton, 2002, Infect. Immun. 70:5547, Boscardin *et al*., 2003, Infect. Immun. 71:2744 and Fralish & Tarleton, 2003, Vaccine 21:3070). To map a CD4 T-cell epitope in the ASP-2 antigen, 8 recombinant proteins were generated as GST-fusion or His-tag polypeptides. These recombinant proteins representing the different domains of ASP-2 antigen were purified and tested in their ability to be recognized by polyclonal antibodies or to stimulate *in vitro* spleen cells of BALB/c or A/Sn mice immunized with a plasmid containing the *asp-2* gene. In preliminary experiments, we determined that spleen cells from DNA-immunized BALB/c or A/Sn mice secreted interferon-gamma when re-stimulated *in vitro* with recombinant proteins representing the amino acids 67-260 or 261-500 of ASP-2, respectively. We are currently pursuing further the epitope mapping of these T cell determinants using the recombinant proteins.

Supported by FAPESP and CNPq.

IM46 - $CD4^+CD25^+$HIGH REGULATORY CELLS IN PERIPHERAL BLOOD OF CHAGAS DISEASE PATIENTS

Vitelli, D.M.1,2,4, Lage, P.S.1, DIAS, J.C.3, Elói-Santos, S.1,5, Correa-Oliveira, R.2,4 & Martins-Filho, O.A.1

1. Laboratório de Doença de Chagas – Centro de Pesquisas René Rachou – FIOCRUZ – Brazil; 2. Laboratório de Imunologia Celular e Molecular – Centro de Pesquisas René Rachou – FIOCRUZ – Brazil; 3. Laboratório de Triatomínios – Centro de Pesquisas René Rachou – FIOCRUZ – Brazil; 4. Departamento de Imunologia e Bioquímica – ICB/UFMG - Brazil; 5. Departamento de Propedêutica Complementar – Faculdade de Medicina/UFMG – Brazil

Despite the identification of two major $CD4^+$ T-cells providing the insights that distinct Type 1 and Type 2 cytokine patterns may regulate immune responses, it is clear that populations of T-cells could also mediate immune responses by cell contact in the absence of cytokine secretion. Experiments demonstrating that $CD4^+CD25^+$ T-cell function as key regulatory effectors in mice have provided

XXX ANNUAL MEETING ON BASIC RESEARCH IN CHAGAS DISEASE - XIX MEETING OF THE BRAZILIAN SOCIETY OF PROTOZOOLOGY - HOTEL GLÓRIA, CAXAMBU, MG, BRASIL - 10-12 NOVEMBER 2003. **Rev. Inst. Med. trop. S. Paulo, 45**(Suppl. 13), November, 2003.

important information about a specific cell population that performs immune regulation through suppression of self response. Whereas the entire population of $CD4^+CD25^+$ T cells exhibit regulatory function in mouse, only the $CD4^+CD25high^+$ population displays a similarly strong regulatory function in humans. The critical importance of identifying the involvement of these regulatory T-cells as a bridge for immune regulation of pathology during chronic human infection by *T cruzi* prompt us to search for a differential frequency of $CD4^+CD24high^+$ T-cells in peripheral blood of chagasic patients presenting different clinical forms of the disease. Here, we report that asymptomatic indeterminate patients showed higher levels of $CD4^+CD25^+$high T-cells in comparison to those symptomatic patients presenting cardiac or digestive forms of disease. Thus, regulatory T-cells expressing high levels of the IL-2 receptor seems to be associated with immunoprotective mechanisms, providing the opportunity to determine whether alterations on these regulatory T-cells are involved in the induction of pathological mechanisms of human Chagas disease.

Financial Support: CNPq, CPqRR/FIOCRUZ

IM47 - CHARACTERIZATION OF $CD4^+$ T CELL HYBRIDOMAS SPECIFIC FOR *TRYPANOSOMA CRUZI TRANS*-SIALIDASE.

Hiyane,I.M., Boscardin, S.B., and Rodrigues, M.M.

Departamento de Microbiologia, Imunologia e Parasitologia, Universidade Federal de São Paulo-Escola Paulista de Medicina, Rua Botucatu 862 6th floor, São Paulo, S.P., Brazil, 04023-062.

BALB/c mice immunized with a DNA plasmid encoding the *Trypanosoma cruzi Trans*-sialidase (TS) developed $CD4^+$ Th1, $CD8^+$ Tc1 and protective immune response against infection[1]. From DNA-vaccinated mice, we obtained $CD4^+$ Th1, which displayed remarkable anti-parasitic activity *in vitro*[2]. The aim of the present study was to obtain T cell hybridomas specific for TS protein that would allow us to further characterize the specificity and function of the $CD4^+$ Th1 cells. For that purpose, lymph node cells from BALB/c mice immunized with the recombinant TS protein was fused to cells AKR thymoma BW 1100.129.237. Twenty-five hybridomas were obtained. Twelve of them were antigen specific, secreting more than 1 ng/ml of IFN-γ when re-stimulated with recombinant TS protein. None of them secreted either IL-4 or IL-10 upon *in vitro* re-stimulation. By flow cytometry analysis, all hybridomas were positives for CD3 and eight of them were highly positive for CD4 T cell marker. Four of five hybridomas tested were also positive for the TCRb chain. One hybridoma positive for the TCRb chain was choused and RNA from the hybridoma clone 09 (H09) was isolated. We employed the nonpalindromic adaptor-PCR (NPA-PCR) method to amplify T-cell receptor (TCR) alpha- and beta-chain transcripts described by Lin WL *at al*., 1998. The NPA-PCR method has been specifically designed for the amplification of transcripts with variable or unknown 5' ends, such as TCRs and immunoglobulins (Ig). The amplifications of products were cloned in *pMOSblue* vector and were sequenced. The variable alfa and beta chains of T cell receptor (TCR) were identified as TCRAV18S2 and TCRBV6S1, respectively. The specific primers to variable alpha and beta chain were constructed. These primers were used to amplification reactions using genomic DNA of H09 as template. The presence of introns in sequences of TCR is important to expression of TCR. The amplified products were cloned in *pMOSblue* vector and were sequenced. The next step the alfa and beta chain will be cloned in pTalfacass and pTbetacass vectors[4], which will be used to express rearranged T cell receptor genes in transgenic mice.

References

1- Costa, F., *et al*., 1998. Vaccine 16, 768-774.

2- Rodrigues, M.M., *et al*., 1999. Infect. Immunity, 67, 3855-3863.

3- Lin, W.L.*et al*.,1998. Hematopathology. and Molecular. Hematology, 11(2),73-88.

4- Kouskoff, V., *et al.,* 1995. J. Immunol. Methods, 180, 273-280.

Supported by grants from FAPESP, PADCT, CNPq, PRONEX and FINEP.

IM48 - IDENTIFICATION OF CTL EPITOPES IN KMP11 PROTEIN FROM *TRYPANOSOMA CRUZI*

Diez H[1], Gonzalez JM[1],Lopez MC[2],Thomas MC[2],Guzman F[3],Rosas F[4],Velazco V[4],Puerta C[1].

[1]-Pontificia Universidad Javeriana, Bogota, Colombia; [2]-Instituto de Biomedicina y Parasitologia Lopez Neyra, Granada,España; [3]-Fundacion Instituo de Inmunologia de Colombia,Bogota, Colombia; [4]-Clinica Shaio, Bogota,Colombia

The Kinetoplastid membrane protein-11(KMP11) is found in several parasites belonging to the *Leishmania* and *Trypanosoma* family. This protein seems to contain epitopes that induce immune cellular responses (Tolson, et al 1994), and it is thought to have a role in protective immunity (Mukhopadhyay, et al 1999). Recently, it was demonstrated that the *T. cruzi* KMP11 is located mainly in the parasite´s flagellar pocket where it is associated with the cytoskeleton. Therefore, its function is critical for the parasite mobility and for its attachment to the host cells (Thomas, et al 2001). Experimental *T. cruzi* infection using the murine model demonstrated that $CD8^+$ T lymphocytes were essential in controlling parasite dissemination. These cells presented cytotoxic activity specific for *T. cruzi* antigens. CD8 T lymphocytes specific for *T. cruzi* antigens were also found in peripheral blood of Chaga´s patients (Wizel, et al 1998). Current data indicates that these cells could play an important role in protection (Reis, et al 1997). In the present study, it was assessed if *T. cruzi* KMP11 could induce CD8 T lymphocytes responses. The aim of this study was to determine the presence of cells specific for the KMP11 synthetic peptide (tleefsakl) in HLA-A2 chagasic patients using γ-IFN production measured by ELISPOT assay. PBMC were isolated using density gradients and cells were typed using specific HLA-A2 monoclonal antibody. Five patients out 17 studied were HLA-A2 positive (29,4%). CD8 T lymphocytes of those patients were purified using magnetic beads and plated for γ-IFN ELISPOT in the presence of peptide pulsed antigen presenting cells. Responses to the well defined HLA-A*0201 restricted peptide from the influenza matrix protein (flu 58-66) were found in 4 out 5 patients with average of frequency of 167.5 $x10^6$ cells. Two of the HLA-$A2^+$ *T.cruzi*-infected individuals tested showed a $CD8^+$ specific response to the peptide with a relative frequency of 110 and 230 $x10^6$ cells. Our dates indicate that KMP11 acts as a T-cell immunogen during Chagas disease but its role in protection has to be defined.

IM49 - EARLY MECHANISM OF COMPLEMENT-MEDIATED LYSIS ARE DIFFERENT AMONG *T. CRUZI* LINEAGE

Cestari I.S., Freitas J.C., Daliry A., Evans-Osses I. S. &Ramirez M.I.

Depto. de Imunologia, Centro de Pesquisas Aggeu Magalhães-FIOCRUZ. Av. Professor Moraes Rego SN, Recife, PE.

Trypanosoma cruzi, the etiological agent of Chagas' disease, is a protozoan that infect about 18 million people in Latin America (WHO, 2003). It has been demonstrated through molecular and biochemical markers (Miles et al, 1978; Zingales et al, 1996) that these parasites can be divided in two main lineage, lineage 1 with domestic life cycle, and lineage 2 with a sylvatic life cycle (Fernandes et al, 1998). Mechanisms of innate immunity can be different among *T.cruzi* strains, and this would strengthen the hypothesis of the different origins proposed by Briones and Zingales, 1998. To infect host cells, *T.cruzi* needs to resist the complement mechanisms, which can be activated by classical or alternative pathway. The time of complement activation and susceptibility to

XXX ANNUAL MEETING ON BASIC RESEARCH IN CHAGAS DISEASE - XIX MEETING OF THE BRAZILIAN SOCIETY OF PROTOZOOLOGY - HOTEL GLÓRIA, CAXAMBU, MG, BRASIL - 10-12 NOVEMBER 2003. **Rev. Inst. Med. trop. S. Paulo, 45**(Suppl. 13), November, 2003.

lyse of parasites in contact with Normal Human Serun (NHS) could be associated with the capacity of parasite evasion in the host immune system. With the objective to determine the differences among *T.cruzi* lineage related to the resistance and activation of the complement system, we have characterized four *T.cruzi* strains, two of lineage 1 (Y and CL Brener) and two of lineage 2 (Colombiana and Dm28c). We determinated: i) the limite dilution; minimun concentration of NHS capable to lyse 50% of the parasites; ii) and the time necessary for the activation of the classic and alternative pathway, through kinetic with different times (30 seconds, 1 minute, 2,5 min, 5 min, 10 min. e 30 minutes). The limite dilution was similar for Y and CL Brener strain (25% of NHS) while Colombiana and Dm28c strains were more sensible (around 6,25% and 12,5%). The complement-mediated lysis activated by classic and alternative pathway resulted in 70% and 71% of alive parasites to Y and CL Brener strain respectively, at 2,5 minutes, while Colombiana and Dm28c, 11% and 35% respectively. When the classic pathway was blocked with EGTA, it was determined that the parasites were equally resistant to alternative pathway, and only its action was not enough to lyse *T.cruzi* strain, with 63% and 35% of alive parasites at 10 minutes. These datas show that lyse is mediated by a sinergic action of classic and alternative pathway. The alternative pathway is not enough to lyse parasite in a short time and this fact is relevant considerating the rapid process of cell invasion. Moreover, the complement-mediated lyse could be a marker to diferenciate the lineage of differents *T.cruzi* strains. *T. cruzi* Y and CL Brener strains are more resistant than Colombiana and Dm28c strains, and this fate could be related with the different enviroment where lineage 1 parasite had evolved. The understading of these mechanisms could contribute to new approaches to control the disease.

Supported by CNPq, FACEPE, FIOCRUZ

IM52 - VACCINATION OF BALB/C MICE WITH DNA ENCODING A CYSTEINE PROTEINASE OF *L. (L.) CHAGASI* AMASTIGOTES

Dias, S.S., Katz, S., Lopes, R.A.M., Santos, M.R.M. & Barbiéri, C.L.

Disciplina de Parasitologia, Escola Paulista de Medicina, Universidade Federal de São Paulo, Rua Botucatu, 862, Caixa Postal 20342, 04023-062, São Paulo, SP, Brasil. suzi.dias@bol.com.br

An antigen of apparent molecular mass of 30 kDa (p30) was identified in *L. (L.) chagasi* amastigotes and showed to induce lymphoproliferative responses mediated by $CD4^+$ Th1 and a partial protection against challenge with *Leishmania (L.) chagasi* in BALB/c mice (Pinto et al., 2000, Int. J. Parasitol. 30:599-607). One antigen of 30 kDa identified in *L. (L.) amazonensis* amastigotes was also implicated in protective responses against homologous infection and characterized as a cysteine proteinase (Beyrodt et al., 1997). Although biochemical characterization of *L. (L.) chagasi* p30 revealed that it is deprived of proteolytic activity, two cysteine proteinase genes, *Ldccys1* and *Ldccys2*, were identified in *L. (L.) chagasi* and promastigotes transfected with these genes expressed proteolytic activity of apparent molecular masses of 30 and 43kDa (Omara-Opyene and Gedamu, 1997). Previously, we have isolated the *Ldccys1* gene by PCR amplification using genomic DNA from *L. (L.) chagasi* amastigotes and primers corresponding to the ORF sequence of the *L. (L.) chagasi Ldccys1* gene published in GeneBank. Cloning of this gene in pHis vector resulted in a recombinant protein of 47 kDa which was recognized by a monoclonal antibody directed to p30 from *L. (L.) amazonensis* (MoAb 2E5D3). This result and previous data showing that the MoAb 2E5D3 reacts with p30 from *L. (L.) chagasi* indicate that the 47 kDa recombinant protein corresponds to the p30 antigen from *L. (L.) chagasi*. The aim of the present work was the cloning of p30 gene from *L. (L.) chagasi* in a mammal constitutive expression vector and characterization of the immune responses triggered after the immunization of BALB/c mice with the recombinant DNA. The *Ldccys1* gene was subcloned in pcDNA3 vector and BALB/c mice were immunized with three doses with 14 days interval of 100 µg of either pcDNA3 or plasmid encoding the p30 antigen (pcDNA3-p30) in the quadriceps. Two weeks after the third dose, sera from all animals were tested by ELISA using the recombinant p30 and Immunobloting of *L. (L.) chagasi* amastigotes and bacterium extracts. Only mice immunized with pcDNA3-p30 presented high antibody titles against proteins of molecular weights of 30 and 47 kDa from parasite and bacterium extracts, respectively. Preliminary results showed that the recombinant p30 and amastigote extract induce secretion of IFN-γ in supernatants of lymphocyte cultures from BALB/c mice previously immunized with pcDNA3-p30. Active immunization assays of BALB/c mice by use of the recombinant *Ldccys1* gene and p30, as well as the characterization of protective immune responses induced by these immunization schedules are currently in progress.

This research is sponsored by CAPES

IM53 - A VACCINATION PROTOCOL USING BCG, CYCLOPHOSPHAMIDE AND MICROSOMAL FRACTION PROTECTS MICE AGAINST *LEISHMANIA (L.) AMAZONENSIS*

Zaverucha do Valle, T.[1]; Calabrese, K.S.[1]; Tedesco, R.C[2]; Barbieri, C.L.[3] Mortara, R.A.[3]; Gonçalves da Costa, S.C.[1]

1 - Instituto Oswaldo Cruz, Depto. de Protozoologia, Lab. de Imunomodulação.Av. Brasil 4365 Pav. Carlos Chagas 3º andar, Manguinhos, Rio de Janeiro, RJ, Brasil, CEP: 21045-900 taniazv@ioc.fiocruz.br. 2 – Escola Paulista de Medicina, UNIFESP, Depto. de Morfologia. 3 – Escola Paulista de Medicina, UNIFESP, Disciplina de Microbiologia, Imunologia e Parasitologia

We have developed a vaccination protocol using BCG, cyclophosphamide and microsomal fraction of *Leishmania (L.) amazonensis* that is able to protect mice against an infection by virulent amastigotes or promastigotes. Protection was evidenced by a reduction of the lesion of vaccinated mice when compared to non-vaccinated control ones. The imunological mechanisms involved in this protection are being studied. We have demonstrated that vaccinated mice present a strong T-cell immunity, as it was shown by a high DTH response. Immunohistochemistry was performed in order to characterize the cellular population in the lesion and draining lymphnode. Immune serum was used to mark amastigotes while monoclonal antibodies were used to highlight $CD4^+$, $CD8^+$ and macrophages. Confocal analysis reveled a huge amount of both $CD4^+$ and $CD8^+$ in the lesion and lymphnode of vaccinated mice. On the other hand, lesions of control mice presented macrophages, but rare $CD8^+$ and $CD4^+$ cells. The amount of these lymphocytes in the lymphnode was smaller than in vaccinated animals. Antibody titration was performed in mice sera. Vaccinated animals presented lower titers of IgG when compared with control ones. Cytokine titration has demonstrated that vaccinated mice present an early IFN-gamma peak, which may be contributing to the control of the parasite. T-cell immunoblotting essays are being carried out in order to study which Fmic proteins are responsible for the protection obtained. When promastigotes are used to infect mice this protection is even more remarkable, than when amastigotes are used.

Apoio financeiro: IOC

IM54 - INTRAMUSCULAR AND SUBCUTANEOUS IMMUNIZATIONS WITH P36(LACK) DNA VACCINE INDUCE A TH1 RESPONSE BUT DO NOT PROTECT AGAINST *L. (L.) CHAGASI* INTRAVENOUS CHALLENGE

Silva EAM[1], Coelho EAF[3], Fernandes AP[4], Tavares CAP[3], Afonso LCC[1], Rezende SA[1,2]

XXX ANNUAL MEETING ON BASIC RESEARCH IN CHAGAS DISEASE - XIX MEETING OF THE BRAZILIAN SOCIETY OF PROTOZOOLOGY - HOTEL GLÓRIA, CAXAMBU, MG, BRASIL - 10-12 NOVEMBER 2003. **Rev. Inst. Med. trop. S. Paulo, 45**(Suppl. 13), November, 2003.

[1]Laboratório de Imunoparasitologia, DECBI/NUPEB/UFOP, Campus Morro do Cruzeiro and [2]Departamento de Análises Clínicas, Escola de Farmácia, Universidade Federal de Ouro Preto, 35400-000, Ouro Preto, MG, Brasil, [3]Departamento de Bioquímica e Imunologia, Instituto de Ciências Biológicas and [4]Departamento de Microbiologia, Escola de Farmácia, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brasil

American Visceral Leishmaniasis in Brazil is a zooanthroponosis formerly restricted to rural and peri-urban areas. With the emergence of new foci in urban areas, this disease has assumed an important role in public health and vaccination is an important mechanism of protection. Several vaccination protocols have been tested for different kinds of leishmaniasis and the use of DNA vaccines is an interesting approach due to its capability to induce a long-term cellular immunity. Furthermore, these vaccines stimulate humoral, helper and citotoxic responses, being the last two very important to induce a protective response in murine models of visceral leishmaniasis. In our study, we tested a vaccination protocol where BALB/c mice were primed and boosted via subcutaneous or intramuscular route with PBS, pCI-neo plasmid or pCI-neo-p36(LACK) DNA vaccine (100 mg/dose and 100 mg/dose in 25% of sucrose solution for subcutaneous and intramuscular immunizations, respectively) and challenged intravenously with 1 x 10^7 *L. chagasi* promastigotes. LACK (*Leishmania* homologue of receptors for activated C kinase) is a 36 kDa protein highly conserved between different life cycle stages and species of *Leishmania* and was previously shown to protect against cutaneous infection by *L. (L.) major*, by redirecting pathogenic Th2 to protective Th1 response when administered as a 24 kDa truncated version. Initially, we tested the capability of the vaccine to induce immune response by measuring the production of IFN-gamma and IL-4 by spleen cells stimulated with *L. (L.) chagasi* antigen (50, 100 and 150 µg/mL of soluble antigen and 50 µg/mL of freeze-thawed antigen) or p36(LACK) protein (25 µg/mL) - measured by ELISA - without challenge. In addition, we tested the vaccine capability of protection through determination of parasite burden in spleen and liver by quantitative limiting-dilution culture, four weeks after challenge (time related with the peak of liver parasite burden). Our results show that intramuscular vaccination with p36(LACK) DNA induces a Th1 response (IFN-gamma but not IL-4 production) when spleen cells are stimulated with 50, 100 or 150 µg/mL of *L. (L.) chagasi* soluble antigen, 50 µg/mL of freeze-thawed promastigotes or 25 µg/mL of p36(LACK) protein. In addition, subcutaneous immunization with p36(LACK) only induces Th1 response if spleen cells are stimulated with either 150 µg/mL of *L. (L.) chagasi* soluble antigen or 25 µg/mL p36(LACK) protein. However, this vaccination does not protect against intravenous challenge with 1 x 10^7 *L. (L.) chagasi* promastigotes at 30 days or 12 weeks after booster.

This research is sponsored by: FAPEMIG and PROPP/UFOP

IM55 - NASAL VACCINATION WITH HSP65 DNA-LOADED MICROSPHERES INDUCES IFN-G AND PROTECTS MICE AGAINST LEISHMANIASIS.

Rayol, A[1]; Pinto, E.F.[1]; Lima, K.M.[2]; Rodrigues Jr., J.M.[2]; Silva, C.L.[2] and Rossi-Bergmann, B. [1]

1- Institute of Biophysics, Federal University of Rio de Janeiro, Brazil. 2- Faculty of Medicine of Ribeirão Preto (FMRP), University of São Paulo (USP), Brazil.

Leishmaniasis shares various pathological and immunological features with tuberculosis. Especially by the fact that both are caused by intracellular pathogens, and that the protection for both diseases would be provided by cellular immune response. The highly conserved *M.leprae* 65-kDa heat shock protein (HSP65) codified by plasmid DNA (HSP65-DNA) has been shown to effectively control tuberculosis in mice when administered by the intramuscular route. The DNA vaccine was further ameliorated by association with the adjuvant trehalose dimycolate (TDM) followed by entrapment in biodegradable poly-DL-lactide-co-glycolide (PLGA) microspheres. In this work we evaluated the effectiveness of the HSP65-DNA/TDM/PLGA to protect BALB/c mice against cutaneous leishmaniasis using the mucosal (nasal) route of vaccination. The results showed that animals nasally vaccinated with HSP65-DNA/TDM/PLGA microspheres significantly controlled lesion development throughout the infection with fluorescent *L. (L.) amazonensis*, while none of the controls were effective. The parasite loads measured by tissue fluorescence were also significantly smaller in the vaccinated animals. Nasal HSP65-DNA/TDM/PLGA did not affect the basal production of IFN-γ in the mucosa-draining lymph nodes, but significantly increased IL-10 production. Protection was accompanied by a 7-fold enhancement in IFN-γ production in the lesion-draining lymph nodes as compared with non-vaccinated animals. This work showed that nasally instilled DNA codifying *M. leprae* HSP65 entrapped in DMT/PLGA microspheres could effectively protect BALB/c mice against *L. (L.) amazonensis* infection. These preliminary results highlight the feasibility of developing a nasal DNA vaccination against cutaneous leishmaniasis.

Support by: Brazilian Network of Nanobiotechnology (CNPq), REDE-TB (CNPq), FAPERJ and FAPESP.

IM56 - VACCINATION OF SWISS ALBINO MICE AGAINST KALA-AZAR WITH THE FML-VACCINE. USING A SAPOGENIN ADJUVANT

Casas C.P.[1], Paraguai de Souza E.[1], Palatnik M.[3], Parente J.P.[2] da Silva BP[2], Tinoco LW[4] and Palatnik de Sousa, C.B.[1]

Instituto de Microbiologia "Prof. Paulo de Góes" [1], Fac. Medicina-HUCFF [2], Núcleo de Pesquisas em Produtos Naturais [3], CNRNM-Bioquimica [4], UFRJ, CP68040, CEP 21941-590, RJ, RJ.

The FML-vaccine using Riedel De Haen saponin as adjuvant, developed a significant protective effect in the isogenic CB hamster (87.7%, $p<0.01$) and the Balb/c (84.4%, $p<0.001$) models. The FML+Quil-A saponin vaccine also induced protection against infection with *L. (L.) donovani,* in the canine experimental model. Although the adjuvant activity of saponins seems to be extensively proved, an undesirable hemolytic effect has been pointed out as the main restriction to their use in human vaccination. Removal of the glicidic moiety of several saponins abolished their hemolytic effect. In the present work we isolated the Riedel de Haen total sapogenin fraction through acid hydrolysis (2 N H2SO4 at 100 °C for 1h) and filtration. The residue was subjected to column chromatography on silica gel (10 g, 0.6 × 10 cm), eluted with chloroform-methanol (97.5 : 2.5, v/v) (5 ml each eluent). The fractions 40-45 ml showed similar thin layer chromatography profiles in the above described solvent system (R_f 0.40). The product (96 mg) was identified as quillaic acid by spectroscopic methods. The chemical removal of saponin glicidic moieties gave rise to its sapogenin fraction. Its ^{1}H NMR spectrum showed the presence of two signals (d 9.226 and 9.236). The intensity of the signals suggested two conformational isomers of sapogenin R in the ratio 53% of equatorial aldehyde group to 47% of axial aldehyde group. We immunized Swiss females with three doses of 150 mg FML + 100 mg Riedel sapogenin, through the sc route. Control animals received saline or sapogenin R. Thirteen days after the third doses, sera were collected and analyzed for the presence of anti-FML antibodies by the FML-ELISA assay. The levels of anti-FML specific antibodies are expressed as $\log_2$ titers as follows:

Treatment	Total IgG	IgG2a	IgG2b
SALINE	7	5	8
SAPOGENIN R	12	10	15
FML+SAPOGENIN R	18	14	18

XXX ANNUAL MEETING ON BASIC RESEARCH IN CHAGAS DISEASE - XIX MEETING OF THE BRAZILIAN SOCIETY OF PROTOZOOLOGY - HOTEL GLÓRIA, CAXAMBU, MG, BRASIL - 10-12 NOVEMBER 2003. **Rev. Inst. Med. trop. S. Paulo, 45**(Suppl. 13), November, 2003.

Significant and specific increases in antibody levels (more than two dilutions) were detected in animals vaccinated with FML+ sapogenin in all subclasses of immunoglobulins. Both saponin and sapogenin potentialyzed the synthesis of both IgG2a and IgG2b immunoglobulins related to complement fixation and protection against infection. The IDR to promastygote lysate was slightly higher in FML treated animals than in controls, although non significant differences were founded between the groups. The chemical treatment abolished the saponin slight *in vivo* toxicity, did not affect their aldehyde contents, but gave rise to an enriched axial aldehyde-containing sapogenin R with enhanced potential on antibody humoral response.

Support: CNPQ-UNIVERSAL, FINEP, CEPG-UFRJ, FUJB-UFRJ, FAPERJ.

IM57 - SAPONIN FRACTIONS IN IMMUNOTHERAPY AGAINST EXPERIMENTAL VISCERAL LEISHMANIASIS WITH THE FML-VACCINE.

Casas C.P.[1], Paraguai de Souza E.[1] and Palatnik de Sousa, C.B.[1]

Instituto de Microbiologia " Prof. Paulo de Góes" [1], Fac. Medicina-HUCFF [2], Universidade Federal do Rio de Janeiro, Caixa Postal 68040, Rio de Janeiro, 21941-590, RJ, Brasil.

The chemotherapy used in control of the human leishmaniasis doesn't guarantee the cure of canine disease. A vaccine therapy against the disease might be an alternative treatment, avoiding dog sacrifice and reducing the reservoir of *L. (L.) donovani* (LD1S) infection. Besides, it could give rise to data about an alternative therapy for human disease. In a previous work, was demonstrated the potential immunoprotective effect of the FML-saponin vaccine, in the isogenic CB hamster (87.7%, $p<0.01$), in the Balb/c (84.4%, $p<0.001$) and Swiss albino mice (85%, $p<0.01$) models. In this work we analized the possible immunotherapeutic effect of the FML- vaccine using purified saponin fractions on Swiss Albino mice infected with *L. (L.) chagasi.* 2-4 months old females were infected with 2 x 10^8 *L. (L.) chagasi* amastigotes by the i.v. route. Fifteen days after infection, animals were treated with three weekly s.c. doses of FML antigen (150 µg) combined with Riedel De Haën saponin or its fractions (100ug each) obtained through silica gel-column chromatography, using $CHCl_3$:MeOH: CH_3COOH:H_2O (15:9:1:2): F1 (tubes 1-2), F2 (tubes 3-14) and F3 (tubes 15-21). F1 (apolar) and F3 (polar) are composed of one different saponin each. Apparently, F2 contains the former two saponins. Fifteen days after the third dose, the delayed type of hypersensitivity (DTH) against *L. (L.) donovani* f/t promastigote lysate antigen was evaluated. The DTH response was significantly higher in mice vaccinated with FML + Riedel De Haën saponin over the saline control (0.42-0.35mm), both at 24h ($p<0.005$) and 48h ($p<0.005$) after injection. Mice treated with FML + F2 fraction showed an even higher response (0.58mm) at 24h post injection ($p<0,005$) suggesting that F2 contains the main active component of Riedel De Haen saponin. and the LDU in liver are in process.

Support: CNPQ-UNIVERSAL, FINEP, CEPG-UFRJ, FUJB-UFRJ, FAPERJ.

IM58 - EFFECTIVE IMMUNOTHERAPY AGAINST CANINE VISCERAL LEISHMANIASIS WITH THE FML-VACCINE

Gagini, T.B[1].,Borja-Cabrera, G.P[1], Cruz Mendes, A [1], Paraguai de Souza, E [1], Hashimoto Okada, LY [2], Trivellato, FA [2], Kawasaki, JKA[2], Cerqueira Costa, A [2], Barbosa Reis, A [3], Genaro, O [4 #], Palatnik, M [5] and Palatnik-de-Sousa, CB*[1].

Instituto de Microbiologia, "Prof. Paulo de Góes" [1], Universidade Federal do Rio de Janeiro (UFRJ), CCS, Cidade Universitária, Ilha do Fundão. CP 68040. CEP 21941-590. Rio de Janeiro, RJ, Brasil. Clínica Veterinária de Cães de Gatos [2], Araçatuba, SP, CP Renée Rachou-Fiocruz,MG-Universidad Federal de Ouro Preto [3], Departamento de Parasitologia [4], Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil. Faculty of Medicine-University Hospital " Clementino Fraga-Filho" [5], Federal University of Rio de Janeiro, Rio de Janeiro, RJ, Brazil. [#]In memorian.

American visceral leishmaniasis, is an important canine zoonosis against which there is not efficient treatment. Seropositive infected dogs are sacrificed for epidemiological control. Previous studies with the FML-vaccine showed protection against experimental and field kala-azar. Recently we demostred the potential effect of the FML-QuilA saponin vaccine on immunotherapy in five mongrel dogs experimentally infected with *L. (L.) donovani*. A strong protective effect was obtained in 3/5 immunotherapy treated dogs that remained asymptomatic, IDR positive, parasite free and with normal proportions of CD4 and CD21 lymphocytes. CD8 proportions were significantly increased as expected for a *Quillaja* saponin vaccine treatment. In the present work we analized the immunochemotherapy effect of FML-QuilA saponin vaccine in two symptomatics naturally infected Rotweiller dogs that were previously treated with glucantime and allopurinol. The clinical signs only disappeared after the complete vaccination. The immunochemotherapy treated dogs also remained asymptomatic, IDR positive and parasite free, up to 3 years after vaccination and with a significantly reduced ($p<0.05$) time for cure and intensity of symptoms when compared with immunotherapy treated group. Furthermore, the FML-saponin R was used on 21 naturally infected dogs when seropositive to FML but completely asymptomatic. They showed stable anti-FML IgG1 levels, increasing IgG2 levels and 79-95% of positive DTH response, during the whole experiment. Twenty-two months after complete vaccination, 90% of these dogs were still asymptomatic, healthy and parasite free, indicating that the FML-saponin R formulation was effective in the immunotherapy against visceral leishmaniasis of asymptomatic infected dogs.

Support: PCDEN and PNUD-FNS; FINEP; CNPQ; CAPES; MCT/PRONEX; CNPQ; RHAE-CNPQ; FUJB-UFRJ; FAPERJ; CEPG-UFRJ, Brazil.

IM59 - SWISS ALBINO MICE VACCINATION WITH THE RECOMBINANT NH36 NUCLEOSIDE HYDROLASE OF *LEISHMANIA (L.) DONOVANI*.

Gagini, TB, Paraguai de Souza E., Borja-Cabrera, GP, Motta, FN., Lobato, LS, Mendes, CO, Teixeira, EB, Dardo RS and Palatnik de Sousa CB.

Instituto de Microbiologia "Prof. Paulo de Góes", Universidade Federal do Rio de Janeiro. Cidade Universitária, Ilha do Fundão. CP 68040. CEP 21941-590. Rio de Janeiro, RJ, Brazil. Email: immgcpa@micro.ufrj.br

The 36-kDa glycoprotein fraction (GP36) of FML is recognized specifically by sera from human kala-azar patients and by monoclonal antibodies from mice immunized with FML. It protects Balb/c mice from experimental kala-azar (68%). The gene that encodes an antigenic protein component of GP36 was cloned on the basis of a partial peptide sequence. Based on the predicted open reading frame, the single-copy gene was identified as a nucleoside hydrolase (*Ld*NH) with significant similarity to family members identified from other kinetoplastids. NH enzymes play an important role in the acquisition of preformed purines from host sources. The NH36 gene PCR product was inserted downstream from the *malE* gene of *E. coli*, which encodes maltose-binding protein (MBP), resulting in the expression of an MBP fusion protein. In previous work we showed that NH36 protein (5,10 or 16 µg) in combination with Riedel De Haen saponin (100µg) induces a significant enhancement of the IgG2a and IgG2b subtype of antibodies, the *in vitro* proliferative and the DTH responses suggesting the triggering of a protective immune response. In the present work. We immunized Swiss females with three doses of 32 mg of P70 fusion protein (composed of both NH36 and the P40-Maltose Binding Protein MBP) in combination with 100 mg Riedel saponin, through the sc. route. Control animals received either saline, or saponin, or P40-MBP in combination with saponin or P40-MBP alone.

XXX ANNUAL MEETING ON BASIC RESEARCH IN CHAGAS DISEASE - XIX MEETING OF THE BRAZILIAN SOCIETY OF PROTOZOOLOGY - HOTEL GLÓRIA, CAXAMBU, MG, BRASIL - 10-12 NOVEMBER 2003. **Rev. Inst. Med. trop. S. Paulo, 45**(Suppl. 13), November, 2003.

Thirteen days after the third dose, sera were collected and analyzed for the presence of anti-FML antibodies by the FML-ELISA assay. The levels of anti-FML specific antibodies are expressed as log_2 titers as follows:

Treatment	IgG		IgG2a	
	Title	Log_2	Title	Log_2
Pre immune	1/16	4	1/16	4
Saline	1/32	5	1/32	5
P40	1/16	4	1/64	6
P70 + saponin	1/16384	14	1/512	9
P40 + saponin	1/2048	11	1/64	6

As expected from previous experiments with purified NH36, significant and specific increases in antibody levels were detected in animals vaccinated with P70 + saponin in IgG and IgG2a. immunoglobulins. However, the P40-MBP saponin control itself induces an immunogenic response indicating a degree of cross reactivity between the leishmanial antigen and the MBP control. An infective challenge with 2x 10^8 amastigotes of *L.(L.) chagasi* was performed. The evaluation of the possible vaccine effect on the reduction of parasitic load is under progress.

Support: CNPQ-UNIVERSAL, FINEP, CEPG-UFRJ, FUJB-UFRJ, FAPERJ.

IM60 - SERUM AND STOOL HUMORAL RESPONSE AND ANTIGEN-DRIVEN PEYER´S PATCHES LYMPHOCYTE PROLIFERATION IN MICE AFTER ORAL IMMUNIZATION WITH IRRADIATED *TOXOPLASMA GONDII* TACHYZOITES.

Galisteo Jr, A.J. [12], Hiramoto, R.M.[1], Carmo C.V. [1], Terentowicz, H.C. K. [1] & Andrade Jr., H.F.[1]

1. Instituto de Medicina Tropical de São Paulo – Lab. Protozoologia. 2. Supervisão de. Radiobiologia/IPEN CNEN SP

Toxoplasma gondii is a protozoan that infects mammalians and birds, causing usually benign disease in humans, except in intrauterine fetal infection or in immune deficient patients. The infection is acquired by ingestion of water and food contaminated with oocysts of feline feces or raw meat contaminated with tissue cysts. Thus, the study of the intestinal immunity in this disease is crucial for the vaccine production. In this work, RH strain tachyzoites of *T. gondii* were irradiated with 255 Gy in the 60-cobalt. Mice were immunized with 10^7 tachyzoites, for oral route, with three doses, suspended in milk (anti-peptic) or aluminum hydroxide (anti-acid) or both, for parasite preservation in the stomach. Specific ELISA for IgA and IgG detection was performed in collected feces and blood samples at weekly interval. Proliferation of Peyer´s patch lymphocytes from C57Bl/6j mice immunized with 255 Gy irradiated tachyzoites was compared to splenocytes from same mice, using infected mice with 10 cysts of Me49 strain as controls. After 15 days of the last oral dose, Peyer´s patches or spleen lymphocytes were suspended in RPMI 1640 medium supplemented with 10% FBS and antibiotics, and 2 x 10^5 cells/well were plated in 96 well plates and stimulated with *T. gondii* antigen (10 µmg/ml). After 48 h of incubation at 37ºC under 5% CO_2, cultures were pulsed for 18 h with [^{3}H] thymidine, harvested and their DNA radioactivity determined. There are few differences between the three challenged groups according to the preservative medium. The production of IgA and IgG in the serum was clearly seen after the first oral dose, increasing thereafter, more intense in the IgG response, but immunized mice produced smaller levels of serum antibodies as compared to chronically infected mice. Fecal IgG production was absent after immunization but IgA production was higher than controls during the first doses but decays after this, suggesting tolerance, with chronically infected mice presented higher levels of IgA in feces. However, studies in cell proliferation of Peyer´s patches lymphocytes shows a great response in immunized mice, specially at the 1st and 2nd doses, similar or higher than chronically infected mice, but, after the 3rd dose, this proliferation subsides. Challenging studies are in progress. Those data shows clearly that the development of mucosal immunity could be attained after oral immunization with sterile parasites, but more improvements must be attained before a complete understanding of mucosal immunity in toxoplasmosis. Our approach would be a good tool for studies in vaccine development in this model, especially for the first scrutiny of oral vaccines that could be used in field for free-living or stray cats, one of the main sources of *Toxoplasma* infection of ruminants.

This work was supported by FAPESP (99/04926-6), LIMHCFMUSP-49, CNPq and CAPES

IM61 - DETECTION OF ANTI-LACK ANTIBODIES IN VISCERAL LEISHMANIASIS CANINE SERA

1-Kádima Nayara Teixeira, 1-Eduardo Antônio Ferraz Coellho, 1-Carlos Alberto Pereira Tavares e [+]Ana Paula Fernandes

1-Department of Biochemistry and Immunology and [+]School of Pharmacy, Federal University of Minas Gerais, 31270-910, Belo Horizonte, MG

Current serologic tests for the diagnosis of leishmaniasis, such as direct agglutination test (DAT), immunofluorescent-antibody test (IFAT) and the enzyme-linked immunosorbent assay (ELISA) use crude antigen preparations and are limited in terms of both specificity and assay reproducibility. Thus, emphasis has been placed on the characterization of *Leishmania* antigenic components as a tool for obtaining specific diagnosis. The LACK (*Leishmania* homolog of receptors for activated C kinase) antigen is a 36 KDa protein highly conserved and expressed in promastigote and amastigote forms of different *Leishmania* species. Here, we have investigated the presence of anti-LACK antibodies in a panel of canine American Visceral Leishmaniasis (VL) sera. A PCR product spanning the *L. (L.) chagasi* LACK coding region was cloned into the pPROEX vector for expression in *Escherichia coli* and the LACK protein containing a tag of six histidin residues (LACK-HIS) was purified by Nickel affinity chromatography. IFAT Negative sera from asymptomatic dogs were used as negative controls and to establish cut-off values. Anti-LACK antibodies were detected by ELISA in a high proportion of sera of symptomatic, IFAT positive dogs, that have also tested positive in the parasitological evaluation. Anti-LACK antibodies were also detected in sera of asymptomatic dogs, but that tested positive in the parasitological evaluation. Our preliminary findings suggest that LACK protein is a potential tool for the serological diagnosis of VL in the New World.

Financial Support: FAPEMIG and CNPq-PRONEX

IM62 - DNA VACCINATION WITH A PLASMID CONTAINING THE GP72 GENE OF *TRYPANOSOMA CRUZI*.

PADILLA, A.M.[1], RODRIGUES, M.M.[2], BOSCARDIN, S.B.[2], PÉREZ BRANDÁN, M.C.[1], DIOSQUE, P.[1] and M.A. BASOMBRIO[1].

[1] Laboratorio de Patología Experimental, FCS, Univ Nac Salta. marpadill@hotmail.com; [2] Dep. de Microbiologia, Imunologia e Parasitologia. Univ Fed São Paulo. Brasil.

The GP72 is a glicoprotein of *Trypanosoma cruzi* involved in the adhesion of the flagellum to the parasite's body. The deletion of the gp72 gene produces atypical forms of reduced infectivity in mice. Here we tested the immunogenicity of a gp72 containing-plasmid as a DNA vaccine against the *T. cruzi* infection. Female Balb/c mice were immunized with four doses of 100 ug of the plasmid. The control group received same doses with the empty vector plasmid pcDNA3.

XXX ANNUAL MEETING ON BASIC RESEARCH IN CHAGAS DISEASE - XIX MEETING OF THE BRAZILIAN SOCIETY OF PROTOZOOLOGY - HOTEL GLÓRIA, CAXAMBU, MG, BRASIL - 10-12 NOVEMBER 2003. **Rev. Inst. Med. trop. S. Paulo, 45**(Suppl. 13), November, 2003.

After the vaccination schedule, mice's sera were taken in order to perform ELISA assays. The delayed type hypersensibility (DTH) reactions were recorded and the mice were challenged with Tulahuén blood-trypomastigotes. The DTH reactions of the immunized mice were increased ($p<0.05$) compared with the control's reactions, although the optical densities of the sera tested by ELISA were not different between the groups. After the challenge, the immunized mice displayed a slightly lower level of parasitemia than the control group. On the basis of the present results we will attempt to improve the vaccine's immunogenicity employing the gp72-containing plasmid, testing different immunization schedules or multi-component vaccines.

This work was supported by CONICET and FAPESP.

IM63 - IMMUNIZATION WITH THE A2 ANTIGEN AGAINST *LEISHMANIA (L.) AMAZONENSIS* INFECTION: COMPARISON OF THE PROTECTION INDUCED BY PROTEIN OR DNA VACCINATION.

*Eduardo Antonio Ferraz Coelho; **Ana Paula Salles Moura Fernandes; *Ricardo Tostes Gazzinelli; ***Fernando Aécio de Amorim Carvalho; *Kadima Nayara Teixeira; ** Bruna Araújo Gomes; ** Josilene Pereira Costa; *Carlos Alberto Pereira Tavares

*DEPARTAMENTO DE BIOQUÍMICA E IMUNOLOGIA/ICB/UFMG; **DEPARTAMENTO DE ANÁLISES CLÍNICAS E TOXICOLÓGICAS/ FACULDADE DE FARMÁCIA/UFMG; ***UNIVERSIDADE FEDERAL DO PIAUÍ/ UFPI

Successful host protection in experimental leishmaniasis involves a complex Th1 cell-dependent, multicytokine–mediated mechanism. This response, that is probably initiated by IL-12 and then driven primarily by IFN-γ, culminates with the macrophage activation and intracellular killing of parasites. Vaccination with plasmid DNA encoding a specific leishmanial antigen has been shown to be more effective than vaccination with leishmanial protein plus IL-12 in sustaining the Th1 responses required for long-term protection. Besides, recombinant proteins are more expensive and time consuming to produce in relationship to plasmid DNA. Here, we report experiments comparing the efficacy of vaccination with A2 protein and A2 DNA against *L. (L.) amazonensis* infection. The A2 gene was cloned into the pET-16b and pCDNA3 vectors, for expression in *Escherichia coli* and eukariotic cells, respectively. Groups of female BALB/c mice were immunized *subcutaneously* with 2 doses, at 3 weeks intervals, with A2-HIS protein (50 µg) alone or in combination with rIL-12 (1 µg) or 250 µg of *Corynebacterium parvum*. For DNA immunization, animals received the A2-pCDNA3 plasmid (100 µg) alone or in combination with the IL-12-pCI plasmid (100 µg) by *intramuscular* injection in the tibia muscle. Mice were infected 4 weeks after the last dose with $1x10^5$ stationary phase promastigotes of *L. (L.) amazonensis.* Our findings demonstrated that A2–HIS protein induced a parasite specific Th1 immune response, characterized by the high levels of IFN-γ and low levels of IL-4 or IL-10 and provided protection against challenge infection only in the presence of Th1 adjuvants, including *C. parvum.* A2 DNA vaccination induced a specific Th1 immune response and provided protection against challenge infection with *L. (L.) amazonensis* in the absence of IL-12 DNA.

Support: CAPES, PRPq/UFMG.

IM64 - IMMUNE RESPONSES INDUCED BY *LEISHMANIA (L.) DONOVANI* A2, BUT NOT BY LACK ANTIGEN, ARE PROTECTIVE AGAINST EXPERIMENTAL *LEISHMANIA (L.) AMAZONENSIS* INFECTION

*Eduardo Antonio Ferraz Coelho; **Ana Paula Salles Moura Fernandes; *Ricardo Tostes Gazzinelli; ***Fernando Aécio de Amorim Carvalho; *Kadima Nayara Teixeira; *Carlos Alberto Pereira Tavares

*DEPARTAMENTO DE BIOQUÍMICA E IMUNOLOGIA/ICB/UFMG; **DEPARTAMENTO DE ANÁLISES CLÍNICAS E TOXICOLÓGICAS/ FACULDADE DE FARMÁCIA/UFMG; ***UNIVERSIDADE FEDERAL DO PIAUÍ/ UFPI

Leishmania (L.) amazonensis is one of the major etiologic agents of a broad spectrum of clinical forms of leishmaniasis and has a wide geographical distribution in Americas, which overlaps with the areas of transmission of many other *Leishmania* species. The LACK and A2 antigens are shared by various *Leishmania* species. A2 was previously shown to induce a potent Th1 immune response and protection against *L. (L.) donovani* infection in BALB/c mice. LACK is effective against *L. major* infection, but no significant protection was observed against *L. (L.) donovani* infection, in spite of the induction of a potent Th1 immune response. In an attempt to select candidate antigen for American leishmaniasis vaccine, we investigated the protective effect of these recombinant antigens (rLACK and rA2) and interleukin 12 (rIL-12) against *L. (L.) amazonensis* infection in BALB/c mice. As expected, immunization with either rA2/IL-12 or rLACK/IL-12 was able to induce a robust Th1 response prior to infection. However, only the BALB/c mice immunized with rA2/IL-12 were protected against infection. A sustained IFN-γ production, high levels of anti-A2 antibodies and low levels of antiparasite specific antibodies were detected in these mice, after infection. In contrast, mice immunized with rLACK/IL-12 displayed decreased levels of IFN-γ and high levels of both anti-LACK and parasite specific antibodies. Curiously, the association between rA2 and rLACK antigens in the same vaccine completely abrogated the rA2 specific IFN-γ and humoral responses and, consequently, the protective effect of the rA2 antigen against *L. (L.) amazonensis* infection. We concluded that A2, but not LACK fits the requirements to compose a safe vaccine against American leishmaniasis.

Support: CAPES, PRPq/UFMG.

IM65 - PROTECTIVE IMMUNITY AGAINST *TRYPANOSOMA CRUZI* INFECTION IN A HIGHLY SUSCEPTIBLE MOUSE STRAIN FOLLOWING SIMULTANEOUS ADMINISTRATION OF PLASMIDS CONTAINING GENES EXPRESSED IN DIFFERENT DEVELOPMENTAL STAGES.

Vasconcelos JR[1], Boscardin SB[1], Marinho CRF,[2] Alvarez JM[2] and Rodrigues MM[1]

[1]Universidade Federal de São Paulo (UNIFESP-EPM), São Paulo, Brazil, [2] ICB, Universidade de São Paulo, São Paulo, Brazil. E-mail address: ronnie@ecb.epm.br

Genetic immunization of BALB/c or C57BLl/6 mice with plasmids containing a single *Trypanosoma cruzi* gene induced protective immunity against lethal parasitic infection. In contrast, similar immunization of the highly susceptible mouse strain A/Sn did not promote mouse survival after challenge. Here, we tested whether the protective efficacy of genetic vaccination in this mouse strain could be improved by combining two different plasmids in the same immunization regimen. We used plasmids containing genes encoding the catalytic domain of *T. cruzi trans*-sialidase or the Amastigote Surface Protein-2. These antigens are expressed on the surface of trypomastigote or amastigote forms, respectively. In spite of the fact that these two antigens share some structural identity, we observed that immune responses were specific for the homologous recombinant antigen. After challenge with trypomastigotes of the Y strain, a significant proportion (>85%) of A/Sn mice immunized simultaneously with both plasmids survived the infection. Mice immunized with individual plasmids had a variable survival and 100% of control mice died. Histological

XXX ANNUAL MEETING ON BASIC RESEARCH IN CHAGAS DISEASE - XIX MEETING OF THE BRAZILIAN SOCIETY OF PROTOZOOLOGY - HOTEL GLÓRIA, CAXAMBU, MG, BRASIL - 10-12 NOVEMBER 2003. **Rev. Inst. Med. trop. S. Paulo, 45**(Suppl. 13), November, 2003.

studies performed in the surviving mice 100 days after challenge revealed a reduced inflammatory response in most tissues with the exception of the liver.

Our results provided evidence that a significant proportion of the highly susceptible A/Sn mice can develop protective immunity against lethal infection if immunized simultaneously with plasmids harboring genes expressed in different mammalian developmental stages of the parasite.

Supported by FAPESP and CNPq.

IM66 - RESPONSE OF IL-12P40$^{-/-}$ C57BL/6 AFTER VACCINE-INDUCED IMMUNITY AGAINST *LEISHMANIA (L.) AMAZONENSIS* INFECTION

Hernández S., M. X., Barçante, T., Afonso, L. C. C., Vieira, L. Q.

Departamento de Bioquímica e Imunologia - Instituto de Ciências Biológicas - UFMG

Protozoa of the genus *Leishmania* are intracellular parasites and have been identified from patients with diverse clinical forms, including cutaneous leishmaniasis, diffuse cutaneous leishmaniasis (DCL), and visceral leishmaniasis. Infection of mice with *L. (L.) major* leads to the development of either a Th1 or a Th2 response. While activation of cells secreting IL-4 promotes a Th2 response, production of IL-12 induces secretion of IFN-γ by natural killer cells and T cells and favors Th1 cell differentiation and proliferation. A Th1 response plays a key role in controlling the infection. In agreement with these findings, adoptive or vaccine-induced protection against leishmaniasis is largely dependent on cell-mediated immunity, Th 1 lymphocytes and IFN-γ. Induction of a Th1 response is dependent on the presence of IL-12 whilst lymphocytes are activated. This study was aimed at evaluating the immunogenicity of a vaccine composed of killed *Leishmania (L.) amazonensis* promastigotes (Leishvacin®), produced by Biobrás, since the role of Th1 cytokines in vaccine-induced immunity with Leishvacin® in experimental models or in humans is not yet elucidated. To evaluate the role of IL-12 in the vaccine-induced immunity against *L. amazonensis* infection, C57BL/6 Interleukin-12-Deficient Mice (IL-12p40$^{-/-}$ C57BL/6) and wild type controls (WT) were vaccinated in the base of the tail. Each animal received two inoculations at an interval of seven days, each dose containing 100 µg of protein vaccine plus 250 µg of *Corynebacterium parvum*. Twenty-eight days after the second dose, the animals received a further 10 µg of vaccine, without adjuvant. Seven days after this booster, the animals were challenged with *L. (L.) amazonensis* in the left hind footpad. Lesion size was measured for 10 weeks. As described previously, this protocol of vaccination protected C57BL/6 mice against infection: these mice showed smaller lesions and smaller parasite numbers than non-vaccinated controls. IL-12p40$^{-/-}$ mice were more susceptible to infection than WT, developing large and progressive lesion. Comparison between vaccinated and non-vaccinated IL12p40$^{-/-}$ mice showed a statistically significant difference from 3 to 10 weeks post infection. Lymph node and spleen cell cultures from C57BL/6 vaccinated mice presented higher levels of IFN-γ when compared to non-vaccinated mice. Cells from IL-12p40$^{-/-}$ mice produced practically undetectable levels of IFN-γ *in vitro*, regardless of vaccination. IL-4 was not detected in supernatants from lymph node or spleen cell cultures. Our results show that the early control of de *L. (L.) amazonensis* infection conferred by vaccination is independent of IL-12. It is possible that IFN-γ from CD8^{+} cells is playing a role in controlling parasite growth in vaccinated IL-12p40$^{-/-}$ mice.

Support: CAPES and FAPEMIG

IM67 - ISOLATION, SEQUENCING AND EXPRESSION OF GENES FROM *L. (VIANNIA) BRAZILIENSIS* ENCONDING PROTECTIVE ANTIGENS AGAINST CUTANEOUS LEISHMANIASIS.

Salay, G., Dorta, M.L., Barbiéri, C.L. & Rodrigues, M.M.

Disciplina de Parasitologia, Universidade Federal de São Paulo-Escola Paulista de Medicina, São Paulo, SP, Brasil. salay@ecb.epm.br

IMmunization with genes encoding antigens *Leishmania* Activated C Kinase (*lack*), Thiol-Specific Antioxidant (*tsa*), *Leishmania* Elongation Initiation Factor (*leif*) and *Leishmania (L.) major* Stress Inducible Protein 1 (*lmsti1*) from *Leishmania (Leishmania) major* induces potent immune response mediated by CD4 Th1 cells and protective immunity against *L. (L.) major* infection in highly susceptible BALB/c mice (reviewed by Reed & Campos-Neto, 2003, Curr Opin Immunol. 15:456). The aim of the present study was to isolate, sequence and express these genes from *L. (V.) braziliensis*, the specie widely distributed in Brazil and implicated with cutaneous and mucocutaneous leishmaniasis. The genes were isolated by PCR amplification using genomic DNA from *L. (V.) braziliensis* strain M2903 and primers corresponding to the ORF of each gene, cloned in *pMOS* vector, and sequenced. The predict amino acid sequence analysis of *lack*, *tsa*, *leif*, and *lmsti1* showed, respectively, 96%, 83%, 98%, and 92% of identity to that described in *L. (L.) major*. Northern blot analysis showed that these genes hybridize with transcripts present in both promastigotes and amastigotes of *L. (V.) braziliensis.* The genes were also subcloned in prokaryotic expression vector PET-22b (+) or the eukaryotic expression vector pcDNA3 in fusion with the nucleotide sequence encoding the signal peptide of the mouse Ig kappa chain. The bacterial recombinant proteins were successfully purified. The plasmids harboring these genes isolated from of *L. (V.) braziliensis* are currently been tested in their ability to induce immune responses in mice.

Supported by FAPESP, CNPq and CAPES.

IM68 - *TOXOPLASMA* ENVIRONMENTAL SPREAD EVALUATED BY STRAY DOG SEROLOGY IN COASTAL AREAS FROM SOUTHERN BRAZIL.

Terentowicz H. C. K.[1], Inoue M. T.[2], Meireles L. R.[1],. Galisteo Jr. A. J.[1], Hiramoto R. M.[1] & Andrade Jr. H. F.[1]

1 – Laboratório de Protozoologia do Instituto de Medicina Tropical de São Paulo/USP; 2 – CECAN – Centro Comunitário de Apoio aos Animais de Caraguatatuba/SP

In São Paulo, nearly 60% of adults were infected by *Toxoplasma gondii*, a protozoan that can cause human disease in minor segments of the infected people, specially when congenital infection or immune disease coexists with the infection. The disease is transmitted orally by ingestion of cysts in raw meat or oocysts from cat stools, with spreading to the environment. The detection of those infective forms are difficult and depends on sample size, as the agent had an exquisitely highly efficient transmission. In domestic animals, like dogs, toxoplasmosis was usually asymptomatic, despite some reports of occasionally severe disease, more frequent in younger dogs. There are few reports of an ocular involvement associated to the toxoplasmosis in dogs. Both *Toxoplasma* infective forms infected dogs, usually omnivorous and carnivorous. Recently, there are reports of the seroprevalence of this infection in stray dogs, inferring that this measure could represent the environmental risk of *Toxoplasma* contamination. Based on this meaning, we studied 151 blood samples colleted from stray dogs from Caraguatatuba, at northern coast of São Paulo State in Southern Brazil, that were captured usually to control other zoonotic diseases, as rabies or leptospirosis. The sero-prevalence of *T. gondii* infection was determined by a specific IgG ELISA,

XXX ANNUAL MEETING ON BASIC RESEARCH IN CHAGAS DISEASE - XIX MEETING OF THE BRAZILIAN SOCIETY OF PROTOZOOLOGY - HOTEL GLÓRIA, CAXAMBU, MG, BRASIL - 10-12 NOVEMBER 2003. **Rev. Inst. Med. trop. S. Paulo, 45**(Suppl. 13), November, 2003.

with inconclusive results confirmed by Western-blot strips from *T.gondii* saline extracts. We found a prevalence of 55.1% (47.0 – 63.0% 95%CI) in those dogs, similar to those reported in other costal areas as Trinidad & Tobago and also in urban areas of São Paulo State. Those data suggests that there is a high environmental contamination in those areas, especially when the incidence was corrected to the short life span of dogs, resulting in a almost 8-10% year conversion. Those data provides an indirect but useful tool that could help in the evaluation of environmental measures to reduce toxoplasmosis incidence in risk areas.

HCK Terentowicz is a fellow of FUNDAP and this research was supported by CECAN, LIMHCFMUSP.

IM69 - USE OF A CYSTEINE PROTEINASE FROM *LEISHMANIA (L.) CHAGASI* IN SERODIAGNOSIS OF AMERICAN VISCERAL LEISHMANIASIS

Dias, S.S., Dorta, M.L., Katz, S., Santos, M.R.M. & Barbiéri, C.L.

Disciplina de Parasitologia, Escola Paulista de Medicina, Universidade Federal de São Paulo, Rua Botucatu, 862, Caixa Postal 20342, 04023-062, São Paulo, SP, Brasil. suzi.dias@bol.com.br

Visceral leishmaniasis (VL) is a parasitic disease associated with high mortality, caused by *Leishmania (L.) donovani*, *L.(L.) infantum* and *L. (L.) chagasi*. Parasitological diagnosis, characterized by demonstration of parasites in tissue samples, is extremely limited by low sensibility and reproducibility (Singh et al., 2003). Serological tests, currently used in VL diagnosis, are based on different methods of antibody detection which include indirect immunofluorescence (IFI) (Pappas et al., 1983), direct agglutination test (DAT) (Harith et al., 1986), immunoblot analysis (Hoerouf et al., 1992), and enzyme-linked immunosorbent assay (ELISA) (Badaró et al., 1996). However, the use of whole parasite extract limits the test specificity due to the ocurrence of cross-reaction with other patogens such as *Trypanosoma cruzi*, *Echinococcus granuloses* and mycobacteria (Badaró et al.,1986; Chiller et al., 1990; Reed et al,1987), leading to the misinterpretation of the serological assays. Thus, the use of recombinant purified antigens has been emphasized as a tool for obtaining more specific serological tests. In the present work we evaluated the use of a recombinant cysteine proteinase from *L. (L.) chagasi* amastigotes (R30) in ELISA for diagnosis of American visceral leishmaniasis. The gene encoding the cysteine proteinase was previously isolated by PCR amplification, cloned in pHis vector and the resulting recombinant antigen was purified by affinity chromatografy in a His-tag column. ELISA was performed with serum samples from Brazilian areas endemic for kalazar and our preliminary data showed a sensibility of 71% when R30 was used as antigen. The test specificity was evaluated by use of serum samples from patients with tuberculosis, cutaneous leishmaniasis and Chagas' disease and showed a specificity of 92% with R30 as antigen, in contrast to that displayed with *L. (L.) chagasi* whole amastigote and promastigote extracts which was of 42 and 31%, respectively. These data suggest that the *L. (L.) chagasi* recombinant cysteine proteinase is an attractive antigen for diagnosis of VL. The evaluation of the sensibility and specificity of the *L. (L.) chagasi* recombinant protein as antigen in ELISA is currently extended to a higher number of kalazar samples, as well as with sera from patients with other diseases.

This research is sponsored by CAPES

IM70 - WESTERN BLOT IDENTIFICATION OF *PHYTOMONAS SERPENS* ANTIGENS RECOGNIZED BY HUMAN CHAGASIC SERA.

Menolli, R. A.[1*]; Graça, V.K.[1]; González A.D.[1]; Carvalho G. S.[1]; Itow Jankevicius S.[1]; Jankevicius J.V.[2]

[1]Universidade Estadual de Londrina, Londrina, PR, Brasil; [2]Universidade Norte do Paraná, Londrina, PR, Brasil. [*]E-mail: ramenolli@hotmail.com

Chagas' disease or American trypanosomiasis affects about 16 million people in Latin America and have great interest on account of the intense cross reactivity of Chagas' disease patients sera with sera from patients with other diseases, mainly caused by other flagellates, in the serologic diagnosis.The search of cheaper, safer and more efficient antigens for the discrimination of Chagas' disease patients has been the objective of many research projects, mainly with recombinant antigens. The immune cross reactivity of *Trypanosoma cruzi* with other Trypanosomatidae family members was early largely demonstrated and in the present work some antigens of fruit flagellates (*Phytomonas serpens*) was demonstrated to be involved in immune cross reactions with *T. cruzi*. *P. serpens* proteic bands recognized by antibodies present in Chagasic and American tegumentary leishmaniasis patients sera was determined by Western blot technique.The results showed the bands of 250, 30 and 26 Kda as the most frequently recognized proteic bands of *P. serpens* by chagasic huma sera and 62, 53 and 43 kDa the most frequently recognized by leishmaniotic human sera. The same reaction was done with *T. cruzi* antigens and human chagasic and leishmaniotic sera.The results also showed strong cross reaction between these Trypanosomatids.The intense cross reactivity among Trypanosomatids and the possibility of induction of immunity by oral ingestion of plant flagellates suggest the possible participation of antigens of *P. serpens* in the evolution of Chagas' disease immunity.

Financial support: CNPq, CAPES and CPG/UEL.

IM71 - *TRYPANOSOMA RANGELI* AND *TRYPANOSOMA CRUZI*: COMPARATIVE STUDY OF SEROLOGICAL CROSS-REACTIVITY OF IMMUNE MICE SERUM AGAINST EPIMASTIGOTE AND TRYPOMASTIGOTE FORMS

Moraes M.H., Girardi F.P., Rodrigues J. B., Koerich L.B., Steindel M., Grisard E.C.

Departamento de Microbiologia e Parasitologia (MIP/CCB), Universidade Federal de Santa Catarina (UFSC), Caixa postal 476, Florianópolis, SC, 88040-900. E-mail: grisard@ccb.ufsc.br

Trypanosoma rangeli and *Trypanosoma cruzi*, the etiological agent of Chagas disease, are hemoflagellate protozoan parasites which infects a variety of mammals, including humans, in both Central and South America. Despite the non-pathogenic nature of *T. rangeli* to mammal hosts, this parasite plays an important role in Chagas disease epidemiology since triatomine vectors and mammalian reservoirs are shared in a wide geographical area. Comparative studies of the antigenic composition of culture epimastigotes of *T. cruzi* and *T. rangeli* revealed that these parasite species shares ate least 60% of their soluble antigenic composition. Thus, the antigenic overlap and the sympatry of these parasites have major influence on serological diagnosis of Chagas disease. The aim of this study was to evaluate the serological cross reactivity of immune mice serum infected with *T. cruzi* (Y strain) or *T. rangeli* (SC-58 and Choachi strains) against homologous and heterologous antigens (culture epimastigotes and trypomastigotes) by indirect immunofluorescence assays (IFA). Experiments were performed in duplicate using a anti-Mouse IgG conjugate labelled with FITC (Sigma). Our results showed that anti-*T. cruzi* serum was able to recognize both forms of either homologous and heterologous antigens. Anti-*T. rangeli* serum of both studied strains strongly reacted with homologous trypomastigotes and epimastigotes but weakly recognized heterologous trypomastigote forms. Moreover, anti-*T. rangeli* serum showed negative results when assayed with *T. cruzi* epimastigotes. These results suggest that infection by *T. rangeli* produces antibodies with higher specificity when compared with antibodies produced in response to *T. cruzi* infection. Also, the herein reported differences may be

XXX ANNUAL MEETING ON BASIC RESEARCH IN CHAGAS DISEASE - XIX MEETING OF THE BRAZILIAN SOCIETY OF PROTOZOOLOGY - HOTEL GLÓRIA, CAXAMBU, MG, BRASIL - 10-12 NOVEMBER 2003. **Rev. Inst. Med. trop. S. Paulo, 45**(Suppl. 13), November, 2003.

attributed to antigenic variation of the studied strains and forms. Further Western blot assays will be performed in order to compare the antigenic profiles of both epimastigote and trypomastigote forms of each parasite species.

Supported by UFSC and CNPq.

IM72 - CANINE VISCERAL LEISHMANIASIS IN ARAÇATUBA (SP): PARASITOLOGICAL, IMMUNOLOGICAL AND MOLECULAR DIAGNOSIS.

[1]Moreira, M.A.B.; [1]Luvizotto, M.C.R.; [1]Garcia, J.F.; [2]Corbett, C.E.P.; [2]Laurenti, M.D.

[1]Laboratório de Patologia do Curso de Medicina Veterinária da UNESP – Araçatuba (SP); [2]Laboratório de Patologia de Moléstias Infecciosas (LIM-50) da FMUSP (SP), Brasil. e-mail: mdlauren@usp.br

Aiming the improvement of the diagnosis of canine visceral leishmaniasis (CVL) in an endemic area at the northwest of São Paulo state – Brazil, the efficacy of parasitological, immunological and molecular diagnosis methods were studied. Animals, with and without clinical signs of the disease and with direct search of parasites positive in lymph nodes smears and/or antibodies detection by ELISA were selected for the study. According to the clinical signs of the disease, 89 dogs attended in the Veterinary Hospital of UNESP in Araçatuba (SP) were divided into three groups: symptomatic, oligosymptomatic and asymptomatic. Twenty-six dogs from non-endemic area of visceral leishmaniasis were used as negative controls. Fine-needle aspiration biopsy (FNA) of popliteal lymph node was collected, stained by Diff-Quick®, direct immunofluorescence and immunohistochemistry, as well as parasite DNA were amplified and detected by PCR in those samples. The dogs were classified as: symptomatic, 35%; oligosymptomatic, 22%, asymptomatic, 20%; and negative control, 23%. The sensitivity was 75.61% for direct search of parasite in FNA stained by Diff-Quick®, 92.68% for direct immunofluorescence, 92.68% for immunohistochemistry and 100% for PCR in the symptomatic group; 32%, 60%, 76% and 96% for the oligosymptomatic and 39.13%, 73.91%, 100% and 95.65% for the asymptomatic group, respectively. The control group, dogs from non-endemic area of visceral leishmaniasis, presented negative results in all the studied methods, reflecting a specificity of 100% of the methods used in the present study. The correlation between different clinical groups for each methodology showed the direct search of parasites and direct immunofluorescence as the best method for diagnosis of symptomatic cases. ELISA and the immunohistochemistry in FNA showed best results in the diagnosis of the asymptomatic cases. The results showed high sensitivity when immunolabelling techniques were used in relation to the direct search of parasites and the immunohistochemistry had higher sensitivity than the immunofluorescence. PCR presented the highest sensitivity among all the methods used, however, the method detects the parasite DNA and not the parasite itself and it does not reflect the stage of the infection and/or the disease.

Supported by LIM-50 HC/FMUSP, UNESP and FAPESP.

IM73 - CROSS-REACTION BETWEEN *LEISHMANIA (L.) CHAGASI* WHOLE ANTIGEN AND ANTIBODIES OF DOGS FROM NON ENDEMIC AREA INFECTED WITH *EHRLICHIA CANIS*, *TOXOPLASMA GONDII*, *BABESIA CANIS AND DIROPHILARIA IMMITIS* IN ELISA ASSAY USING PROTEIN A.

Dossi A.C.S,. Lima, V.M.F.

Department of Clinic, Surgery and Animal Reproduction, Veterinary Medicine - UNESP, Araçatuba, Sao Paulo - Brazil

Leishmaniasis is a disease caused by a *Leishmania* protozoa. This disease has great importance in public health because dogs are the domestic reservoirs. These protozoa can cause self-healing diseases and lethal visceral leishmaniasis. Infected dogs may develop a severe syndrome characterized by chronic evolution of viscerocutaneous signs, which result from *Leishmania* multiplication in macrophages of spleen, liver, bone marrow, lymph nodes and skin. ELISA is a useful method of diagnosis because of its high sensitivity and specificity. However, this method presents limitations due to cross-reaction with *Ehrlichia canis,* and *Babesia canis*. Serum from symptomatic mixed breed dogs from a region of high incidence of visceral leishmaniasis in Brazil were examined for the presence of antibodies using peroxidase conjugate of either protein A or anti-dog IgG in ELISA assay. The presence of cross-reaction between *L. (L.) chagasi* whole antigen and serum from dogs from areas non-endemic for leishmaniasis and infected with *Toxoplasma gondii* (6), *Ehrlichia canis* (15) e *Babesia canis* (10) and *Dirophilaria immitis* (19) was also investigated in both systems. The results showed that protein A ELISA system is more sensitive than anti-IgG to detect positive animals. In direct comparison with anti-immunoglobulin conjugate, enzyme-linked protein A resulted in higher absorbance values for positive sera without a corresponding increase in absorbance values for sera from non infected dogs. The effect was an increase in the distance between positive and negative values, which aided in the interpretation of the results. In addition, no cross-reaction occurred between *L. (L.) chagasi* whole antigen and serum tested. The ELISA optical density values of the tested serum were similar to those found in healthy dogs from non-endemic areas. These results are important and indicate that ELISA assay using *L. (L.) chagasi* whole antigen from promastigotes associated with protein A can be useful to serological diagnosis of visceral leishmaniasis in dogs.

Supported by FAPESP and FUNDUNESP.

IM74 - THE USE OF THE *TRYPANOSOMA CRUZI* RECOMBINANT COMPLEMENT REGULATORY PROTEIN TO EVALUATE THERAPEUTIC EFFICACY FOLLOWING TREATMENT OF CHRONIC CHAGASIC PATIENTS

Wendell S. F. Meira[1,4], Lúcia M. C. Galvão[1], Eliane D. Gontijo[2], George L. L. Machado-Coelho[3], Karen A. Norris[4], Egler Chiari[1]

Departamento de Parasitologia, Instituto de Ciências Biológicas[1]; Faculdade de Medicina[2] Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais; Escola de Farmácia,Universidade Federal de Ouro Preto[3], Ouro Preto, Minas Gerais, Brasil and Department of Immunology, University of Pittsburgh[4], Pittsburgh, Pennsylvania, USA

One of the greatest concerns in Chagas' disease is the absence of reliable methods for the evaluation of chemotherapy efficacy in treated patients. The available tests, which are able to evaluate cure after the specific treatment, are the complement mediated lysis (CoML) and flow cytometry, but they are not feasible for routine clinical use. In this study, we evaluated an ELISA test based on the recombinant *Trypanosoma cruzi* complement regulatory protein (rCRP) as a method to determine parasite clearance in comparison to the CoML and other methods such conventional serology, hemoculture and PCR in sera samples of 31 patients collected before and after the treatment, followed for an average of 27.7 months after chemotherapy. The results showed that the percentage of patient samples that were positive by rCRP ELISA was reduced from 100% to 70.3%, 62.5%, 71.4% and 33.4% in the first, second, third and fourth years after treatment respectively, while the samples positive by CoML were reduced to 85.2%, 81.2%, 71.4% and 33.4% during the same period, demonstrating the same significant tendency in the reduction of positive samples. On the other hand, the conventional serology (CS) tests did not present this reduction. The percentage of samples positive by PCR was initially 77.4%, and decreased to 55.5%, 68.7%, 47.7% and 50.0% at the fourth year after treatment, confirming

XXX ANNUAL MEETING ON BASIC RESEARCH IN CHAGAS DISEASE - XIX MEETING OF THE BRAZILIAN SOCIETY OF PROTOZOOLOGY - HOTEL GLÓRIA, CAXAMBU, MG, BRASIL - 10-12 NOVEMBER 2003. **Rev. Inst. Med. trop. S. Paulo, 45**(Suppl. 13), November, 2003.

the drastic clearance of circulating parasites after treatment. Our results strongly suggest that the rCRP ELISA was capable of detecting the early therapeutic efficacy in treated patients, and confirmed its superiority over the CS tests and parasitologic methods.

Supported by FAPEMIG and CNPq

IM75 - THE CLINICAL SPECTRUM IN CANINE VISCERAL LEISHMANIASIS AND THE CHANGES IN THE ISOTYPES PATTERNS OF IMMUNOGLOBULINS

Reis, A. B[1,3].; Vale, A.M.[2].; Corrêa-Oliveira, R[1].;Giunchetti, R.C[2]; Lage, S.R; Mayrink, W[2]; Genaro, O[2] & Martins-Filho, O. A[4].

1- Laboratório de Imunologia Celular e Molecular CPqRR-FIOCRUZ 2- Laboratório de Leishmanioses ICB-UFMG, 3-Laboratório de Parasitologia e Histopatologia NUPEB-UFOP, 4- Laboratório de Doença de Chagas CPqRR-FIOCRUZ. alexreis@cpqrr.fiocruz.br

Dogs are considered the most important vertebrate reservoir of the disease due to its greater prevalence and the frequency of *Leishmania* amastigotes in the skin of these animals. In CVL, the immunological mechanism underlying the susceptibility or resistance to severe disease remains for less defined. Policlonal activation of B-cells leading to high titers of circulating antibodies are found in the course of *L. (L.) chagasi* infection and the detection IgG ant-*Leismania* antigens is an important diagnostic to in identifying case of CVL. In the present study were evaluated 40 naturally infected dogs by *Leishmania (L.) chagasi* with different clinical features and 20 non-infected dogs as a control group. The infected animals were classified according to their clinical symptoms in three groups: asymptomatic, oligosymptomatic and symptomatic dogs. These animals were submitted for a detail analysis of serological parameters by Enzyme Linked Immunosorbent test (ELISA). Serum samples were tested from a 1:80 until 1:327,000 dilutions to determine the title limit of each sera by a display of specific monoclonal anti-canine isotype antibodies (IgA, IgM, IgE, IgG, IgG1 and IgG2), employing a soluble *L. (L.) chagasi* antigen. The results show an association between higher levels of IgG1 with asymptomatic animals. In the other hand, higher levels of IgG, IgG2, IgA and IgE were observed in oligosymptomatic and symptomatic dogs. Our results emphasize that progression of disease in dogs is characterized by appearance of specifics isotypes of immunoglobulins (mainly IgG2), which may contribute to aggravation of the clinical status of the infected dog. Furthermore, the high production of IgG2, IgA and mainly IgE in oligosymptomatic and symptomatic group, might suggest an association of this clinical feature with a type 2 immune response.

Supported by: FAPEMIG, CNPq, FIOCRUZ and UFMG.

IM76 - AVALIAÇÃO DE DESEMPENHO DA PESQUISA DE ANTICORPOS IGG ANTI-PROMASTIGOTAS VIVAS DE *LEISHMANIA (V.) BRAZILIENSIS,* DETECTADOS POR CITOMETRIA DE FLUXO, PARA A IDENTIFICAÇÃO DE LEISHMANIOSE TEGUMENTAR AMERICANA ATIVA.

Rocha RDR1, Gontijo CMF1, Elói-Santos SM2, Carvalho AT3, Ferrari TC2, Corrêa-Oliveira R1, Marques MJ4, Mayrink W2, Martins-Filho OA1.

1Centro de Pesquisas René Rachou, Belo Horizonte, Brasil. 2Universidade Federal de Minas Gerais, Belo Horizonte, Brasil. 3Universidade Federal de Ouro Preto, Ouro Preto, Brasil. 4Universidade Federal de Juiz de Fora, Juiz de Fora, Brasil.

Recentemente desenvolvemos um método de detecção de anticorpos IgG anti-formas promastigotas vivas (AAPV-IgG) de *Leishmania (V.) braziliensis* por citometria de fluxo. Para avaliar a aplicabilidade do novo método em identificar LTA em atividade clínica, foram submetidos à AAPV-IgG e à reação de imunofluorescência indireta (RIFI), soros de 145 indivíduos de área endêmica, classificados em dois grupos quanto a ausência/presença de lesão (L-, n=67; L+, n=78). Os resultados da AAPV-IgG foram expressos sob a forma de percentual de parasitos fluorescentes positivos (PPFP) na diluição do soro 1:1024, no intervalo de 0-100%. Os resultados da RIFI foram expressos em títulos de IgG, considerados como positivos títulos iguais ou superiores a 1:40.. O desempenho de ambos os testes, foram avaliados segundo diferentes índices, incluindo sensibilidade, especificidade, valores preditivos, acurácia, índice J de Youden, "receiver operating characteristic curve" (curva ROC) e a razão de verossimilhança (RV). Na análise do desempenho da AAPV-IgG, três pontos de corte foram selecionados, incluindo PPFP de 20%, 50%, 60%. O limiar de PPFP £20% mostrou-se de grande valor diagnóstico, uma vez que exclui a possibilidade de LTA ativa (RV=0,07). O ponto de corte de 50% distinguiu 92% (72/78) dos pacientes L+ como positivos e 81% (54/67) dos indivíduos L- como negativos. No entanto, a análise da curva ROC indicou o estabelecimento de 60% de PPFP como o ponto de corte, no qual a AAPV-IgG apresentou um ganho real em relação à RIFI ³1:40, incluindo sensibilidade (88/92%), especificidade (57/87%), valor preditivo positivo (70/89%) e negativo (81/91%), probabilidade de doença pós-teste negativo (19/9%), acurácia (74/90%) e índice J de Youden (51/80%). Além disto, valores de PPFP>60% (RV=7,98) demonstrou que a AAPV-IgG pode contribuir para o esclarecimento diagnóstico de LTA ativa. Por outro lado, o RV=2,41 para a RIFI foi desprovido de valor diagnóstico. Em conjunto, nossos resultados demonstraram que a AAPV-IgG, por citometria de fluxo, apresenta-se como um novo instrumento para o diagnóstico sorológico da LTA.

IM77 - THE IMPORTANCE OF USING DIFFERENT SEROLOGICAL APPROCHES IN CLINICAL STUDIES AFTER ETIOLOGIC TREATMENT OF CHAGAS' DISEASE.

[1]WENDLING, A.P.B.; [1]VITELLI, D.M.; [1]ELOI-SANTOS, S.M.; [1]PINTO-DIAS, J.C.; [2]DE LANA, M. & [1]MARTINS-FILHO, O.A.

[1]Laboratório de Doença de Chagas – Centro de Pesquisas René Rachou – FIOCRUZ/Belo Horizonte – MG. [2]Departamento de Análises Clínicas – UFOP/ Ouro Preto - MG

Objectives: Aiming to evaluate the performance of different serological approaches to identify post-therapeutic cure during chronic Chagas'disease, we have analyzed the serological status of Chagas' disease patients after specific etiologic treatment during chronic infection, using conventional and alternative methods.

Methods: Sera samples from 60 chronic Chagasic patients living in Berilo/ MG, who have been previously submitted to specific etiologic treatment, were comparatively screened for their IgG reactivity by conventional serology (CS) by RIFI and ELISA – EIE (both from BioManguinhos - FIOCRUZ), besides three alternative tests, including recombinant EIE – EIEr (BioManguinhos - FIOCRUZ), PAGIA (DIAMED) and by flow cytometry analysis of anti–fixed epimastigotes antibodies (FC-AFEA), described by Cordeiro et al, 2001, referred as non-conventional methods (NCS).

Results and Conclusions: Using the positivity on both RIFI and EIE as the criterion for classic serological diagnosis of Chagas'disease, we observed that 87% (52/60) of the treated patients remained positive after treatment; whereas 8%(5/60) and 5% (3/60) of them became serologically indeterminate or negative, respectively. Comparative analysis confirmed that individuals with negative (3/ 3) or indeterminate (5/5) results by CS presented negative results by NCS. Interestingly, 25% (13/52) and 2% (1/52) of individual with positive CS showed negative results on EIEr and FC-AFEA, respectively. Taken together, the EIEr results reached 31.5% (18/57) of sero-dissociation between positive and indeterminate CS. These data showed post-therapeutic serological status

XXX ANNUAL MEETING ON BASIC RESEARCH IN CHAGAS DISEASE - XIX MEETING OF THE BRAZILIAN SOCIETY OF PROTOZOOLOGY - HOTEL GLÓRIA, CAXAMBU, MG, BRASIL - 10-12 NOVEMBER 2003. **Rev. Inst. Med. trop. S. Paulo, 45**(Suppl. 13), November, 2003.

consistent with those previously reported by Krettli and Brener 1976 for chronic Chagas' disease, with serological cure observed in 5% of treated patients besides 30% of sero-dissociation conventional serology and the alternative methods. Future studies are currently under investigation in our laboratory in order to validate the EIEr findings with those previously presented by Martins-Filho et al 1995 using anti-live trypomastigotas antibodies for cure assessment after treatment of chronic Chagas'disease. Financial Support: PIBIC/FIOCRUZ.

IM78 - CANINE VISCERAL LEISHMANIASIS: HISTOPATHOLOGICAL AND IMMUNOHISTOCHEMICAL STUDY OF EAR SKIN BIOPSIES

Fernanda Nobre AMARAL[1]; Vitor Márcio RIBEIRO[2]; Marilene Suzan Marques MICHALICK[3], Washington Luiz TAFURI[1]; Wagner Luiz TAFURI[1].

1- Instituto de Ciências Biológicas, Departamento de Patologia Geral, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brasil; 2- Clínica Veterinária Santo Agostinho, Belo Horizonte, Minas Gerais, Brasil; 3- Instituto de Ciências Biológicas, Departamento de Parasitologia, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil

Canine visceral leishmaniasis is an endemic disease in Brazil and it has been increased dramatically in the suburban areas of large cities. Naturally infected dogs with *Leishmania (Leishmania) chagasi* were obtained from Santo Agostinho Clinical Veterinary, Belo Horizonte, MG, Brazil. Forty-four naturally infected animals were clinical classified in asymptomatic, oligosymptomatic and symptomatic. Serological exams of all animals (IFAT, Complement Fixation and ELISA) were positive. Skin fragments of ears of all animals were collected during the clinical exams by veterinarian biopsies (punch of 4mm). All the ear skin tissues were fixed in a solution of buffered formalin 10%. Then, these tissues were analyzed for histopathological and immunohistochemical studies. The main lesion observed in the skin of all infected animals was a diffuse chronic inflammatory reaction in the upper dermis and in focus around vessels and/or glands in the deep dermis. The cellular exudate was composed by mainly macrophages, plasmocytes and lymphocytes. The histopathological picture observed was similar among all cases and it was independently considering the clinical aspects. The streptoavidin-peroxidase technique was carried out to detect intracellular amastigotes forms of *Leishmania* in the paraffin tissues. Our results have indicated an increasing positivism when we consider the immunohistochemical results. In fact, 15,9% positive cases observed in conventional histology (H&E) increased to 34% of positive cases after immunohistochemical observation.

Our histological and immunohistochemical previous results (Xavier et al., 2001) showed asymptomatic and oligosymptomatic dogs with similar ear skin tissue parasitism as the symptomatic dogs. Moreover, *Leishmania* intracellular amastigotes forms were observed independently of the intensity of the cellular exudate. In this work we have observed the similar results. Also, the immunohistochemistry technique has been confirmed an useful tool for epidemiological, clinical, and histopathological studies.

Supported by FAPEMIG, CNPq and UFMG

IM79 - AN ALTERNATIVE IMMUNOHISTOCHEMICAL METHOD TO DETECT *LEISHMANIA* AMASTIGOTES IN PARAFFIN-EMBEDDED CANINE TISSUES

Wagner Luiz TAFURI[1], Renato de Lima SANTOS[2], Rosa Maria Esteves ARANTES[1], Fernanda Nobre Amara1[1], Maria Norma de MELO[3], Ricardo GONÇALVES[1], Marilene Suzan Marques MICHALICK[3], Washington Luiz TAFURI [1]

[1]Departamento de Patologia Geral, Instituto de Ciências Biológicas, UFMG; [2]Departamento de Clínica Veterinária, Escola de Veterinária, UFMG; [3]Departamento de Parasitologia, UFMG, Belo Horizonte, MG, 31270-901.

Canine visceral leishmaniasis (CVL) is a zoonosis and a chronic systemic disease of the dog caused by a protozoan of the genus *Leishmania infantum* in the Old World and *Leishmania chagasi* in the New World. Several methods are currently employed for diagnosis of CVL, including microscopic detection of the parasite in bone marrow and lymph node aspirates, demonstration of specific antibodies anti-*Leishmania* in sera from infected animals, isolation of the parasite by "in vitro" culture or by laboratory animal inoculation. However, a definitive diagnosis is based on the actual detection of the parasite, which is conventionally achieved by examining Giemsa stained smears or histopathological sections stained by hematoxilin and eosin. These methods have a low sensitivity and therefore they are often inconclusive. This is particularly true in canine organs that have a low level of parasitism such as kidneys, lungs and guts or in some cases, in the skin. The technique for immunohistochemical detection of leishmanial amastigotes in canine tissues has been reported previously and it is proven to be undoubtedly efficient for the diagnosis. In this work we describe an ease and inexpensive immunohistochemical approach for *Leishmania* detection in formalin-fixed paraffin-embedded canine tissues. Amastigotes forms of *Leishmania* are easily observed within macrophages in several organs from naturally infected dogs using the streptoavidin-biotin immunohistochemical method with canine hyper immune serum as primary antibody. In addition, the second antibody used was not specific to canine immunoglobulin characterizing a cross immune reaction.

The immunoperoxidase protocol employed in this study, which is based on the use of serum from naturally infected dogs, is inexpensive and readily available, when compared to monoclonal or polyclonal anti-*Leishmania* antibodies (Bourdoiseau et al., 1997; Livini et al., 1983). Although the secondary antibody (LSAB+ Kit, Dako) is not specific to the dog serum ("crusade immunoreaction"), this method proved to be as specific as the use of monoclonal or polyclonal anti-*Leishmania* antibodies. Furthermore, the use of canine serum resulted in a low or absent background staining, and no staining was observed when the canine serum was replaced by PBS or serum from an uninfected dog, clearly indicating that the secondary antibody reacts to the canine serum from an infected dog used in this study as a primary antibody. Our results indicate that this technique could be an useful tool for epidemiological, clinical, and histopathological studies.

Apoio Financeiro: FAPEMIG, CNPq, UFMG

IM80 - RECOGNITION OF *PLASMODIUM VIVAX* VARIANT ANTIGENS (VIR) BY HUMAN ANTIBODIES INDUCED DURING NATURAL INFECTION.

Oliveira, T.R.[1], Fernandez-Becerra, C.[2], Cômodo, A.N.[1], Soares, I.S.[1] & del Portillo, H.A.[2]

[1]Departamento de Análises Clínicas e Toxicológicas, Faculdade de Ciências Farmacêuticas, Universidade de São Paulo, São Paulo, Brasil. [2]Departamento de Parasitologia, Instituto de Ciências Biomédicas, Universidade de São Paulo, CEP 05508-900, São Paulo, Brasil. Correspondence: hernando@icb.usp.br

Variant antigens in *Plasmodium vivax* are encoded by members of the multicopy *vir* gene family. *vir* genes can be subdivided into different sub-families based sequence similarities (del Portillo et al. 2001. Nature, 410:839). We expressed seven glutathione S-transferase fusion proteins corresponding the four *vir* sub-families (A, B, C, and E) obtained from parasites of a single patient from the Amazon Region. Recombinant proteins were purified by affinity chromatography and used in ELISA assays to analyze the naturally acquired antibody responses of individuals during patent *P. vivax* infections. Sera were collected from individuals living in different endemic areas from the north of

XXX ANNUAL MEETING ON BASIC RESEARCH IN CHAGAS DISEASE - XIX MEETING OF THE BRAZILIAN SOCIETY OF PROTOZOOLOGY - HOTEL GLÓRIA, CAXAMBU, MG, BRASIL - 10-12 NOVEMBER 2003. **Rev. Inst. Med. trop. S. Paulo, 45**(Suppl. 13), November, 2003.

Brazil pertaining to the States of Pará and Rondônia. These same sera were also tested in their ability to recognize two recombinant proteins representing two merozoite surface antigens of *P. vivax*: the merozoite surface protein 1 (MSP1) and the apical membrane antigen 1 (AMA-1). Anti-VIR antibodies were detected against each recombinant protein tested and yet the prevalence of such antibodies was significantly lower than the prevalence of antibodies against MSP1 and AMA1. Studies are now in progress to evaluate the presence of cross-reactive epitopes among the antigens encoded by the different *vir* sub-families using sera of mice immunized with each recombinant protein.

Supported by FAPESP and CNPq.

IM81 - COMPARISON OF THE REACTIVITY BETWEEN ANTIGENS OF *LEISHMANIA (L.) CHAGASI*, *L.(L.) AMAZONENSIS* AND *LEISHMANIA SP.* (BIO-MANGUINHOS) IN THE SERO-DIAGNOSIS OF VISCERAL LEISHMANIASIS BY THE INDIRECT IMMUNOFLUORESCENCEC TEST.

Lima, L. V. R.; De Souza, A. A. A.; Jennings, Y. L.; Corrêa, Z. C. de J.; de Jesus, R. C. S.; Everdosa, D. R.; Ayres, M.;. Silveira, F. T.

Instituto Evandro Chagas (SVS), Belém, Pará, Brasil.

Introduction: antigenic specificity still represents a controversial point of discussion with regards the standardization of an antigen for use in the serodiagnosis of human visceral leishmaniasis (HVL) by the indirect immnofluorescence antibody test (IFAT). For this reason we have sought to compare the reactivity between antigens of *L.(L.) chagasi* (amastigotes and promastigotes), *L.(L.) amazonensis* (amastigotes) and a *Leishmania sp.* (promastigotes) from Bio-Manguinhos. Objective: to standardise an antigen for the diagnosis of HVL by the IFAT. Material and methods: *Sera*: 90 serum samples from patients with a previous serological diagnosis of HVL were randomly selected, together with 30 samples from individuals resident in Belém, Pará, with no previous evidence of infectious.Antigens were prepared from the promastigotes of *L.(L.) chagasi* (strain MCAO/BR/1998/M18011, Imperatriz, Maranhão State), amastigotes of *L.(L.) amazonensis* (strain IFLA/BR/1966/PH8, Belém, Pará), and promastigotes of the *Leishmania sp.* from Bio-Manguinhos. The antigens of amastigotes were made on IFAT slides by dab-smears of pieces of liver, spleen and skin of hamsters infected with the respective parasites. The promastigote antigen of *L.(L.) chagasi* was prepared from stationary phase cultures in Difco B45 medium, with a suspension of 3 x 10^6 parasites/ml. The 3 antigens were distributed on IFAT slides, fixed with acetone, and preserved at -20 ºC. The Bio-Manguinhos antigen was used following the maker's instructions. *Serological test*: the IFAT was carried out using anti-IgG (Bio-Manguinhos) for the 4 antigens, with positive sera considered to be those with a titre equal or above 80. *Statistical analysis*: we used the screening-test and curve ROC (IC 95%, of the programme Bio-Estat 2.0) to evaluate the sensibility and specificity, and the Dunnett (ANOVA) ($p < 0,01$) to evaluate differences between the averages of the antigen titres. Results: the amastigote antigen of *L.(L.) chagasi* atained a 100% sensibility and specificity level. That of *L.(L.) amazonensis* amastigotes achieved a 87% sensibility efficiency and a 93% specificity efficiency. The *Leishmania sp.* (Bio-Manguinhos) antigen gave sensibility and specificity efficiencies of 88% and 90%, respectively. ROC curve values were d=0,00 for amastigotes of *L.(L.) chagasi*; d=0,17 for promastigotes of the same parasite; d=0,15 for amastigotes of *L.(L.) amazonensis*; and d=0,16 for promastigotes of the *Leishmania sp.* (Bio-Manguinhos). With regards differences between averages of the reacting sera, it may be noted that the amastigote antigen of *L.(L.) chagasi* (6.366) was significantly ($p < 0,01$) better than the promastigote antigen of the same parasite (3.712); that of *Leishmania sp.* Bio-Manguinhos was 1.299; and that of amastigotes of *L.(L.) amazonensis* 1.070. Conclusion: the results show that the *L.(L.) chagasi* amastigote antigen is the antigen of choice for the sero-diagnosis of HVL and for monitoring chemotherapy of this disease.

IM82 - EVALUATION OF IGG SUBCLASSES ANTI-*LEISHMANIA* BY ELISA IN DOGS WITH AMERICAN VISCERAL LEISHMANIASIS (AVL) IN RIO DE JANEIRO/RJ.

[2]FREITAS, APTB, [1]Nascimento, LD, [2]Ribeiro, FC; [1]Madeira, MF, [2]Schubach, TMP, [2]Marzochi, MCA, [1]Mouta-Confort, E.

[1]Departamento de Ciências Biológicas, ENSP- FIOCRUZ - RJ.[2]Departamento de MicroImunoParasitologia, Serviço de Parasitologia - Centro de Referência em Leishmanioses, IPEC - FIOCRUZ-RJ.

Introduction: The dog is the main reservoir of the AVL in urban areas. High levels of IgG are detected in canine AVL, and also in asyntomatic animals with high degree of parasitism in the health skin and viscerals. Deplazes et al. (1995), suggest that the titles of IgG1 and IgG2 are safer indicators for the status of the illness than the IgG. In this work we investigate, through ELISA, the seroprevalence of IgG and subclasses IgG1 and IgG2 anti-*Leishmania* in dogs of AVL endemic area, evaluating their importance for the diagnosis of the illness. Methodology: It was used in ELISA a partially soluble antigen of promastigotas forms of *L. (L.) chagasi*. For this study the samples of serum had been classified in the following groups: group I - 20 serum of dogs with positive parasitologic diagnosis (8 of symptomatic dogs and 12 of asyntomatics); group II -16 dogs without parasitologic diagnosis, with positive serologic; group III- 3 dogs with negative parasitologic and group IV - control group of healthy animals. Results: In group I the seroprevalence in ELISA for the asyntomatics animals was 8.3% (1/12) for IgG1 and 100% (12/12) for IgG2 and for the symptomatic animals was 12,5%(1/8) and 100% (8/8) respectively; in group II the seroprevalence for IgG1 was 43,7% (7/16) and 100% (16/16) for IgG2; in groups I and II the seroprevalence for IgG was 100%; in groups III and IV all the sera had been not reactors for IgG and its subclasses. Conclusions: The IgG2 was prevalent and detected in high levels in dogs with AVL, however, in these same animals, IgG1 was detected in low levels. On the contrary to many authors, significant difference was not observed between the levels of IgG1 and IgG2 when correlating these subclasses with the presence or absence of clinical signals. The sensibility and specificity of ELISA for IgG2 detection were higher than IgG detection, and the agreement with the indirect immunofluorescense, seems to strengthen the safe use of this test for the diagnosis of the canine AVL.

Support: Secretaria de Vigilância em Saúde

IM83 - IMMUNE RESPONSES AND PROTECTION INDUCED BY A COMBINED LACK AND *MYCOBACTERIUM* HSP65 DNA VACCINE AGAINST *LEISHMANIA (L.) MAJOR* INFECTION

Eduardo A. Ferraz Coelho[1], Karla Melo Lima[2], José Maciel Rodrigues Junior[2], Célio Lopes Silva[2], Carlos Alberto Pereira Tavares[1] e Ana Paula Fernandes[1]

[1]Faculdade de Farmácia e Departamento de Bioquímica e Imunologia - ICB - Universidade Federal de Minas Gerais. [2]Centro de Pesquisa em Tuberculose, Faculdade de Medicina de Ribeirão Preto, Av. Bandeirantes 3900, CEP 14049-900. Ribeirão Preto – SP (Email jmrj@rdbiotec.com.br).

The LACK (*Leishmania* homolog of receptors for activated C kinase) antigen is a 36 KDa protein highly conserved and expressed in promastigote and amastigote forms of *Leishmania.* Immunization of BALB/c mice with a truncated (24-kDa) version of LACK, protein or DNA, confers strong protection against *L. (L.) major* infection. Here, we compared the protective effect of na encapsulated LACK DNA vaccine against *L. (L.) major* and *L. (L.) amazonensis* infection in BALB/c mice. Development of Th1 immune responses are essential for protection against *Leishmania* infection. *Mycobacterium* HSP65 shares high homology with *Leishmania* HSP proteins and is able to induce high levels of IFN-γ, when tested as a DNA vaccine against *Mycobacterium* infection. Thus, *M. leprae*

XXX ANNUAL MEETING ON BASIC RESEARCH IN CHAGAS DISEASE - XIX MEETING OF THE BRAZILIAN SOCIETY OF PROTOZOOLOGY - HOTEL GLÓRIA, CAXAMBU, MG, BRASIL - 10-12 NOVEMBER 2003. **Rev. Inst. Med. trop. S. Paulo, 45**(Suppl. 13), November, 2003.

HSP65 and interleukin 12 (IL-12) genes were evaluated as adjuvants. Eukariotic cells expression vectors contaning the LACK, HSP or *IL-12* coding regions were encapsulated in microspheres of lactic and poliglicolic acids (PLGA) by the method of multiple emulsions in a ratio of 6 ug of DNA/mg of polimer. Groups of eight BALB/c mice were immunized with two intramuscular injections (30 days interval) of 2,5 mg of microspheres and challenged 30 days latter with with 10^6 metacyclic promastigotes of either *L. (L.) major* or *L. (L.) amazonensis* in the hind footpad. Control groups were immunized with vector DNA or IL-12 DNA. Mice immunized with either LACK, HSP or a combination of these two DNA vaccines and challenged with *L. (L.) major* were significantly protected as indicated by reduction of edema in the infected footpad as compared with control groups. No significant differences were observed among groups immunized with LACK or HSP DNA and those immunized with LACK/IL-12 or LACK/HSP combined DNA vaccines. No significant protection was observed in mice immunized with IL-12 or vector DNA alone or in mice challenged with *L. amazonensis*. Protection was accompanied by significant reduction of parasite loads in the infected footpad and increased specific IgG2a antibody levels in sera of mice. Splenocytes of protected mice produced increased levels of IFN-γ and TNF-α and decreased levels of IL-4, as compared with mice immunized with IL-12 or vector DNA. We also observed that low doses of encapsulated DNA are required for induction of protection and specific Th1 immune responses. In addition, we demonstrated a significant cross-protection against *L. (L.) major* infection by immunization with *M. leprae* HSP65 DNA vaccine.

Financial supporte: FAPEMIG and CNPq-PRONEX

IM84 - VISCERAL LEISHMANIASIS NEW IMMUNODIAGNOSTIC ALTERNATIVE.

Marchán, E[1], Hernández, A.G.[3]; Simoni, Z.J[1,2]; Figuera, L.J[1]; Guevara, L.[3] y Dagger, F.[3]

[1]Instituto de Investigaciones en Biomedicina y Ciencias Aplicadas (IIBCA), [2]Postgrado de Biología Aplicada, Núcleo de Sucre / Universidad de Oriente. [3]Instituto de Biología Experimental (IBE), Universidad Central de Venezuela. edgar_marchan@hotmail.com

Visceral leishmaniasis (VL) is a severe world public health problem with 12 millions people infected, an incidence of 500000 and annual mortality of 80000 cases (WHO, World Health Report, 1998). Although multidisciplinary efforts carried out to eradicate this disease it remain without effective treatment making indispensable the rapid diagnosis of disease to increase the probability of successful cure. Nowadays there are not experimental immunoassay or commercial kit for VL diagnostic to be reliable, with low cost and easy application. In this sense the present study consist of Standardization of dot blot-ELISA technique for Visceral leishmaniasis immunodiagnostic. The antigen was a pool of surface glycoproteins ($gp_{27,}$ gp_{50} and $gp_{67)}$ purified from promastigotes of *Leishmania (L.) mexicana*, NR strain. Serum of patients with the different clinical manifestations of leishmaniasis treated or no treated with Glucantime®, Chagas' disease or malaria from endemic areas of Sucre state located at northeastern of Venezuela were assayed. We found between 11 serum of VL patients without treatment that 9 of them were positives whilst only 3 of 13 serum of VL patients treated with antimonial pentavalent were positives. These results are in agreement with the pointed out by Da Matta *et al*, J. Clin. Lab. Anal.,2000,14(1):5-12 who reported that type and subtypes antibodies levels decreasing after administered treatment. There were not cross reactivity with Chagas' disease and Malaria. Efficacy of immunodiagnostic test employed in present study using criterions of Pozo, Med.Clin.,1998,90:779-85, showed sensibility of 82% and specificity of 83%. In summary these findings suggest that dot blot-ELISA assay using the surface glycoproteins antigen of *L. (L.) mexicana* promastigotes is a method of high sensitivity, specificity and reproducibility, which will be implemented for visceral leishmaniasis diagnostic, regarding of course, clinical complementary information of the patient. Furthermore is an easy application method useful for field epidemiological study and valuable tool for rapid diagnostic and appropriate treatment.

Financial support: Universidad de Oriente: CI-5-1901-0848/99 and Universidad Central de Venezuela: CDCH- PTD-03-10-4197-98

IM85 - PROFILE OF ANTI-*TOXOPLASMA GONDII* IGG AVIDITY IN EXPERIMENTALLY INFECTED RABBITS

Meireles, L. R. [1], Vicentine, M. P.[2], Cardoso, R.P.A.[2], Galisteo Jr., A, J.[3], Andrade Jr., H. F[1].

1- ICB & IMTSP/USP, 2 - IMTSP/USP and 3 - IPEN & IMTSP/USP. Contacts: E-mail lrmeirel@usp.br

Toxoplasmosis is one of the more common parasitic zoonoses worldwide. The causative agent *Toxoplasma gondii* infects most warm-blooded animals including man, with significant veterinary and medical importance, because it may cause abortion or congenital disease in its intermediate hosts. As well as other mammals, the rabbits are also susceptible to *T. gondii* and fatal disease has been reported in this specie especially in wild life. The main objective of this work was to standardize and to evaluate the prognostic efficiency of the ELISA avidity in the temporal diagnosis of rabbit toxoplasmosis. We screened New Zealand rabbits with approximately 45 days of age and negative specific IgG by ELISA. These animals were inoculated subcutaneously (s.c.) with 10^7 irradiated tachyzoites of the RH strain of *T. gondii*. The animals were maintained at the Institute of Tropical Medicine/USP, receiving commercial food and water *ad libitum* and observed by 6 months, a period during which it did not present any clinical signs of the infection, with normal growth. Blood was collected biweekly and analyzed for anti-*T. gondii* IgG by ELISA and avidity determination using a 6 M urea washing. Antibody titer increased during the first weeks of infection achieving a plateau after 60^{th} day of infection, but avidity maturation (100%) was only achieved at day 150^{th} of infection, presenting a linear correlation with infection time until this time. The ELISA avidity was confirmed as a useful tool in the diagnosis of acute *T. gondii* infection in rabbits and could be predictive of the stage of infection and used in epidemiological studies. IgG avidity maturation appears to be a universal phenomenon in mammals.

This work was supported by LIMHCFMUSP and CAPES.

IM86 - STANDARDIZATION AND EVALUATION OF THE IGG AVIDITY IN THE DIAGNOSIS OF TOXOPLASMOSIS IN CATS AND ITS RELATIONSHIP WITH OOCYST EXCRETION

Meireles, L. R.[1], Freire, R. L.[2], Andrade Jr., H. F[1].

1- ICB & IMTSP/USP, 2 - Veterinary Medicine School, Univ. Est. Londrina. Contacts: E-mail lrmeirel@usp.br

Felids play a major role in the epidemiology of toxoplasmosis, since they are the definitive hosts of *Toxoplasma gondii*. Cats are also the only domestic animals in which the parasite completes the sexual stage of the life cycle, producing oocysts that are excreted in the feces and infect other hosts. As cats are the key element in the transmission of *T. gondii* to animals and humans, we decide to study the anti-*T. gondii* IgG maturation in this specie, looking for markers of environment contamination by oocysts, that are mainly excreted in the acute stage of this infection. We screened young cats breed in captivity, selecting those negative for specific IgG by ELISA. These animals were feed with brain from experimentally infected mice containing 1200 cysts of the AS-28 strain *T. gondii*. The animals

XXX ANNUAL MEETING ON BASIC RESEARCH IN CHAGAS DISEASE - XIX MEETING OF THE BRAZILIAN SOCIETY OF PROTOZOOLOGY - HOTEL GLÓRIA, CAXAMBU, MG, BRASIL - 10-12 NOVEMBER 2003. **Rev. Inst. Med. trop. S. Paulo, 45**(Suppl. 13), November, 2003.

were maintained at the Veterinary Hospital/UEL, receiving commercial food and water *ad libitum* and observed by at least 3 months. Blood was collected weekly and analyzed for anti-*T. gondii* IgG by ELISA and avidity determined using a 6 M urea washing. The feces were collected daily and analyzed qualitatively and quantitatively for oocysts, which were excreted during the first few weeks after infection. Antibody titer increased during the infection and IgG avidity achieved 50% only after the day 60th of infection, presenting a linear correlation with infection evolution. During oocyst shedding, serum samples presented low IgG avidity (<50%). The avidity maturation of IgG antibodies in cats was similar to other mammals and this test could be used to discriminate acute infections in those animals, allowing both the determination of incidence of this disease in cats and estimation of oocysts shedding in feces.

This work was supported by LIMHCFMUSP and CAPES.

IM87 - AMERICAN CUTANEOUS LEISHMANIASIS IN A RURAL AREA OF GOIÁS STATE, BRASIL.

Dorta ML[#], Gosch CS[#], Dorta RO[+], Pereira LLA*[#], Pereira AJSC[*] & Ribeiro-Dias F[#]

[#]Laboratório de Imunobiologia das Leishmanioses do DMIPP/IPTSP, [+]Instituto de Estudos Sócioambientais da Universidade Federal de Goiás e Hospital Anuar Auad de Goiânia, GO. mdorta@iptsp.ufg.br

Increasing of leishmaniasis incidence around the world represents a serious problem of public health. Current available data indicate that 1.5 million cases of leishmaniasis are diagnosed every year and more than 80% of the total 12 million cases live in development countries, particularly Iran, Afghanistan, and Brazil. Over the last three years (2000-2002) autochthonous cases of American cutaneous leishmaniasis (ACL) have been identified in an area of rural workers belonging to the landless movement (MST) in Goiás state, Brazil. These workes have occupied forest areas near to riverheads which represent a very suitable habitat for several species of *Lutzomyia*, the leishmaniasis vector, widely distributed in those areas. In Goiás state two *Leishmania* species, *Leishmania (L.) amazonensis* and *Leishmania (V.) braziliensis*, have been incriminated in diagnosed cases of cutaneous leishmaniasis. The aim of the present work was to characterize *Leishmania* species isolated from MST people with ACL as well as to follow up these patients after treatment. The present report describes twenty two cases of patients with ACL which were attended in the outpatient department for leishmaniasis of Anuar Auad Hospital in Goiânia, the capital of Goiás state. Diagnostic procedures included clinical symptoms evaluation, exposure history, direct microscopy visualization of lesion biopsies, growth of transforming promastigotes in axenic medium cultured with material isolated from lesions, hystopathological analysis of lesion thin sections, immunological methods such as the Montenegro skin test, detection of antibodies against *Leishmania* by IFA and ELISA, and detection of *Leishmania* DNA. Fifteen isolates obtained by punch biopsy were processed and the *Leishmania* species were characterized by polymerase chain-reaction (PCR). Amplification was achieved by use of total DNA extracted from biopsies and primers from kinetoplast minicircles of *Leishmania* published in GeneBank. The *Leishmania* species isolated from 93.3% patients was identified as *Leishmania (V.) braziliensis*, whereas 6.3% were infected with *L. (L.) amazonensis*. These results show that the PCR technique by use of kDNA is useful for identification of *Leishmania* species and represents a very good support for the other methods currently used for *Leishmania* characterization. Treatment data showed that 93.3% of ACL patients responded to Meglumine antimoniate (Glucantime), whereas one patient which did not respond to Glucantime was succesfully treated with Amphoterecin deoxycholate. Extension of diagnosis by PCR amplification to a higher number of patients with ACL from the same area is now in progress, however our preliminary results described in the present work permit us to conclude that unprojected settlements represent a very relevant and serious risk of increasing human cases of ACL, leading to a significant change in the epidemiology of *Leishmania (V.) braziliensis*, the causal agent of disfiguring mucocutaneous leishmaniasis in Brazil.

Supported by FUNAPE/UFG/GO and CAPES.

IM88 - SURVEY OF *PLASMODIA* SEROEPIDEMIOLOGY AND DETECTION OF ASYMPTOMATIC MALARIA CASES AMONG RESIDENTS OF ATLANTIC FOREST AREAS IN ESPÍRITO SANTO STATE, BRAZIL.

Cerutti Jr, C [1]; Malafronte, R. S [2]; Boulos, M [3]; Coutinho, A. F [4]; Rezende, H. R [5]; Falqueto, A[1]; Amaro, P [2]

1- UFES, Depto de Medicina Social, 2- USP, IMT/Lab. de Protozoologia, 3- FMUSP, Depto de Moléstias Infecciosas e Parasitárias, 4- Secretaria da Saúde do Estado do Espírito Santo, Setor Entomologia, 5- Secretaria da Saúde do Estado do Espírito Santo, Setor de Malária.

Several malaria cases have been notified during the last few years in regions surrounded by the Atlantic Forest in Espírito Santo State. The aim of this study was to determine the exposure of human subjects to *Plasmodium* parasites (sexual and asexual stages) by serological tests and to determine the asymptomatic malaria profile in areas with low endemicity. Results of a seroepidemiological survey and the detection of asymptomatic malaria cases in the Espírito Santo State are reported here. Aliquots of total blood were obtained from 1.600 subjects from the following Municipalities: Santa Tereza, Santa Maria de Jetibá, Domingos Martins, São Roque do Canaã, Santa Leopoldina and Alfredo Chaves. The prevalence of IgM and IgG antibodies against malaria asexual stages was evaluated by IIF (Indirect Immunofluorescence) with crude *P. vivax and P. malariae* antigens. ELISA tests using synthetic peptides corresponding to the CSP (circunsporozoite protein) of *P.vivax* complex and *P. malariae* were used to detect IgG antibodies against the sexual stages of the parasite. PCR reactions, for detection of *P. vivax*, *P. falciparum* and *P. malariae* specific DNA, were carried out using multiplex primers. IIF for the detection of anti *P. malariae* antibodies was proceeded only for sera that gave positive results in ELISA and PCR. The IgM/IgG seroprevalence for *P. vivax* and *P. malariae* antigens were 29.1%/44,4% and 1.7%/14.7% respectively. The seroprevalence of antibodies against the CSP synthetic peptides were 30.7% (88/286) (classic *P. vivax*); 7.1% (20/280) (*P.vivax* VK247); 14.1% (39/276) (*P.vivax* like) and 9.1% (26/285) (*P. malariae*). Asymptomatic *P. vivax* and *P. malariae* infections were detected in 3 and 9 individuals respectively and a mixed *P. malariae/P. falciparum* infection was detected in one sample. These studies were carried out with the purpose of evaluate malaria transmission in a unique ecological niche (Atlantic Forest, Espírito Santo State). Our results indicate that a more comprehensive study is necessary and may be of great help in the design of effective intervention strategies for the control of the disease.

Financed by Fapesp: 02/03869-3 and 02/12211-1.

IM89 - CHARACTERIZATION OF BRAZILIAN HUMAN *GIARDIA DUODENALIS* ISOLATES BY USING IMMUNOLOGICAL AND BIOCHEMICALPARAMETERS

Míriam O. Rocha[a,*], Vicente de Paulo Coelho Peixoto de Toledo[a], Tania Mara Pinto Dabés Guimarães[a], Elúzia Castro Peres[a], Edna Maria Pires[b], Edward F. Silva[b]

[a]School of Pharmacy, Federal University of Minas Gerais, Belo Horizonte, MG, Brazil; [b]Department of Parasitology, Federal University of Minas Gerais, Belo Horizonte, MG, Brazil; * morocha@farmacia.ufmg.br

XXX ANNUAL MEETING ON BASIC RESEARCH IN CHAGAS DISEASE - XIX MEETING OF THE BRAZILIAN SOCIETY OF PROTOZOOLOGY - HOTEL GLÓRIA, CAXAMBU, MG, BRASIL - 10-12 NOVEMBER 2003. **Rev. Inst. Med. trop. S. Paulo, 45**(Suppl. 13), November, 2003.

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) of the trophozoites extracts, evaluation of the immune humoral response in rabbits, cross-reactivity of the immune sera to the homologous and heterologous antigens by using the immunofluorescent test (IFT) and western blotting were used to characterize three Brazilian human isolates of *Giardia duodenalis* (syn: *Giardia lamblia*, *Giardia intestinalis*) and its clones. The Portland-1 strain (ATCC 30888) was included in the study as a reference pattern.

The Brazilian isolates were axenized from cysts obtained from the feces of patients showing different clinical characteristics. The BHRA93 was isolated from a symptomatic patient while the BHRF92 was isolated from an asymptomatic one. The BHLF93 was isolated from an asymptomatic patient who presented a persistent *G. duodenalis* infection despite the different anti-giardial therapies. The reference pattern was the Portland-1 strain (ATCC 30888), axenized in 1971 from the duodenal aspirate of a symptomatic woman in the United States of America. One clone of each isolate was included in the study.

Antigenic differences among the isolates were observed by SDS-PAGE, western blotting and IFT. Greater antigenic heterogeneity was observed between Portland-1 and Brazilian isolates. Several protein bands ranging from 15 to 200 kDa were identified in the SDS-PAGE. All the isolates induced the production of anti-*Giardia* specific antibodies in rabbits immunized with whole antigenic extract of the trophozoites. Cross-reactivity of the anti-sera was greater with the homologous antigen than with the heterologous ones. Portland-1 and BHRA93, both symptomatic isolates induced higher titers of antibodies sera.

Little difference was detected between the parental isolates and the clones.

These data represent a significant advance on the current knowledge about *G. duodenalis* in Brazil.

Supported by: FAPEMIG and CNPq

IM90 - OCCULAR TOXOPLASMOSIS: ROLE OF RETINAL PIGMENT EPITHELIUM MIGRATION IN INFECTION

Tedesco, R.C.[1,3], Smith, R.L.[3], Corte-Real Faria, S.[1], Calabrese, K.S.[2]

1. Departamento de ultra-estrutura e biologia celular, laboratório de ultra-estrutura celular, Instituto Oswaldo Cruz - FIOCRUZ. 2. Departamento de Protozoologia, laboratório de imunomodulação, IOC - FIOCRUZ. 3. Disciplina de anatomia descritiva e topográfica, Universidade Federal de São Paulo, Escola Paulista de Medicina.

We used a murine experimental model of ocular toxoplasmosis. Our aim was to study the migration of retinal pigmented epithelium (RPE) into the retinal layer during infection of C57BL/6 mice with *Toxoplasma gondii*. Eyes from infected and non-infected animals were analyzed on the 60th day of infection by light and transmission electron microscopy. Non-infected showed the typical normal morphology. In contrast, in infected eyes we observed free parasites in the retinal vasculature, presence of mononuclear inflammatory infiltrate (MNII) and parasites in the vasculature of choroids. No inflammatory infiltrate was observed; RPE cells were identified near the MNII in nuclear and plexiforme layers. RPE cells were also found on ganglionar and in outer segments of the photoreceptor. The morphology showed that RPE cells caused a discontinuity of the nuclear and plexiforme layers. Clusters of parasites were found surrounded by RPE cells and MNII in the inner plexiforme layers. Ultrastructural analysis showed that RPE cells migrated through the epithelium into the inner retinal layers. We did not observe Toxoplasma cysts in many eyes in which pathological changes were detected. Only 8.3% of the animals presented Toxoplasma cysts in the inner nuclear layer in the absence of inflammatory cells. Migration of RPE cells can be triggered by disruption of the RPE monolayer or injury to the neural retina as in the case of toxoplasmosis.

Apoio financeiro: IOC

IM91 - CARACTERIZATION OF CLINICAL PARAMETERS OF VISCERAL LEISHMANIASIS IN DOGS NATURALLY INFECTED WITH *L. (L.) CHAGASI.*

Lage[1], R.S.; Reis, A.B[1,5].; Vale[2], A.M.; Martins-Filho, O.A[3].; Corrêa-Oliveira, R[1].; Carvalho, M.G[4].; Mayrink, W[2].; Giunchetti, R.C.[2].; & Genaro, O.[2]

1- Laboratório de Imunologia Celular e Molecular CPqRR-FIOCRUZ, 2-Laboratório de Leishmanioses ICB-UFMG, 3-Laboratório de Doença de Chagas CPqRR-FIOCRUZ,4-Laboratório de Hematologia Clínica EF-UFMG 5-Laboratório de Parasitologia e Histopatologia NUPEB-UFOP. alexreis@cpqrr.fiocruz.br

Dogs are the domestic reservoirs of zoonotic visceral leishmaniasis caused by *Leishmania (L.) chagasi*. Have been considered to be most important than human infection due to its greater prevalence and the frequency of *Leishmania* amastigotes in the skin of these animals. In this study two groups of adult mongrel dogs were used. The first group with 40 naturally infected animals (immunofluorescence test-IFAT positive and parasite isolation) each classified according to their clinical symptoms (asymptomatic, oligosymptomatic and symptomatic); the second group (20 dogs) was the control, serologically and parasitologically negative for *L. chagasi*. The dogs were submitted to detailed serological, parasitological and biochemical-hematological parameters. The study on IgG antibodies was performed by IFAT and ELISA which was detected increase of levels and titles of IgG according to clinical evolution of CVL. In the parasitological studies (mieloculture, impression smears of skin biopsies and sternal bone marrow puncture for evaluation of parasite burden by "Leishman Donovan Units" – LDU) was detected *Leishmania* amastigotes in 90% symptomatic dogs in different tissues showing a positive correlation in to the disease progression. The parasitism in the bone marrow and skin was higher in the symptomatic animals in comparison to the infected dogs. Analysis of biochemical parameters showed hypergammaglobulinemia with reversal of the Albumin/Globulin ratio in oligosymptomatic and symptomatic animals. Hematological changes were observed in symptomatic dogs with anemia and leukopenia (lymphopenia, monocytopenia and eosipenia). Our data suggest that changes in laboratorial parameters (serological, parasitological and biochemical-hematological) might play an important role in the accompaniment of CVL porgnostic, can be used in the evaluation of clinical trials, since specific changes occur in each clinical symptoms groups.

Support: CNPq, FAPEMIG, FBB, UFMG, FIOCRUZ (CPqRR).

IM92 - CANINE VISCERAL LEISHMANIASIS: A REMARKABLE HISTOPATHOLOGICAL PICTURE OF ONE ASYMPTOMATIC ANIMAL REPORTED FROM BELO HORIZONTE, MINAS GERAIS, BRAZIL.

Wanderson Geraldo de LIMA[1], Danielle Maria Fonsceca MAIA[2], Marilene Suzan Marques MICHALICK[3], Washington Luiz TAFURI[2], Wagner Luiz TAFURI[2],

[1]Departamento de Anatomia Patológica e Medicina Legal, Faculdade de Medicina da UFMG. [2]Departamento de Patologia Geral, Instituto de Ciências Biológicas, UFMG; [3]Departamento de Parasitologia, UFMG, Belo Horizonte, MG, 31270-901.

Human and canine visceral leishmaniasis in the New World are caused by *Leishmania (Leishmania) chagasi* which is transmitted by the phlebotomine *Lutzomyia (Lutzomyia) longipalpis*. *Leishmania* is an intracellular protozoan parasite that is delivered to its vertebrate host by the bite of an infected sandfly. Following injection into the skin, the extracellular promastigote form of the parasite must rapidly enter its host cell, the macrophage, and later transform into the intracellular amastigote form. Visceral leishmaniasis (VL) remains a serious public health problem in the world and dogs (*Canis familaris*) are the

XXX ANNUAL MEETING ON BASIC RESEARCH IN CHAGAS DISEASE - XIX MEETING OF THE BRAZILIAN SOCIETY OF PROTOZOOLOGY - HOTEL GLÓRIA, CAXAMBU, MG, BRASIL - 10-12 NOVEMBER 2003. **Rev. Inst. Med. trop. S. Paulo, 45**(Suppl. 13), November, 2003.

peridomestic reservoir host (Anderson, 1980; Grimaldi et al., 1989; Tesh, 1995). In Brazil, VL is highly endemic in the semiarid northeastern poor states of Ceará, Bahia, Maranhão, Piauí, Pernambuco, Rio Grande do Norte and Paraíba. In all states there is an association with infected dogs and abundant *L. longipalpis* (Chagas et al., 1938; Deane & Deane, 1962; Guedes et al., 1978). Canine VL appears to be spreading further in Brazil and outbreaks have recently been reported in regions as City of Belo Horizonte (MG), (Genaro et al., 1988; Michalick et al., 1993), Teresina (PI), São Luiz (MA), Fortaleza, (CE), Rio de Janeiro (RJ) (Marzochi et al., 1994) and Bahia (BA) (Cunha et al., 1995; Carvalho et al., 1996; Ashford et al., 1998). Serological exams complement fixation reaction (CFR), indirect imunofluorescense (IFAT) and enzyme-linked immunosorbent assay (ELISA) were carried out in ICB/UFMG. The parasitism of all organs, expect skin tissues, was evaluated by the *Leishmania Donovani* Units (LDU) indices. An asymptomatic dog was sacrificed with a lethal dose of 33% Thiopentalâ (intravenous). After the necropsy samples of liver, spleen, cervical, axillary and popliteal lymph nodes, and skin (ear, nose and abdomen) were collected and fixed in 10% neutral buffer formalin solution. All tissues samples were dehydrated, cleared, embedded in paraffin, cut (4-5mm thickness) and stained by Hematoxylin and Eosin (H&E) and immunocytochemistry to detected parasites in paraffined tissues (Tafuri et al., 2003). Immunolabelled parasites were quantified by morphometrical analysis (KS300 software - Zeiss). Under optic microscopical observation all organs showed an intense parasitism associated to a severe pathological picture. All lymph nodes had conspicuous histological architecture alterations. Lymphocytes were substituted for macrophages stuffed with an intense number of amastigotes forms of *Leishmania*. The lymphoid nodules (without germinal centers) and the mantle zones in the cortex that surround the follicles were markedly attenuated. Livers showed small intralobular granulomas composed by macrophages loaded with amastigotes. Spleens had an intense depression of the white polp whereas the lymphocytes were replaced by parasitized macrophages. All fragments of different anatomical region showed a chronic inflammation characterized by plasmocytes, macrophages and lymphocyte. Intracellular parasites were ease found in macrophages in the dermis. Taken together of the morphometrical analysis data of all organs we observed higher numbers than others asymptomatic dogs that we have been analyzed in our laboratories. This asymptomatic dog had average of 16,86 to 33,49 μm^2 parasites/field. In contrast, others asymptomatic dogs had shown averages of 0,55μm^2 to 9,41$\mu m2$ parasites/field. These parasitological results and general histopathological features of this asymptomatic animal indicated an anergic immune response. However, we did not observe any clinical sign of the disease at the necropsy time.

IM93 - INFLUENCE OF SALIVA FROM *LUTZOMYIA LONGIPALPIS* IN INITIAL EVENTS OF INFECTION BY *LEISHMANIA (L.) CHAGASI* USING AN *IN VITRO* PRIMING SYSTEM.

Souza-Neto, S. M.; Afonso, L.; Clarêncio, J.; Barral, A.; Brodskyn, C. & Barral-Netto, M.

Fiocruz-CPqGM- LIP, Waldemar Falcão 121, Brotas, Salvador, BA, Brazil. e-mail:.smsouzaneto@cpqgm.fiocruz.br

Infection by *Leishmania (L.) chagas*i and the initial immune responses against this parasite are not completely understood. Interactions between the parasite, APC and lymphocytes may determine the outcome of the infection. Furthermore salivary components from *Lutzomyia longipalpis* can influence immune responses against the parasite. We evaluated the initial immune response against *Leishmania (L.) chagasi* (MHOM/BR2000/MER2) from PBMC of normal donors by using an *in vitro* priming system (lVP). Brief1y, PBMC were separated by ficoll gradient and cultured in 24 well plates at 5 x 10^6 cells/mL and subsequently infected with *Leishmania (L.) chagasi* promastigotes stationary phase (1x10^6 parasites/mL) plus 2 pair/mL of *Lutzoymia longipalpis* salivary gland sonicate (SGS). Macrophages served as APC for second stimulation and were infected 24 hours before the incubation with previously stimulated lymphocytes. Supernatants from the first round of stimulation were harvested in order to perform cytokines quantification. Blast cells were adjusted to 10^6/mL and used in the second stimulation and for flow citometry analysis. After 72 hours, supernatants from the second stimulation were harvested for ELISA and cytokine quantification. This system al1owed us to detected cytokines and surface markers, which can be modulated by the parasite and salivary gland sonicate. We tested the capacity of the *Leishmania (L.) chagasi* alone or simultaneously with SGS to stimulate the cel1s in the IVP. We found that the *Leishmania chagas*i plus SGS stimulated weak IFN-gamma production on the 1st stimulation, whereas in the second stimulation a higher production of IFN-γ was observed. Expression of $CD4^+CD25^+$: 12,5% ± 3,2 and 16,5% ±2,5 and $CD8^+CD25^+$: 8,3 ±1,5 and 10,42 ±2,3 after 1st and 2nd stimulation respectively. Actually we are looking for different markers of activation and trying to identify the influence of saliva in the immune response against *Leishmania (L.) chagasi.* The phlebotomine saliva components seem to affect the functions of the main cells involved in this response, altering cytokine profile and the expression of costimulatory molecules which are important to drive cell differentiation, and in this sense a protective immune response against *Leishmania*.

Supported by: FAPESB and TMRC-NIH

IM94 - THE INFECTIVITY OF *LEISHMANIA (VIANNIA)* SPP. TO CULTURED MACROPHAGES OF BALB/C MICE

Campos, M. B[1].; Gomes, C[2].; Pires, R. N[1].; Brandão, J. A[1].; Martins, A. F. P[1].; Corbett, C. E.[2]; Lainson, R[1].; Silveira, F. T.[1]

1- Instituto Evandro Chagas (SVS), Belém, PA; 2-FMUSP /LIM-50, São Paulo, SP.

Introduction: the infectivity of *Leishmania* may vary within the same species or strain of parasite (Pearson & Souza, 1996), and in the Old World this variation has been demonstrated for *L. (L.) major* and *L. (L.) infantum* (Liew *et al*., 1990; Mendez *et al*., 1996). Up till now, however, there have been few observations regarding such variation for New World species, in particular those of the Amazon Region where seven species have been shown to be the etiological agents of cutaneous leishmaniasis. Objective: to determine, *in vitro*, the infectivity of strains of *Leishmania (Viannia)* in cultures of peritoneal macrophages of BALB/c mice, using infection-rate as the parameter. Materials and methods: 18 strains of the following 5 species of *Leishmania (Viannia)* were studied: *L. (V.) shawi, L. (V.) braziliensis* (cutaneous and mucosal leishmaniasis), *L. (V.) lainsoni, L. (V.) naiffi and L. (V.) guyanensis*. Parasites used were stationary phase promastigotes from NNN (Difco B45) cultures. The inoculum into the cell cultures was in the proportion of 4 parasites/per macrophage. The cultures were incubated at 35^{0}C with 5% CO_2 and, 24 hours following inoculation, the slides were stained by Giemsa's method to determine the infection-rate (percentage of macrophage infected x number of parasites per macrophage). Results: the infection-rates of the species *L. (V.) shawi* (300,8); *L. (V.) braziliensis* (358,5) from cases of mucosal leishmaniasis and *L.(V.) naiffi* (289,6) were similar, and greater than the infection-rates of *L. (V.) guyanensis* (224,1), *L. (V.) lainsoni* (229,2) and the strains of *L. (V.) braziliensis* (167,1) from cases of localized cutaneous leishmaniasis The strains of *L. (V.) braziliensis* from cases of mucosal leishmaniasis were more infective ($p< 0,05$) than those from localized cutaneous leishmaniasis caused by the same parasite, and the other species, with exception of *L. (V.) shawi*. Conclusion: the difference of infection-rate among strains of *L. (V.) braziliensis* from patients with localized cutaneous leishmaniasis may have been influenced by the fact that this parasite develops slowly in culture compared with other species. In this way, the population of promastigotes used for the inoculation of the macrophage cultures was only at the beginning of the stationary phase and therefore with less infective forms than the other species which have more rapid

XXX ANNUAL MEETING ON BASIC RESEARCH IN CHAGAS DISEASE - XIX MEETING OF THE BRAZILIAN SOCIETY OF PROTOZOOLOGY - HOTEL GLÓRIA, CAXAMBU, MG, BRASIL - 10-12 NOVEMBER 2003. **Rev. Inst. Med. trop. S. Paulo, 45**(Suppl. 13), November, 2003.

growth. With regards to the difference seen between the strains of *L. (V.) braziliensis* from localized cutaneous and mucosal leishmaniasis, we suggest that this is due to a greater virulence of strains from patients with the latter disease.

IM95 - THE EVASION MECHANISM OF *TOXOPLASMA GONDII* FROM THE MICROBICIDAL ACTIVITY OF ACTIVATED MACROPHAGES IS BASED ON PHOSPHATIDYLSERINE EXPRESSION THAT INDUCES SECRETING OF TRANSFORMING GROWTH FACTOR-β CAUSING AN AUTOCRINE EFFECT.

Seabra, S.H. *, de Souza, W.* DaMatta, R.A.†

* Laboratório de Ultraestrutura Celular Hertha Meyer, Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, 21944-970, Rio de Janeiro, RJ, Brazil. †Laboratório de Biologia Celular e Tecidual, Centro de Biociências e Biotecnologia, Universidade Estadual do Norte Fluminense, 28013-600, Campos dos Goytacazes, RJ, Brazil. renato@uenf.br

Toxoplasma gondii is an obligate intracellular protozoan, which infects different cell types. Activated macrophages express inducible nitric oxide (NO) synthase (iNOS) that produce NO, a microbicidal agent that controls *T. gondii* growth. However, active invasion of *T. gondii* inhibits NO production. To understand how this parasite inhibits this microbicidal agent, mice peritoneal macrophages were seeded over cover slips, cultured in Dulbecco's Modified Eagle's Medium (DMEM) with 5% fetal bovine serum and activated with interferon-γ (IFN-γ) and lipopolysaccharide (LPS). Tachyzoites, RH strain, were obtained by peritoneal washes of infected mice. Activated macrophages were infected with a 10 to 1 tachyzoite macrophage ratio and some were treated with anti- transforming growth factor-α (TGF-β) IgY (6 µg/ml) during the 2h interaction period and until 48 h. Culture supernatants were collected after 24 and 48 h for nitrite evaluation by the Griess reagent. For localization of iNOS, filamentous actin and Smad 2 and 3 (intracellular mediators of TGF-β signaling), macrophages were labeled with anti-iNOS, phalloidin and ant-Smad, respectively. PS expression of tachyzoites was analyzed in a flow cytometer after annexin-V-FITC labeling; parasites were also treatment with annexin V after interaction. Infected macrophages presented reduced expression of iNOS and filamentous actin. This result was reverted after treatment with anti-TGF-β. Further evidence for TGF-β involvement was the increase of phosphorylated Smad 2 and 3 in infected macrophages. Tachyzoite presented 50% of its population expressing PS. Furthermore, PS blockage by annexin-V abolished NO production inhibition and parasite survival. These results indicate that the evasion mechanism of *T. gondii* is based on the surface expression of PS that induces in infected macrophages secretion of TGF-α. This factor causes an autocrine effect that induces actin filament depolymerisation, iNOS degradation and NO production inhibition as a consequence. This evasion mechanism mimics the anti-inflammatory state caused by the uptake of apoptotic cells and is similar to the mechanism used by *Leishmania* (de Freitas Balanco *et al*. Curr. Biol. 11, 1870-1873, 2001) both based on PS expression leading to TGF-β secretion.

Supported by: CNPq, FAPERJ, PRONEX.

IM96 - MODULATION OF EFECTOR FUNCTION OF MURINE MACROPHAGES BY GLUTATHIONE

Vianna P[1], Rocha-Vieira E[1], Dutra WO[2] & Gollob KJ[1].

1. Lab. Lymphocyte Biology, Dpt. Biochemistry & Immunology and 2. Lab. Cell-Cell Interactions, Dpt. Morphology, UFMG, Belo Horizonte, MG - Brazil

Leishmania are obligate intracellular protozoan parasites that infect host macrophages. The murine model of *L. (L.) major* infection has been extensively 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 and killing of the parasites living within macrophages. Promastigotes bind to specific receptors on macrophages and are internalized by receptor-mediated phagocitosis. This 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. (L.) major* infected BALB/c mice are treated with N-acetyl-cystein (NAC), a GSH precursor, the histopathologic outcome of disease is greatly improved, characterized by less intense tissue vacuolization and reduced parasite load (Rocha-Vieira *et al*., Immunol., 2003). Considering that macrophages are the main effector cells controlling parasite replication at the site of infection, 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 (60U/mL) and LPS (10ng/mL) 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 and the nitric oxide production were evaluated. 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.

Financial Support (WHO/TDR,PRONEX/CNPq). E-mail: privianna@hotmail.com etelrv@mono.icb.ufmg.br and kjgollob@mono.icb.ufmg.br.

IM97 - EVIDENCES OF APOPTOSIS IN MACROPHAGES INFECTED WITH *LEISHMANIA (L.) AMAZONENSIS* AND *LEISHMANIA (V.) GUYANENSIS*

DaMata, J.P., Sousa-Franco, J., Lima-Santos, J. and Horta, M.F.

Departamento de Bioquímica e Imunologia- Instituto de Ciências Biológicas-UFMG.

Leishmania is a gender of intracelular protozoan parasites of vertebrate animals, including man. It replicates inside macrophages, which end up rupturing and releasing the parasites, infective for neighboring cells. Being an amplifying step, host cell death may be a key point in the development of diseases. In the murine model, BALB/c is susceptible to most species of *Leishmania (L.) amazonensis*, for instance, cause a growing lesion at the site of infection and, eventually the death of the host. However, *L. (V.) guyanensis* produce no lesion following infection. Using MTT assay, we have observed a sudden reduction in the viability of peritoneal BALB/c macrophages infected with *L. (V.) guyanensis*, but not in cells infected with *L. (L.) amazonensis*. To investigate whether *L. guyanensis*-infected macrophages were dying from apoptosis, we examined the DNA fragmentation through agarose gels and TUNEL technique. In agarose gels, we have observed in both *L. (L.) amazonensis* and *L. (V.) guyanensis*-infected cells the fragmentation of DNA that appeared as a ladder pattern with fragment sizes multiples of ~200 bp, typical of apoptotic cells. Using the TUNEL technique, we have also observed that both *L. (L.) amazonensis* and *L. (V.) guyanensis*-infected cells had their nuclei labeled. The percentage of apopotic nuclei was higher in macrophages infected with *L. (V.) guyanensis* than in cells infected with *L. (L.) amazonensis*. These results suggests that programmed cell death occurs in macrophages infected with *Leishmania*. It remais to be investigated whether macrophage death through apoptosis contributes to a less severe outcome of the disease.

XXX ANNUAL MEETING ON BASIC RESEARCH IN CHAGAS DISEASE - XIX MEETING OF THE BRAZILIAN SOCIETY OF PROTOZOOLOGY - HOTEL GLÓRIA, CAXAMBU, MG, BRASIL - 10-12 NOVEMBER 2003. **Rev. Inst. Med. trop. S. Paulo, 45**(Suppl. 13), November, 2003.

IM98 - ELIMINATION OF *LEISHMANIA (V.) GUYANENSIS* BY MURINE MACROPHAGES: POSSIBLE INVOLVEMENT OF REACTIVE OXYGEN INTERMEDIATES IN APOPTOTIC DEATH OF THE PARASITES

Sousa-Franco, J., Mendes, E. A., Da Mata, J. P., Lima-Santos, J. and Horta, M. F.

Departamento de Bioquímica e Imunologia - Instituto de Ciências Biológicas-UFMG.

Murine leishmaniasis have different outcomes determined by either the species of *Leishmania* or the mouse strain. In the present work, we show that BALB/c mice, incapable of healing *L. (L.) major* or *L. (L.) amazonensis* lesions, do not develop lesion when infected with *L. (V.) guyanensis*. Accordingly, the percentage of macrophages infected *in vitro* with *L. (V.) guyanensis*, but not with *L. (L.) amazonensis*, decreases significantly in 72 h reaching almost null values in 96 h. Using the TUNEL technique, we have shown that, in 24 hs after infection, 25% of *L. (V.) guyanensis*-infected macrophages had parasites with DNA fragmentation, whereas 8% of *L. (L.) amazonensis*-infected cells had stained parasites. In 100 macrophages observed, around 35 positive *L. (V.) guyanensis* amastigotes were found as oposed to 9 positive *L. (L.) amazonensis* amastigotes. These results suggest that *L. (V.) guyanensis* amastigotes die through apoptosis inside the macrophage. Since *L. (V.) guyanensis*-infected macrophages do not produce detectable levels of NO, we have looked at the ability of *L. guyanensis* to induce the respiratory burst. We have found that *L. (V.) guyanensis*, but not *L. (L.) amazonensis*, induces the respiratory burst of BALB/c macrophages, as determined by chemoluminescence. Inhibition of respiratory burst impaired the capacity of BALB/c macrophages to eliminate *L. (V.) guyanensis*. We have also shown that H_2O_2 is able to kill *L. (V.) guyanensis* in a dose-dependent manner. Our results suggest that the elimination of *L. (V.) guyanensis* by BALB/c macrophages is probably through apoptosis, which may be due to reactive oxygen intermediates generated during infection.

Support: CNPq, FAPEMIG, PRONEX and PADCT

IM99 - PRODUCTION OF OXIGEN REACTIVE SPECIES BY MACROPHAGES INFECTED WITH *LEISHMANIA (L.) MAJOR*

H. C. Santiago, L. A. V. D. R. Santos; F. A. Barbosa; L. Saraiva; L. Q. Vieira1

1 Departamento de Bioquímica e Imunologia, ICB, UFMG, CP 486, 30 161-970, Belo Horizonte, MG, Brazil

Leishmania (L.) major is an intracellular parasite of human macrophages that also infects mice under experimental conditions. Natural resistance to this parasite is characterized by the development of small lesions at the site of infection that spontaneously heal, although parasitological cure does not occur naturally. Resistance, in the murine model, is characterized by production of high levels of interferon-gamma (IFN-γ), which activates macrophages to produce nitric oxide (NO). This radical is the ejector molecule that kills the parasite inside the macrophage. Several studies have dismissed other oxygen reactive species as having any effect on *L. (L.) major*. However, we have observed that IFN-γ knockout mice are more susceptible to infection with *L. (L.) major* than mice that lack the inducible nitric oxide synthase (iNOS). Hence, it is possible that in the absence of NO, macrophages resort to a different control mechanism that is still dependent on IFN-γ. Here, we investigate if oxygen reactive species are produced by macrophages from wild-type, IFN-γ knockout or iNOS knockout mice in response to infection with *L. (L.) major*. Macrophages were infected in vitro with *L. (L.) major* in the presence or absence of IFN-γ or phorbol miristate acetate (PMA). We found that both knockout mice produced oxygen reactive species (as detected by reaction with luminol) at a much higher level than the wild type mice, in the absence of any stimulus. When stimulated with *L. major*, wild-type mice produced very low levels or oxygen reactive species, while both iNOS and IFN-γ knockout mice produced 10 times as much. The same profiles were obtained when macrophages from knockout and wild-type mice were stimulated with PMA or zymosan. iNOS knockout macrophages were able to control growth of *L. (L.) major* when stimulated with IFN-γ and PMA. We suggest that, in the absence of NO production, macrophages resort to production of high levels of reactive oxygen species, which are capable of some control of parasite growth.

IM100 - NITRIC OXIDE PATHWAY FROM *L. (L.) AMAZONENSIS* PARTICIPATES ON PARASITE-MACROPHAGE INTERATION

Marcelo Genestra, Léa-Cysne-Finkelstein, Leonor L Leon

Department of Immunology, Oswaldo Cruz Institute, Oswaldo Cruz Foundation/ FIOCRUZ, Brazil.

Analysis of the interaction of *Leishmania* promastigotes with the target host cell suggests that both parasite and host molecules are involved in cell adhesion. The main function of macrophages is to destroy intracellular pathogens, but the manner in which *Leishmania* and other intracellular parasites are able to survive and replicate within this ostensibly hostile intracellular milieu is an important question in cell biochemistry and immunology (Bogdan *et al*., 1996). Nitric oxide (NO) a free radical derived from molecular oxygen and the guanidine nitrogen of L-arginine, is involved in a variety of biological functions in different cells, and is an important anti-microbial effector molecule in macrophages against intra- and extra cellular pathogens (Moncada & Higgs, 1995). The present work provide evidence of the participation of NO pathway from *L. (L.) amazonensis* in infection mechanism, through assays realized with the L-aginine analogs L-NAME (Nw-nitro-L-arginine methyl esther) in order to verify the importance of L-arginine metabolism to macrophage-parasite interaction. *L. (L.) amazonensis* (MHOM/BR/77/LTB0016 strain), were maintained in Schneider's Insect Medium supplemented with 10% of heat inactivated FCS at 26^{0}C and pH 7.2. Murine resident peritoneal macrophages obtained from BALB/c mice were collected in cold serum free RPMI 1640 medium and incubated for 2 hours at 37°C in an atmosphere containing 5% CO_2. The cells ($5x10^5$ cells/well) were maintained in the same culture conditions for 24 hours before the infection. *L. (L.) amazonensis* promastigotes were pre-incubated with 0.5mmol/L L-NAME for 24 hours and parasites were harvested fro the medium in the log late phase of growth. Parasites were incubated overnight with peritoneal adherent cells (10 parasites/cell, $5x10^6$ parasites/well), in a CO_2 incubator at 37°C. Infected macrophages were maintained in RPMI 1640 with 5% HIFCS for 24 and 48 hours. Coverlids were fixed in methanol and stained with Giemsa's solution and culture supernatants were collected until the assay for measurement of nitrite by the Griess method (Green *et al*., 1982). It was possible to observe the interference of L-NAME on metabolic NO pathway of parasites, at the point to interfere on macrophages infection. The infection range of the murine macrophages by *L. (L.) amazonensis* pre-cultured with L-NAME decreases significantly from 93,3% to 61% in 24 hours and was still lower (19%) at 48 h post infection. The NO production also was seriously affected. These facts pointed strongly that the promastigotes-NO pathway exerts a fundamental role to establishment of the infection; and this data corroborated with studies of Ballestieri *et al.* (2002) such stated out that the increase of the parasites number on macrophage phagolysosom, in these times evaluated, takes to downregulation of $NO^{\cdot}$ production by iNOS.

Supported by PDTIS, PAPES and IOC/FIOCRUZ

XXX ANNUAL MEETING ON BASIC RESEARCH IN CHAGAS DISEASE - XIX MEETING OF THE BRAZILIAN SOCIETY OF PROTOZOOLOGY - HOTEL GLÓRIA, CAXAMBU, MG, BRASIL - 10-12 NOVEMBER 2003. **Rev. Inst. Med. trop. S. Paulo, 45**(Suppl. 13), November, 2003.

IM101 - LEISHMANICIDAL ACTIVITY AND NITRIC OXIDE SYNTHASE EXPRESSION BY HUMAN MACROPHAGES ARE DEPENDENT ON INTRACELLULAR GSH LEVELS.

Rocha-Vieira E[1], Vianna P[1], Dutra WO[2] & Gollob KJ[1].

1. Lab. Lymphocyte Biology, Dept. Biochemistry & Immunology and 2. Lab. Cell-Cell Interactions, Dept. of Morphology, UFMG, Belo Horizonte, MG - Brazil

Glutathione (GSH) is the major intracellular redox buffer. It plays an essential role in protecting cells against oxidant damage and modulates the expression of several genes. Moreover, it is critical for T cell proliferative response to mitogens and can modulate the pattern of cytokine secretion during cellular immune responses. We are interested in how gluthathione modulation could be employed to improve immune responses. For this purpose we evaluated the response of human macrophages to *Leishmania (V.) braziliensis* infection and IFN-γ and LPS stimulation in the presence of glutathione modulating agents. Reducing intracellular GSH levels in macrophages with dethyl-maleate (DEM) led to an increased frequency of infected macrophages, which was further correlated with a reduced expression of nitric oxide synthase, in response to IFN-γ and LPS stimulation. These findings indicate that reduced GSH levels are detrimental to the nitric oxide dependent leishmanicidal activity of human macrophages. We are know investigating whether increasing macrophage GSH levels could be used as a strategy to improve the host response to leishmania infection.

Financial Support (WHO/TDR and PRONEX/CNPq). E-mail:etelvieirav@terra.com.br and kjgollob@mono.icb.ufmg.br.

IM102 - IFN-γ PRODUCTION IN MUCOSAL LEISHMANIASIS PATIENTS IS NOT ASSOCIATED TO A+874T POLYMORPHISM IN A PILOT STUDY

Bittar, R.C.[1], Nogueira, R.[1], Mattos G[2], Silva-Filho VF[2], Covas, C.J.[1], Amato, V.S.[4], Mattos, M.[3], Oliveira-Neto, M.P.[3], Coutinho, S.G.[1], Sampaio, E.P.[2], Moraes, M.O.[2#], Da-Cruz, AM[1]*.

[1]Lab. Imunidade Celular e Humoral, Dept. Imunologia/IOC, [2]Lab. Hanseníase, Dept. Medicina Tropical/IOC, [3]IPEC/FIOCRUZ, [4]Dept. de Doenças Infecciosas e Parasitárias, FCM-USP. *alda@ioc.fiocruz.br, [#]mmoraes@fiocruz.br

Interferon-gamma (IFN-γ) is a cytokine, which is believed to play a key role in controlling the intracellular pathogens but can also trigger inflammatory complications of many infectious diseases. High levels of IFN-γ and TNF-α are implicated in the development of tissue damage in mucosal leishmaniasis (ML) (Da-Cruz et al. 1996, Bacelar et al. 2002, Amato et al. 2003). Recent results of our group pointed that two subgroups of IFN-γ producers are found among long-term cured ML patients: high and low responders although those differences were not significant at that time. These results raised the hypothesis that those two IFN-γ profiles could be associated with the beneficial clinical evolution or reactivation of the disease (Da-Cruz et al., 2002). Our aim was to investigate if single nucleotide polymorphisms (SNPs) A+874T of IFN-γ gene (that have been previously associated with low/high production of the cytokine in tuberculosis) are associated to the clinical course of ML and the levels of IFN-γ production *in vitro*. Methodology and patients: Twenty-six ML and 15 cutaneous leishmaniasis patients were studied. The cellular immune responses were analyzed *in vivo* by Montenegro skin test (MST). For *in vitro* studies, lymphoproliferative response assays (LPR) of peripheral blood mononuclear cells (PBMC) stimulated with *Leishmania (V.) braziliensis* antigens (Lb-Ag) were performed. The culture supernatants were tested for IFN-γ production by ELISA tests. The genomic DNA was obtained from frozen PBMC and the detection of genotype for IFN-γ polymorphism by polymerase chain reaction-amplified refractory mutational system (PCR-ARMS). Results: As expected, ML patients produce higher levels of IFN-γ than patients from the cutaneous form. The production of IFN-γ was directly related to the period of illness (r=0,65, p=0.002), but not to the MST diameter. We have observed TT genotype (12,1%), that was normally accounted for high IFN-γ producer, only in ML but the number of patients did not allow statistical analysis. The levels of IFN-γ in culture supernatants were not statistically significant (p=0.43) among the different genotypes either in ML (AA mean= 8.216±7.377,5 pg/ml; TA mean=10.550±8.801pg/ml; TT mean=3.167±3.665pg/ml) or cutaneous leishmaniasis (AA mean= 993±1.655 pg/ml and TA mean= 2.517±2.353 pg/ml). Interestingly, IFN-γ secretion among TT patients was lower than in the other genotypic groups. Conclusions: The preliminary results suggest that IFN-γ genotype could influence the outcome of ML, but once the status ML vs. CL is achieved other levels of regulation might be at place to define IFN-γ secretion in leishmaniasis patients.

Apoio Financeiro: PAPES III/VPPDT e IOC - FIOCRUZ, FAPERJ.