IM-1
A FUSION PROTEIN SPECIFIC FOR *TRYPANOSOMA CRUZI* AMASTIGOTES INDUCES ANTIBODIES THAT CROSS-REACT WITH A HOST'S HEART PROTEIN

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The protozoan *Trypanosoma cruzi*, is the causative agent of Chagas disease, which is endemic in many Latin American countries. The finding of a T cell-rich inflamatory mononuclear cell infiltrate in the presence of extremely few parasites in tissue lesions can doubt on the direct participation of *T. cruzi* in the pathogenic mechanism, and suggested the posible involvement of autoimmunity. Such an anti-tissue autoimmune response could be triggered either by molecular mimicry with some parasite antigens homologous to tissue proteins, or by the display of intracellular sequestred tissue proteins. Antibodies to *T. cruzi* have been found to cross-react with host components, and T cells directed against cardiac and nervous sistem have been found in mice with experimental Chagas disease. T cells directed against *T. cruzi* antigens which crossreact with nervuos tissue have been found to recreate nervous tissue pathology when transferred to naive animals. These features suggest to the hypothesis of an autoimmune process leading to chronic disease where *T. cruzi* antigenically mimics host tissues. In order to investigate whether amastigote specific recombinant protein induces antibodies that cross-react with host components, the DNA insert of an amastigote specific cDNA clone (Am230), was subcloned in an expression vector and fusion protein (pMAL-Am230) purified by affinity chromatography. Polyclonal antibodies against pMAL-Am230 localizes the native protein (66 kDa) on the surface's parasite, and cross—react with a cardiac tissue protein of 97 kDa. By tissue immunoperoxidase staining tha anti-pMAL-Am230 antibodies reacted with miocardics fibers. The above results suggest that the 66 kDa amastigote specific protein could be a candidate antigen for autoimmune mimicry leading to cardiac tissue pathology.

IM-2
A NEW FOCUS OF CANINE VISCERAL LEISHMANIASIS IN SÃO GONÇALO PERI-URBAN AREA, RIO DE JANEIRO

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A two years old mongrel female dog showing progressive loss of weight, cachexia, hind legs hemiplegia, single small crusted ulcers (mainly in thorax and elbows), generalized loss of hair, onychogryphosis, apathy, edema and intestinal disturbs was examinated by our team at the Policlínica veterinaria-UFF. Preliminary laboratorial studies showed in serum an inverted albumin/globulin index (2.1/5.7g/l). Hemocytozoa (*Ehrlichia* sp. and *Babesia canis*) were absent from peripheral blood. The presence of antibodies to *Leishmania (L.) donovani* was demonstrated by the FML-ELISA assay, (Abs 492nm= 0.778/ cut-off 0.435) in this animal and in other three assymptomatic dogs from the neighborhood. Sera samples of three of these dogs (the symptomatic one among them) were positive (1/80) by the Bio-Manguinhos Immunofluorescent assay (Fiocruz) and negative by both, an Immunofluorescent and an ELISA assays using *L.(L.) mexicana* antigen. The FML-ELISA assay previously showed 100% sensibility and 96-100% specificity in diagnosis and of human and canine Kala-azar, respectively. Recently, the test proved to be highly predictive recognizing as seropositive the sera of 21 assymptomatic dogs of a kala-azar endemic area that developed the fatal disease in a 6-month period. The symptomatic dog was removed from São Gonçalo and further monitored in our lab. 40 days after dog remotion, anti-FML antibodies were still present in serum (0.643± 0.123), recognizing the FML-GP36 antigen in Western blot membranes. Hematocryt decreased from 40 to 31%; platelets counts ranged from 350 to 54 x 10^3ml, and monocytes increased 960 to 5.100x 10^3/mm^3. The albumin/globulin ratio keep abnormal (2.52 ± 0.32/ 5.32 ± 0.7 g/l). An increase in serum amylase (1616 U/l) disclosed a discrete pancreatitis. DTH reaction to promastigote was negative, as well as parasite evaluation in bone marrow and lymphnode punction, NNN culture, ear distal edge biopsy and ulcerated skin. Amastigotes were found in Giemsa stained spleen smears after splenectomy. Hepato-splenomegaly was patent. *In vitro* mononuclear cell proliferation was significantly increased after incubation with FML antigen. A screening for determination of anti-*Leishmania* seroprevalence in São Gonçalo dogs is underway. To our knowledge this is the first report of a case of zoonotic canine visceral leishmaniasis in the periurban area of São Gonçalo, in the State of Rio de Janeiro.

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IM-3
A PRELIMINARY STUDY OF THE INFLAMMATORY RESPONSE INDUCED BY *LEISHMANIA MAJOR* AFTER IMPLATING PARAFFIN-TABLETS SUBCUTANEOUSLY IN BALB/C MICE

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The present study has evaluated the kinetics of the inflammatory response in skin of BALB/c mice infected with *L. major* after implanting paraffin-tablets. The paraffin tablets (220,0mg x 1,0cm x 0.5cm) were subcutaneously implanted in the dorsal region of mice. Immediately after the implanting, mice received an injection of 1 x 10^6 promastigotes of *L. major*/ 0.1 ml subcutaneously at the same anatomical region. Mice infected with promastigotes of *L. major* without paraffin-tablets were used as control. All animals were sacrificed 7, 14 and 21 days after infection. Tissue samples (all the skin around the paraffin-tablets) were collected for histopathological and electron microscopic studies.

After the 7^{th} day, the microscopical analysis of skin of animals in which the paraffin-tablets were implanted showed a delicate granulation tissue (fibroblasts, small blood vessels and leukocytes) that became mature by day 21. However, in animals in which *Leishmania* was inoculated after the paraffin-tablets, the skin samples showed an intense and diffuse inflammatory reaction composed of lymphocytes and macrophages loaded with *L. major* amastigotes. Moreover, this inflammatory reaction was more intense than the group infected with *L. major* alone. The preliminary electronic microscopy results have shown intracellular amastigotes without the "parasitophorus vacuole" and the majority were degenerated. Taken together, these results suggest that paraffin is able to stimulate monocyte-macrophages to become effector cells despite being host cells. Immunocytochemistry and immunological studies are being carried out to better understand these results.

IM-4
A RELIABLE AND SPECIFIC ELISA METHOD TO CAPTURE IGM FROM HUMAN CHAGASIC SERA USING FIXED EPIMASTIGOTES OF *TRYPANOSOMA CRUZI*

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Chagas' disease, a zooanthroponosis caused by hemoflagellate *Trypanosoma cruzi*, was considered by OPAS/ WHO as the most important parasitic disease in Latin America. However, in human, the acute phase of this illness is not well understood. In this work, a rapid, sensitive and reliable direct ELISA was stablished to determine the levels of anti-*T. cruzi* IgM in acute chagasic sera (ACS). We have used this method to screen an epidemiologic survey of Chagas' disease in a hyperendemic area in Bolivia. The ELISA method was compared to both indirect immunofluorescence (iIF) and parasite DNA detection by PCR. The standard protocol developed in our experiments involves coating wells with 10^6 formaline killed *T. cruzi* epimastigotes, incubation with a 1/250 mixture of the patient's serum, previously treated with a rheumatoid factor (RF) neutralizing reagent, and horseradish peroxidase-conjugate goat anti-human IgM. Comparing the ELISA method to others, 95% and 71% of the results correlated to PCR and to iIF, respectively. At the serum dilution applied, RF did not influence the results and the samples with Leishmaniasis or with mixed *Leishmania* and *T. cruzi* infection could also be discarded from ACS. Highly specific and reliable results were obtained, a great number of the sera could be tested in only one assay, and a quantitative index of variation could be calculated, without serial titration. Using test samples in triplicates, the method offers an useful tool to detect early acute phase of *T. cruzi* infection in humans.

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IM-5
AMERICAN TEGUMENTARY LEISHMANIASIS (ATL): ANTIGENS REACTIVE T-CELLS DERIVED FROM LESIONS

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We have already shown that in ATL patients a preferential induction of blood $CD4^+$ *Leishmania*-reactive T-cell and a mixed type 1 (IFN-g) and type 2 (IL-4, IL-5) cytokine pattern is associated with active lesions, while $CD8^+$ cells and a type 1 cytokine production are observed during the healing process. Immunopathological studies of leishmanial lesions have contributed to characterize the *in situ* inflammatory infiltrate. Our objective is to analyze the *in vitro* responsiveness of those infiltrating cells to the parasite antigens. Mononuclear cells (LeMC) obtained from lesions of 39 cutaneous leishmaniasis patients (CL), 04 mucosal patients (ML) and 05 patients with disseminated lesions (DissL) were phenotypically characterized for B, T, $CD4^+$, $CD8^+$, gd, macrophages, IL-2 receptor (IL-2r) and IFN-gr by flow cytometry. The percentages of the T-cell subpopulations were similarly distributed in the inflammatory infiltrates of CL, ML and DissL patients ($CD4^+$=31.3±4.3; $CD8^+$=30.6±5.4). Higher percentages of IL-2r (X=87.2±5.2%) and IFN-gr (X= 81.3±6.6%) were observed in ML as compared to CL and DissL. Assays of lymphocyte proliferative responses (LPR) of LeMC induced *in vitro* by *L. braziliensis* (Lb) antigens as well as by concanavalin A (Con-A), *T. gondii* (Tg) and *T. cruzi* (Tc) were performed. Positive LPR to Lb antigens (stimulation indices – SI ³ 2.5 over the background cultures) was seen in 12 out 23 CL patients (X Dcpm x 10^{-3}=5,2±2). LeMC from 10 out 16 CL patients seroreactive to *Toxoplasma*, proliferate in response to Tg antigens (Dcpm x 10^{-3}=8,3±5). The LeMC from seven patients with no serological evidence of past *T. gondii* infection did not proliferate to Tg antigens. The LPR to *T. cruzi* was positive in only 01 out 18 CL patients (SI=3.4). The supernatants of those cultures were harvest for type 1 and type 2 cytokine quantification. IFN-g production was detected in LeMC cultures stimulated with Con-A, Lb and Tg. Nor IL-4 neither IL-5 production were detected in the Lb-stimulated culture supernatants. Lb-reactive cells after four days in cultures were also separated in a Percoll gradient for $CD4^+$ and $CD8^+$ phenotypic analysis. The percentages of $CD4^+$ and $CD8^+$ Lb-reactive cells were variable: 03 patients had $CD4^+$ > $CD8^+$ and 03 had $CD8^+$ > $CD4^+$. It was seen that T-cells from leishmanial lesions are able to proliferate and produce IFN-g not only in response to leishmanial antigens but also after stimulation with other non related parasite antigens (Ec: Tg). So cell populations reactive to other antigens can also migrate to the site of the leishmanial lesions. Their role for healing or aggravation of the lesions in not well determined.

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IM-6
ANALYSIS OF HUMAN LYMPHOCYTE RESPONSES TO FOUR RECOMBINANT *LEISHMANIA* ANTIGENS

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The ability of purified leishmania antigen to induce human immune response is an important step for selection of molecules candidate to vaccine against leishmaniasis. In the present study the human T cell responses to four recombinant leishmania antigens (HsP83, H_3, H_2A, KmP_{11}) was evaluated. H_3 and H_2A are histones of the nucleosomas and the KmP_{11} is a protein of 11KD found in flagely of the promastigotes. These antigens are high immunogenic in dogs. The cytokine profile (IFN-g, IL-5 and IL-10) on supernatants of lymphocyte cultures stimulated with the recombinant antigens of 11 leishmaniasis patients with cutaneous leishmaniasis and four controls were determined by ELISA. None of the recombinant antigens induced significant cytokine production in cells from healthy donnors. The mean IFN-g levels were: crude leishmania Ag (1067 ± 572pg/ml), HsP83 (239 ± 314pg/ml), H_3 (274 ± 628pg/ml), H_2A (300 ± 456pg/ml), KmP_{11} (100 ± 126pg/ml). Although there was no significative IL-5 production in cultures stimulated with the recombinant antigen, IL-10 production was high in three of the four Ag tested: HsP83 (656 ± 105pg/ml), H_3 (251 ± 257pg/ml), H_2A (24 ± 39pg/ml) and KmP_{11} (789 ± 59pg/ml). Depletion of CD4+Tcells abrogated IFN-g production to all antigens. Although all 4 antigens induced IFN-g production, 3 of them also stimulate IL-10 secretion. The H_2A antigen was the only antigen able to induce IFN-g production and no IL-10.

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IM-7
ANALYSIS OF MAST CELL EXPRESSION AND FUNCTION IN HUMAN AMERICAN TEGUMENTARY LEISHMANIASIS

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Mast cell can participate in chronic inflammation since they can be activated for cytokines release by parasites components through conserved receptors. This study was designed to examine the influence of mast cell in the immunopathogenesis of human leishmaniasis. We performed a morphometric analysis by cytologic detection of naphtol

AS-D chloroacetate esterase using a commercial kit (Sigma, USA) in lesions from patients with localized cutaneous leishmaniasis (LCL) and mucosal leishmaniasis (ML). The number of mast cells was significantly higher (p=0.04) in 15 LCL patients (39,19 ± 24.8 cells/mm^2) than those observed in mucosal biopsies of 8 ML patients (19,65 ± 20,71 cells/mm^2). Number of mast cells was significantly elevated in lesions from patients with a Montenegro skin test reaction < 20mm (p=0.03) as compared to those that show reactions higher than 40 mm. In order to verify the activation of mast cell by *Leishmania braziliensis* promastigote total antigen we used the human cell line HMC-1. The cells were cultured with procyclic and metacyclic antigens at different times (30 sec, 1 min, 5 min and 15 min) at 37ºC. After stimulation, the cells were stained for naphtol esterase or immunostaining using anti-triptase monoclonal antibody (Dako, Denmark) and classified as degranulated (staining alteration or granules discharge) or normal. The procyclic antigen was able to activated an expressive percentage of cells (range from 30,7% to 64%) while metacyclic antigen induced activation in a much lower degree (range from 19,8% to 40,6%). We also performed a RT-PCR to analyse the expression of IL-4 and p40 IL-12 transcripts in HMC-1 cells stimulated with *Leishmania* procyclic or metacyclic antigens. Procyclic antigen was able to induce the expression of IL-4 and IL-12 transcripts, while no expression of these cytokines mRNAs were found in non-stimulated cells. The metacyclic antigen induced only the expression of IL-12, but not IL-4. These data suggest that mast cells are likely to be a cytokine source in *Leishmania* lesions and therefore should be considered to have a role in the pathogenesis of human leishmaniasis.

Supported by Faperj.

IM-8
ANTIBODIES AGAINST SPOROZOITE OF *PLASMODIUM GALLINACEUM*(PG) IN SERA FROM INDIVIDUALS WITH PAST ACUTE MALARIA IN ENDEMIC AREAS OF *PLASMODIUM FALCIPARUM* (PF) AND *P. VIVAX* (PV) AND IN FOCAL AREAS OF *P. VIVAX* TRANSMISSION

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Molecular approaches recently used to classify malaria parasites (instead of the traditional use of blood stage morphology, biology and vertebrate susceptibility) places the avian malaria Pg close to the human Pf malaria. Monoclonal antibodies to the circumsporozoite protein (CS) of Pg (Rocha et al. 1990 *Braz J Med Biol Res 23*) has allowed cloning and sequencing this gene showing homologies between Pg and Pf (McCutchan et al. 1996 *PNAS 93*). These two parasites may cause death of the vertebrate host by cerebral malaria, although through different developmental stages - in the case of Pf blood forms block the capillary vessels of the brains; with Pg exoerythrocytic forms block the vessels. In any case, there are neurological signs of parasitism and intense morbidity, not well understood. The possibility that Pg sporozoites may represent a good experimental model for such malaria studies has inspired our work and the Pg sporozoite reactivity with human sera from malarial patients has been studied. Most patients were from areas with Pf and Pv transmission (Apiacas/MT, n= 411); a few were diagnosed in Belo Horizonte/MG (BH) (n=23) with imported malaria from the Amazon; others had Pv only (Belem/PA n= 80; Mantena, MG, n= 120, a focal area of Pv (Fontes et al. 1991 *Am J Trop Med Hyg 44*). Such reactivity was evaluated by indirect immunofluorescence assay (IFA) and by western blot (WB). In areas of Pf we observed 70% of reactivity by IFA. Patients classified accordingly to the last parasites (in thick blood smears confirmed by microscopy) had IFA positivity of 82% for Pf and 49% for Pv. We assume that the latter group had been infected by Pf as well, since all Pv sera were negative. Control sera from non-exposed individuals living in BH were always negative with Pg sporozoites. By WB, only sera from Pf patients (positive by IFA) reacted with a sporozoite protein of low molecular weight which however, did not correspond to the CS protein recognized by the anti-CS mAb (MW 76 and 64 kDa); none of the anti-Pv sera or normal sera bound the low molecular weight protein recognized by the Pf sera. It is tempting to conclude that only sera from human malaria caused by Pf react with Pg sporozoites, in support of a phylogenetic closeness. We still need to find areas of focal Pf transmission, to confirm that such Pg reactivity is indicative of previous exposure to Pf sporozoites. Results of IFA using blood stage Pg antigens showed a similar reactivity (>83%) with either Pf or Pv human malaria species. Now we are attempting to characterize further this sporozoite antigen as to make it available for an ELISA test, valuable for epidemiological evaluations of previous contact with Pf sporozoites.

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IM-9
ANTIBODY RESPONSES OF AMERICAN CUTANEOUS LEISHMANIASIS PATIENTS TO HEAT SHOCK PROTEIN 70 KD AND DERIVED PEPTIDES

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The heat shock proteins (HSPs) are some of the most dominant antigens recognised during the immune response to a wide range of pathogens. They are highly conserved from prokaryotic to eukaryotic organisms including humans. However, the major concern about the HSPs is the possibility that they induce autoimmune responses to cross reactive epitopes. On the other hand, it is well established that the antibody titers against *Leishmania* are very high in the severe forms of ACL (diffuse, LCD and mucocutaneous leishmaniasis, LCM), This may suggest that they do not mediate protection. Members of the HSP70 kD family have been cloned for *Leishmania sp* and many other pathogens and show clear sequence homology with the mycobacterial, *Escherichia coli* and human HSP70 genes. Therefore, we examined the possible association of antibody levels to HSP70 kD and derived peptides in severe forms of ACL. We measured using ELISA, the serum levels of IgG to recombinant HSP70 kD from *M. tuberculosis* and peptides containing *Leishmania / Mycobacterium* specific or conserved sequences between both microorganisms and human. A total of 30 ACL patients and 14 healthy volunteers were studied. The patients were diagnosed at the "Instituto de Biomedicina" by established clinical, by epidemiological and by histopathological criteria as either localised cutaneous leishmaniasis (LCL, n=16), MCL (n=14) and DCL (n=10). ACL patients showed higher titers of IgG anti-HSP70 kD compared by t test to healthy volunteers ($0.001<p<0.05$). Interestingly, DCL and MCL patients had lower ($p<0.05$) levels of IgG to human specific HSP70 residue 88-103 versus control individuals and LCL patients. In contrast, there was not observed differences between patient groups when the same sequence in *Leishmania sp* HSP70 kD was considered. Responses in LCL patients had much higher levels of anti- residue 109-123 (*Leishmania* HSP70 kD sequence) compared with DCL patients and healthy volunteers. As for the residue 88-103, a human sequence containing residue 109-123 did not induce different levels of IgG in ACL patients. In summary, HSP kD was recognised by ACL patients but it does not seem to be implicated in the pathology of ACL, since human residues elicited high levels of IgG in the resistant form of the disease (LCL). Further studies are require to extend this analysis to antibody subtypes reflecting T helper subset activity.

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IM-10
ANTIBODY RESPONSE TO THE N- AND C-TERMINAL REGIONS OF *PLASMODIUM VIVAX* MSP1 ARE RELATIVELY SHORT-LIVED

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The Merozoite Surface Protein 1 of *Plasmodium* sp. have been intensively studied as candidate for the development of a vaccine against malaria. The present study was designed to determine the persistence of antibody response to the N- and C-terminal regions of *P.vivax* MSP1 (PvMSP1) after infection *with P. vivax* in individuals from the north of Brazil. The IgG and IgM antibodies were estimated by ELISA using as antigen $PvMSP1_{19}$ containing the most C-terminal 111 amino acids representing the two EGF-like regions of PvMSP1. We also used a recombinant protein spanning 506 amino acids of the N-terminal region (ICB2-5). Both recombinant proteins were expressed in *E. coli* as GST fusion proteins. The frequency of individuals with patent infection who have IgG antibody that recognize recombinant proteins ICB2-5 or $PvMSP1_{19}$ were 63,8 and 75%, respectively. After only two months following treatment, there was a significant reduction in the frequency of responders to ICB2-5 and $PvMSP1_{19}$ (38,9 and 47,2%, respectively). Among responders to the C-terminal region, 44,4% became serologically negative and 44,4% had their antibody titers reduced. Only 11,2% of individuals had their antibody titers maintained during that period. The decay in the antibody response to the recombinant protein representing the N-terminal region of PvMSP1 was also noted, however it was not as dramatic. In sera from individuals with patent infection, IgG1 and IgG3 were the predominant subclasses of antibodies that recognized both recombinant proteins. In addition, we found that the frequency of serum samples containing IgM specific for $PvMSP1_{19}$ was higher in individuals during their first malaria episode when compared to individuals during their second or third infection. In contrast, the frequency of individuals with IgM to recombinant protein ICB2-5 was high in all three groups. This fact indicates that a high frequency of individuals with antibodies to ICB2-5 was unable to switch properly from IgM to IgG during their second or third contact with parasite antigens. The fast decline in the antibody levels to the C-terminal region of PvMSP1 might contribute to the high risk of re-infection in these individuals.

IM-11
ANTIMONIUM TREATMENT DECREASES *LEISHMANIA*-INDUCED TNF-α PRODUCTION BY HUMAN MACROPHAGES

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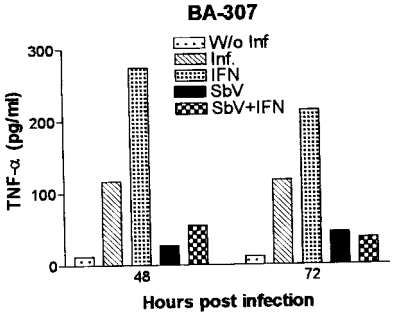
Leishmania infection stimulates TNF-α production by human macrophages. Such production is dependent on the presence of IFN-γ. Pentavalent antimonium (Sb^v)salts are the drugs of choice in the treatment of leishmaniasis.

This report deals with the influence of antimonium on the initial response of the human cell to the parasite. We have cultured either monocytes (obtained by Percoll gradient cultured for 3 days and infected) or macrophages (PBMC left to adhere for 12 hours, washed, cultured for 7 days and then infected). Infection was performed at a 10:1 parasite:cell ratio, with either *L. amazonensis* or *L. donovani*. Sb^v (10 mg/ml) was added immediately after infection and kept for 72 hr, supernatants were then harvested and TNF-a levels determined by ELISA. In some cultures IFN-g (10 IU/ml) was added simultaneously to the Sb^v.



Antimonial treatment sharply decreases TNF-a production by leishmania-infected macrophages, even on the presence of IFN-g.

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IM-12
ANTI-*TRYPANOSOMA CRUZI* VACCINE: PARTICIPATION OF HUMORAL IMMUNITY AND LYMPHOCYTE SUBSETS IN PROTECTION

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The mechanisms that act to control *T. cruzi* infection in normal mice have been intensively exploited in the last ten years, and various mechanisms were demonstrated to participate in parasitemia control. Herein, we sought to determine which immune mechanisms confer protection to animals vaccinated with live clone CL-14 trypomastigotes. These animals do not show histo- or immunopathology, and do not die or develop patent parasitemia when challenged with virulent *T. cruzi*. Our results show: (1) *in vivo* depletion of CD4 or CD8 subsets from CL-14-immune mice did not abolish protection; (2) passive serum transfer conferred efficient protective immunity; (3) challenged-vaccinated mice presented higher plasma titers of anti-*T. cruzi* IgG1 than vaccinated or infected controls; (4) challenged-vaccinated mice depleted of either CD4 or CD8 subsets had less increased IgG1 titers and diminished capacity to confer protective immunity as donors in passive serum transfer assays. The discrepancy between these results and our previous data showing that adoptive transfer of immunity with splenocyte suspensions could be abolished by previous depletion of CD8 cells, indicate that the humora-l response mounted by CL-14-immune mice can not be reconstituted with splenocyte transfer. The results shown herein indicate that CD4 and CD8 subsets participate in the generation of a protective humoral response.

Supported by WHO, Pronex, CNPq, Finep, Faperj and FUJB.

IM-13
ANTI-*TRYPANOSOMA CRUZI* ANTIBODIES AFTER REVERTION OF THE CHRONIC PHASE OF THE INFECTION TO THE ACUTE PHASE

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The chronic phase of infection with *Trypanosoma cruzi* is characterized by a low parasitemia and by the observation that experimental reinfection with the parasite even with a large dose fails to generate a second acute phase. The control of parasitemia and survival in the chronic phase of the infection is supposed to be due to the presence of antibodies. When mice chronically infected with *T. cruzi* are submitted to procedures that suppress antibody production the infection reverts to the acute phase, the parasitemia returns to a high level and the animals die soon after. We report here that the total antibody content and the serum lytic ability did not change significantly after reagudization of the infection induced by a non-lethal dose of gamma radiation but that the level of clearance

antibody decreased. These results suggest that clearance antibodies are important for the survival of the host during the chronic phase of infection. In terms of the interaction between parasite and host one question may be raised, for instance, whether the parasitemia and mortality observed after irradiation of chronically infected mice are only due to a decrease in the clearance antibodies production. Our results do not exclude the possibility that cytokines may also be involved since activation of effector cells by cytokines has been shown by many authors to be essential for the resistance in different infectious diseases including *T. cruzi* (Aliberti et al. 1996 *Infect Immun* 64: 1961, Holscher et al. 1998 *Infect Immun* 66: 1208).

IM-14
APOPTOSIS AS IMMUNOSUPRESSIVE MECHANISM IN VISCERAL LEISHMANIASIS IN HAMSTERS

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During the active phase of visceral leishmaniasis imunosuppression occurs which mechanism is not completely known. It has been related to the presence of soluble factors in the serum and in the hamsters lipid has been appointed as the suppressive component. We previously reported the generation of lipid peroxidation products during visceral leishmaniasis in hamsters (Lindoso et al. 1996 *Mem Inst Oswaldo Cruz 91* Suppl: 175). Oxidized products of lipids, mainly oxidized low density lipoproteins have been shown to have either stimulatory or cytotoxic effect on cells. Since it has been reported that this cytotoxicity can involve apoptotic mechanism (Yuan et al. 1997 *Atherosclerosis 133*: 153) we studied the presence of apoptosis in the spleen and the liver in hamsters intraperitonelly infected with 2×10^7 *Leishmania (L.) chagasi* at 15, 30, 45, 60 and 90 days post-infection (PI). We used the TUNEL method to evaluate the presence of apoptosis in the sections from parafin-embedded tissue samples. We observed a presence of apoptosis in inflammatory cells and in Kupffer cells in the liver in the initial phase until 45 days PI. In the spleen since apoptosis is present also in normal hamsters similar aspect could not be observed. However no apoptosis of any cell was observed in the spleen from 30 days onward. We conclude that the presence of apoptosis of inflammatory cells can be a mechanism of imunossupression in the initial phase of experimental visceral leishmaniasis. In contrast the disappearance of apoptosis in the spleen cells and in the Kupffer cells with progression of the infection can be another mechanism that favors the parasite growth by protecting the infected macrophages from programmed cell death.

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IM-15
ASSESSMENT OF A RECOMBINANT *LEISHMANIA* HSP70 ELISA FOR THE SERODIAGNOSIS OF CANINE VISCERAL LEISHMANIASIS

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Visceral leishmaniasis is endemic in Brazil's northeastern region. Dog culling is mandatory for seropositive dogs, diagnosed by indirect immunofluorescence on *Leishmania* promastigotes. The test has been shown to present cross reactions and low sensitive when compared to western blots and PCR. To circumvent these problems, we developed a test, based on the ELISA technique, having as antigen the carboxi-terminal (78%) portion of the recombinant *Leishmania chagasi* HSP-70. Results obtained in this new ELISA (rec.ELISA) were compared with those from a conventional methodology (with sonicate of promastigote) and in some cases, with western blots. Serum samples of 421 dogs from the cities of Itamaracá-PE (43), Goiana-PE (10) and Natal-RN (368) were used. For the rec.ELISA, sonicates of DH5a *Escherichia coli* expressing the recombinant clone were used to coat the plates (3mg/well) and sera (in 1:50 dilution) were previously immunoadsorbed against a sonicate of wild type *E. coli* (100mg/ml). For conventional ELISA (conv.ELISA), the plates were coated with promastigote sonicate (0,4 mg/well), and sera used in the dilution of 1:400. Western blots were done on SDS-PAGE promastigote antigens and sera on a single 1:400 dilution. Positive (3) and negative (3) controls were used in all tests. Of the 421 sera tested, 320 (76.0%) agreed in the ELISA tests (rec. and conv.), 12 (2.85%) being positive in both tests and 308 (73.15%) being negative in both. Of the 101 remaining sera, 76 (18.05%) were negative for the rec.ELISA and positive for conv.ELISA and the other 25 (5.94%) were positive for the rec.ELISA and negative for conv.ELISA. This last group was evaluated in western-blot, resulting in 9 positive sera and 16 negative, although 11 recognized a band of approx. 70kDa. These bands may be HSP70, which is admittedly antigenic and recognized early in the infection. Our results show a good performance of the rec.ELISA, with the possibility of specific and precocious identification of infected dogs.

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IM-16
AUTOANTIBODIES OF SISTEMIC LUPUS ERYTHEMATOSUS PATIENTS REACT WITH PLAMODIAL ANTIGENS IN THE ABSENSE OF MALARIAL INFECTION

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Several studies have been shown that the immune response during malarial infection is accompanied by autoantibodies formation (Aabs), such as antinuclear antibodies, classically observed in autoimmune diseases. In addition, it was also observed that malarial infection protects against autoimmune disease development. However, the inverse mechanism, that is, a possible protection effect of autoimmune response in the malarial infection, has not been studied. In the present work, we aimed at verifying the antiplasmodial activity of autoantibodies, by analyzing the reactivity of the sera from patients with Systemic Lupus Erythemathosus (SLE) - prototype of autoimmune pathology - against *Plasmodium falciparum* antigens. Blood samples were withdrawn from 80 SLE patients, 50 with and 30 without lupus activity. Individuals without SLE and who had never been to malaria endemic areas were used as normal controls (n=30). Serum samples of *P. falciparum* and *P. vivax*-infected individuals from the Brazilian Amazon were also assayed. Sera were tested by ELISA against *P. falciparum* antigens (PfAg) and synthetic peptides: Nt47 (p126), R0 e R2 (GLURP). Preliminary results have shown that: from the 80 SLE patient sera, 18 (22.5%) reacted with PfAg - being 12 (67%) with active lupus; 14 (17.5%) reacted with Nt47; 3 (3.7%) with R0; and none of the sera reacted with R2. All normal controls were negative and most malaria patients were positive for all antigens. Sera are currently being tested by indirect immunofluorescency assay. These preliminary results have shown that patients with autoimmune process can produce antiplasmodial antibodies in the absense of malarial infection.

IM-17
AVALIATION OF ANTIGENS FROM AXENIC AMASTIGOTES FORMS OF *LEISHMANIA AMAZONENSIS* TO THE AMERICAN TEGUMENTARY LEISHMANIASIS DIAGNOSIS IN ELISA AND IFI

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Many serological tests uses as antigens the entire parasite or total parasitic extracts from promastigotes forms that can be easily obtained axenic culture in Schneider insect. However antigens obtained from the evolutive form when used in immunodiagnosis reaction of the tegumentary leishmaniasis presents a significative number of false-negatives results. Antigens from amastigotes forms in the host-vertebrates can be constituted in molecules of high immunogeinicity, resulting in sensitives reactions. For being intracellular growing forms, the amastigotes are difficult to obtain, being less used to the diagnosis. In this work we used amastigotes forms of the *L. amazonensis* growed in axenics Schneider insect culture medium to be evaluated as antigens in immunoenzimatic assays (ELISA) and Indirect- Immunofluoescence (IFI) to the tegumentary leishmaniasis diagnosis. It was analysed 158 serum of individuals from endemic areas of LTA in which we observed a sera-prevalence of 48,8% and 25,94% respectively. When promastigotes forms were used we observed a sera-positivity of 24,68% in ELISA and 17,08%. The preliminary results obtained in this study suggest that amastigotes forms used in immunodiagnosis assay shows higher results from the others obtained with the reference test and can be used as antigens to the LTA diagnosis.

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IM-18
BINDING OF CHAGASIC PATIENT'S IGGS TO THE MUSCARINIC ACETYLCHOLINE RECEPTORS FROM PORCINE ATRIUM

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Reports using the functional Langendorf setup show that sera from chronic Chagasic (CrCh) patients induce bradycardia and in some cases, AV conduction blockade, suggesting a possible muscarinic mediated effect. To study the direct interaction of the IgG fraction from the sera of CrCh patients at the muscarinic acetylcholine receptors (subtype m2) (mAChR-m2), radioligand binding assays were performed using the microsomal fraction (0.03-0.06 mg of total

proteins) obtained from density gradient (13%–28% sucrose) centrifugation of homogenates from right porcine atrium. The muscarinic selective antagonist [^{3}H]*N*-methylscopolamine ([^{3}H]-NMS) at concentrations that resembled the K_D for the ligand or a saturating condition (150 or 500 pM) and 250 µg of IgGs purified by DEAE ion-exchange chromatography from normal blood donors (NBD) and CrCh patients (classified as group II or III by the Los Andes criteria for the Chagas' disease) were used in the binding assays. When 150 pM of [^{3}H]-NMS is assayed including 1 mM Mg^{2+}, a significant inhibitory effect of CrCh IgGs is observed (74.95 ± 4.82 % of inhibition specific binding as compared with a control assay in the absence of antibodies) whereas, in the presence of NBD IgGs a smaller inhibitory effect is observed (36.30 ± 4.83 % of inhibition specific binding). Similar conditions without Mg^{2+} elicit a smaller inhibitory effect when CrCh IgGs are used (50.49 ± 6.65 %) while NBD IgGs under this conditions do not show specific binding to the mAChR-m2 (4.61 ± 12.93 %). Under saturating concentrations of [^{3}H]-NMS (500 pM) and 1 mM Mg^{2+} no inhibition was seen with NBD (6.34 ± 6.98 %), while CrCh IgGs had a significant effect over binding (41.38 ± 2.40 % of inhibition specific binding without IgGs). To further study the inhibitory effect of CrCh IgGs on [^{3}H]-NMS binding, saturation isotherms were performed in the equilibrium, showing no change in K_D, and a significant decrease in total receptor occupancy (Bmax) from 480.4 ± 25.10 fmol/mg of total proteins for the control assay, to 174.4 ± 12.37 fmol/mg when CrCh IgGs are included. Our results suggest that total IgGs purified from CrCh patients' sera specifically displace muscarinic antagonists and bind to mAChR-m2.

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IM-19
BINDING OF *TRYPANOSOMA CRUZI* TRANS-SIALIDASE TO HEART CELLS *IN VIVO*

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Trypanosoma cruzi trypomastigotes express on their membrane a trans-sialidase (TS) that catalyses the transfer of sialic acid residues from host molecules to parasite surface glycoproteins. The TS is readily released from the parasite membrane, even within the host cell. When the infected cell bursts, the enzyme spreads in the surrounding interstice. As it has been shown that *T. cruzi* antigens bind to normal heart cells, possibly sensitising them to immunological effector mechanisms, the possibility that TS binds to murine heart cells *in vivo* was investigated as described below. Cryostat sections from heart tissues of *T. cruzi*-infected mice, untreated or previously washed with sucrose-containing phosphate-buffered saline, were stained by an immunoperoxidase technique. Primary antibodies were polyclonal and monoclonal anti-TS, and rabbit and mouse polyclonal anti-*T. cruzi* antibodies. The washing was performed to eliminate possible artifacts due to fixation of by-standing antigens to tissue sections by the fixation procedure. As a control of the washings, normal mouse heart tissue was stained for IgG with anti-mouse IgG antibodies. TS adsorbed to heart cells in the vicinity of *T. cruzi* nests could be observed in both washed and non-washed tissues. On the other hand, a rabbit anti-*T. cruzi* serum stained only non-washed tissues. This antiserum recognised several *T. cruzi* antigens with Mr below 106 kD, but did not recognise TS. As expected, IgG could be detected only in non-washed sections. As described above, TS binds to heart cells *in vivo*. The binding is strong enough to persist after overnight washings of the tissues. It may play a role in the pathogenesis of Chagas' disease, since TS antibodies are the first, or one of the first, to be formed during *T. cruzi* infections. These antibodies could well lyse TS-sensitised heart cells and initiate an inflammatory reaction that, through alteration of normal molecules, would eventually lead or contribute to autosensitisation.

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IM-20
cAMP-INDEPENDENT PATHWAY INVOLVED IN THE REDUCTION OF L-TYPE CALCIUM CURRENT BY A MONOCLONAL ANTI-M2 RECEPTOR ANTIBODIES IN GUINEA PIG VENTRICULAR MYOCYTES

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Circulating autoantibodies with ß-adrenergic and muscarinic activities have been detected in patients with chagasic cardiomiopathy. Reports using pharmacological and immunological methods show that these autoantibodies recognize the second extracellular loop of G-protein coupled receptors. To study further muscarinic effects of some of these autoantibodies, found in chagasic patients, monoclonal antibodies were directed against a peptide corresponding to the second loop of the M2 acetylcholine receptor. The physiological effects of one of these monoclonal

antibodies, B8E5, that recognized the epitope corresponding to the N-terminal part of the second extracellular loop of the M2 receptor (V-R-T-V-E-) were studied on the L-type Ca2+ current (I_{Ca}) of guinea pig ventricular myocytes using the whole-cell patch clamp technique. B8E5, similar to carbachol and chagasic autoantibodies, reduced the isoprenaline-stimulated I_{Ca} by 42.8 ±.6.6 % (n = 7). The electrophysiological parameters of isoprenaline-stimulated I_{ca} were not modified in presence of B8E5. The half-inactivation voltage was -38.8±1.3 mV before and -40.2 ± 0.7 mV after aplication of B8E5. The half-activation was -26.7± 2.9 mV before and -28.0 ± 1.1 mV after B8E5. The reduction of I_{Ca} amplitude induced by B8E5 could not be explained by the modification of time constant of recovery from inactivation which was 159±12 ms in ISO and 162±16 ms with B8E5 in presence of ISO. The inhibition of I_{Ca} by B8E5 was still observed when intracellular cAMP was either enhanced by forskolin, or maintained constant by using non-hydrolysable cAMP or by applying the phosphodiesterase inhibitor, IBMX. A guanylyl cyclase inhibitor (LY 83583, 30 µM) attenuated the effect of the B8E5. The results suggest that the monoclonal anti-M2 muscarinic receptor antibodie (B8E5) antagonises the b-adrenergic response of the L-type calcium current through activation of the M2- muscarinic receptor but not via the classical pathway of decreasing intracellular cAMP.

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IM-21
CD 54 AND HLA-DR BASAL AND INTERFERON GAMMA STIMULATED LEVELS ARE DOWN MODULATED IN *LEISHMANIA (L.)CHAGASI* INFECTED HUMAN MACROPHAGES. INFECTION INDUCES LOSS OF ADHERENCE IN HUMAN MONOCYTES

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Macrophages and monocytes are the preferential host cells for *Leishmania* spp. *L(L)chagasi* initially inoculated in skin migrates inside the host cells to spleen and liver causing severe and sometimes fatal disease with strong depression of cell mediated immunity. Our hypothesis is that *L(L)chagasi* infection induces changes on macrophages surface molecules that may be related to macrophage adhesion (involved in dissemination?) and macrophage antigen -presenting function to lympyhocytes. The very early initial steps of infection can only be explored *in vitro*. Monocytes were obtained using Percoll gradients (>90% of purity) and left to differentiate in 24 well plastic plates with RPMI medium,10% human serum and 5% CO_2 at 37ºC. Macrophages and monocytes were infected at 10:1 parasite/human cell ratio and scrapped for FACS analyses after 48 hours .The mean of 5000 events were taken per sample /condition. The table below summarizes results of cell surface molecules examined in infected cells related to non infected controls.

	CD 54	HLA-DR	CD11b	CD86	CD49e	HLA-BC
Macrophages	ß 9/10*	ß6/6	Ý3/5	Ý3/3	Ý1/1	ß3/3
Monocytes	ß3/4	ß3/3	ß4/4	——-	ß3/3	Ý1/1

ß 9/10 means decreased expression in 9 out of 10 donors whose cells were infected with *L(L)chagas*.

IFN-g(10 U/ml;n=5) and LPS (10ng/ml;n=3) raised the macrophage basal levels of CD 54 and HLA-DR in all samples tested .The infection had opposite effect in all donors with mean reduction values between 22% to 48% .Infected monocytes had reduced adherence to plastic in 3/3 experiments(exp.) and to fibronectin 2/2 exp. with mean reduction values between 5% and 85%. Infected macrophages increased adherence to plastic in 2/2 exp. with a mean increase of 24%. In conclusion, *L(L)chagasi* modulates adhesion and costimulatory molecules in human monocyte-macrophage lineage. Monocytes could be the cells involved in parasite dissemination as they loose adhesiveness when infected.

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IM-22
CELL DEATH (APOPTOSIS AND NECROSIS) OF CD4+ AND CD8+ T CELLS DERIVED FROM LESIONS OF AMERICAN CUTANEOUS LEISHMANIASIS PATIENTS: A FLOW CYTOMETRIC STUDY

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A flow cytometric method was used to quantify apoptotic or necrotic cells within CD4+ or CD8+ T cell populations obtained from lesions of patients with human cutaneous leishmaniasis. Quantitative analysis of cell death in

lymphocyte subsets has shown the presence of non-viable cells that exhibit a spectrum of changes when stained by 7-amino-actinomycin D (7-AAD). These changes ranged from features typically seen in apoptotic cells to those seen in necrotic cells. In order to study cell death events in T lymphocytes, mononuclear cells obtained from lesions of patients with active disease or spontaneous healing were stained with 7-AAD, and simultaneously surface receptors were stained with anti-CD4 and anti-CD8 monoclonal antibodies. Taking into account the total lymphocyte population, high levels of apoptosis were observed in cells obtained from lesions of patients with active disease in comparison with cells from lesions of patients with spontaneous healing. Low levels of necrosis were observed in the cells of all patients studied. In the group of active disease patients, higher levels of apoptotic CD8+ T cells were detected as compared with apoptotic CD4+ T cells. Patients with spontaneous healing showed low levels of apoptotic CD8+ T cells, while the levels of apoptotic CD4+ T lymphocytes were similar to active disease patients. It is possible to speculate that, during the immune response, differences in apoptotic events of CD4+ and CD8+ T cell subsets could be responsible for changing the CD4/CD8 balance, thus leading to healing or maintenance of disease. In this context, the low levels of apoptotic CD8+ T cells observed during the spontaneous healing process suggest that this T lymphocyte subpopulation may play an important role in the disease outcome. Additionally, the flow cytometric method adapted for this study can be used as an important tool to evaluate the cell death events in different cell subsets involved in the cellular immune response.

IM-23
CELLULAR CYTOTOXICITY AGAINST MACROPHAGES INFECTED WITH AMASTIGOTE FORMS OF *TRYPANOSOMA CRUZI*

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Trypanosoma cruzi, the causative agent of Chagas' disease, infects about 16-18 million people in South and Central America. During the chronic phase of the disease, the parasite-specific antibodies that activate the classical complement pathway, and kill trypomastigote forms, are though to be the main effector molecules responsible to parasite growth control. In this study, we investigated the in vitro cytotoxic activity of spleen cells from BALB/c mice chronically infected with *T. cruzi* (Y strain) against peritoneal macrophage infected with the parasite. Ours results demonstrated that spleen cells from chronically infected mice, but not spleen cells from normal mice, when stimulated with rMuIL-2, lysed ^{3}H-deoxiuridine-labeled peritoneal macrophage cells infected with *T. cruzi.* The flow cytometry analysis revealed low levels of CD95 (Fas) expression in spleen cells from normal or chronically infected mice. On the contrary, CD95 expression was significantly enhanced when the splenocytes from chronically infected but not from normal mice were cultured with trypomastigote forms or parasite lysate. The CD95 expression was not changed by simultaneous IL-2 addition. Differently, CD95L (FasL) expression in spleen cells from normal or infected mice was always high, independently of parasite or IL-2 addition. These results suggest that CD95/CD95L interaction may regulate T cells interaction against macrophages infected with *T. cruzi.* We are investigating whether the presence of anti-CD95L antibodies may inhibit the cytotoxic activity of spleen cells from infected mice. These studies may contribute to explain the mechanisms by which the intracellular parasite growth and cell damage are controlled in the chronic phase of Chagas' disease.

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IM-24
CELLULAR DIFFERENTIATION IN TRYPANOSOMATIDS ISOLATED FROM PLANTS: USEFULNESS OF MORPHOLOGICAL CHARACTERS TO IDENTIFY *PHYTOMONAS*

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In the present work we studied at the morphobiological level eight isolates of plant trypanosomatids, which have been confirmed as *Phytomonas* or *Herpetomonas* by at least two of the following approaches: isozyme analysis, reactivity with selected monoclonal antibodies or lectins, arginase detection, analysis of restriction sites in some rDNA sequences, and by molecular hybridizations using probes derived from kDNA, rDNA or SL genes. The *Herpetomonas* were: *H. davidi* (previously named *P. davidi*) and the isolate of *Euphorbia hyssopifolia*. The *Phytomonas* were: *P. serpens*, the isolates obtained from *Euphorbia pinea*, *Jathropha macrantha*, *Allamanda cathartica*, *Citrus bergamia*, as well as the so-called *"H." mcgheei.* Three well-characterized *Herpetomonas* species isolated from insects were also studied as references. We were looking for a character only shared by the *Phytomonas* isolates, and which could be considered of diagnostic value. Then, we followed the cellular differentiation of these isolates in LIT medium at 27.3^{o}C, at 24 hr intervals, from 48 to 120 hr. The cultures were started with 5x10^{5} cells/

ml seeded in 4 ml-volumes of medium distributed in 16x150mm screwcap tubes. The percentage of the different evolutive stages was determined by examining about 300-500 randomly chosen cells in Giemsa-stained smears. Paramastigotes and opisthomastigotes were detected only in isolates identified as *Herpetomonas*. On the other hand, in all *Phytomonas* cultures occurred both a type of promastigote presenting a very short flagellum and similar forms without an apparent flagellum. These aflagellated forms are very peculiar and were found at rates ranging (mean) from 1.9% (*"H". mcgheei*) to 51.4% (*Phytomonas* from *Allamanda cathartica*), but they were not seen in the *Herpetomonas.* They had already been seen in *P.serpens*, *"H". mcgheei* (in the plant) and other *Phytomonas* spp., but have not been reported in other genera. Then, we believe they are useful morphological markers to identify *Phytomonas.* It is worthy mentioning that these aflagellate forms are distinct from amastigotes of *Leishmania* and *Trypanosoma*, as well as from "cysts" of *Leptomonas*. In the majority of *Phytomonas* cultures we also observed a type of division producing one cell with flagellum and another without it; it seems possible that this division would be a source of aflagellated forms. Otherwise, although long twisted promastigotes are common in *Phytomonas* in the host plants, they were rare or not seen in the majority of the cultures examined herein. Our data also evidenced that the presence of paramastigotes in trypanosomatids from plants should be considered a clue, to be confirmed, that the isolate can be a *Herpetomonas* sp.

IM-25
CHEMOKINE EXPRESSION IN CENTRAL NERVOUS SYSTEM DURING EXPERIMENTAL *TRYPANOSOMA CRUZI* AND *TOXOPLASMA GONDII* INFECTION: ANALYSIS BY RT-PCR

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The central nervous system (CNS) is considered an immunoprivileged site able to restrict the entrance of immune surveilling cells. In this way, the CNS constitutes a target for intracellular pathogens, such as virus and parasites. However, during systemic inflammatory processes, such as parasitic infections, the blood brain barrier is disrupted and inflammatory lesions are established. In Chagas' disease, about 10% of *T. cruzi*-infected individuals show neurological alterations. *Toxoplasma gondii* infection is usually controlled by the host immune system, resulting in an asymptomatic chronic infection mantained by dormant parasitic cysts, mainly in the CNS. However, in immunocompromised individuals both infections can lead to severe CNS damage. Further, some studies showed the presence of pro-inflammatory and regulatory cytokines able to modulate the specific immune response and inflammatory reactions during these infections. Chemokines (CK) are a group of multifunctional cytokines produced by different cellular types and involved in leukocytes recruitment to inflammatory sites. In order to characterize the inflammatory cells and to investigate the presence of CK in the CNS during chagasic and toxoplasmic infections, C3H/He and C57Bl/6 were infected with 100 blood trypomastigotes of *T. cruzi* (Colombian) and 15 cysts of *T. gondii (*ME49*),* respectively. Edema, enlargement of perivascular spaces and irregular distribution of inflammatory infiltrates were observed during acute phase of both infections. The distribution of these infiltrates was not related to the presence of *T. cruzi* antigens and *T. gondii* cysts. The immunohistochemical characterization of cells present in these lesions showed that in both parasitic infections they consisted mainly of macrophages and $CD8^+$ cells, although a few $CD4^+$ cells were also found. The IFN-g-induced CK (Mig, Crg-2) and RANTES mRNAs were all expressed in high levels in the CNS of *T. cruzi* and *T. gondii*-infected animals during both acute and chronic phases. Also, MIP-1a was detected in these tissues, corroborating our immunohistochemical data showing the predominance of $CD8^+$ over $CD4^+$ cells in the CNS lesions. Altogether, these results suggest that CK play an important role in the modulation of the inflammatory response observed in the CNS during *T. cruzi* and *T. gondii* experimental infections.

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IM-26
CLEAVAGE BY PI-PLC OF GPI-ANCHORED SURFACE PROTEINS OF *TRYPANOSOMA CRUZI* TRYPOMASTIGOTES TRIGGERS ITS TRANSFORMATION TO AMASTIGOTES-LIKE FORMS

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The transformation of trypomastigotes of *Trypanosoma cruzi* into amastigotes is an essencial step in the adaptation of this parasite to the vertebrate host. This process can be followed *in vivo* by host cell infection with trypomastigotes, which transform in amastigotes and, finally, are released by rupture of the host cell. Previously, we

have reported that trypomastigotes treated with phosphatidylinositol phospholipase C (PI-PLC) are induced to differentiate into amastigote-like round forms. In the present report, using confocal microscopy we were able to show that trypomastigotes treated with PI-PLC initiate the process of flagellum remodeling by 30 sec after the contact with the enzyme. Parasite amastigote-like round forms are detected 10 min after PI-PLC treatment. These forms show round nucleus and cigar-shaped kinetoplasts and, similar to amastigotes, they show from none to slight reactivity with the trypomastigote-specific 3C9 Mab. By FACS analysis, the Ssp-4 amastigote-specific epitope can only be detected on the surface of the parasite 24 hours post-treatment with PI-PLC. The analysis of the flagellum with the Mab 4D9 show heterogeneous labeling among the parasites, suggesting a remodeling of these molecules. By cleaving the GPI anchor of *T. cruzi* surface molecules, PI-PLC releases GPI-tethered proteins such as those containing the Ssp-3 epitope and generates diacylglycerol (DAG) that may function as a second messenger to trigger the parasite remodeling to amastigote.

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IM-27
CLONING OF THE *LEISHMANIA AMAZONENSIS* KMP-11 CODING GENE TO IDENTIFY POTENTIAL IMMUNOGENS

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Cutaneous leishmaniasis normally occurs accompanied by parasite-driven T cell-mediated immune responses. The knowledge of the mechanisms involved in those responses may be exploited for the development of appropriate vaccine strategies. Previous work from our laboratory has demonstrated that the T-cell stimulation previously thought to be induced by lipophosphoglycan, the predominant glycolipid on the promastigote surface, was in fact due to tightly associated protein contaminants. These proteins have been identified and termed kinetoplastid membrane protein (KMP-11), as they are present in all Kinetoplastid genera. Studies with human and murine T lymphocytes have demonstrated the high antigenicity of this molecule indicating that it can be considered as a vaccine candidate. Cytoplasmic RNA from promastigotes of *L. amazonensis* (IFLA/BR/67/PH8), the strain used in the composition of a leishmaniasis vaccine in Brazil, was isolated and used in RT-PCR with oligonucleotides specifically constructed. The DNA fragments obtained were purified (QIAEX, QIAGEN, Switzerland), inserted into the pZErO^{TM} vector (Invitrogen, USA), and partially sequenced. Sequence-alignment analysis using the FASTA algorithm identified a high level of homology (about 95%) with sequences described for *L. infantum* and *L. donovani*. The cloning and sequencing of the KMP-11 gene from a *Leishmania* strain already used in the composition of a human vaccine stand for an important prospect to the development of biochemically defined antigens for prophylactic or diagnostic use.

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IM-28
CO-INFECTION WITH *TOXOPLASMA GONDII* SWITCHES THE EXPECTED TH2 IMMUNE RESPONSE INTO TH1, LIMITING INFLAMMATION AND TISSUE PARASITSM IN BALB/C MICE INFECTED WITH *LEISHMANIA MAJOR*

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There is a growing interest on concomitant infections and their reciprocal effect in altering the immune response and resistance to each pathogen. In the present study we co-infected BALB/c mice with two distinct parasites, i.e. *Toxoplasma gondii* and *Leishmania major*, and studied the mutual effect of each infection on the synthesis of cytokine, parasite specific antibody isotypes, expression of chemokines mRNA and protozoa induced immunopathology. In our first experiment, mice were infected i.p. with *T. gondii* and five days after challenged with *L. majo*r in footpad. Our results demonstrated that when infected acutely infected with *T. gondii* susceptible BALB/c mice were able to control the lesion and produce high level of IFN-gin draining lymph node in response to *L. major.* Further characterization of the immune response showed that dually infected animals also produced lower level of anti-leishmania antibodies of IgG1. The smaller footpad swelling in co-infected infected animals was related to a smaller inflammatory infiltrate and tissue parasitism as indicated by histologic analysis and confirmed by analysis of cytokine mRNAs expression as well as the presence for parasite specific GP63 gene in the tissue sections. Thus, in agreement with footpad measurements and histopathology at 4-5 weeks post-infection with *L. major* promastigotes, our RT-PCR experiments showed that the expression of different chemokines (mainly CRG-2, MCP5, MIP1-a, MIP1-b, MIP2) are down-modulated when comparing dually infected animals with mice infected with *L. major* alone. Interestingly, our studies revealed that when

BALB/c mice were challenged with *L. major* during the chronic toxoplasmosis (i.e. seven weeks post-infection with *T. gondii)*, the BALB/c mice were unable to either control lesion development due to *Leishmania* infection nor augment the expression of IFN-gby the draining lymph node. In this latter experiment, the quantitation of parasites through PCR, showed no difference among experimental and control groups. These results suggest that activation of innate immune response, but not acquired immunity, during *T. gondii* infection is responsible for control of *L. major* replication and immunopathology observed in dually infected BALB/c mice.

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IM-29
COMPARATIVE STUDY OF GLYCOSYLPHOSPHATIDYLINOSITOL ANCHORED MOLECULES ISOLATED FROM *TRYPANOSOMA CRUZI* TRYPOMASTIGOTES, *TOXOPLASMA GONDII* TACHYZOITES, AND *LEISHMANIA* SP. PROMASTIGOTES ON THEIR ABILITY TO INDUCE CYTOKINE AND NITRIC OXIDE SYNTHESIS BY MACROPHAGES

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Different studies demonstrate that most of the surface molecules from intracellular protozoa are anchored to the parasite membranes via glycosylphosphatidylinositol (GPI)anchors. Our recent studies suggest that glycosylphosphatidylinositol (GPI) anchors isolated from GPI-anchored mucin-like glycoproteins purified from *T. cruzi* trypomastigotes are potent stimulators of nitric oxide (NO) and cytokine synthesis by macrophages. The present study was undertaken to compare the ability of the major GPI-anchored molecules from *T. cruzi* (trypomastigotes), *T. gondii* (tachyzoites), *L. amazonensis* and *L. chagasi* (promastigotes: exponential and stationary phase) on their ability to stimulate macrophages. For this purpose we used a method previously employed to fractionate *T. cruzi* membrane components (Almeida, et al. J. Biochemistry 304:793, 1994). Parasites (1 x 10E10) were obtained from culture, washed several times with PBS, dried in a lyophilizer and submitted to a chloroform, methanol and water (C:M:W, 5:10:4). Aqueous and organic phase were obtained after centrifugation at 5000 x g. The organic phase was then dried in a speed vacuum and them resuspended in a buthanol:water (2:1) mixture. The organic and aqueous phase were termed F1 and F2,respectively. The aqueous phase from C:M:W extraction was also dried in a speed vacuum and then re-extracted with 9% buthanol and centrifuged at 5000 x g. The supernatant and pellet were termed F3 and F4, respectively. The fractions F2 and F3 were pooled, dried, resuspended into 1 ml of water, and loaded to a column containing a octyl-Sepharose. The content of octyl-Sepahrose was eluted in propanol gradient, and tested in their reactivity with sera from patients infected with one of the various parasites, or mAbs specific for known antigens of each parasite. Our results, show that several fractions from *T. cruzi* trypomastigotes or *T. gondii* tachyzoite were reactive with sera from infected patients. In the case of *Leishmania* sp. several fractions were reactive with mAbs against agalactose or LPG. The different fractions eluted from octyl-Sepahrose were dried and had their content of *myo*-inositol measured. Finally the samples were tested on their ability to stimulate cytokine and NO synthesis by macrophages. Only the tachyzoite and trypomastigote fractions released at 25 and 36% of propanol were able to activate cytokine syntheis and NO synthesis by macrophages, and were recognized by mAbs specific for GPI anchors and a-galactose, respectively. In contrast, non of the eluted material obtained from *Leishmania* parasites were able to trigger macrophage functions. These results suggest that not all GPI anchors from protozoa are able to activate macrophages. Thus, specific structural features appears to be required so the protozoan GPI anchors can trigger cytokine synthesis and microbicidal activity by macrophages.

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IM-30
COMPARATIVE STUDY OF GLYCOSYLPHOSPHATIDYLINOSITOL ANCHORED MUCIN-LIKE GLYCOPROTEINS ISOLATED FROM *TRYPANOSOMA CRUZI* TRYPOMASTIGOTES, ENDOTOXIN FROM *ESCHERICHIA COLI* AND LIPOPROTEIN FROM *MYCOPLASMA FERMENTANS* ON THEIR ABILITY TO INDUCE CYTOKINE AND NITRIC OXIDE SYNTHESIS BY MACROPHAGES

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We have recently identified a glycosylphosphatidylinositol-anchored mucin-like glycoprotein (GPI-mucin) as a main membrane component responsible for induction of cytokines by macrophages exposed to *T. cruzi* trypomastigotes. However, a possible contamination by bacterial products is an important consideration in the field

of macrophage activation. In the present study we compared GPI-mucins, lipopolysaccharide from *E. coli* (LPS) and the macrophage-activating lipopeptide (MALP) from *M. fermentans* on their ability to activate macrophage, as measured by synthesis of cytokine or nitric oxide. Macrophages were obtained from different mouse strains using two protocols, i.e. intraperitoneal injection of thyoglicolate or subcutaneous injection of polyacrilamide beads. We also used a macrophage cell line, named RAW. Macrophages were primed or not with IFN-g (100 units/ml) and stimulated with either GPI-mucin (1 pmol/ml), LPS (100 ng/ml) or MALP (50-10 pmol/ml). The unprimed macrophages, regardless of their source, produced TNF-a low levels of IL-12 and no NO upon stimulation with GPI-mucins. Macrophage priming with IFN-g resulted on enhancement of TNF-a and dramatic increase of IL-12 and NO synthesis after stimulation with trypomastigote glycoconjugates. No major quantitative difference was observed among macrophages obtained from different mouse strains, i.e. C3H/eJ, BALB/c and C57BL/6, in terms of cytokine and NO synthesis after simulation with GPI-mucins. Our kinetic results showed that TNF-a, IL-12 and NO peaked at 8, 24 and 48h post stimulation of IFN-g primed macrophages with parasite glycoconjugates. Interestingly, macrophages from C3H/HeJ mice which are hiporesponsive to LPS, mounted a strong cytokine and nitric oxide response to *T. cruzi* glycoconjugates. In addition, experiments with Polymixin B using macrophage from BALB/c mice completely inhibited cell activation by LPS but with GPI-mucins. Because macrophage activation by LPS requires a serum component in the culture medium, we also studied the requirement of serum for macrophage activation by GPI-mucin. Surprising, serum was shown to be necessary for activation of C57BL/6 but not C3H/HeJ macrophages. The above results demonstrate a distinct activity for LPS and GPI-mucins. However, MALP has been shown to be a strong activator of nitric oxide and different pro-inflammatory cytokines by inflammatory macrophages from C3H/HeJ mice primed with IFN-g. Therefore we performed a quantitative analysis of MALP, GPI-mucins and GPI anchors (tGPI) purified from trypomastigote derived GPI-mucins. These results show that highly purified tGPI were as potent as GPI-mucin and at least 100 fold more active than MALP in their ability to induce NO, TNF-a and IL-12 by IFN-g primed macrophages. Together with the extremely clean profile on the mass spectrometry analysis of tGPI mucins, our results suggest that is unlikely that the activity of GPI-mucin or tGPI is due to contamination with bacterial products.

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IM-31
CROSS REACTIVITY BETWEEN TOTAL PROTEINS OF LOWER TRYPANOSOMATIDS AND ANTIBODIES IN SERA OF PATIENTS WITH CHAGAS' DISEASE AND AMERICAN CUTANEOS LEISHMANIASIS BY WESTERN BLOTTING

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The intimate serologycal relation shown by trypanosomatids isolated from plants, leishmanias and *Trypanosoma cruzi*, all belonging to the order Kinetoplastida, suggests that commom antigens may be easily found among these species. Serological studies using whole serum have shown high levels of cross reactivity among these different species. The trypanosomatids studied were *Phytomonas serpens* isolated from tomatoes, *Herpetomonas macgheei* isolated from corn, 415Ga isolated from salivary glands of *Leptoglossus* sp in Londrina/PR,1990, 268Tb isolated from tomatoes. They are able to parasitize many plants without apparent pathology. Sometimes, however, they can cause pathologies in plants of economic importance. The prevalence of Chagas' disease and leishmaniasis are still significantly high in Brazil. Although these infectious agents have some common biological features, they produce distinct pathologies with broad clinical and epidemiologic importance. These endemic diseases are major public health problems, causing physical disability and severe economic burden on the rural population of South America. This stimulated us to characterize by western blotting specific proteins of trypanosomatids isolated from plants and insects recognized by human sera that could be used to diagnose human leishmaniasis and Chagas' disease. The usual preparation of antigens for the diagnose of those diseases, generally derived from leishmania and *T. cruzi* cultures, is expensive, easily contaminated and potentially infective for laboratory workers. The strains were cloned and mantained in GYPMI complex medium. An antigenic suspension was prepared. The protein was quantified according to Bradford, 1970. The electrophoresis (SDS-PAGE) was carried out such as in Laemmli, 1970. Mini-gradient gel, 7.5-15% with dimension of: 5cm of height, 7cm of width and 1mm of thickness. It was run for 4 to 6 hours at 50volts, 15 ampers and 1watt. The M.W. markers used were carbonic anidrase, 29 KDa, egg albumin, 45KDa, and bovine albumin, 66KDa. Fifteen microliters of each sample were transferred to the wells. Gel was transferred to nitrocelulose membranes in Biotech 30volts, 400mA for 2 hr and western blotting performed with sera from leishmaniasis and Chagas' disease patients of Hospital Universitário Regional do Norte do Paraná (HURNP), and others without any apparent pathologies. Preliminary results showed complex reactivity because some proteins were just recognized by chagasic patients's sera or patients with ACL and other proteins showed cross reactivity. Some proteins reacted with sera from healthy patients. The pattern of positive profiles are in determination.

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IM-32
CUTANEOUS LYMPHOCYTE-ASSOCIATED ANTIGEN AND b1-INTEGRINS IN AMERICAN CUTANOUS LEISHMANIASIS

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An inflammatory process involves attraction, retention/proliferation and recirculation of leukocytes to the areas of antigenic insult. Costimulatory factors and adhesion molecules such as the b1-integrins that mediate the cellular interactions with extracellular matrix components and the epithelial basement membrane (fibronectin, laminin, collagen). In skin inflammatory responses, the expression of cutaneous lumphocyte-associaed antigen (CLA) identifies a subgroup of leukocytes that are capable of selectively migrates to the epidermis. Thus, CLA expression is closely associated to leukocyte activation where molecules such as CD69 are involved. In the present study, we attained an in situ characterization of CLA, CD69, and the b_1-integrins a_2b_1, a_3b_1, a_4b_1 y a_5b_1 in lesions of localized cutaneous leishmaniasis (LCL) (n=7) and intermediate cutaneous leishmaniasis (ICL) (n=6), using an avidin-biotin immunoperoxidase method on frozen sections. The results were as follows: CLA+ cells represented a low percentage of the total leukocyte population with a density of 557 cells/mm^2 (13.9%) in LCL, and 767 cells/mm^2 (19.2%) en ICL (non-significant statistical differences). Moreover, CD69+ cells were more abundant in LCL (1441 cells/mm^2) (36.0%) than in ICL (548 cells/mm^2) (13.7%) ($p<0.001$). In addition, the density of cells expressing a_2b_1 in LCL was 267 cells/mm^2 (6.7%) and 729 cells/mm^2 (18.2%) ($p< 0,01$) in ICL, whereas the number of positive cells for a_3b_1 was 588 cells/mm^2 (14.7%) in LCL and 89 cells/mm^2 (2.2%) in ICL. Marked immunoreactivity for a_4b_1 and a_5b_1 was observed in most dermal infiltrating cells and extracellular matrix components. These molecules were seldom expressed by the keratinocytes. The high numbers of infiltrating cells expressing leukocyte common antigen (LCA) and the lower density of activated CD69+ cells in ICL indicated that this granuloma comprised proinflammatory cells with a potent migratory capacity but a diminished efector potential. The differences in the expression of b_1-integrins in the two clinical entities analyzed may be the result of distinct leukocyte migratory pathways involving defects in epidermal accessory signaling and cytokine patterns.

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IM-33
CYTOKINE PROFILE BY RT-PCR IN LYMPH NODE CELLS IN THE EARLY AND LATE CLINICAL STAGES OF THE CUTANEOUS LEISHMANIASIS

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Human leishmaniasis includes a spectrum of diseases of viriable severety of clinical manifestations wich ranging from localized cutaneous ulcer to fatal visceral disease. Recovery from and resistance to disease in leishmaniasis are strongly associated with a effective cellular immune response of the host associated with T cell. Regional lymphadenopathy has been documented in patients with cutaneous leishmaniasis. Previous studies have shown that activated T-cells present in these lymph nodes, may be important as initial T cell response to *Leishmania* infection. In this study we performed a semi-quantitative RT-PCR assay to determine the cytokine profile of these cells. We analyzed lymph node cells from 20 patients in different stages of the disease. The first ten patients were included in this study before skin lesion development. Three of ten patients never developed ulcer. Ten other patients has duration of the disease varied from 15 days to 90 days. A variable mRNA expression for IFN-g was documented in 6 of 10 patients, in contrasting to a consistent expression of RNA IL-4 documented in all sample except in one. Th0 cytokine pattern expressing IFN-g and IL-4 in cells was observed in lymph nodes. None of the patients expressed RNA for IL-10. Cytokine evaluation of remained ten patients and RT-PCR to IL-12, TGF-b and TNF-a are undergoing. Further studies will be necessary to better define immunological markers in Cutaneous Leishmaniasis.

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IM-34
CYTOKINE REGULATION OF RESISTANCE TO *TRYPANOSOMA CRUZI* AT THE INITIAL PHASE OF INFECTION

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C57Bl/6 (B6) mice are resistant whereas BALB/c and C3H/hepas are highly susceptible to *T.cruzi* infection. This work aims to investigate whether the observed variations in susceptibility among these mouse strains are related to quantitative or qualitative differences in the production of macrophage activating or regulatory cytokines. To date, there are no comparative data on the synthesis of IL-12, IL-18 and TGF-b by *T.cruzi*-infected resistant and susceptible mouse strains. IL-18 is a recently characterized cytokine, largely produced by macrophages, with many analogous functions to IL-12. We investigated the production of IL-12 and IL-10 and the expression of mRNA for TGF-b, IL-12 and IL-18 in the spleen and in lymph nodes (LN) draining the subcutaneous (s.c.) inoculation site. Spleen and LN were obtained from mice inoculated respectively by the intraperitoneal and s.c. routes with 200,000 tissue culture trypomastigotes. Comparison of mRNA levels for the different cytokines was done by a competitive, semi-quantitative RT-PCR assay. As early as twenty-four hours after infection, four-fold higher levels of TGF-b mRNA were detected in BALB/c mice than in B6 mice. Interleukin-12 message levels in spleen and LN were similar in the three analyzed mouse strains. However, B6 spleen and LN synthesized higher levels of IL-12 in comparison with the susceptible strains. Message for IL-18 was detectable at all time points of infection, until day 16, in all the mouse strains. Interleukin-10 was only detected from day 3 of infection; on days 6 and 10, IL-10 production was higher in C3H and BALB/c than in B6 mice, coincident with the rise and peak of parasitemia seen respectively at these time points.

These results suggest the importance of TGF-b and IL-10 , as possible determinants of susceptibility to infection: TGF-b would act very early in the course of infection whereas IL-10 is active from the third day of infection on.

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IM-35
DETECTION OF HIGH LEVELS OF IL-12 P40 IN THE SERUM OF PATIENTS WITH VISCERAL LEISHMNAIASIS. CORRELATION WITH CLINICAL FORMS OF DISEASE

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American visceral leishmaniasis (AVL) is a disease that exhibits an immunosuppression characterized by absence of cellular immune response that can be mediated by serum molecules such as IL-2R and immune complexes. By contrast, in American tegumentary leishmaniasis (ATL) there is a potent cell-mediated immune response. IL-12 is a heterodimeric cytokine that induces IFN-g secretion and promotes growth of activated T and NK cells. It has an important role in the immune modulation of leishmaniasis were it is related to protection against the parasite. IL-12 is composed of two chains : p35 and p40. The subunit p40 was shown to form homodimers that interact with the IL-12R and functions as an antagonist. The aim of this study was to investigate if IL-12 p40 plays a role as serum suppressor agent in human leishmaniasis. Using ELISA, we compared the plasmatic concentration of IL-12 p40 in 15 patients with AVL, including 9 after treatment, 10 patients with ATL and 15 controls. Median IL-12 p40 levels were 283 pg/ml for AVL, 109 pg/ml for ATL and 89 pg/ml for normal controls. The high levels of IL-12 p40 observed during active AVL were greatly reduced after treatment (p=0.01). These results suggest that IL-12 p40 may function as an immunosuppressive agent in human leishmaniasis since it is elevated only in AVL, the unresponsive pole of the disease and not in ATL patients. The fall to normal levels of IL-12p40 after effective treatment also reinforces such hypothesis.

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IM-36
DETECTION OF MALARIAL ANTIBODIES IN MAN BY FLUORESCENT ANTIBODY TEST USING *P. CHABAUDI* AS ANTIGEN

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Indirect fluorescent antibody test (IFAT) were standardized and evaluated using *P. chabaudi* as an alternative in the serological diagnosis of human malaria. Conventional IFA using *P. falciparum* as antigen were used as reference tests. To obtain *P. chabaudi* antigen, ten groups of C57Bl/6 mice were inoculated i.p. with 10^7 parasites and they were killed between 6^{th} and 7^{th} day after infection when parasitemia levels were above 10% infected erythrocytes. Parasitized red blood cells were utilized as antigen in IFA in anti-malarial IgG antibody detection at the same time, *P. falciparum* was cultered “in vitro” for preparing antigens for IFAT development. IFAT sensibility, specificity and reproducibility, employing *P. chabaudi* were compared with those using *P. falciparum* in detection of IgG plasmodial antibodies. Sensitivity of *P. chabaudi* IFA (428 sera) from individuals with past/present malaria was 89,5% while *P. falciparum* IFA in the same sera was 91,58%. Specificity was 100% with both antigens, when IFA was

assayed in 62 sera from healthy blood donors of blood bank as well as 76 sera from individuals with other diseases tests reproducibility study showed and agreement serum titers. On account of the low cost and similar results of IFA with *P. chabaudi* antigen in the detection of anti-malarial antibodies, this parasite antigen can be used as an inexpensive alternative for the serological diagnosis of human malaria.

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IM-37
DEVELOPMENT OF A DOT-ELISA FOR THE SIMULTANEOUS DETECTION OF CS PROTEIN FROM *PLASMODIUM FALCIPARUM* AND *P. VIVAX* IN ANOPHELINE MOSQUITOES

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To develop a DOT-ELISA capture for simultaneous detection of *Plasmodium falciparum* (Pf) and *Plasmodium vivax* (Pv) in infected mosquitoes, nitrocellulose membrane (NCM) was used as a solid phase binding support for malaria species specific monoclonal antibodies (mAbs) Pf2A10 and PvNSV3. To sensitize the NCM, the diluted monoclonal antibodies were applied using a computer controlled pen plotter to produce parallel lines. The sensitized NCM was blocked with 3% skimmed milk in PBS (BB), washed in PBS, dried, cut into small strips (1.5 x 0.5cm) and stored at 4^{o}C. Assays were conducted in 24 well culture plates containing the sensitized NCM strips using PfR32tet32 and PvNS1v20 recombinants circumsporosoite protein (RCs) diluted in BB to establish the optimum reaction parameters. A mixture of peroxidase conjugated mAbs diluted in BB was added to wells and, after washing, TMB substrate membrane was added. The appearance of a dark line indicated the presence of recombinant circumsporosoite protein. The DOT-ELISA sensitivity was 25 pg for both PfR32tet32 and PvNS1v20 RCs protein. To evaluate the specificity, we compared the DOT-ELISA with a ELISA test using triturate mosquitoes (in BB with NP-40) infected with Pf or Pv parasites. A total of 42 mosquitoes infected with Pf and 37 with Pv were positive in both the DOT-ELISA and ELISA test. Up to 22 non-infected control mosquitoes were negative in both techniques. No cross reaction was observed between the Pf and Pv circumsporosoite protein (Cs). DOT-ELISA total assay time is about 3 hours. Mosquitoes can be tested simultaneously for both Pf and Pv Cs protein using a single NCM strip which eliminates the necessity of using two microtiter plate wells, when using the ELISA test. The DOT-ELISA test can be carried out in field conditions because it is simple, involves easily handled reagents and permits the visual detection of the CS protein without the need of a reader equipment.

IM-38
DIFERENTIAL PATTERNS OF CD44 EXPRESSION, CELL DEATH AND PROLIFERATION AMONG PERIPHERAL LYMPHOID ORGANS IN EXPERIMENTAL CHAGAS' DISEASE

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Cell migration, classified as a dynamic and selective process, has been related to intrinsic factors, to the cell type and to the microenvironment towards which the cell migrates. Increasing literature shows that extracellular matrix components participate in cell positioning and lymphocyte activation.In this work studied the expression patterns of a molecule related to cell migration and activation, CD44, in distinct secondary lymphoid organs, in the model of experimental Chagas' disease. Our results demonstrate a variation in cellularity and CD44 expression in the various lymphoid organs, in parallel with the polyclonal activation previously described. In the mesenteric lymph nodes (mesenteric chain, gastric and infra-hepatic), cell number was decreased, whereas CD44 expression was increased in these same cells. By contrast, in spleen and subcutaneous lymph nodes (inguinal, axially and brachial), there is an increase in cellularity together with a decrease in CD44 expression. Considering the data showing a polyclonal activation in the lymph nodes of *Trypanosoma cruzi* infected animals in the acute phase of the disease, we evaluated the hypothesis that cells known to be activated, expressing IL2-receptors, could express low levels of CD44 and that was the case. Morevover, we evaluated *ex-vivo* spontaneous cell proliferation and we evidenced an augmented number of cells in the S and G2 phases in the organs with increased cellularity, thus compatible with the presence of polyclonal activation.

Finally, intending to define the phenomenon responsible for the disappearance of cells in the mesenteric lymph nodes, we studied apoptosis in different tissues. We observed that in the 7th day after infection there is a clearcut

increase in the number of apoptotic cells in the mesenteric lymph nodes of infected animals, with the other tissues being comparable to the control animals. By contrast, in parasitaemia peak, the mesenteric lymph nodes, which where already diminished, showed a decrease in apoptotic cell number, whereas the other lymphoid organs exhibited an increase in the number of apoptotic cells. The data presented here suggest that *T. cruzi* infection promotes a differential pattern of cellularity among peripheral lymphoid organs, wich can be related to CD44expression and apoptotic death.

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IM-39
DIFFERENCES BETWEEN CBA MICE MACROPHAGE INFECTION BY *LEISHMANIA MAJOR* OR *LEISHMANIA AMAZONENSIS*

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CBA mice are resistant to *Leishmania major* (*Lm*) infection but susceptible to *Leishmania amazonensis* (*La*). There is evidence that the events occurring in the early stages of infection are crucial to the course of the disease. Macrophages (mf) play a central role in *Leishmania* infection, as they are the cells that harbor parasites and are one of the antigen-presenting cells to specific T lymphocytes. Mf phagocytizes *Leishmania* promastigotes and once activated can destroy parasites by a NO-dependent killing mechanism. We are interested to know how CBA mice can establish differential immune response when infected with *Lm* or *La*. In the present report we examined the differential capacity of CBA peritoneal mf to destroy *Lm* or *La in vitro*. We estimated the percentage of infected cells pretreated or not with rIFN-g. In order to evaluate the parasite survival and multiplication inside mf we enumerated parasites per infected mf after different periods of infection. Activation of mf to kill parasites was determined by NO production in IFN-gtreated as compared to control cells. Our results demonstrated that between 90 min and 6 hours after the promastigote addition, the proportion of infected mf and the number of parasites/mf were similar. Interestingly, 12 to 24 hours later the percentage of *La* infected mf was almost 2.0 times higher in comparison to *Lm* infected cells. These differences maintained during the next 48 to 72 hours after infection. At these time points the number of parasites per cell was 4.0 times higher in *La* infected cells. In addition, between 12 and 24 hours in both *Lm* or *La* infected cells IFN-ginduced a decrease in the percentage of infected mf but did not decrease the number of parasites per cell. NO was similar at 12 hours after *Lm* or *La* infection. Twenty-four hours later mf infected with *Lm* presented 1.5 times higher NO production in comparison with *La* infected mf. In summary, until 12 to 18 hours after infection CBA mf displayed similar infection capacities by both leishmanias. The kinetic studies showed that the percentage of infected cells and parasite load were higher in mf infected with *La* as compared to *Lm* promastigotes. Production of NO upon IFN-g activation was higher in mf infected with *Lm* as compared to *La* infected. These differences may be important to determine resistance or susceptibility *in vivo*.

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IM-40
DIFFERENT BIODEMES OF *TRYPANOSOMA CRUZI* EXPRESS THE SAME LEVELS OF TRANS-SIALIDASE AND SIALIDASE ACTIVITY

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Trypanosoma cruzi trypomastigotes synthesise a trans-sialidase (TS) that catalyses the transfer of sialic acid from host molecules to parasite surface glycoproteins. In the absence of an acceptor for sialic acid, the enzyme acts as a sialidase, i.e., it transfers sialic acid residues to water. It has been reported that different strains *T. cruzi* differ up to 200 fold in levels of sialidase activity (Mol. Biochem. Parasitol. 20:183, 1986). Unpublished data from our laboratory, however, fails to show any significant differences in the levels of trans-sialidase activity from a few tested strains. This discrepancy could be explained by *T. cruzi* strains having TS with different ratios of trans-sialidase/sialidase activities or by the existence, in some strains, of both TS and a strict sialidase. To quantify levels of TS and sialidase activities in strains of *T. cruzi* with different biological behaviours (biodemes). Blood trypomastigotes from seven *T. cruzi* strains were obtained from LLC-MK_2 cell monolayers. All cultures received the same numbers of host cells and trypomastigotes. Parasites and culture supernatants were harvested 5 to 7 days after cell infection. Fixed numbers of trypomastigotes and their corresponding supernatants were analysed for amount of TS by an enzymatic assay based on the transfer of sialic acid residues to ^{14}C-lactose and for sialidase activity by measuring the hydrolysis of the fluorogenic substrate methylumbeliferyl N-acetyl neuraminic acid. Levels of TS and sialidase differed less than one order of magnitude among strains. There was also a good correlation between TS

and sialidase activities in different strains. Strains of different biodemas had similar TS/sialidase activities. The data described above does not support the hypothesis that some *T. cruzi* strains would synthesise an enzyme possessing strict or preferential sialidase activity. Contrary to what has been previously reported, there is not a correlation between TS/sialidase activities and the biological behaviour of the *T. cruzi* strains tested. This indicates that the differences in infectivity, virulence and pathogenicity of different *T. cruzi* strains is not dependent on TS.

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IM-41
DIFFERENTIAL IMMUNOSCREENING OF *TRYPANOSOMA CRUZI* AMASTIGOTE ANTIGENS

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The majority of persons chronically infected with *T. cruzi* are asymptomatic and remain in the so called indeterminate phase of the disease. In 20-30 % of infected individuals however, cardiac and gastrointestinal manifestations typical of chronic Chagas disease occur. As a long term goal for the present study, we propose to evaluate a large number of recombinant antigens derived from *T. cruzi* amastigotes, according to the humoral and cellular immune responses they elicit in two groups of chronically infected chagasic patients: asymptomatic persons and patients with chagasic cardiopathy. Using an amastigote cDNA library and pools of sera from these two groups of patients, we have isolated 30 positive clones. The vast majority of them react equally with antibodies from both pools. In this fisrt screening however, where 50,000 p.f.u. were analysed, two clones seem to react more strongly with pool of sera from patients with cardiophathy and one shows a more strong reaction with sera from asymptomatic persons. The cDNA inserts in various positive clones have been characterized by restriction mapping and partial nucleotide sequences of eight cDNAs have been determined so far. As expected, some clones were found to encode ribosomal proteins and heat shock proteins, but sequence comparison of four cDNA clones showed no homology with previously known sequences. These recombinant antigen are now in the process of being tested by Western blotting to provide a better estimation of the levels of reactivity with serum from each patient. We hypothesize that two classes of antigens could be found: (*i*) "protective" antigens that stimulate B and or T- cell responses controlling the parasitic infection in asymptomatic patients; (*ii*) "cross-reactive" antigens containing epitopes shared by the parasite and mammalian cells that will be more likely identified using sera from cardiac patients which may present autoimmune reactions.

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IM-42
DOWN REGULATION OF COSTIMULATORY B7 AND MHC CLASS II MOLECULES IN J774 G8 MACROPHAGES INFECTED WITH *TOXOPLASMA GONDII*

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The *Toxoplasma gondii* is an obligate intracellular parasite, able of infect different tissues from mammal's organism (Kasper and Mineo, 1994 / Veronesi, 1990). The activation of T cell subsets is the center of immune response to toxoplasmosis and begins by interaction of T cell receptor with MHC proteins. The interaction of proteins present in T cell membrane, with costimulatory molecules exert an aditional signal to T cell effective activation. In this work we investigate the expression of some co stimulatory molecules in J774 G8 macrophages infected by RH strain of *Toxoplasma gondii*. The infected and control group of macrophages were stained with anti-B7, CD69, MHC class II and MHC class I. Our result shows a down regulation of 25% in expression of MHC class II and B7 costimulatory molecule in *Toxoplasma gondii* infected cells, the other molecules analyzed, CD69 and MHC class I, did not exhibit significative expression variation. This down regulation if occurs *in vivo*, can contribute to *Toxoplasma gondii* 's escape from host's immune response. The reduction in MHC class II molecule level, in infected cells, did not compromised the capacity of this protein to present peptides distinct of observed in control group.

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IM-43
DOWN REGULATION OF RECEPTOR AND CORRECEPTOR MOLECULES IN CD8$^+$ T CELLS AT THE EARLY CHRONIC PHASE OF EXPERIMENTAL CHAGAS' DISEASE

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TCR interaction with peptide-MHC complexes drives T cells to activation pathways which include clonal expansion and effector differentiation with functional polarization, development of memory status, acquisition of anergic or suppressed phenotypes and death by apoptosis. Cells entering these different pathways may change phenotypically, displaying new sets of molecules and altering the expression of pre-existing ones. In this paper, we studied the phenotypic changes in spleen CD8$^+$ T cells at the early chronic phase of *T. cruzi* infection, when parasitemia is already under control and when the lymphoid compartments gradually revert from massive expansion at the acute phase. A/J mice infected with 1000 bloodforms of *T. cruzi* (Y strain) were treated with benzonidazol at day eight to avoid death at the acute phase. FACS analysis of spleen cells were done using three colors. At the end of the acute phase, the number of B and CD4$^+$ T cells gradually returned to normality. This did not occur for CD8$^+$ cells, which showed spleen frequencies significantly higher than controls, both at day 26 (19,1% vs. 12,7%, respectively) and day 64 (23,4% vs. 11,9%, respectively). While CD8$^+$ T cells from normal mice were uniformly positive for membrane expression of CD8 molecules, a considerable fraction of CD8$^+$ cells of mice at the early chronic phase showed downregulation in the expression of this correceptor. CD8 downregulated cells increased in number from day 26 to day 64 of infection, when they became 50% of total CD8$^+$ cells and when they constituted a distinct subset from conventional CD8$^+$ cells. A detailed analysis of the CD8^{LOW} population revealed they had a strong downregulation in CD45RA, CD45RB, and CD45RC expression. Interestingly, the CD8^{LOW} subpopulation also showed reduced levels of TCR ?? receptors, but normal levels of Thy-1. These cells did not show signals of apoptosis or necrosis when tested by the incorporation of Propidium Iodide and 7 – AminoActinomicin D. Moreover, after stimulation with PMA and Ionomycin, CD8^{LOW} cells produced IFN-g at levels similar to those of conventional CD8$^+$ cells, which indicated that they were functionally active

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IM-44
EFFECT OF GANGLIOSIDES *IN VITRO* ON MITOGEN STIMULATED LYMPHOCYTES FROM PATIENTS WITH CHAGASIC CARDIOPATHY AND ON THEIR ASSOCIATION IN ROSETTES WITH MACROPHAGES

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In human Chagas'disease previous work showed the occurrence of a T-lymphocyte CD4-positive population, high producers of PAS-positive glycoproteins, with evidences suggesting a role in production of damages in myocardium and in neural structures in chagasic heart disease (ChHD) (Cabral and Braxs, Medicina (BsAs) 42, 415, 1982; Cabral, Robert and Novak, Mem Inst Oswal Cruz, Rio de Janeiro, Suppl II vol 84, p71 IM-6, 1989; Cabral and Novak, Mem Inst Oswal Cruz, Rio de Janeiro, Suppl I vol 90, p157 IM-017, 1995). Other workers take into consideration such facts and employed gangliosides (biological substances with neurotrophic and immunomodulatory properties) in chagasics with chronic cardiomyopathy and disautonomic signs, and obtained an improvement of functional signs and a decreasing of the number of PAS+ lymphocytes (Iosa et al, Am Heart J, 122,775, 1992). In the present work we have studied the effect of mixed gangliosides (Cronassial) on cell cultures of total leukocytes or on mononuclear cells prepared through Ficoll-Hypaque. Blood was obtained from 17 patients with ChHD. Experiments were undertaken to assess the effect of policlonal mitogens Phytohaemagglutinin (Phy) and Concanavalin A (Con A) on blastic transformation, estimated by cell size and cytologic study at 48, 72, 96 and 120 hours. Besides, the production of PAS+ substances by the lymphocytes and blast were assessed. On the other hand, it was studied the effect of gangliosides on the cell-cell association in rosettes between macrophages and T-lymphocytes maintained in cell cultures (RML) (Cabral and Novak, Mol Biol Cell, vol 7 Suppl. ASCB, 603a, 1996; Cabral and Novak, Mol Biol Cell, Suppl. ASCB, vol 8, p 414, 1997). Gangliosides were added at final concentrations of 100 mg/ml or 200 mg/ml. Cell viability was assessed by means of Trypan blue test. With respect to blastic transformation, results showed a significative decreasing in the cultures that received gangliosides 48 hours before of mitogen administration, as compared with controls ($p<0.01$) (both for Phy and Con A). On the other hand, the production of lymphocytic PAS+ substances decreased in the cultures of chagasics in which gangliosides were added. Some of these results confirmed previous findings on the matter (Cabral and Novak, Mem Inst Oswal Cruz, Rio de Janeiro, vol 91, Suppl., p 251, 1996). With respect to formation of rosettes between macrophages and T-lymphocytes, in the

gangliosides-treated cultures there were both less rosettes, and adhered lymphocytes ($p<0.01$). The facts suggest that gangliosides can modulate same lymphocytic activities in the chagasics. Besides, the reduction of RML occurrence, suggests in that cell-cell association, a CD4 receptor might be involved.

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IM-45
EFFECTS OF BLOCKERS OF L-ARGININE-NITRIC OXIDE PATHWAY ON THE ELECTROCARDIOGRAPHIC CHANGES INDUCED BY ACUTE CHAGAS' DISEASE IN RATS

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In acute phase of Chagas' disease, the l-arginine-nitric oxide (NO) pathway is activated due to the induction of nitric oxide synthase type 2 (iNOS) type 2. The NO seems to perform cytotoxic and parasiticide actions. The aim of this study was to evaluate the effect of the inhibition of NO production on the electrocardiograph (ECG) changes induced by acute Chagas' disease in rats. Eighteen male Wistar rats (70-80 g) were divided in four groups: control group (CON, treated with saline, n=4), chagasic group (CHG, inoculated with 2×10^6 blood forms of the Y strain of *Trypanosoma cruzi* and treated with saline, n=5), chagasic + aminoguanidine group (CH-AM, same inoculation and treated daily with Aminoguanidine , 50 mg/kg, n=5) and chagasic + nitroarginine group (CH-NA, same inoculation and daily treated with nitro-L- arginine, 50 mg/kg, n=4). After 20 days, under anaesthesia (tribromoethanol, 250mg/Kg), the animals had their ECGs recorded using nine leads (six classical frontal and three precordial leads) and an acquisition data system (Gould instruments) on a personal computer. The following parameters were analysed: heart rate (HR), RR interval (RRi), amplitude (aP) and duration (dP) of P wave, PR interval (PRi), QRS duration (QRS), QaT interval (QaTi), J point changes, blocks and arrithmias. In the end, serum NO2+NO3 measurements were performed to test the efficacy of treatment. Mean values of HR, RRi, aP, dP, QRS and J point were not different among groups. Mean values (±SE) of PRi (ms) and QaTi (ms) in CON, CHG, CH-AM and CH-NA groups were, respectively: PRi: 57,2±3,9, 65,5±2,7*, 69,4±5,2*, 61,0±1,8* and QaTi: 30,1±1,8, 39,8±2,0*, 42,0±4,4*, 39,6±1,5* (* $p < 0,05$ versus CON). The incidence (%) of first degree AV block and enlargement of QaTi was respectively: 0, 80*, 75*, 75* and 0, 80*, 100*,100* (*$p<0,05$ vs. CON). Arrithmias and other changes were not found. Serum NO2+NO3 measurements were, respectively: 32±5, 343±59, 98±13, 247±98 mM. Conclusions 1) acute Chagas' disease in rats was characterized by the following ECG changes: PRi and QaTi enlargement, probably associated with conduction defects and myocarditis. 2) these changes were not prevented by blockers of L-arginine-NO pathway.

IM-46
EFFECTS OF LPS ON MICE INFECTED WITH *TRYPANOSOMA CRUZI*

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Septic shock, a clinical syndrome responsible for the majority of death in UTI has been traditionally recognized as a result of gram-negative bacterial infection. LPS, the major inducer of septic shock, interacts with macrophages inducing the production of several pro-inflammatory cytokines which acts on several tissues and cells, frequently leading to multiple organ failure. Hyperreactivity towards LPS in protozoan infections was previously described, warning to the danger of overlapping protozoan and bacterial infections. To investigate the role of LPS on *T.cruzi* infected mice, we evaluated its effect on death, the cytokine production by shocked mice, and the correlation parasite load X mortality. Herein, we show that: (1) doses capable of killing all infected mice do not kill control animals; (2) inoculation with LPS produce higher mortality two weeks post-infection, but chronically infected animals also show higher sensitivity than matched adult controls; (3) inoculation with LPS induces early production of high plasma levels of TNF-a in infected mice, followed by high levels of IL-6 and IFN-g. This imbalance of cytokine production by infected mice could be responsible for its higher susceptibility to endotoxic shock. Studies are being developed to measure the production of other cytokines involved in the downregulation of toxic shock syndrome such as IL-10.

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IM-47
EPITOPE FINE MAPPING OF S4 PEPTIDE ANALOG OF *TRYPANOSOMA CRUZI* B13 PROTEIN

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The immunodominant recombinant antigen B13 is a tandemly repeated domain of the *T. cruzi* 140/116 kDa protein. The recombinant B13 antigen is recognized by sera from 98% of patients with Chagas' disease and is formed by 16 tandemly repeated motifs composed by 12 amino acid residues [P(L)S(P)P(L)FGQAAA(E)G(A)DK]. Previous studies, where the antigenicity of B13-derived synthetic peptides was screened on a limited number of sera suggested that the peptide FGQAAAGDKPS-NH_2 contained the immunodominant epitope of B13 and that the sequence AAAGDK is the "core" of that epitope. We synthesized 5 peptides with single deletion on N and C terminal of the peptide and tested them in competitive ELISA with B13 as the solid phase antigen to perform the fine epitope mapping of B13 protein, using sera from six Chagas' disease patients. (see table below)

Peptide	FGQAAAG DK-NH_2	GQAAAG DK-NH_2	QAAAGD K-NH_2	AAAGDK- NH_2	AAAGDKP -NH_2	QAAAGD KPS-NH_2
% of inhibition ± SD	93 ± 8	67 ± 28	56 ± 31	2 ± 2	68 ± 25	55 ± 26

These results show that the sequence AAAGDK may be necessary but not sufficient for optimal antibody recognition and the flanking amino acids could somehow modulate the ability of the antibody to recognize the epitope core and therefore be related to the peptide antigenicity. This is in accordance with the previous reports of secondary structure that forms in the AAAGDK region in the presence of a minimum number of amino acids. (Duranti et cols, XXIV Annual Meeting on Basic Research in Chagas' Disease).

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IM-48
EQUINE TRYPANOSOMOSIS IN THE PANTANAL OF NHECOLANDIA, BRAZIL: A SMALL SURVEY DEVELOPED DURING THE OUTBREAKS OF 1994 AND 1995

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Equine trypanosomosis caused by *Trypanosoma evansi* is known as "Mal de Caderas" in the Pantanal and other subtropical areas of Argentina. In the Pantanal, this parasite has been found in horses, dogs, coatis (*Nasua nasua*), small wild rodents (*Oryzomys* sp.) and capybaras. Since 1894, the disease has been reported in horses from Nhecolandia subregion of Pantanal. The study on seroprevalence was made using equine serum samples frozen at -20º C and colleted in 1993, 1994 and 1995. A total of 22 sera samples from each year were tested using the Indirect Immunofluorescence Antibody Test (IFAT). All samples belong to same ranch. Our study showed the following prevalences: 0, 79.2% and 68% in 1993, 1994 and 1995, respectively. The high prevalence observed in 1994, probably was caused by several outbreaks that occured in the subregion with a mortality rate of 51% in some ranches. In December of 1995 some outbreaks occured in the region, but with less intensity. The zero prevalence observed in 1993, probably because the samples were collected in August when the populational peak of tabanids is very low coinciding with the dry season of the Pantanal. Franke et al. (1994 Acta Tropica 58: 159-169) found a *T. evansi* prevalence of 4.1% in 364 horses from the Pantanal of Poconé region using enzyme-linked immunosorbent assay Ag-Elisa, using (Ab)-Elisa 9.6%, using card agglutination test for trypanosomiasis (CATT/T.evansi) 14.6% and using standard parasitological methods 0.3%. Monzón et al. (1988 Arg. Bras. Ved Vet Zoot 40: 279-285) using the IFAT, reported a prevalence of 19.3% of *T. evansi* in horses from the Formosa department, Argentina, from 1983 to 1987.

The present study showed preliminary data about the incidence of *T. evansi*, in horses from the Pantanal during the outbreaks of 1994 e 1995. Further studies are needed for a better understanding of the disease in the region.

IM-49
EVALUATION OF A MALARIAL ANTIBODIES TO *PLASMODIUM FALCIPARUM* USING SIMULTANEOUSLY CRUDE ANTIGENS AND RED BLOOD CELL CONTROL ANTIGENS BY ELISA

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Serology to blood-stage malarial antigens is valuable for epidemiological surveys, monitoring the effectiveness of control measures and screening blood donors in malaria non-endemic areas. Thus, *P. falciparum* antigen from cultures used in indirect immunofluorescence (IFI) is the choice method, highly sensitive. Nevertheless, its disadvantages are detection of malarial antibodies after parasitological cure and the cross-reactivity among the malaria species and other protozoa (*Leishmania*, *Trypanosoma cruzi*) (Abramo et al., Am.J.Trop.Med.Hyg. 53(2): 202-5,

1995). We have evaluated an ELISA for malaria, based upon duplicate testing of serum samples on both crude *P. falciparum* antigens and red blood cell control antigens. The final optical density (OD at 492nm) was calculated by subtracting the OD with test and control antigens. The threshold of positivity was an OD value of 0.1 based on the mean plus three standard deviations of the sera reactivity from the 40 healthy non-exposed subjects. Fifty-five sera from convalescent subjects, who had recovered from clinical malaria (confirmed by positive thick smears), were used to cross-evaluate ELISA and IFI. The results showed that titers in ELISA were highly correlated to titers obtained in IFI, i.e. 94.5% were concordant positive results (95%CI Kappa 0.86-1.00). Among 68 sera from convalescent patients who had had a single episode of *P.vivax* malaria, one (1.5%) was positive for anti-*P.falciparum* antibodies. We also determined the frequency of cross-reactions between blood-stage antigens and antibodies present in sera of individuals with visceral leishmaniasis (n=19), cutaneous leishmaniasis (n=20), toxoplasmosis (n=20) and Chagas' disease (n=29). Positive reactivity by ELISA was detected only in three samples from individuals with visceral leishmaniasis. This high degree of species-specificity using *P.falciparum* crude antigen suggests that the ELISA is sufficiently sensitive and specific more than IFI is and can be used in epidemiological studies and to screen at-risk blood donors.

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IM-50
EVALUATION OF EXCRETORY-SECRETORY ANTIGENS OF DIFFERENT *TRYPANOSOMA CRUZI* STRAINS AT DIFFERENT TIME POINTS CULTURE

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The fact that trypomastigotes spontaneously realease into the medium polypeptides of different molecular weights which are recognized by chagasic sera has led several groups to evaluate the usefulness of these antigens for immunodiagnosis of Chagas´ disease. The present work aim to analyse the antigenic profile of excretory-secretory antigens of different *T. cruzi* strains, recognized by chronic individuals. Excretory-secretory antigens of *T. cruzi* strains (Y=Type I, WSL and 12SF=Type II, and Colombiana=Type III) were obtained from the supernatant of Vero cells previously infected with 5×10^6 trypomastigotes. Four days post-infection the cells were washed and reincubated for 24, 48 e 72 h at 37ºC in 5% CO_2, on RPMI 1640 medium without fetal calf serum. The supernatant were recovered, centrifuged at 2,800 x g for 10 min at 4ºC, filtered and dialysed. Protein concentration was determined and 18 mg of each batch were loaded into individual wells of a 7,5% polyacrylamide gel (SDS-PAGE). Separated antigens were transferred to a nitrocellullose membrane (pore size=0.45mm) and incubated with a pool serum from 5 individuals proved (clinically, eletrocardiographically, serologically and epidemiologically) to have Chagas' disease (cardiac, asymptomatic and digestive clinical forms). The antigenic profile was more reactive when the antigens were obtained as following: 5 days post-infection for the Y strain, 6 days for the 12SF and Colombiana strains, and 7 days for the WSL strain. It was found that these sera recognized more strongly 3 bands (180, 97 and 56 kDa) in the 12SF, WSL and Colombiana strains, and 2 bands (180 and 97 kDa) in the Y strain. In contrast, sera from nonchagasic individuals, including patients with visceral and mucocutaneous leishmaniasis, were not reactive. The detection of these antigens may be indicative of *T. cruzi* infection. Further studies are in progress to better characterize these antigens.

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IM-51
EVALUATION OF SPECIFIC SYNTHETIC PEPTIDE COMBINATIONS IN SERODIAGNOSIS OF CHAGAS' DISEASE

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Chagas' disease still is one of the most important tropical diseases in the Americas. The programs for the control of *T. cruzi* transmission in the endemic areas are very promising and in several areas natural transmission rate has dropped over 60%. Even though natural transmission could be interrupted, millions of infected people would probably survive until the next century. The most efficient diagnostic method of *T. cruzi* infection remains the serological tests. Most available kits among the large variety of serological methods utilize as source of antigen, extracts of proteins from the hemoflagellates forms of *T. cruzi* . Often, discrepancies in sensitivities and specificities are reported with similars methods. Several antibody binding epitopes of *T. cruzi* have been identified by phage library screening including the recently identified and patented TcD by Corixa Corporation W.A., USA. This report presents the ELISA evaluation of the tripeptide and tetrapeptide in the detection of *T. cruzi* infection. Sera were collected from 50 individuals with *T. cruzi* infection living in an endemic area of Chagas' disease located 100Km

from the capital city of Salvador – Bahia – Brazil. In order to test the specificity of the peptide assays, 143 serum samples were collected from normal indivudual and tropical diseases endemic in Bahia. ELISA was used to test the peptides. Both Tri and Tetrapeptides reacted with all 50 chagas'disease sera with dilutions of 1/50 and 1/25. To evaluate the specificity of the Tri and Tetrapeptides were test with a panel of control 143 sera including visceral leiishmaniasis(25), tegumentary leishmaniasis(25) and tuberculosis(25).

Cross-reactivity was noted with serum from mucocutaneous leishmaniasis (12% and 8% for Tetra and Tripeptide respectively). Only one serum from a tuberculosis patient reacted with both antigens. Overall both the Tri and Tetrapeptides had a sensitivity of 100% and also specificity greater than 92% in the population groups studied. In Healthy individuals tested the specificity was 100%.

Supported by Pronex, CNPq and CADCT.

IM-52
EVALUATION OF THE IMMUNE RESPONSE AND PRODUCTION OF INTERFERON IN DOGS NATURALLY INFECTED WI TH *LEISHMANIA CHAGASI*

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Studies on the role of T-cells and cytokines in dogs, one of the *Leishmania*'s natural reservoirs, are incomplete but suggest that the resistance to infections is mediated by mechanisms similar to the ones observed in the murine model. Two animal groups were employed in this study: Control animals, made up of 5 serologically and parasitologically negative for *L. chagasi*, and test animals, made up of 22 naturally infected dogs, from Belo Horizonte, MG, Brazil. The animals were tested for immunofluorescence, lymphocyte proliferation and interferon (IFN) bioassays by using serum and supernatants of the mononuclear-cell cultures, respectively. Supernatants of mononuclear cells, collected at 24, 48 and 72 hours, were quantitatively tested for the presence of IFN. The supernatants were tested for their ability to inhibit the cytopathic effect of the Vesicular Stomatitis Virus (VSV) over Madin-Darby canine kidney cells (MDCK) according to STEWART (1979). MDCK were cultivated in D-MEM supplemented with 10% fetal calf serum in 96-well plates at an initial population density of $3.5x10^4$ cells/well. After 18 hours of incubation with the supernatants of the mononuclear cell cultures the MDCK cells were infected by VSV at a volume of 100ml viral suspension/well. IFN activity was expressed in standard units/ml, representing the inverse of the maximum dilution capable of protecting 50% of the infected cells, by visual estimation. We demonstrate here that asymptomatic naturally infected dogs, present lesser antibody titers ($p< 0,001$) than animals with more advanced leishmaniasis. The proliferative lymphocyte response to *Leishmania* could only be detected in asymptomatic or oligosymptomatic dogs. The IFN production was detected in supernatants of peripheral blood mononuclear cells in response to stimulation by *L. chagasi* antigen in 55,5% of oligosymptomatic and in 12,5% of symptomatic animals. This cytokine was not detected in symptomatic and control groups. Thus, we conclude that the absence of symptoms in dogs is due to the early infection and it doesn't have relationship to the increase of cellular immune response once this response showed more stressed in oligosymptomatic animals, disappearing with the increasing of clinical symptoms.

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IM-53
EVALUATION OF THE POTENCY AND STABILITY OF A CANDIDATE VACCINE AGAINST AMERICAN CUTANEOUS LEISHMANIASIS

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Immunization of the population at risk to contract cutaneous leishmaniasis (CL) appears to be the more cost-effective prophylactic measure against CL. Mayrink's vaccine is composed by a suspension of killed promastigotes forms in phosphate buffer saline preserved in thimerosal and, therefore, can suffer proteolytic degradation after storage. This study aimed to compare the immunogenicity and to establish the stability of different preparations of a single strain killed candidate vaccine. Healthy adult volunteers living in the rural areas of Caratinga, Brazil, received one of the following recently produced vaccine preparations: a) autoclaved vaccine, b) lyophilized vaccine and, c) non-autoclaved vaccine. Twelve months later, another group of volunteers, from the same geographical region, received the vaccine preparations that have been stored at 4ºC. The candidate vaccine was produced by

BIOBRAS, under the GMP guidelines, using promastigote forms of *Leishmania amazonensis*. All volunteers were Montenegro Skin Test-negatives (MST), the groups were comprised by 30 individuals each. Two doses of 360 mg of nitrogen were given at 21 days intervals. Forty days after the second dose, blood was collected for *in vitro* assessment of proliferative response to *L. amazonensis* antigenic stimulus, gama-interferon (g-IFN) and interleukin 4 (IL-4) were measured in the supernatant. At this time, MST conversion was also assessed. The table shows the results found.

Vaccine preparation	MST conversion (%)	Individuals producing g-IFN (%)	Levels of g-IFN (Mean ± SD)
Autoclaved recent	25/28 (89.3)	13/28 (46.4)	145.7 (68.9)
Non-autoclaved recent	25/28 (89.3)	13/28 (46.4)	131.4 (38.7)
Lyophilized recent	23/25 (92.0)	14/25 (56.0)	174.5 (71.9)
Autoclaved 12 mos.	8/30 (26.7)*	11/29 (37.9)**	62.2 (113.1)***
Non-autoclaved 12 mos.	28/30 (93.3)	17/26 (65.4)	171.8 (166.7)
Lyophilized 12 mos.	24/30 (80.0)	18/24 (75.0)	293.3 (209.7)

* $p<0.005$ - ** $p= 0.005$ - *** $p<0.005$

The autoclaved preparation showed a significant decrease in its immunogenicity after 12 months of storage at 4ºC. The non-autoclaved and the lyophilized preparations preserved their immunogenicity after the storage period. IL-4 production was not detected in any of the volunteers. In future field trials of killed *Leishmania* vaccines either the thimerosal-preserved or the lyophilized shall be used instead of the autoclaved preparations.

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IM-54
EVALUATION OF THE ROLE OF IL-12 AND IL-15 AS IMMUNOREGULATORY MOLECULES IN HUMAN LEISHMANIASIS

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The resistance to leishmania infection is mediated by cellular immune response characterized by macrophage activation and production of cytokines as IL-2, IL-12 and IFN-g. American visceral leishmaniasis (AVL) is characterized by absence of cellular immune response. Tegumentary leishmaniasis includes a spectrum of diseases that varies from the unresponsive diffuse cutaneous leishmaniasis (DCL), to mucocutaneous leishmaniasis (MCL), the hyperresponsive pole. IL-15 has biological activities similar to IL-2 including growth stimulation of activated T and NK cells. The aim of this study was to determine the role of IL-15 in the immune response of human leishmaniasis and its potential synergism with IL-12. We studied PBMC of 19 AVL, 19 MCL and 3 DCL patients. After a five day culture in the presence of leishmania antigen, cytokines (IL-12 and IL-15) or mAbs a-IL-12 or a-IL-15 we evaluated lymphoproliferation, IFN-g production by ELISA and cytotoxicity. PBMC of AVL patients, as well as those from normal controls, showed a dose-response mitogenic response to IL-15. IL-15 did not augment IFN-g production in AVL or LCD patients nor synergize with IL-12. In the same manner, IL-15 and in a less extend, IL-12, induced an increased NK cell cytotoxicity. As was previously demonstrated, PBMC of MCL patients showed a high of cytotoxic response after stimulation with leishmania antigen. This response was heterogeneous and was not negatively regulated by neutralization of IL-12 or IL-15. Lymphoproferation and IFN-g production were not abrogated by a-IL-12 or a-IL-15. Taken together these results suggest that IL-15 mediates cytotoxicity and lymproliferation in a unspecific manner in AVL and it does not restore the immunossupression. The exacerbated immune response in MCL is not reversed with the blockage of IL-12 and/or IL-15.

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IM-55
EVALUATION OF THE TEST QUALICODE™ CHAGAS/LEISHMANIA FOR ANTIBODIES RESEARCH IN SERUM

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Serological tests for Chagas disease are still problematic for clinical laboratories and blood banks. The capacity of the *Trypanosoma cruzi* presenting different antigens and the presence of several circulating strains, these are some of the reasons of the occurrence of false positive and negative results. Besides in leishmaniasis endemic areas, the crossed reactivity among these two parasites is quite intense, and the use of parasitological tests in association to

clinical symptoms is necessary to establish the correct diagnosis. However, there is a possibility of obtaining all these information by the laboratory. The tests most used for research leishmaniasis and Chagas disease are the indirect imunofluorescence (IFI) and ELISA. IFI can be used as confirmatory test, although its good specificity it is a difficult test and its reading is quite subjective. Related to ELISA, the appropriate antigens obtaining is still looked to maintain the sensibility without committing the specificity. With the objective of evaluating a new test for diagnosis between *T. cruzi* and *Leishmania chagasi* the kit QualicodeTM Chagas/Leishmania was tested. Recombinant antigens of the two parasites are applied at nitrocellulose ribbons paper besides specific antigens of *T. cruzi* by electrophoresis. Thirty six samples were tested with known results for both diseases through IFI and ELISA. All the 14 positive serums for *T. cruzi*, presented at least 3 important bands for the diagnosis and negative serums didn't recognize important bands. Six calazar positive serums recognized the *L. chagasi* antigen while none of the 8 cutaneous leishmaniasis positive serums recognized the same antigen, this is a good specificity indicative. The test is simple to handle and the bands are visualized easily, that turns it advantageous in relation to IFI. It can be considered that it is adapted for differential diagnosis of both protozoosis although other samples are being tested to obtain a more complete evaluation as the sensibility and specificity.

IM-56
EXPERIMENTAL *TRYPANOSOMA CRUZI* INFECTION: EXTRACELLULAR MATRIX PROTEINS, ADHESION MOLECULES AND CHEMOKINES INVOLVED IN HOMING OF T CELLS TO THE HEART

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The experimental infection of C3H/HeJ mice with *Trypanosoma cruzi* induces an acute phase with high parasitemia and polyclonal activation, and a chronic stage with low number of circulating parasites. In both acute and chronic phases intense inflammatory infiltrates spread throughout the cardiac tissue are observed. These infiltrates are mainly composed of $CD8^+$ TCRab^+ cells, although $CD4^+$ and macrophages are also found. Recent works have shown that the expression of extracellular matrix components including laminin (LN) and fibronectin (FN) is enhanced during the chronic chagasic infection. Moreover, it was demonstrated that LN and its receptor VLA-6 play an important role in the migration of splenic $CD4^+$ T cells obtained from chronically *T. cruzi*-infected mice to cardiac tissue. Also, it was demonstrated that antibodies recognizing VLA-4 and VLA-5 inhibit the binding of T cells to FN, and that VLA-4 and VLA-5 increase the anti-CD3-induced T cell adherence to FN. These data raise the possibility that FN and its receptors VLA-4 and VLA-5 could be involved in the genesis and perpetuation of the myocarditis observed in chagasic patients and infected animals. The immunohistochemical assay revealed an increased expression of FN in the myocardium during the acute and chronic infection and the cytofluorimetric analyses showed that all $CD4^+$ and $CD8^+$ T-cells isolated from blood and inflamed myocardium are VLA-4^+ during the acute and chronic infection. Chemokines (CK) are important cytokines that stimulate leucocyte motility and directed movement to the of inflammatory site. Further, it is proposed that these cytokines play a critical role in T cell adhesion to ECM proteins. The analysis of CK by RT-PCR showed increased expression of RANTES, MIP-1a, MIP-1b, MIG, CRG-2 and JE in myocardium, spleen and blood cells. Taken together, these results suggest that VLA-4, FN and chemokines could be involved in the entrance of circulating mononuclear cells into the myocardium during *T. cruzi* infection. Presently we are investigating the biological consequences of the interaction of these VLA-4-bearing T cells isolated from inflamed myocardium with the ECM components.

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IM-57
EXPRESSION OF A COMPLEMENT CONTROLLING MOLECULE ON THE SURFACE OF *LEISHMANIA AMAZONENSIS* PROMASTIGOTES

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In humans, a variety of cell membrane molecules have been described that inhibit homologous complement activation on the cell surface. Among others, CD59 blocks complement lytic activity by interfering with the C9 polymerization in the cell membrane. We have recently described that promastigotes of *L.amazonenesis* express a 46 kDa glycoprotein that regulates human complement in a manner similar to human CD59. Recently, we have purified this complement regulatory molecule from *L. amazonensis* promastigotes and produced monoclonal antibodies (Mab) against this protein. Here we report the use of IgG Mab and FACS to follow the expression of this

molecule on the surface of promastigotes cultured *in vitro*. Hybridomas were prepared as described (see abstract by Ferraz, D.B. in this issue) and the clones were expanded. The supernatants shown to be positive by ELISA were concentrated by NH_2SO_4 precipitation and ultrafiltration with Centricon 30. IgG molecules were affinity purified by using a Protein G High Flow column. Purified IgG was used to follow the expression of the complement regulatory membrane protein on formalin-fixed promastigotes during their growth *in vitro*. Consistently with our previous findings that promastigotes of *L. amazonensis* become gradually resistant to complement with a peak at the late logarithmic phase, our results indicate that the complement regulatory protein is displayed with increasing intensity from day one throughout day five (late log phase), decreasing thereafter. We are now investigating the ability of these Mabs to block complement inhibitory activity on the surface of *L. amazonensis* promastigotes.

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IM-58
EXPRESSION OF ADHESION AND ACTIVATION MOLECULES IN HUMAN MONOCYTES/ PRE-DENTRICTIC CELLS IS AFFECTED BY INFECTION WITH *TOXOPLASMA GONDII*

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The *Toxoplasma gondii* is an obligate intracellular coccidian that has an extremely wide host range and can survive in all nucleated cells. Its normal life cycle induces potent cell mediated immunity (CMI) response, leading to host resistance. Studies on the interaction of this parasite with cell of the immune system provide a unique approach for identifying events that selectively lead to evasion of the immune response. Recent study demonstrated that *T.gonddi* induce down-regulation of MHC class II and inability to up-regulate class I molecules in murine macrophages after infection (Luder CG et al., Clin Exp. Immunol. 1998). In this report we investigate the expression of several adhesion/activation molecules on human monocytes recently isolated from PBMC, infected or uninfected with *T.gondii* for 24h, by flow cytometry. We developed a model of culture of adherent cells where 3 populations can be identified according to FS x SS and expression of some markers: pro-lymphocytes, monocytes and pre-dendritic cells. We used a panel of monoclonal antibodies as follow: anti- CD1a, anti-CD4, anti-CD11a, anti-CD14, anti-CD29, anti-CD40, anti-CD44, anti-CD106 and anti-HLA-DR,DQ,DP. The expression of CD11a, CD44 e CD1a was down-regulated (15-30%), while the expression of CD14 and CD106 was up regulated. The regulation is distinct among the adherent population, and the most drastic effects were obtained over pre-dendritic cells. Our data suggests that this may be an important strategy for evasion from the host immune response and for intracellular survival of the parasite.

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IM-59
EXPRESSION OF FIBRONECTIN AND ITS RECEPTORS IN THE INFLAMED MYOCARDIUM DURING MURINE *TRYPANOSOMA CRUZI* INFECTION

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Trypanosoma cruzi infection of C57Bl/6 resistant mice induces an acute phase characterized by low parasitemia and sparse inflammatory infiltrates in the myocardium. During the chronic infection, circulating parasites are absent or hardly detected and a mild inflammation is observed in the myocardium. Initial studies have shown that these infiltrates are composed of mononuclear cells. The characterization of these cells by cytofluorimetric assay showed the predominance of $CD8^+$ T cells during the acute (42 days post-infection) and chronic (120 d.p.i.) phases, although $CD4^+$ T cells and macrophages were also detected (Table 1). Several studies showed an intense increase in the expression of fibronectin (FN) in the myocardium of chronic chagasic patients and *T. cruzi*-infected mice in the acute and chronic phases of the infection. Moreover, it was shown that VLA molecules play a major role in the migration of activated T cells to inflammatory sites. Therefore, we studied the distribution of the FN receptors VLA-4 and VLA-5 in the inflamed myocardium in both acute and chronic phases of the infection. By immunohistochemical assay we showed the presence of $VLA\text{-}4^+$ and $VLA\text{-}5^+$ cells in the inflammatory infiltrates during acute and chronic infection. Aiming to characterize the VLA-bearing cells cytofluorimetric assay was performed (Table 2).

Table 1- Cytofluorimetric characterization of inflammatory cells in *T. cruzi* infection (%)

	CD4	CD8	Mf
Acute	8	27	25
Chronic	16	28	50

Table 2- Percentual of VLA-bearing cells in acute *T. cruzi* infection

	$CD8^+$ $VLA\text{-}4^+$	$CD8^+$ $VLA\text{-}5^+$	$CD4^+$ $VLA\text{-}4^+$	$CD4^+$ $VLA\text{-}5^+$
Myocardium	93	26	76	30
Blood	95	8	33	5
Spleen	92	17	47	18

The majority of $CD8^+$ T cells present in the myocardium, blood and spleen of *T. cruzi* infected mice are VLA-4^+ . Interestingly, a preferential localization of $CD4^+$ VLA-4^+ T cells was observed in the myocardium. Altogether our results suggest that VLA-4 play an important role in the entrance/localization of activated T cells in the myocardium during chagasic infection. It remains to be solved the participation of these VLA-4^+ cells in the perpetuation of the myocarditis.

IM-60
EXPRESSION OF GALECTIN-3 AND TENASCIN IN THE THYMUS FOLLOWING EXPERIMENTAL ACUTE *TRYPANOSOMA CRUZI* INFECTION

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Acute *Trypanosoma cruzi* infection induces several alterations in the thymus gland. Increasing evidence reveals that extracellular matrix (ECM) components can be regarded as mediators in intrathymic T cell migration and differentiation. In this respect the adhesive proteins fibronectin and laminin are enhanced in thymus of acutely *T. cruzi*-infected animals. Moreover, we have recently suggested that thymocytes from infected animals may have an imbalance in their intrathymic migration, with escape of immature cells to the periphery. Thus, one can predict alterations on events involved on adhesion/de-adhesion in the thymus of these animals. This prompted us to investigate the intrathymic content of galectin-3 (a b-galactoside binding protein) and tenascin (an ECM protein), both molecules presenting de-adhesive properties in different models of cell interactions. Here, we observed that the expression of both galectin-3 and tenascin is increased in the thymus of infected animals. Galectin-3 expression was enhanced in the cortex and also in the medullary regions. In the same way, tenascin expression, which is normally restricted to the medulla, cortico-medullary junction and blood vessels, formed a much denser network including the cortex. Additionally, investigating the expression of galectin-3 and tenascin in a thymic epithelial cell line, as well as in primary cultures of thymic nurse cells, we demonstrated a marked enhancement of both proteins in cultures examined after *in vitro T. cruzi* infection. Additionally, *T. cruzi*-infected cultures of phagocytic cells of the thymic reticulum, showed an increased immunoreactivity for tenascin. In conclusion, the data presented herein reveal an enhancement in the intrathymic content of galectin-3 and tenascin following acute experimental *T. cruzi* infection. Considering the anti-adhesive and immunomodulatory properties of these proteins, it is possible that they may be related to the imbalance of thymocyte migration and differentiation, possibly related to the appearance of immature T cells in the pheriphery of *T. cruzi*-infected mice, with potencial consequences for the immunopathology of Chagas' disease.

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IM-61
FLOW CYTOMETRIC ANALYSIS OF HEART INFLAMMATORY CELLS DURING ACUTE INFECTION WITH *TRYPANOSOMA CRUZI*

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Mice experimentally infected with *T. cruzi* develop varying degrees of cardiac inflammatory infiltration in both acute and/or chronic phases of the disease depending on the genetic background. C3H/He mice present high mortality during acute phase whereas DBA/2 mice show low mortality rates and progress into chronic phase. In this study we have investigated the phenotype of mononuclear cells from several organs of C3H/He and DBA/2 mice infected i.p. with 10^2 blood trypomastigotes (Colombian strain). Hearts, peripheral blood, spleens, thymuses and lymph nodes were harvested, homogenized by passing through a metal mesh and single cell suspensions were centrifuged over a discontinuous Percoll gradient. Mononuclear cells from the 40%-80% Percoll interface were collected, washed and stained with different combinations of monoclonal antibodies for 3-color flow cytometry analysis. In both mouse strains, $CD4^+$ and $CD8^+$ T cell populations were increased in comparison with uninfected littermates, with predominance of $CD8^+$. A marked increase of gd^+/$CD69^+$ T cells in heart infiltrates of C3H/He mice was detected by 21 days after infection. Furthermore, a significant percentage of $CD8^+$ and $CD4^+$ co-expressed CD69 in this strain. Conversely, infected hearts from DBA/2 mice had no increase in gd^+ T cells or CD69 expression. In peripheral organs of both mouse strains, the number of recently activated cells was low, similar to control (uninfected) mice. These data are in accordance with our previous report for chronically infected Balb/c mice and suggest early migration of T cells to heart tissues during acute infection, accompanied by extensive activation *in situ.* These

activated cells, specially $\gamma\delta^+$ T lymphocytes, might play a pivotal role in the pathogenesis of aggressive myocarditys of C3H/He mice. On the other hand, myocarditis in DBA/2 mice appears to have a delayed onset, possibly due to later migration or activation of T cells that would contribute to the chronic phase myocarditis.

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IM-62
FLOW CYTOMETRIC ANALYSIS OF IGG ANTIBODY ISOTYPES REACTIVES TO LIVE TRYPOMASTIGOTE AND FIXED EPIMASTIGOTE IN HUMAN CHAGAS DISEASE

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Different IgG antibodies isotypes have been associated with distinct CD4+ T cell subpopulations and their cytokine production. As Chagas disease presents a spectrum of clinical and immunological manifestations that seems to be correlated with the nature of the host CD4+ T cell response, the analysis of IgG subclasses in chagasic patients is relevant to evaluate the relationship between cellular and humoral immune response. IgG isotype response has been performed basically by immunoassays using soluble or fixed whole antigen preparations. In this study, 76 sera from chagasic patients with chronic disease included Indeterminate (IND), Cardiac (CAR) and Digestive (DIG) clinical forms and 20 sera from healthy individuals were evaluated by flow cytometry. Analysis of the anti-*Trypanosoma cruzi* IgG isotype profile was performed using live (trypomastigotes-TRIPO) and fixed (epimastigotes-EPI) parasites, to evaluate the antibodies reactivities to membrane and intracellular antigens on morphologically intact cells. Immunofluorescence staining was carried out using anti-human IgG antibodies labeled with FITC for total IgG measurement and biotin/avidin-phicoerytrin for IgG isotypes. The results are expressed as mean fluorescence channel since both TRIPO and EPI preparations have an unimodal distribution on flow cytometry histograns. IgG1 is the predominant specific antibody isotype reactive to TRIPO and EPI, with no detectable differences between the clinical groups evaluated. IgG2 antibodies reacted significantly with EPI but not with TRIPO, however no correlation with clinical forms was also observed. The levels of IgG3 were low or undetectable in most sera, with a slightly higher reactivity to TRIPO. Anti-EPI IgG4 was absent with only some individuals presenting IgG4 reactivity to TRIPO, although at very low levels. In conclusion, our study showed no direct relationship between isotype profile and clinical forms of the disease. However, analysis of the IgG isotype response to TRIPO and EPI suggested that IgG1 seems to be the most specific isotype to *T. cruzi*. Considering the nature of the binding of IgG2 antibodies to antigens, binding of this isotype to the EPI seems to occur through carbohydrates epitopes abundantly present on this parasite form.

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IM-63
FLOW CYTOMETRY AS A TOOL TO ANALYZE OF ANTI-LIVE PROMASTIGOTES ANTIBODY REACTIVITY IN PATIENTS WITH AMERICAN CUTANEOUS LEISHMANIASIS

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Several studies have reported the protective role of the Th1-type immune response in patients with american cutaneous leishmaniasis (ACL). However, the contribution of antibodies in protective as well as pathogenic mechanisms is still controversial. The conventional serology (CS) using soluble antigens and fixed parasite preparations demonstrate low levels of antibody reactivity with no correlation with active disease. Moreover the major limitation of CS is the cross-reactivity between species of Trypanosomatidae. We have developed a new immunoflorescence approach to evaluated the humoral immune response of patients with ACL using flow cytometry as a tool to access the reactivity of anti-live promastigotes antibodies. This methodology will allow an *ex vivo* analysis of intact parasites simulating the *in vivo* reactivity, avoiding the interference of antibodies directed against intracellular structures and antigens that would be the major source of undesired cross-reactivity of sera from patients with other protozoan infections. Moreover, once established the experimental procedures the analysis of immunoglobulins classes and sub-classes patterns will permit the establishment of a relationship between the profile of humoral response and the different clinical forms of the disease. We have tested three different stages of parasite growth (4,7 and 10 days after *in vitro* culture) of LIT cultured *Leishmania (V.) braziliensis* promastigote to search for specific antibodies, and the late log phase parasites (10) have been selected as the more reactive preparation with low cross-reactivity with sera from healthy donors. The number of promastigotes in suspension, the temperature and appropriated dilutions for incubation with sera and fluorocrome-conjugated anti-human IgG antibodies were also optimized. Initially we have analyzed sera sample from 48 individuals from Araçuaí - MG an endemic area for ACL, subdivided in 6 groups

based on Montenegro Test, Indirect Immunofluorescence and Presence of Lesion. Preliminary results demonstrated a positive correlation between the percentage of fluorescent parasites and the presence of lesion, suggesting that this method may would also have a prognostic value.

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IM-64
GEOGRAPHIC DIFFERENCES IN SERUM REACTIVITY TO B13 ANTIGEN: POLYMORPHISM AT THE IMMUNODOMINANT REGION?

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The recombinant protein B13 contains 12 amino acid tandem repeats that correspond to the immunodominant region of *T. cruzi* 140/116 kDa antigen (Gruber & Zingales, 1993). B13 protein has been evaluated for serodiagnosis of Chagas' disease. Analysis of approximately 260 chronic chagasic sera from nine countries of Central and South America showed variation in the median values (Me) of B13 ELISA according to the analysed country: (*eg* Argentina, Me=2.0; Brazil, Me=1.4; Honduras, Me=1.0) (Umezawa et al., in preparation). Such variability may be the result either of genetic differences among *T. cruzi* strains or of the host immune response. It has been reported that the single copy gene corresponding to the 140/116 kDa antigen contains different numbers of the 12 amino acid antigenic repeats in two *T. cruzi* strains. In order to verify the genetical heterogeneity of the strains in the immunodominant region of B13 protein a PCR assay was standardized with primers flanking the amino acid repeats. DNA from 23 *T. cruzi* strains derived from different hosts and countries was submitted to PCR with $Vent_R^{\circ}$ DNA polymerase and the amplified products were analysed by gel electrophoresis and Southern blot. Amplification was observed in 11 isolates (50%). Within individual isolates one or two amplified fragments in the 1500-3700 bp range were observed. Previous studies indicated that the immunodominant epitope core of B13 protein is the hexapeptide AAAGDK (Cunha-Neto et al., 1995). Competitive ELISA with recombinant B13 protein in the solid phase and two nonapeptides - S4 (FGQAAAGDK) and S5 (QAAAGDKPS) was performed with 40 chronic chagasic sera from Brazil. The median percent inhibition was 86% for S4 and 68% for S5. These nonapeptides will be tested with chagasic sera from different countries in order to verify possible variations in the antigenic recognition.

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IM-65
GLYCOSYLPHOSPHATIDYLINOSITOL ANCHORED MUCIN-LIKE GLYCOPROTEIN FROM *TRYPANOSOMA CRUZI* TRYPOMASTIGOTES ENHANCES MICROBICIDAL ACTIVITY OF IFN-γ PRIMED MACROPHAGES AGAINST PARASITES FROM GENUS *LEISHMANIA*

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Different studies suggest that infection of macrophages with different intracellular protozoa results on activation or impairment of signaling pathways involved on macrophage functions such as cytokine synthesis and microbicidal activity. Here we study the ability of IFN-γ primed or unprimed macrophages to produce nitric oxide (NO) and display microbicidal activity when infected with culture promastigote (stationary phase) or trypomastigote parasites of the genus *Leishmania* or *Trypanosoma cruzi*, respectively. IFN-γ primed macrophages were infected with either *L. major, L. braziliensis, L. chagasi, L. amazonensis* or *T. cruzi* at a ratio of five parasites per macrophage in the presence or absence of aminoguanidine, a specific inhibitor of NO synthesis from degradation of L-arginine by the *inducible nitric oxide synthase* (iNOS). To measure the levels of nitrite, tissue culture supernatants were collected at 24, 28 and 72 h post macrophage infection with *Leishmania sp* or *T. cruzi*. In order to evaluate the microbicidal activity of macrophages, the percentage of infected macrophages as well as number of amastigotes per 100 macrophages were analyzed at 72 h after infection. Our results showed that IFN-γ primed but not unprimed macrophages were able to synthesize NO and display microbicidal activity when infected with various parasites. We also observed a gradual increase of nitrites, during infection with various *Leishmania* or *T. cruzi*, reaching the highest nitrite levels at 72 h post-infection. The aminoguanidine almost abolished the nitrite accumulation in the macrophage culture supernatants, indicating that the nitrites are most likely derived from NO generated by iNOS. The microbicidal activity of IFN-γ primed macrophages was also largely inhibited by the addition of aminoguanidine to the macrophage cultures. Interestingly, we found that in general *T. cruzi* was a better stimuli than different species of *Leishmania* for NO synthesis and microbicidal activity by IFN-γ primed macrophages, as measured by the levels of nitrite in the culture supernatants, percentage of infected cells and number of amastigotes per 100 macrophages.

Because glycosylphosphatidylinositol anchored mucin-like glycoprotein from *T. cruzi* trypomastigotes (GPI-mucin) have been shown to stimulate NO synthesis by IFN-g primed macrophages, we tested its ability to enhance the microbicidal activity against *Leishmania sp.* Our results show that in fact GPI-mucin were a potent stimulator of NO synthesis and microbicidal activity of macrophages infected with *Leishmania.* Interestingly, based in a correlation between nitrite levels in the culture supernatants, percentage of infected cells and number of amastigotes per 100 macrophages, we also found that *Leishmania* parasites in addition to be poorer stimulator of microbicidal activity by macrophages, appear to be more resistant then *T. cruzi* to high levels of nitric oxide released by IFN-g/GPI-mucins by activated macrophages.

Supported by Fiocruz, Papes, CNPq

IM-66
HEART-SPECIFIC MONOCLONAL AUTOANTIBODIES RECOGNISING *TRYPANOSOMA CRUZI*

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Heart-reactive T cells present in *Trypanosoma cruzi* mice are sufficient, in the absence of parasite antigens, to cause lesion in normal hearts. The autoreactivity could be theoretically elicited by altered self-antigens, formed during heart inflammation, and/or by *T. cruzi* antigens cross-reacting with self. There are indeed reports in the literature of cross-reactivities between *T. cruzi* and mammalian cells. Antibodies could be used as tools to disclose such cross-reactivities. Obtain heart-reactive monoclonal antibodies (mAbs) from *T. cruzi*-infected mice and investigate their specificity. Hybridomas were obtained by fusing spleen cells from BALB/c mice, infected 6 months earlier with *T. cruzi*, with SP2/0 myeloma cells. Two mAbs reacted both with autologous heart and with *T. cruzi*. They had, however, distinct specificities. One of them (Tc10) produced a sarcolemmic pattern in heart muscle fibers by indirect immunofluorescence, reacted with three bands in Western blots of *T. cruzi* trypomastigote lysate (two bands with apparent molecular weights above 200 kD, one of approximate 40 kD), and with a 200 kD band in Western blots of heart lysate. The other mAb (Tc8) produced sarcolemmic and sub-sarcolemma patterns, reacted with two bands in Western blots of *T. cruzi* trypomastigote lysate (one with molecular weight above 200 kD, one of approximate 33 kD), and did not react with heart antigens in Western blots (indicating that it recognizes a conformational determinant in heart muscle). The autoreactivity was heart-specific, in that none of the two mAbs reacted with liver, skeletal muscle, brain or kidney. Two distinct cross-reactivities of heart with *T. cruzi* are reported herein. The existence of these cross-reactivites, as well as other reported in the literature, allow us to speculate that the heart-specific autoreactive response present during the chronic phase of the *T. cruzi* infection may be intensified or even triggered by multiple parasite-host cell cross-reactivities. The nature of the antigens recognised by monoclonal autoantibodies in the present study is currently being investigated.

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IM-67
HIGH PERCENTAGE OF CD3+ CELLS EXPRESSING IFN-g IN THEIR CYTOPLASM CORRELATES WITH THE CARDIAC FORM OF CHAGAS' DISEASE

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We have previously shown that IFN-g and IL-10 production by peripheral blood mononuclear cells (PBMC) from cardiac (CARD) and indeterminate (IND) chagasic patients after *in vitro* stimulation with *T. cruzi* antigens have different secretion kinetics. While IFN-g secretion has its peak on 6th day of culture, IL-10 secretion is significantly earlier, being observed after 6hs with the peak of secretion at 24 hs of culture. We have also demonstrated that 83% of CARD and 49% of IND are high IFN-g producers, suggesting that the IFN-g may be an important factor on the pathology of Chagas'disease. In this study using a flow cytometric immunofluorescence staining for intracellular cytokines and cell surface markers we have initiated our studies to identify the cell phenotype producing IL-10 and IFN-g in cultures stimulated with *T. cruzi* antigens. Preliminary results showed that in PBMC from chagasic patients the majority of the IL-10 producing cells were monocytes/macrophages ($CD14^{High}$+ cells) whereas the major source of IFN-g were T lymphocytes (CD3+ cells), both gamma/delta or alpha/beta. While in the CARD group the majority of the CD3+ cells were IFN-g+, in the IND group the IL-10+ cells were predominantly CD14+. A strong relationship was observed between these findings and our ELISA data using antigen-stimulated cell cul-

ture. These results suggest a relationship between the production of IFN-g by CD3+ cells and morbidity in Chagas'disease, and that IL-10 is an important regulatory component of IFN-g, therefore involved in controlling the immunopathology of the disease. We hypothesize that production of IFN-g and IL-10 are good indicators of development of clinical form of Chagas'disease.

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IM-68
HUMAN ANTIBODIES SPECIFIC FOR A *PLASMODIUM VIVAX* VACCINE CANDIDATE RECOGNIZE EPITOPES CONSERVED BETWEEN POLYMORPHIC ALLELIC FORMS OF THE PROTEIN

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Recently, we have evaluated several immuno-epidemiological aspects of the naturally acquired human immune responses to the MSP1 of *P. vivax*, the most prevalent human malaria parasite in Brazil. We found that a high frequency of Brazilians during natural infection had IgG antibodies and T-cell reactivity to a recombinant protein based on the 19 kDa C-terminal region of PvMSP1 ($PvMSP1_{19}$). The fact that ~75% of the individuals had IgG antibodies to $PvMSP1_{19}$ suggested that this region is highly conserved among the different isolates of *P. vivax*. Nevertheless, some individuals fail to develop antibodies to $PvMSP1_{19}$ even in the presence of high antibody titers to other polypeptides of PvMSP1. The lack of reactivity observed in these individuals could be explained by strain variations. The extension of the polymorphism of $PvMSP1_{19}$ in Brazil has not been explored. Also, it is unknown whether a single amino acid substitution present in some Asian strains would affect recognition by human antibodies to $PvMSP1_{19}$. The present study was designed to address these two questions. Initially, we sequenced the DNA corresponding to the 19 kDa region of *P. vivax* MSP1 in 28 Brazilian isolates. Eighteen of these isolates were collected in the state of Rondônia, 7 in the state of Pará and 3 in the state of Maranhão. A 314 bp fragment corresponding to $PvMSP1_{19}$ gene was amplified using PCR and the products were cloned into the pMOS*Blue* plasmid vector. Eighty-eight amplified gene fragments were sequenced and compared with the original Belém sequence of the *P. vivax* MSP1 gene. The data demonstrated that the sequences encoding the $PvMSP1_{19}$ from Brazilian isolates were conserved.

Subsequently, we studied the reactivity of human antibodies to recombinant proteins representing two known allelic forms of $PvMSP1_{19}$ (Belém and Asia). Antibodies from 80 patients of the State of Pará, Brazil, infected with *P. vivax* equally recognized both recombinant proteins by ELISA. Therefore we concluded that the antibody responses to $PvMSP1_{19}$ during *P. vivax* infections are directed primarily against B cell epitopes conserved between the two alleles of the protein. Our results strongly suggest that $PvMSP1_{19}$ should continue to be explored as a candidate to a vivax malaria vaccine.

IM-69
HUMAN $CD8^+$ CYTOTOXIC T LYMPHOCYTE RESPONSES TO *TRYPANOSOMA CRUZI* TRANS-SIALIDASE PROTEINS

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The critical role of $CD8^+$ T cells in resistance to *T. cruzi* has been demonstrated using the murine model of Chagas' disease. $CD8^+$ T cells dominate inflammatory foci in parasitized tissues and their elimination from mice leads to uncontrolled parasite replication and subsequent death of the infected host. These cells are presumed to protect mice via their recognition and killing or inactivation of parasite-infected cells. A trypomastigote surface antigen, TSA-1, and two amastigote surface molecules, ASP-1 and ASP-2, were recently identified as targets of $CD8^+$ cytotoxic T lymphocytes (CTL) in *T. cruzi*-infected mice. This finding provided the tools to evaluate CTL responses in human *T. cruzi* infections. For this purpose, HLA-A2.1 motif-bearing peptides representing sequences from these 3 trans-sialidase molecules were synthesized and A2.1 binding peptides were tested for CTL recognition by immune splenocytes from *T. cruzi*-infected and peptide-immunized $A2.1/K^b$ transgenic mice and by PBMC from $HLA\text{-}A2^+$ *T. cruzi*-infected humans living in Chagas' disease endemic areas of Guatemala. Twenty three of 145 motif-bearing peptides bound with high to intermediate binding affinity to A2.1 molecules. Using the transgenic system, antigen-specific CTL responses were reproducibly detected for 6 ASP-1, 2 ASP-2 and 4 TSA-1 peptides, suggesting that these peptides could represent A2.1-restricted epitopes in $HLA\text{-}A2^+$ *T. cruzi*-infected humans. Of 20 peptides used to stimulate PBMC from 24 $HLA\text{-}A2^+$ *T. cruzi*-infected volunteers, 5 ASP-1, 2 ASP-2 and 4 TSA-1 peptides generated effectors with lytic activity in 21 patients. In all cases tested, CTL activity was antigen

specific, A2-restricted and $CD8^+$ T cell-dependent. No CTL activity was detected against these peptides when using PBMC from HLA-$A2^+$ volunteers with no evidence of *T. cruzi* infection. Demonstration of human CTL responses against *T. cruzi* and against target molecules identified using the murine model provides important information for the optimal design and evaluation of vaccines to prevent or ameliorate Chagas' disease.

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IM-70
HUMORAL RESPONSE IN GOATS EXPERIMENTALLY INFECTED WITH *TOXOPLASMA GONDII*

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The objective of this study was to evaluate the serum levels of antibodies to *T. gondii* using the Enzyme Linked Immunosorbent Assay (ELISA), Western blotting (WB) and Indirect Hemagglutination (IHA) tests, in experimentally inoculated goats. Serum samples of five goats inoculated with tachyzoites of the C4 strain of *Toxoplasma gondii* were analyzed using ELISA, IHA and WB techniques. The animals were observed up to 491 days after inoculation. The goats developed IgG antibodies to *T. gondii* from the 12th day until the end of the observation period. Antibodies detected by ELISA showed a peak between the 19th and 62th days after inoculation, persisting throughout the experiment without showing relation with parasitaemia. Using WB, the main antigens detected had molecular weights of approximately 68, 62, 50, 48, 42, 34, 28, 26, 22 and 19 kDa. Antibody titers of 1:256 to 1:32000 were observed using IHA, with a significant drop in activity after treatment with 2-mercapto-ethanol between days 12 and 48, coinciding with the parasitaemic period that occurs between 5 and 64 days after inoculation. One animal was observed throughout two gestations during the experiment. *T. gondii* was not recovered from the organs of the foetus aborted after the first gestation, although antibodies to *T. gondii* were detected in the pleural liquid. The second gestation produced a healthy kid without antibodies to *T. gondii* in the serum obtained before ingestion of the colostrum. The results indicate the applicability of serological tests for the diagnosis of toxoplasmosis in goats. IHA together with 2-mercapto-ethanol application could be used as an auxiliary test for the characterization of acute toxoplasmosis in goats.

IM-71
IDENTIFICATION AND ISOLATION OF MEMBRANE GLYCOLIPIDS FROM TACHYZOITE FORMS OF *TOXOPLASMA GONDII*: USE FOR DETECTION OF ANTIBODIES PRESENT IN THE SERA FROM PATIENTS WITH ACUTE OR CHRONIC TOXOPLASMOSIS

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Different studies demonstrate that the membrane components from the tachyzoite stage of *Toxoplasma gondii* are the major targets for the humoral response from patients with either acute or chronic toxoplasmosis. The present study was undertaken to fractionate different membrane components from tachyzoite parasites that could be used in a serological tests to identify either parasite specific IgM and/or IgG antibodies. For this purpose we used a method previously employed to fractionate *T. cruzi* membrane components (Almeida, et al. J. Biochemistry 304:793, 1994). Tachyzoites (1 x 10E10) were obtained from tissue culture fibroblasts, washed several times with PBS, dried in a lyophilizer and submitted to a chloroform, methanol and water (C:M:W, 5:10:4). Aqueous and organic phase were obtained after centrifugation at 5000 x g. The organic phase was then dried in a speed vacuum and them ressuspended in a buthanol:water (2:1) mixture. The organic and aqueous phase were termed F1 and F2,respectively. The aqueous phase from C:M:W extraction was also dried in a speed vacuum and then re-extracted with 9% buthanol and centrifuged at 5000 x g. The supernatant and pellet were termed F3 and F4, respectively. All fractions (F1 to F4) were dried, ressuspended into 1 ml of PBS, and the protein content measured using the Bradford method. Fractions F1 to F4 were tested for their ability to discriminate sera from uninfected individuals or patients acutely or chronically infected with *T. gondii*. Our results showed that fractions F2 (specificity 92.8% and sensibility 68.9%) and F3 (specificty 93.3% and sensibility 89.7%) yielded the best results being recognized specificaly by IgM and IgG from patients with acute and chronic toxoplasmosis, respectively. Our Western immunoblotting analysis demonstrate that a major single antigen of 14 kDa present on F2 was recognized by IgM from patients with acute toxoplasmosis. In contrast, multiple components with molecular weight varying from 18 to 200 kDa were from F3 were recognized by IgG present in sera from chronic chagasic patients. In order to improve the sensitivity and specificity of our serological tests we decided to further purify the components present on F2 and F3, which are recognized by IgM and IgG antibodies, respectively. Because most components present in tachyzoite surface are linked through a glycosylphosphatidyl (GPI) anchor, we decided to use an hydrophobic resin, named octyl-sepharose. F2 or F3 fraction were loaded into the octyl sepharose and eluted with a proponal gradient. Interestingly the antigen recog-

nized by IgM or IgG eluted in a single peak from octyl-Sepharose resin loaded with either F2 (from 32 to 42% of propanol) and F3 (from of 18 to 32 % propanol), respectively. Interestingly, the glycolipids recognized by IgM but not IgG were also recognized by murine mAbs T33F12 but not T54E10 previously shown to recognize GPI anchors from *T. gondii* tachyzoites (Striepen et al., J. Molec. Biol. 266: 797). Further structural analysis of the glycolipids purified from F2 and F3 are currently being determined.

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IM-72
IDENTIFICATION BY WESTERN BLOT ANALYSIS OF POTENTIALLY DIAGNOSTIC *LEISHMANIA BRAZILIENSIS* ANTIGENS IN HUMAN CUTANEOUS LEISHMANIASIS

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During the past few years, particular emphasis has been given to characterization of *Leishmania* antigenic components as a tool in obtaining specific antigens for diagnosis. In the present study, the antibody response was analysed by immunoblotting in patients with American cutaneous leishmaniasis, patients with other infectious diseases and normal individuals. Soluble and insoluble protein fractions of *Leishmania braziliensis* promastigotes were used as antigens. A specific reactivity profile for *Leishmania* infection was found. The 66, 60, 48, 30, 27, 19 and 16 kDa peptides were specifically recognized by 30%, 33%, 70%, 88%, 91%, 52% and 85% of the leishmaniasis patients's sera, respectively. The recognition of the 27 and 30 kDa soluble antigens was considered specific to cutaneous leishmaniasis and it was not detected in normal individuals or in patients with other infectious diseases (excluding visceral leishmaniasis and Chagas' disease). Western blotting was compared to indirect immunofluorescence and enzyme-linked immunosorbent assay and it was found to be significantly more sensitive and specific.

IM-73
IDENTIFICATION OF INSOLUBLE ANTIGENS BY SERA OF CHAGASIC PATIENTS USING DIFFERENT STRAIN OF *TRYPANOSOMA CRUZI*

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In previous studies we obtained insoluble antigens from the epimastigote forms of 3 different strains of *Trypanosoma cruzi* (Type I - Y, Type II - WSL and Type III - Colombiana). These antigens were analyzed by Western blotting to verify the ability to react with the serum of 22 patients carrying Chagas disease: 10 (44,5%) with the cardiac form-CF, 11 (50%) with asymptomatic form-AF and 1 (4,5%) with the digestive form-DF. Sera of patients with other parasitic diseases (visceral and cutaneous leishmaniasis, toxoplasmosis, amebiasis, schistosomiasis and filariasis) were also analyzed to identify *T. cruzi* cross-reacting antigens. The reactivity pattern showed by patients with Chagas' disease, as well as the intensity of the reaction were variable among the tested sera. An antigenic pattern characteristic of the clinical forms was not found. Cross reactions with all parasitic diseases were observed with several polypeptides, excepting the 46 kDa and 58 kDa antigens that were recognized by all chagasic sera (Pereira et al., *Mem. Inst. Oswaldo Cruz* 92:Suppl I, 1997).

Recently, these polypeptides were purified and the antigenic preparations obtained, named Tc 46 and Tc 58, were tested in ELISA assays. Specificity to CF and AF using Tc 46 antigen were 100%, while the sensitivity showed variation depending on the clinical forms (CF= 90% and AF= 100%). These results suggest application in clinical laboratories. ELISA assays with Tc 58 antigens presented a specificity of 91,3% and a sensitivity of 100% in all clinical forms studied, showing that this antigen could be used in blood banks. The results presented suggest that Tc 46 and Tc 58 may be useful in the serodiagnosis of Chagas' disease. We are currently increasing the number of individuals tested in order to validate the present results.

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IM-74
I-DEPLETION OF NK1.1 CELLS INDUCES SUSCEPTIBILITY TO *TRYPANOSOMA CRUZI* INFECTION IN EUTHYMIC BUT NOT IN THYMECTOMIZED C57BL/6 MICE

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Young C57Bl/6 mice are extremely susceptible to the infection with the Tulahuen strain of *T. cruzi*, whereas aged C57Bl/6 mice are resistant. Depletion of NK1.1 cells causes high levels of parasitemia and mortality in young and aged C57Bl/6 mice infected with *T. cruzi*, thus indicating that NK cells could provide the basis for resistance to *T. cruzi* infection. It was initially proposed that NK1.1 cells were responsible for the early production of large amounts of IFN-gamma, thus resulting in host resistance (Cardillo et al, Infect. Immunity 64:128, 1996). However, the NK1.1 marker is not only expressed on CD3- (Natural Killer) cells, but it is also expressed on a special subpopulation of T cells. Although a large proportion of NK T cells is produced daily in the thymus and migrate to peripheral lymphoid organs, some are produced in the bone marrow and are considered to be thymus independent. In order to eliminate the output of newly generated NK T cells of thymic origin, thymectomy was performed in young and aged C57Bl/6 mice before infection with *T. cruzi*. This procedure would allow the evaluation of the role of peripheral NK1.1+ cells and therefore their role during the acute phase of *T. cruzi* infection. It was found that thymectomy, by itself, decreased parasitemia and increased resistance either in young or aged mice. However, and surprisingly, depletion of NK1.1+ cells by chronic administration of a monoclonal antibody (PK-136) did not cause increase in parasitemia or mortality in young or aged thymectomized C57Bl/6 mice infected with T. cruzi (Tulahuen strain). These results indicate that the main effect of NK1.1 depletion is on the thymus itself and more importantly, susceptibility to *T. cruzi* infection caused by depletion of NK1.1+ cells in euthymic animals is not due to the simple absence of recent thymic emigrant NK T cells or peripheral NK1.1+ cells. In addition, these data suggest that the presence of a functional thymus might contribute for susceptibility to T. cruzi infection by producing suppressors and/or regulatory cells, as recently shown (Cardillo et al, Int. Immunol. 10:107, 1998).

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IM-75
IFN-b INCREASES PARASITE BURDEN IN *LEISHMANIA AMAZONENSIS* AND *L. BRAZILIENSIS*-INFECTED HUMAN MACROPHAGES AND ABROGATES IFN-g-INDUCED? LEISHMANICIDAL ACTIVITY

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Presently, cure or progression of leishmaniasis is considered to depend upon the balance between Th1 (IFN-g, TNF, IL-12) and Th2 (IL-4, IL-10, TGF-b cytokines, both in humans and in experimental animal models. IFN-? plays a central role because of its capacity to induce leishmanicidal activity in macrophages. IFN-ghas been shown to exert a protective role in mice infected with *L. major*, (Shankar *et al*., 1996, Dieffenbach *et al*., 1998) which seems to correlate to induction of NO synthase. We have studied the possible effect of IFN-b in human macrophages obtained from healthy donors, infected with different species of leishmania *in vitro*. We found that treatment of *L. amazonensis*-and *L.braziliensis*-infected macrophages with IFN-b for 24 h strongly increased the cellular parasite load. This increase was time-dependent, reaching a plateau at 72 h of treatment. IFN-g, as previously shown, drastically reduced the percentage of infected cells, as well as the number of parasites/cell. Both the protective effect of IFN-g and the converse effect of IFN-b were found to be dose-dependent. When both IFNs were added simultaneously, IFN-b was able to completely antagonize the protective effect of IFN-g. Pretreating macrophages before infection did not significantly alter the effect of IFN-b but slightly increased the effect of IFN-g. NO production, measured by use of Griess reagent in cell supernatants, was undetectable in either IFN-b or IFN-g-treated cells, argueing against a role of NO synthase in the converse effects of both IFNs. A direct effect of IFN-b on *Leishmania* proliferation, as previously shown for IGF-1 and GM-CSF, is unlikely, since IFN-b had no effect on ^{3}H-thymidine incorporation of axenically grown *L. amazonensis* promastigotes.

Due to its negative role in *Leishmania*-infected human macrophages, we believe human IFN-b might be considered as a potential Th2 type cytokine, in contrast to murine IFN-b suggesting a strong physiological difference between species for this cytokine.

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IM-76
IFN-g PRODUCTION BY CD45RB^{HIGH} AND CD45RB^{LOW} CD4^{+} T CELL SUBSETS ALONG THE ACUTE AND CHRONIC PHASES OF EXPERIMENTAL CHAGAS DISEASE

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CD45 is one of the most abundant leukocyte surface glycoproteins that plays a pivotal role in signal transduction of antigen-stimulated T and B lymphocytes. Expression of different isoforms generated by alternative splicing of exons A, B and C varies according to the lymphocyte types and the functional status of the cells. For CD4^{+} T

cells, the relative expression of exon B molecule distinguishes a CD45RB^{HIGH} and a CD45RB^{LOW} subset. These subpopulations have been claimed to represent IL-2 and IL-4 producing subsets, respectively, but more recent data suggest they correspond to naive and experienced helper lymphocytes. We have evaluated the expression of CD45RB by spleen CD4$^+$ cells at the acute and early chronic phases of murine *T. cruzi* infection and correlated exon B expression with the cells capacity to produce IFN-g spontaneously and after *in vitro* re-stimulation. A/J mice were infected with 1000 bloodforms of *T. cruzi* (Y strain). For chronic phase studies, mice were treated with benzonidazol at day eight of infection to avoid death at the acute phase. FACS analysis was done using three colors. The spleen cells were incubated with labeled-Mabs to CD4, CD45RB (clone 16A) and IFN-g for surface and intracytoplasmic stainings. During the acute phase, when total CD4$^+$ spleen cell numbers drasticaly increased, there was a marked shift in CD45RB profile with predominance of CD45RB^{HIGH} cells. CD4$^+$ total cell numbers and subset distribution reverted to normality by day 26 of infection, but, by day 64, a discrete shift towards CD45RB^{LOW} phenotype was observed. Nine and eleven days after infection aproximately 8% of CD4$^+$ cells spontaneously produced IFN-g most of them being CD45RB^{HIGH}. Re-stimulation with PMA and Ionomycin resulted in minor increase in the frequency of IFN-g producers, and although a small percentage of CD45RB^{LOW} was recruited for secretion, bulk production remained CD45RB^{HIGH}. By day 26, most CD4$^+$ cells spontaneously producing IFN-g were also CD45RB^{HIGH}, but after re-stimulation cells from both of the CD45RB^{HIGH} and CD45RB^{LOW} CD4$^+$ subsets became IFN-g producers. By day 64, a time point where spontaneous production of IFN-g had declined, re-stimulation induced a small increase in IFN-g producing cells restricted to the CD4$^+$ CD45RB^{LOW} phenotype.

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IM-77
IFN-g-DEPENDENT TNF-a PRODUCTION BY HUMAN MACROPHAGES INFECTED WITH *LEISHMANIA*

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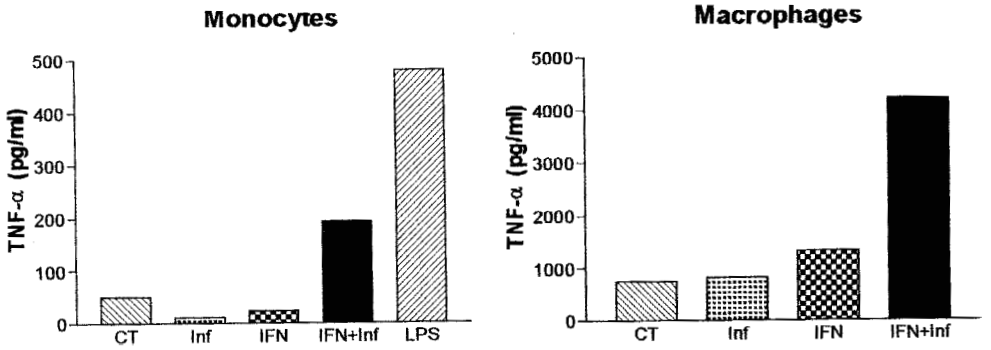
Upon *Leishmania* infection the human macrophage produces different cytokines. Some such as TNF-a is involved in macrophage activation and host protection, whereas others, such as TGF-b, are involved in macrophage deactivation and parasite growth. Understanding these early steps of the response of the human cell to *Leishmania* is elemental in designing rational strategies of protection. We have cultured either monocytes (obtained by Percoll gradient cultured for ... hours and infected) or macrophages (PBMC left to adhere for ... hours, washed, cultured for ... days and then infected). Infection was performed at a ....:1 parasite:cell ratio, with either *L. amazonensis* or *L. donovani*. The presence of IFN-g in the culture media largely increases TNF-a production as shown in the figures.



These data illustrates the necessity of an integrated approach to the comprehension of the cytokine network in the early steps of leishmanial infection.

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IM-78
IGG ANTIBODIES TO *PLASMODIUM FALCIPARUM* 19-kDA C-TERMINAL FRAGMENT OF THE MEROZOITE SURFACE ANTIGEN (*PFMSP1-19*) IN BRAZILIAN GOLD MINE WORKERS WITH ASYMPTOMATIC MALARIA PARASITAEMIA

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Studies on the mechanisms of clinical immunity naturally acquired to *Plasmodium falciparum* in hyper-holoendemic areas in Africa and Asia suggest that after life-long exposure immunity gradually develops. The mechanisms of immunity are unclear, although data have shown that antibodies play a critical role controlling the parasitaemia. In Brazil, since the transmission and morbidity are unstable, studies are required to clarify whether resistance against the infection is being developed. Although asymptomatic malaria has been considered uncommon in our country, recent study has shown the presence of asymptomatic infected subjects living for many years in gold mines located in the north of Mato Grosso State (Fontes, PhD Thesis, 1998). Thus, our study was aimed at delineating the relationship between natural acquired immune response and clinical disease. Thus, we investigated the prevalence and concentration of antibodies to PfMSP1-19, a candidate malaria vaccine. Sera IgG antibody concentrations were measured by ELISA in parasitaemic subjects with clinical symptoms of malaria (symptomatic group, n=49) or no classical symptoms for at least 72 hours after parasite detection (asymptomatic group, n=57). The optical densities were converted to antibody concentrations (ng/ml) by comparison with a standard curve derived from a two-fold serial dilution of the IgG acquired commercially. The threshold of positivity was a concentration of 100 ng/ml based on the mean plus three standard deviations of the sera reactivity from the 40 healthy non-exposed subjects. Anti-PfMSP1-19 antibodies were highly prevalent (86%) in subjects who had no malarial symptoms; however, among the symptomatic patients, 59% were anti-PfMSP1-19 positive, a significantly lower result ($p < 0.05$). Moreover, the highest concentrations of antibodies were also detected for subjects who had had asymptomatic malaria. Among the asymptomatic positive subjects, 63% had concentrations of IgG anti-PfMSP1-19 above 10,000 ng/ml whereas 62% of positive subjects from the symptomatic group had IgG concentrations below that value. Our results showed an association between absence of clinical malaria and high antibody concentration to PfMSP1-19, suggesting a possible semi-immune state of the populations exposed in the Amazon endemic region for many years.

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IM-79
IGG1 AND IGG4 SUBCLASSES SPECIFIC TO *TRIATOMA INFESTANS* SALIVARY GLAND ANTIGENS ARE SELECTIVELLY SECRETED BY PATIENTS EXPOSED TO THE INSECT-BITE

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The blood-feeding bug *Triatoma infestans* is the main vector of *Trypanosoma cruzi* infections in Brasil. The pharmacologically active insect salivary gland proteins, which facilitate the feeding action, can induce immune responses. The role of the specific immune responses against the insect saliva components, injected during blood feeding in humans, remains to be determined. This study reports the quantitative (ELISA) and qualitative (Immunoblotting) humoral immune factors in the populations subjected (Posse, GO) and not-subjected (Brasília, DF) to the triatomine bite.

Specific IgG antibody titers obtained by ELISA with the *T. infestans* salivary gland secreted antigen (SGA) showed statistically significant differences when sera from individuals subjected to the insect bite were compared to those from individuals not-subjected to the insect-saliva injection. In addition, the antibody titers were significantly higher in chagasic than in non-chagasic patients. The presence of antibodies against Reduviid bug saliva antigens in sera from individuals living in Brasilia (not exposed to the insect bite), were due to cross-reactions with other non-related species of blood-feeding arthropods, as shown by absorption studies with antigenic extracts of *Culex quinquefaciatus* female, which is the main hematophagus mosquito in both study areas.

Immunoblott assays showed at least four protein bands (79.7, 77.0, 58.0 and 47.0 kDa) revealed by sera from an average of >50.0% of chagasic patients from Posse, GO. None of these protein bands were revealed by sera from chagasics living in Brasília during more than 15 years. On the other hand, the non-chagasics living in Posse showed frequencies of 4.2%, for the 79.7 and 47 kDa proteins only.

IgG1 and IgG4 have been found to represent the subclasses associated with the immune responses against the *T. infestans* salivary gland antigens. Of interest, IgG1 is related to the activation of the complement system by the classical pathway whereas, IgG4 shows cytotropic features related to allergic reactions induced by mast cell mediators.

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IM-80
II-DEPLETION OF NK1.1 CELLS INDUCES SUSCEPTIBILITY TO *TRYPANOSOMA CRUZI* INFECTION IN EUTHYMIC BUT NOT IN THYMECTOMIZED C57BL/6 MICE: T CELL PHENOTYPES

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Depletion of NK1.1 cells causes high levels of parasitemia and mortality in young and aged C57Bl/6 mice infected with *T. cruzi*, thus indicating that NK cells could provide the basis for resistance to T. cruzi infection. It was initially proposed that NK1.1 cells were responsible for the early production of large amounts of IFN-gamma, thus resulting in host resistance (Cardillo et al, Infect. Immunity 64:128, 1996). Although a large proportion of NK T cells is produced daily in the thymus and migrate to peripheral lymphoid organs, some are produced in the bone marrow and are considered to be thymus independent. In order to eliminate the output of newly generated NK T cells of thymic origin, thymectomy was performed in young and aged C57Bl/6 mice before infection with *T. cruzi*. It was found that thymectomy, by itself, decreased parasitemia and increased resistance either in young or aged mice. However, and surprisingly, depletion of NK1.1+ cells by chronic administration of a monoclonal antibody (PK-136) did not cause increase in parasitemia or mortality in young or aged thymectomized C57Bl/6 mice infected with *T. cruzi* (Tulahuen strain). These results indicate that the main effect of NK1.1 depletion is on the thymus itself. To further study the involvement of other cell phenotypes in this model, we analyzed activation/memory markers on splenic CD4+ and CD8+ T cells from the different experimental groups at different time-points along the infection. Our results showed that there is a decrease in the percentage of CD4+ CD69+ T cells in the first week after infection in susceptible mice when compared to resistant groups. Furthermore, a decrease in CD4+ CD45RB- and CD8+ CD45RB- T cells was observed after 3 weeks post-infection. The diminution of T cell activation was correlated to an accumulation of CD4+ CD44- and CD8+ CD44- "naive" T cells late in acute infection. The latter results indicate that resistance (aged, young thymectomized and thymectomized NK-depleted mice groups) parallels with early activation of CD4+ cells and susceptibility (young euthymic and euthymic NK-depleted mice groups) is correlated with lack of activation or increased numbers of naive T cells late in infection. This findings could be due to the augmentation of suppressors/regulatory and/or naive T cell output by the thymus after NK depletion as previously shown for normal animals (Nomizo et al, Anais do XII Congresso da SBI, S. 16.3: 62, 1997).

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IM-81
IL-12 AS A VACCINE ADJUVANT IN EXPERIMENTAL LEISHMANIASIS FOR *LEISHMANIA AMAZONENSIS*

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IL-12 is a primary citokine in the initial stage of leishmanial infection exhibiting an important function in the differenciation and expansion of T CD4+ Th1 cells. It has been shown that IL-12 may act as an adjuvant in vaccines associated with soluble or purified *L. major* antigens leading to the protection of susceptible mice.

To evaluate the effect of IL-12 as an adjuvant in vaccine against *L. amazonensis* susceptible BALB/c mice were treated with 1mg of IL-12 or 25mg of leishmanial antigen or 1mg of IL-12 + 25mg of leishmanial antigen in two doses with a 7 day interval. Mice were infected days after immunization with 5 x 10^5 promastigotes of *L. amazonensis* and lesions were measured weekly for 9 weeks. There was no difference in lesion size among the groups.

Resistente C57BL-6 mice were similary treated and challenged with 1 x 10^5 promastigotes of *L. amazonensis*. From the 8^{th} to 11^{th} week, mice treated with IL-12 + leishmanial antigen had a 75% reduction in lesion size as compared to PBS-treated animals and those differences were statistically significant. Mice treated with either IL-12 or antigen exhibited a 45% reduction in lesion size than control. From the 12^{th} to 15^{th} week the differences among the groups were not statistically significant.

Our results show that although IL-12 exhibited a potential as a vaccine adjuvant against *L. amazonensis*, its effect was not as important as the reported against *L. major* infection.

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IM-82
IMMUNE PARAMETERS IN THE NON-HUMAN PRIMATE *CEBUS APELLA* DURING A SECONDARY CHALLENGE WITH *LEISHMANIA (LEISHMANIA) AMAZONENSIS*

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Previously we (Garcez *et al*., Mem. Inst. Oswaldo Cruz 91 Suppl: 180, 1996) established a protocol for the induction of cutaneous lesions in laboratory bred monkeys, *Cebus apella*, by subcutaneous injection of stationary phase promastigotes of *L. (Leishmania) amazonensis* (IFLA/BR/67/PH8 strain) . In the present study we analysed the development of the cutaneous lesion in *Cebus apella* during a secondary challenge with the homologous para-

site. We also analysed the development of anti-*Leishmania* IgG antibodies and the 'in vitro' *Leishmania* antigen-dependent lymphoproliferative response and production of IFN-g. We performed two consecutive experiments by firstly using different infection doses (0.5×10^6, 1.0×10^6, 1.6×10^6 and 4.8×10^6) and secondly a single sized dose (1.6×10^6). After cure of the initial infection the challenge with the homologous parasite still resulted in the development of a cutaneous lesion in 73% of animals but with significantly shorter latency, shorter duration and smaller lesion diameter as compared with the primary infection. The anti-*Leishmania* IgG antibody level (ELISA) was higher after the challenge infection. Anti-*Leishmania* IgG antibodies from infected animals recognise different *L. (L.) amazonensis* antigen fractions upon Western blot and more fractions were recognised by sera from animals infected with higher dose of parasites that resulted in larger lesions. A positive *Leishmania* antigen-specific lymphoproliferation associated with IFN-g production was observed one month after the initial infection. The lymphoproliferation index during the secondary infection was higher ($\geq$ 2x). It decreased sharply 45 days post-challenge and at this time the lesions had cured. After this it increased to an even higher level 60 days post-challenge. We conclude that the *L. (L.) amazonensis*-infection (live vaccination) in *Cebus apella* induces specific humoral and cell-mediated immunity that is associated with production of IFN-gand partial protection upon challenge with homologous parasites. These results indicate that the model is suitable for evaluating vaccine candidates.

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IM-83
IMMUNE RESPONSE ASSOCIATED WITH PROTECTION IN DOGS INFECTED WITH *LEISHMANIA CHAGASI*

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Visceral leishmaniasis (VL) is endemic in tropical America. There is strong evidence for dogs infected by *Leishmania chagasi* being the main reservoir for the human infection. In theory, it would be possible to erradicate American VL by protecting dogs with a vaccine, and in this way stopping transmission of the parasite. The final aim of this work is to define the nature of the immune responses associated with susceptibility and resistance to canine VL. Definition of these parameters are important for future assesment of vaccine candidates. Dogs from an endemic area of VL (Jequié-BA) were serologically tested by ELISA and skin tested with *L. chagasi* crude antigens (DTH). The DTH was tested using antigens without thymerosal and confirmed by histological analysis. In addition, splenic tissue collected by needle biopsy was cultivaded to disclose *L. chagasi* infection. According to their immune response to *Leishmania* antigens, three different populations of animals could be recognized: 1) dogs with DTH and low or undetectable levels of specific antibodies; 2) dogs with DTH and high levels of specific antibodies, and 3) dogs without DTH and high levels of specific antibodies. Interestingly, *Leishmania* could be detected in spleen cultures of only 2 out of the 10 dogs with DTH to *Leishmania* antigen, whereas all the 6 dogs with high levels of *Leishmania* antibodies but no DTH had spleen cultures positive for *Leishamania* (statistical significance of the difference: $p = 0{,}007$, Fisher's exact probability test). These data suggest an association between positive DHT and protection against *L. chagasi* infection in dogs. The pattern of the cytokines produced by, and the antigen-specificity of leukocytes from these dogs, as well as their infectivity for sandflies, are presently being determined.

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IM-84
IMMUNE RESPONSE OF *TRYPANOSOMA CRUZI*-INFECTED RHESUS MONKEYS

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Since 1909 several non human primates have been studied as models for Chagas' disease. Aiming to reproduce the clinical aspects of Chagas' disease we have infected 15 rhesus monkeys (*Macaca mulatta*) subcutaneously with 1.5×10^4 metacyclic trypomastigotes of Colombiana strain of *T.cruzi*. All animals showed patent parasitemia assessed by hemoculture and/or xenodiagnosis. (Bonecini-Almeida MG et al. *Mem Inst Oswaldo Cruz* 85:163, 1990). Myocarditis and myositis were present in sacrificed monkeys during acute phase, but the lympho-histiocyte inflammatory infiltrate were not detected after 3-years p.i. (Meirelles, MNL et al., *Mem Inst Oswaldo Cruz* 85:173, 1990). The specific immune response was demonstrated by the presence of anti-*T.cruzi* IgM, IgG and lytic antibodies up to 28 weeks and 3 years p.i, respectively. In the acute phase these animals showed evidence of immunosuppression by inversion of T CD4:CD8 ratio and diminished lymphoproliferative response to *T. cruzi* antigens (Bonecini-Almeida, MG., *in press*). As these animals are thought to represent a suitable experimental model for human Chagas' disease, a long lasting chronic disease, an Institutional project is being carried out aiming to study the evolution of

Chagas' infection in this experimental model. Presently we are re-evaluating the clinical, parasitological and immunological aspects of the infected animals 14 (4 animals) and 17 (3 animals) years p.i. Preliminary results demonstrated that circulating parasites were not detected by PCR and hemoculture (Mangia RHR et al., abstract in this meeting), suggesting the elimination of the parasites. However, lymphoproliferative responses to mitogens (s.i. 42 to 139) and *T. cruzi*-antigens (s.i. 3.2 to 5.5) are present. Altogether, these results suggest that although circulating parasites are absent, memory immune response could be achieved and probably are maintained by the presence of tissue sequestered parasites.

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IM-85
IMMUNE RESPONSE TO *LEISHMANIA* IN HUMANS USING THE PRIMARY IN VITRO (PIV) SYSTEM

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Being an easily performed and controlled *in vitro* correlate with *Leishmania major* (Lm) *in vivo*, the PIV offers the ideal assay in which to test the response of various combinations of cells in the presence or absence of added cytokines. We are using three different *Leishmania* strains in this assay: dhfr-ts- (E-10), a totally avirulent form of Lm; CC1, an attenuated clone from which E-10 was derived and virulent Lm. E-10 was engulfed by macrophages (Mf), transformed in amastigotes within the Mf, survived in mice for two months and elicited a leishmanial specific immune response in the animals. As a result, E-10 treated mice were partially protected from challenge with virulent Lm (Titus et al., 1995). Therefore, we decided to verify the human PIV responses using these parasites, since they are potential candidates for vaccine development Before starting the PIV experiments, we determine the ability of E-10 to survive in the human Mf. We infected human Mf, obtained from PBMC from normal donors, with E-10, CC1 and Lm, at the ratio of 5 parasites to 1 cell. We observed that these parasites were taken up by Mf, where they differentiated into amastigotes. Beyond 24 hr, CC1 and Lm continued to replicate as amastigotes within Mf. In contrast E-10 did not replicate and were slowly destroyed. To explore early immune responses, an *in vitro* model of human *Leishmania* infection was developed. PBL from normal donors were exposed to E-10, CC1 and Lm infected Mf for 7 days. After this period, blast cells were harvested and restimulated using *Leishmania*-infected Mf for 48 hours. We collected the supernatants and determined IFN-gand IL-5 production by ELISA. An analysis of *Leishmania*-specific cytokine production by these PBL revealed that most of individuals developed Th1 or Th0 responses early after infection, independent of the parasite used in the experiments. Addition of recombinant IL-12, during the PIV responses increased the production of IFN-g but the addition of anti-IL-12 or indometacyn did not affect the concentration of these cytokines. In order to explore the mechanism responsible for the cytokine profile observed, experiments have been done analyzing the production of some monokines by *Leishmania*-infected Mf. Preliminary data has showed the production of IL-6 and IL-12 by infected Mf from some donors. Thus, this *in vitro* system can be used to delineate the nature of a protective human immune response against *Leishmania* induced early after infection.

IM-86
IMMUNOAFFINITY PURIFIED 30 KDA ANTIGEN FROM *LEISHMANIA (L.) CHAGASI*: LYMPHOPROLIFERATIVE ASSAYS AND ACTIVE IMMUNIZATION OF BALB/c MICE

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Among several antigens present in amastigote forms of *L. (L.) chagasi* in particular an antigen with molecular mass of 30 kDa (p30) was able to elicit lymphoproliferative responses *in vitro* mediated by $CD4^+$ Th1 and induced significant protection against infection with *L. (L.) chagasi* in BALB/c mice. The p30 antigen was purified by electroelution after fractionation of *L. (L.) chagasi* amastigotes extracts by SDS-PAGE. Characterization of *L. (L.) chagasi* p30 was performed by means of a monoclonal antibody specific to an antigen of 30 kDa from *L. (L.) amazonensis* amastigotes (MoAb 2E5D3). This monoclonal antibody cross reacts with *L. (L.) chagasi* p30. In *L. (L.) amazonensis* amastigotes the p30 possesses cysteine proteinase activity and is present in megasomes (Beyrodt et al., 1997, Infect. Immun. 65, 2052). In contrast, p30 preparations from *L. (L.) chagasi* amastigotes purified by immunoaffinity chromatography did not exhibit proteolytic activity, although this fraction displayed proteolytic activity at a higher molecular mass probably due to associated precursors of the enzyme (47 and 70 kDa). Immunoelectron microscopy observations in thin sections of *L. (L.) chagasi* amastigotes using the MoAb 2E5D3 indicated the predominant distribution of gold particles in the electron dense particles similar to megasomes.

The lymphoproliferative responses to *L. (L.) chagasi* p30 purified by immunoaffinity chromatography were examined in BALB/c mice. The antigen was able to induce significant proliferative responses in lymphocytes from animals previously immunized either with *L. (L.) chagasi* amastigotes or with *L. (L.) chagasi* p30 and the responses were higher than those elicited by p30 purified by electroelution.

These results suggest the comparison of p30 purified by immunoaffinity chromatography and by electroelution in active immunization tests in BALB/c mice.

IM-87
IMMUNO-ANALYSIS OF *TRYPANOSOMA CRUZI* PROTEIN KINASE C (PKC) ISOFORMS

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The protein kinases C (PKC) define a family of serine/threonine-specific kinases that are considered as important regulatory enzymes involved in many cellular responses. They are activated by lipid second messengers, predominantly dyacylglicerol, in response to various extracellular agonists like growth factors, hormones, etc. we observed that PKC might be part of the signaling pathways controlling *T. cruzi* growth, as PKC inhibitors prevented DNA synthesis induced by serum (FCS). We have also observed a time-dependent protein phosphorylation in serine residues after FCS stimuli which also contributed to suggest a serine/threonine kinase in this process. Investigating further the presence of PKC in *T. cruzi* we have used different approaches such as immunochemistry and confocal immunofluorescence microscopy. The anti-PKC-consensus antibody reacted with *T. cruzi* cytoplasmic proteins. In Western blotting analysis we detected two major reactive bands and at least 4 minority ones. Trypomastigotes showed a different pattern of immunoreactive bands, suggesting a differential expression of PKC isoforms between epimastigotes and trypomastigotes.

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IM-88
IMMUNOBLOT ANALYSIS OF *LEISHMANIA BRAZILIENSIS* ANTIGENS IN SERA OF PATIENTS WITH AMERICAN CUTANEOUS LEISHMANIASIS BEFORE AND AFTER CURE

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In the present communication, we analyzed the level and specificity of the antibody response by Western blot analysis, enzyme-linked immunosorbent assay (ELISA) and indirect immunofluorescence (IIF), in three groups of subjects: before treatment, after meglumine antimoniate (Glucantime) chemotherapy and after spontaneous cure. As we have previously demonstrated, when soluble and insoluble protein fractions of *Leishmania braziliensis* promastigotes were used as antigens in immunoblotting analysis, patients with active American cutaneous leishmaniasis developed a specific pattern of reactivity, characterized by the recognition of the 66, 60, 48, 30, 27, 19 and 16 kDa antigens. The 27 and 30 kDa were the most frequently recognized. The levels of specific antibodies decreased after treatment or spontaneous cure, as judged by the lower capacity for detection of these situations by Western blot analysis, ELISA and IFI. There was no change in the pattern of antigen recognition by Western blotting after cure, since the overall antibody specificities were similar in the three groups studied. However, the recognition frequency of all relevant antigens decreased.

IM-89
IMMUNOGLOBULIN SECRETION STIMULATED BY THE GLYCOINOSITOL-PHOSPHOLIPID (GIPL) PURIFIED FROM *TRYPANOSOMA CRUZI*: CHANGE IN THE *IN VIVO* ANTIBODY REACTIVITY PATTERN

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GIPLs are one of the major glycolipids of the *T. cruzi* surface. Previous studies have shown that this glycoconjugate is a potent B cell activator (Bento et al., *J. Immunol*, 157: 4996, 1996). In the present study we characterized the effect of the GIPL on *in vivo* immunoglobulin secretion and on the pattern of antibody reactivity after *in vivo T. cruzi* G strain GIPL injection. Mice were injected iv with either GIPL or its oligosaccharide moiety and were bled at different time points after injection. We observed that iv injection of *T. cruzi* GIPL stimulated an increase in sera IgM levels. This

glycoconjugate was also observed to have an adjuvant effect, since it increased antigen specific IgG responses from polysaccharide immunized mice. We also investigated the specificity of the serum IgM antibodies. The reactivity of these sera upon different protein extracts was tested by immunoblotting and analyzed by multivariate statistics. This technique was previously shown to be highly discriminative since it distinguishes between a normal and an autoimmune repertoire (Ferreira et al, *Scand. J. Immunol.* 45: 331, 1997). We observed that the GIPL stimulated the appearance of a different reactivity profile. The observed reactivity pattern suggests that the GIPL stimulate antibody secretion by B cell clones that are not predominant in the resting, unstimulated, state. Our results indicate, based on the alterations on antibody repertoire reactivity, that the *in vivo* effect of the GIPL on B cells could be, at least in part, responsible for *T. cruzi* infection-associated immunological changes.

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IM-90
IMMUNOLOGICAL MECHANISMS INVOLVED IN *TRYPANOSOMA CRUZI* INFECTION IN THE GENUS *DIDELPHIS* (MARSUPIALIA, DIDELPHIDAE)

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The genus *Didelphis* is considered an important reservoir of *Trypanosoma cruzi*, mantaining a harmonic host-parasite interaction. In order to shed light into the cellular and humoral mechanisms involved in experimental infections in two didelphid species, *D. marsupialis* and *D. albiventris* infected by *T. cruzi*, we followed-up: 1) the kinetics of humoral response by indirect immunofluorescence assay and Western blot; 2) delayed type of hypersensitivity response in th patent and subpatent phases of infection and 3) lymphocyte proliferation of both didelphids in experimental infections. The IFAT and Western blot analysis of *D. albiventris* serum from experimentally infected animals with G645 strain showed that these antibodies can recognize peptides from epimastigotes in the range of 90-14 kDa. The intense recognition of 90 kDa peptide early described in *D. marsupialis* was observed in *D. albiventris* sera. High serological IFAT (1:640) titers were observed in two animals with scent glands parasitism. *D. marsupialis* presented the same results, however all the animals presented the extracellular multiplication cycle in the lumen of the scent glands. Data on the DTH response could not be detected in both species and we did not observe any inflamatory sites in these animals by histological analyses. *D. albiventris* are able to mount humoral response as *D. marsupialis*. These results are at the very begining and further experiments will be done in order to continue studying the immune mechanisms involved in these well balanced interaction: *Didelphis* x *T. cruzi*.

IM-91
IMMUNOSTIMULATION OF HOST T-LYMPHOCYTE FUNCTION BY *TRANS*-SIALIDASE FROM *TRYPANOSOMA CRUZI*

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Trans-sialidase (TSase) from *T. cruzi* was identified as an important molecule for parasite invasion of mammalian cells and as a virulence factor in infected mice. We have investigated the effects of purified native and recombinant TSase on host T-cell function. TSase induces a dose-dependent mitogenic response in $CD4^+$ T cells from normal BALB mice in the presence of a costimulus, and also synergizes with TCR stimulation for induction of polyclonal T-cell activation. By immunoblotting, TSase induces ERK-1/ERK-2 nuclear translocation, and synergizes with TCR signals to enhance MAP kinase translocation. TSase increases IL-2 secretion by TCR-activated $CD4^+$ T cells, and induces maximal TNF-a secretion irrespective of TCR stimulation. Addition of TSase to $CD4^+$ T cells from *T. cruzi*-infected mice also induced co-mitogenic and costimulatory responses, and completely blocked activation-induced cell death (AICD) triggered by TCR ligation. Following infection with *T. cruzi*, surface expression of CD43 (leukosialin, the most abundant lymphocyte mucin) was upregulated in $CD8^+$ T cells and in a subset of $CD4^+$ T cells. Pre-treatment of T cells with TSase blocked the binding of anti-CD43 mAb, but had no effect on binding of mAbs to CD4, CD8 or to CD45, another sialylated protein. Treatment of $CD4^+$ T cells with anti-CD43 also blocked TCR-induced AICD, while anti-CD45 enhanced lymphocyte killing. AICD could be induced in $CD4^+$ T cells from either wild-type or CD43KO mice infected with *T. cruzi*, but TSase failed to rescue only CD43KO T cells from cell death. Together, these results indicate that TSase exerts multiple stimulatory effects on host $CD4^+$ T cells, and that at least some of the effects could be mediated through CD43 binding. We propose that TSase effects on T-cells lead to immunopathology in the course of Chagas' disease.

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IM-92
IMMUNOSUPRESSIVE ACTIVITY OF *KALANCHOE PINNATA* IN LEISHMANIASIS MAY BE RELATED TO NITRIC OXIDE PRODUCTION AND APOPTOSIS

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We have previously shown that the therapeutic effect of aqueous extrat of *Kalanchoe pinnata* plant (Kp) in murine leishmaniasis is related to imunosuppression and production of nitric oxide. In the present work, the association of immunosuppression with apoptosis, NO production and antiamastigote effect was evaluated. For the antileishmanial effect, *L. amazonensis*-infected macrophages were incubated with 500 mg/ml Kp in the presence or absence 100 mM NMMA, a NO synthesis inhibitor, for 48h. Controls were incubated with medium or NMMA alone. To test the NO effect on the immunosuppressive activity *in vivo*, BALB/c mice were treated daily with Kp alone by the oral (8mg) and ip (4mg) routes or simultaneously with 300 mg NMMA ip for three days. Controls were left untreated. The spleen cells were then tested for inhibition of mitogen-induced proliferation, and NO production was measured in the culture supernatants after 48h. For induction of apoptosis lymphonode cells from normal or infected mice were incubated for 17-48h in the presence of 500 mg/ml Kp, 50 mg/ml F12 (an active fatty acid-rich Kp fraction) or 50 mg/ml palmitic acid (major fatty acid present in F12). Their isolated DNA was analysed for fragmentation in agarose gel.

The results showed that the decreased intracellular amastigote growth (60%) induced by Kp was reverted with NMMA. Mouse treatment with Kp suppressed 73% of spleen cell mitogenic responses whereas the NO production was increased about 100-fold. Co-treatment with NMMA inhibitted the capacity of spleen cells to produce NO by 60% and at the same time totally reverted T cell responses, indicating an association of Kp-induced immunosuppression with increased NO production. We observed strong DNA fragmentation in the cultures particularly when the cells were incubated with F12 and palmitic acid. These results suggest that induction of NO production is an important antiparasitic and immunosuppressive mechanism of Kp and that the later may be associated with an apoptotic process.

IM-93
IMPAIRMENT OF SINGLE CHANNEL L TYPE CALCIUM CURRENTS BY CHAGASIC PATIENT'S IGG

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We have previously described perturbations in electrogenesis and conduction in rabbit heart and blockade of macroscopic L type Ca^{2+} currents by IgGs from chronic Chagasic patients. In order to investigate the interactions of Chagasic IgGs with the Ca^{2+} channels of rabbit heart myocytes we assayed single channel activity using the cell attached patch clamp technique.

Single Ca^{2+} currents were recorded in cells incubated in the presence of IgGs purified by DEAE ion-exchange chromatography from normal blood donors and Chagasic patients (80 µg/ml). Cell-attached patch clamp recordings were made with pipettes filled with isotonic Ba^{2+} solution (96 mM BaCl2, 0.01 mM Bay-K-8644 and 10 mM Hepes-NaOH, pH 7.4). The bath solution contained 140 mM DL-Aspartic Acid, 20 mM MgCl2, 10 mM EGTA and 10mM Hepes-NaOH, pH 7.4. The voltage-sensitive Ca^{2+} currents were elicited at 0 mV by depolarizing voltage steps applied for 200 ms, from a holding potential of –40 mV. Each protocol consisted of 100 such voltage steps applied with a pulse interval of 2 sec.

Single calcium channel activity was modified by treatment with Chagasic IgGs. Both, the open probability [Po control, 0.074±0.023 (n=15); Po experimental, 0.0250±0.007 (n=9), $p<0.05$] and mean open time [control t_o, 5.363±0.631 (n=15); experimental t_o, 2.343±0.451 (n=9), $p<0.05$] were decreased significantly by IgGs from chagasic patients applied in the bath solution. In addition, these parameters were unaffected by presence of IgGs from normal blood donors patients (Po control: 0.0298, Po experimental: 0.025; control t_o: 4.1524, experimental t_o: 3.219). The unitary conductance of the channel was unaffected by either IgGs. These findings can be interpreted as indicating that the autoantibodies from chagasic patients act by reducing the kinetic behavior of functional calcium channels and suggest that Chagasic IgGs are not interacting directly with the calcium channels.

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IM-94
IN SITU IMMUNOHISTOCHEMICAL EVALUATION OF CD8+ CELLS IN LESIONS FROM PATIENTS WITH AMERICAN CUTANEOUS LEISHMANIASIS - CORRELATION WITH IFN-g PRODUCTION AND HEALING OF LESIONS

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The cellular immune responses of 36 patients with American Cutaneous Leishmaniasis from an endemic area of the Mato Grosso State, Brazil, were evaluated before and after antimonial therapy. Diagnostic criteria included clinical and epidemiological data, positive Montenegro's skin test, parasitological diagnosis and clinical response to treatment. *In vitro* stimulation of PBMCs induced a significative production of IFN-g which was partially dependent on the presence of IL-12 as shown by a reduction of IFN-g production in the presence of anti-IL-12 antibody. An increased production of IFN-g after treatment was observed in patients with mucosal lesions when compared to the production of this cytokine before treatment onset. On the other hand, no significant change in IFN-g production was observed in cultures of PBMCs from patients presenting only cutaneous lesions. The presence of CD8+ cells in lesion biopsies was evaluated by *in situ* immunohistochemistry using monoclonal antibodies. Our results show that the presence of a heavy CD8+-cell infiltrate correlated with a decreased parasitism in the lesions, while those with less CD8+ cells showed an increased number of parasites. In addition, a positive correlation between CD8+ cells infiltration and IFN-g production before treatment was observed. Furthermore, a correlation of CD8+ cell infiltrate and response to treatment was also observed. Lesions from patients showing heavy CD8+ cell infiltrate tended to heal faster than those from milder CD8+ infiltrates. Thus, our results corroborate previous evidence that, in American Cutaneous Leishmaniasis, the presence of CD8+ cells are instrumental in the control of the disease.

Supported by Fapemig

IM-95
IN VITRO ZINC SUPPLEMENTATION OF HUMAN MACROPHAGES INFECTED WITH DIFFERENT *LEISHMANIA* SPECIES STRONGLY REDUCES INTRACELLULAR PARASITE LOAD

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In spite of the large amount of literature dedicated to the importance of zinc to the immune response *in vitro* and *in vivo* in numerous infectious and non-infectious diseases, no data exist on the possible role of zinc (Zn^{2+}) in leishmaniasis. However, a recent publication describes low serum zinc and iron levels in cutaneous leishmaniasis patients in Turkey (Kocyigit et al., 1998). This zinc deficiency in patients with active disease is of significant interest, considering that zinc is essential to the functioning of cellular immunity, which correlates to resistance and cure in human as well as experimental murine leishmaniasis, while humoral immunity does not. Therefore, we were interested in a possible effect of zinc supplementation *in vitro* on the immune capacity of human macrophages infected with different *Leishmania* species.

Our results show that addition of zinc (as ZnCl2) to macrophages infected with *L. amazonensis* dramatically decreased intracellular parasite load. This leishmanicidal effect of zinc was already observed at 24 h of treatment and gradually increased with time. Reduction in parasite load was maximal at 10 mM of Zn^{2+} and decreased at 30 mM, while there was no significant effect at 100 mM. Similar results were obtained with macrophages infected with *L. braziliensis* or *L. major*, indicating that the action of zinc was not species-specific. Zn^{2+} at 10 or 30 mM slowed down proliferation of axenically grown *L. amazonensis* promastigotes, but did not have an overt cytotoxic effect, suggesting that the observed leishmanicidal effect of zinc is mediated by the host cell. The molecular mechanism through which zinc reduces intracellular parasite burden is currently under investigation.

IM-96
INABILITY TO PRODUCE IL-10 AND HIGH TNF-a PRODUCTION ARE THE MAIN IMMUNOLOGICAL FEATURES OF MUCOSAL LEISHMANIASIS

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Mucosal leishmaniasis is characterized by a marked inflammatory response with destruction of tissue in the upper airway tract. Previous studies have determined that patients with mucosal leishmaniasis have increased promoter for TNF-a and that TNF-a sera levels are increased in this disease. Additionally, response to therapy in mucosal leishmaniasis is associated with decrease in sera TNF-a levels. IFN-g is elevated in mucosal disease and this cytokine upregulates TNF-a production. In contrast, IL-10 is the main cytokine that down-regulates TNF-a and IFN-g production. The objectives of this study were: 1. to determine TNF-a, IFN-g and IL-10 levels by ELISA in supernatants of lymphocyte cultures and by PCR in cells from patients with mucosal leishmaniasis; 2. To evaluate the ability of IL-10 in down-regulating TNF-a and IFN-g production.

High expression of mRNA for IFN-g, TNF-a and IL-10 was detected in mononuclear cells of mucosal leishmaniasis patients. IFN-g (2264 ± 854pg/ml) and TNF-a (828 ± 526pg/ml) production were increased in lymphocyte supernatants of patients with mucosal leishmaniasis and decreased after therapy by 42% and 71% respectivelly. Suppression of the production of both cytokines was documented by addition of IL-10 to lymphocyte culture. However, secretion of IL-10 upon leishmania antigen stimulation was very low 18 ± 15pg/ml in these patients. These data indicate that decreasing in IL-10 production during mucosal leishmaniasis prevent the down-regulation of pro-inflammatory cytokine in this disease.

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IM-97
INCIDENCE OF LEISHMANIASIS CASES IN AN ENDEMIC AREA CORRELATES WITH FREQUENCY OF INFECTED PERIRESIDENCIAL *LUTZOMYIA* SPP.

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We have recently shown that detection of *Leishmania braziliensis* parasites in specimens of *Lutzomyia spp* can be greatly improved by the combination of kDNA minicircle PCR diagnosis and pinpointed capture. By pinpointing we mean capture of phlebotomines in the peridomiciliar area of recently diagnosed cases of leishmaniasis (up to 15 days after diagnosis). Using the same PCR procedure described we examined whether frequency of *Lutzomyia spp* infection correlated with incidence of disease in human beings sharing the same environment. A total of 67 cases enrolled the three month-study, spanning 26 microregions of Corte de Pedra endemic region. As in the previous work, phlebotomines were captured near the homes of cases and were pooled in samples of ten. Each sample was submitted to diagnostic PCR and the correlation between frequency of positive pools and incidence of cases per one hundred dwellers of the same microregion was analyzed. No correlation between those parameters was found. Detection of positive phlebotomines rendered negative in 15 of the 26 microregions with human cases of disease, even using the sensitive diagnostic PCR. Probably, the low density of positive phlebotomines in such areas hindered a precise discrimination among the samples studied, indicating that larger pools should be tested. Furthermore, these results suggest that even at low densities, leishmania positive *Lutzomyia spp* is very efficient in spreading the infection among humans.

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IM-98
INCREASED SUSCEPTIBILITY OF CD95 LIGAND-DEFICIENT *GLD* MICE TO *TRYPANOSOMA CRUZI* INFECTION DUE TO A TH2-BIASED IMMUNE RESPONSE

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In the course of Chagas'disease, CD4 T cells from *Trypanosoma cruzi* infected mice become highly susceptible to die upon activation induced by TCR (T cell receptor) triggering. Activation induced cell death (AICD) is mediated by CD95-CD95L (ligand) interactions resulting in CD95-triggered apoptosis of T cells. Both CD95 and CD95L are expressed and functional in T cells from *T. cruzi*-infected mice, being involved in apoptosis-related immunosuppression in vitro. In order to test whether AICD could also limit the immune response to the parasite in vivo, we studied *T. cruzi* infection in CD95L-defective *gld* mice. In vitro AICD was absent in infected *gld* mice and proliferative responses were not suppressed in CD4 T cell cultures. Moreover, CD4 T cells from infected *gld* mice failed to exacerbate parasite replication in macrophages. Surprisingly, infected *gld* mice developed increased parasitemia compared to CD95L-intact control mice. Much higher amounts of Th2 cytokines, such as IL-4 and IL-10, were

produced by CD4 T cells from infected *gld* mice. Neutralization of IL-4 in vivo by anti-IL-4 treatment markedly reduced parasitemia. These results suggest that in the absence of CD95-CD95L pathway, the host develops increased susceptibility to *T. cruzi* infection due to an uncontroled Th2 response.

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IM-99
INFECTION OF ATHYMIC OR SPLENECTOMIZED MICE WITH THE CL STRAIN OF *TRYPANOSOMA CRUZI* RESULTS IN HIGHER PARASITE LOAD IN THE LIVER THAN IN THE HEART

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During the acute infection with the CL strain of *T cruzi* the heart is the most severely affected organ. Conversely, parasite multiplication in the liver is almost undetectable. We have previously shown that the control of parasite multiplication in the heart is largely dependent on $CD4^+$ T cells. However, in the liver, both $CD4^+$ and $CD8^+$ T cells subsets control parasite growth (Russo et al., Immunol. Letters 49:163.,1996). To further evaluate the role played by T cells in the control of parasite multiplication in these organs we used athymic (nude) mice and splenectomized mice. Balb/c nu/nu or C3H splenectomized mice were infected subcutaneuosly with 10^3 CL strain of *T.cruzi* and 14 days later the organs were removed, fixed and tissue sections stained with hematoxylin/eosin. As control mice we infected BALB/c nu/+ (euthymic) and sham-splenectomized C3H mice. The parasite load was determined by counting the number of parasite nests per mm^2 present in heart or liver sections with the aid of an integration ocular eyepiece. As expected, in BALB/c nu/+ or C3H sham-splenectomized, parasite nests (pseudocysts) were found in heart but not in liver sections mice. In contrast, athymic or splenectomized mice presented parasite nests in both organs. Most importantly, in these mice, the parasite load in the liver was roughly 2-3-fold higher than in the heart. These experiments document that T lymphocytes rather than the strain characteristic, govern tissue distribution of *T. cruzi*. Moreover, that effector T cells generated in the spleen are essential for the control of *T. cruzi* infection in the liver.

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IM-100
INHIBITION OF THE NITRIC OXIDE SYNTHESIS BY *LEISHMANIA (L.) AMAZONENSIS*

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In this study, inhibition of LPS-induced nitric oxide (NO) production was demonstrated in BALB/c and C57BL/6 peritoneal cells and J774 macrophages infected with *L.(L.) amazonensis* promastigotes. This inhibition was not observed in bone marrow macrophages growth in GM- or M-CSF. The down modulation of NO production observed in infected and LPS-stimulated J774 cells was correlated with reduction of the iNOS activity. However reduction of iNOS activity did not occur by inhibition of iNOS mRNA expression. The inhibition of NO synthesis was not due to insufficient substrate or cofactors because additional supplementation with L-arginine or tetrabiopterin did not restore or increase NO production. LPS-induced NO production in peritoneal cells was shown to be dependent on IFN-a/b and TNF-a synthesis. However infection with *L.(L). amazonensis* promastigotes did not reduce these cytokines production when peritoneal cells were stimulated with LPS after infection.

The cytokines IL-10, TGF-b and the lipid mediator-PGE2-had been described as inhibitory molecules on NO production. However none seem to be involved in NO synthesis inhibition observed in our model. Addition of IL-12 or IL-12 plus IFN-g to cultures of infected and LPS-stimulated peritoneal cells increased plus the NO production in 100%. The up regulation of NO synthesis in peritoneal cells stimulated with IL-12 is possibly mediated by IFN-g and other cytokines, secreted by NK cells or T lymphocytes.

IM-101
INTERFERON-GAMMA MODULATES APOPTOSIS BY INDUCING NITRIC OXIDE PRODUCTION AND FAS EXPRESSION DURING THE ACUTE PHASE OF EXPERIMENTAL *TRYPANOSOMA CRUZI* INFECTION

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The protozoan *Trypanosoma cruzi* is the etiologic agent of Chagas' disease. Despite its medical importance, the mechanisms by which the parasite leads to the subversion of host cell for its own survival are scarcely known. In this regard, we have previously shown that splenocytes from *T. cruzi*-infected mice present high levels of apoptosis, which seems to be mediated by nitric oxide (NO). In the present work, we investigated the role of IFN-g in modulating NO production, apoptosis induction and host protection in *T. cruzi* infected mice. The IFN-g-deficient (IFN-g -/-) mice were more susceptible to *T. cruzi* infection, produced lower levels of NO, exhibited fewer apoptotic spleen cells and higher lymphoproliferative response to Con-A, compared to the WT infected mice. Furthermore, during the acute phase of infection the Fas and Fas-L expression in splenocytes was significantly enhanced and higher in WT mice, than in the IFN-g-/- mice. The addition of recombinant murine IFN-g to splenocytes from IFN-g-/- infected mice resulted in increased apoptosis levels, Fas expression and NO production. In the presence of IFN-g and absence of NO (by addition of LNMMA), the apoptosis levels were significantly reduced, but maintained higher than those found in infected control splenocytes, while Fas expression was unchanged. These results suggest that Fas expression is not modulated by the high levels of NO produced during the acute phase of the infection. Moreover, *in vivo*, Fas expression and NO production appears to be independent phenomena, since treatment of WT infected mice with the iNOS inhibitor (aminoguanidine), led to decreased NO and apoptosis levels but not Fas expression. Taken together, these results suggest that besides being of crucial importance in mediating resistance to *T. cruzi* infection in mice, by inducing NO production, IFN-g might play a role in the modulation of immune response, by mediating apoptosis induction and Fas and Fas-L expression during the acute phase of infection.

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IM-102
INTERSTITIAL DENDRITIC CELLS OF THE HEART : SPECIFIC *TRYPANOSOMA CRUZI* ANTIGENS PRESENTATION AND MYOCARDITIS

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The interstitial dendritic cells (IDC) of the heart of dogs, chronically infected with *T. cruzi*,has been previously identified by immunolabeling with anti-S100 Protein, and appeared as a component of the inflammatory infiltrate (Andrade et al. *Mem. Inst. Oswaldo Cruz*, 87(Supl II). Since the IDC are MHC - Class II antigen presenting cells (APC), we investigated the possibility that, in the infection with *T. cruzi* , they maintained the parasite antigens in their membranes, so contributing to the immunological responses, by stimulation of T-lymphocytes compartments in the lymphoid organs. Search for specific antigens in the IDC has been performed by immunohistochemistry, using specific purified anti-*T. cruzi* antibody , in comparison with anti-S100 Protein. A quantitative evaluation of dendritic cells has been performed in all cases. Lymphocyte subsets, present in cardiac infiltrates, were also identified, using specific monoclonal antibodies. Fifteen mongrel dogs were used: a) 02 normal controls ; b) Acute infection : 07 dogs (1.100 to 3.200g), inoculated with 4 x 10^5 trypomastigotes of the 21SF strains of *T. cruzi* , sacrified from 18 to 34 days after infection; c) Chronic infection - 06 dogs infected either with the Colombian strain ($5x10^4$tryp) or the 12SF strain ($6x10^4$ tryp). Duration of infection varied from 140 to 380 days. Sections of the heart were cryopreserved, either unfixed or after paraformaldehyde fixation, or fixed into buffered 10% Formalin and paraffin embedded. Immunolabeling of IDC was done with the Peroxidase technique,using Vectastain ABC kit : 1) primary antibody: rabbit-anti- cow S100 Protein monoclonal antibody); 2) primary antibody: purified anti-*T. cruzi* IgG. Results: positive immunostaining of *T. cruzi* antigens, as granular and dense deposits, were present in the IDC membrane. There was a significant increasing of IDC number in the myocardium, in relation to the inflammatory infiltration. They appeared isolated in the infiltratrates, or forming small clusters of 3 to 5 cells, assoociated with lymphocytes groups, identified as CD4. Quantitative evaluation has shown a significant difference between the number of IDC pmm^2 as compared with normal controls in acute or chronic infection: acute phase - labeling with anti-S 100 Protein ($p<0,0013$) and anti-*T. cruzi* ($p<0,027$) ; chronic phase - anti- S100 Protein ($p<0,006$) and anti-*T. cruzi* ($p<0,012$). These results are worth considering in the interpretation of the pathogenesis of Chagas' disease myocarditis.

IM-103
IS NECROSIS/APOPTOSIS CORRELATED WITH THE EVOLUTION OF CUTANEOUS LESIONS IN THE AMERICAN TEGUMENTARY LEISHMANIASIS?

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In their vertebrate hosts, *Leishmania* parasites replicate within the phagolysossome of macrophages where the antigen processing occurs. Thus, infected macrophages become target for $CD4^+$ and $CD8^+$ T cells. In the last few

years, several studies have pointed out the different mechanisms of cell death, e.g. via the exocytosis of the granular contents of CTL cells (perforin and granzymes) or via recognition of ligants present in the surface of immune cells by their specific receptors such as Fas-FasL or TNF-a-TNFR. Cytotoxic $CD8^+$ T cells have been shown to be implicated in the process of healing of cutaneous lesion (Da-Cruz et al, 1994) or in the protection during reinfection in the murine model (Müller at al, 1992, Conceição-Silva et al, 1994) but also has been correlated with the mucosal form of the disease (Brodskyn et al, 1997). On other hand, Conceição-Silva et al (1998) have shown that, in contrast to the wild type, both B6-GLD (FasL mutation) or B6-LPR (Fas mutation) mice develop a chronic and non-healing lesion at the site of subcutaneous infection. Furthermore, the *in vivo* treatment of B6-GLD with a FasL decreases both lesion size and parasite burden.

In order to verify the possible role of these two pathways of cell death in the evolution of the lesions of human cutaneous leishmaniasis (ACL) caused by *Leishmania braziliensis*, we performed an immunohistochemistry study in 28 cryopreserved biopsies collected during the diagnostic procedure of patients with active ACL before treatment. The patients were divided in two groups, according to the duration of the lesions: less than 3 months (15 biopsies) or more than 6 months (13 biopsies). Indirect immunoperoxidase staining was performed on 3 ?m sections using anti-CD3, or perforin or FasL monoclonal antibodies. The results show that in the group with shorter time of lesion, the number of perforin positive cells was significantly higher than in the group with 6 or more months lesions (6.8 to 28.8% and 1.7 to 13.15% respectively; $p< 0.0083$). The percentage of FasL positive cells in both groups were less than 10%, but the number of positive cells was significantly higher in the group with 6 months lesions ($p< 0.02$). Studies are been now performed in order to study the expression of iNOS and to confirm by PCR amplification the amount of perforin and FasL present in the smears.

These preliminary results strengthen the hypothesis that CTL function has an important role in parasite clearance. After that occurrence, it is likely that the immune response would be limited by apoptosis. Therefore, cells that are no longer necessary, could be eliminated via Fas-FasL, and the tissue repair would take place.

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IM-104
LEISHMANIA AND LIPID MEDIATORS INTERACTIONS

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The aim of the present study was to investigate the role of lipid mediators in the experimental *Leishmania (Leishmania) amazonensis* infection. Mouse peritonial resident macrophages were infected with amastigotes of *Leishmania* and the Infection Index determinated 24 or 48 h later. Evolution of the infection was followed during 5 weeks in mice infected in the paw with promastigotes by measuring the increase in paw volume and the number of parasites in the lymph nodes and spleen. In the "*in vitro*" infection of macrophages, our results shown that PAF have a marked stimulatory effect upon macrophages leishmanicidal activity. PAF antagonists increased the infection and addition of PAF to macrophages cultures inhibited the infection. The effect of PAF stimulating nitric oxide (NO) production was reduced by cyclooxygenase inhibitors but the inhibitory effect of PAF on the infection was unaffected by this treatment. Addition of PGE2 to macrophages cultures stimulated NO production and significantly increased the infection. Thus, macrophages leishmanicidal activity does not always correlate with the levels of NO produces. The tumoral necrosis factor (TNF-a) is also involved, inhibiting macrophage infection. In the "*in vivo*" infection, PAF also plays a relevant role since treatment of mice with PAF-antagonists markedly increased the paw lesion as well as the parasites load in regional lymph nodes and the spleen. These results indicate that PAF also modulates the "*in vivo*" infection controlling the progression of the infection in mice.

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IM-105
LEISHMANIA MAJOR INDUCES TNF AND IL-10 IN MURINE SPLENOCYTE CELLS MAINLY IN RESISTANT STRAIN AND INHIBIT IL-12 AND IL-10 IN SUSCEPTIBLE AND RESISTANT STRAIN

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We have previously shown that the intracellular parasite *L. major* induces cytokine production when cultured in vitro with human peripheral blood mononuclear cells. Cytokine production was most significant when cells were stimulated with promastigotes in the logarithmic (log) phase of growth . On the other hand, *L. major* also inhibits cytokine production by human peripheral blood mononuclear cells stimulated with bacterial products. In this study,

we investigated the effect of *L. major* promastigotes, in the log or stationary phases of growth, on the production of IL-12, TNF-a and IL-10 by murine spleen cells. Spleen cells from mice strain resistant (C3H/HeN) or susceptible (BALB/c) to *L. major* were cultured *in vitro* with *Staphylococcus aureus* Cowan strain (SAC) in the presence of several concentrations of *L. major* promastigotes. As shown for human cells, *L. major* in both growth stages inhibited IL-12 production, but not TNF production similarly in C3H/HeN or BALB/c cells. However, while in human cells *L. major* inhibited IL-10 production, spleen cells from C3H/HeN mice produced similar levels of IL-10 in response to SAC in the presence or absence *of L. major*. Unlike the data obtained with human cells, no IL-12 production could be detected in supernatants of murine spleen cell cultures stimulated by *L. major* promastigotes, even when primed with IFN-g. TNF-a production was induced at higher levels by log phase promastigotes in cells from C3H/HeN mice, and at lower levels in BALB/c mouse cells or C3H/HeN cells stimulated with stationary phase promastigotes. IFN-gpriming increased production of TNF-a by spleen cells and broadened the parasite-to-cell ratio in which TNF-a production could be detected. Surprisingly, IL-10 production induced by *L. major* was higher in cell cultures from C3H/HeN mice than in cultures from BALB/c mice. No differences in the ability of stationary or log phase parasites to induce IL-10 production *in vitro* were noted. In conclusion, we were unable to detect IL-12 production by spleen cells from BALB/c or C3H/HeN stimulated in vitro with *L. major*, although *in vivo* this cytokine has been detected early after infection. More surprisingly, cells for the resistant mouse strain used in this study produced higher levels of IL-10 than the susceptible mouse strain, suggesting a minor role for early IL-10 for the outcome of infection with *L. major*.

IM-106
LEISHMANIN AND TUBERCULIN SENSITIVITY IN HEALTHY RHESUS MACAQUES VACCINATED WITH KILLED LEISHMANIAL PROMASTIGOTES PLUS BCG AND AFTER CHALENGE INFCTION WITH *LEISHMANIA* (*L.*) *MAJOR*

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The leishmanin skin test (LST), which measures the cutaneous delayed-type hypersensitivity (DTH) reaction to *Leishmania*-derived antigens, is an important tool in the epidemiological study of leishmaniasis. In cutaneous leishmaniasis (CL) the LST will become positive 2-3 months after the appearance of the lesion and may remain positive for life (Furtado 1980, *An Bras Dermatol 55*: 81). Moreover, T cell responses correlate with recovery from and resistance to human leishmaniasis, thus a positivity LST associated with protection might be a valuable tool for evaluating vaccine trials (reviewed in Grimaldi & Tesh 1993, *Clin Microbiol Ver 6*: 230). Periodic boosting in humans using inactivated parasites promotes a DTH response to leishmanial antigen, which seems to increase the recipient´s chance of being protect (Mayrink et al. 1979, *Trans R Soc Trop Med Hyg 73*: 385; Castés et al. 1994, *Vaccine 12*: 1041).

This study reports the results of a vaccine trial (see accompaining abstract, Amaral et al.) established to study the cellular immune responses *in vivo* (LST) and *in vitro* (T-cell proliferation assays) to both lesihmanial and mycobacterial antigens following vaccination of healthy adult rhesus macaques (*Macaca mulatta*) with killed leishmanial promastigotes vaccine plus BCG, Bacille Calmette-Guerin [N= 7; group A] or BCG alone [N= 7; group B]. The skin-test reactivities to parasite antigen and PPD were reassessed at 4-10- and 14-17 week follow-ups. The results of this trial demonstrated that the combined vaccine [group A] and BCG [group B] were not able to induce skin-test conversion in the study animals, but a high percentage of vaccinees (>90%), manifested lymphocyte proliferation *in vitro* to leishmanial antigen; in comparison to PPD-stimulated cultures, lower proliferative responses of T lymphocytes to parasite antigen was detected in animals receiving BCG alone (see accompaining abstract, Amaral et al.). However, the LST positivity rate (LST reaction size = 6-15 mm) in vaccinated and non-vacinated control (N= 4; group C) monkeys after a subsequent challenge infection with virulent *L. major* was very high (in > 90% of animals at 8 weeks p.i.) Further studies are required to determine whether the presence of proliferative (and/or IL-2, IFN-gresponses) in the absence of a skin test response are sufficient indicators of potential vaccine success.

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IM-107
LONG TERM EFFECTS OF A TREATMENT WITH A GANGLIOSIDES MIXTURE ON AUTONOMIC CHAGASIC CARDIOPATHY- EVOLUTION OF ANTI-GM1 ANTIBODIES

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Chagas' disease determines cardiac deterioration, initially autonomic disturbances affecting heart rate (HR) and blood pressure (BP). At this stage, disminished HR responses to atropine and propranolol are observed. Previous studies showed improved responses to these agents after a short term treatment with gangliosides but no information

is available on long term effects of gangliosides and on the evolution of anti-GM1 antibodies titers. *Objectives:*1) to observe the effects of a long term treatment with gangliosides on autonomic tests in patients with chagasic cardioneuropathy; 2) to study the evolution of anti-GM1 antibodies titers. *Material and Methods:* 90 patients (57 men, 33 women; age: 25-60 years) were incorporated, with positive serology for Chagas'and ECG showing sinus bradycardia and incomplete blockage of right bundle, without cardiomegaly, with autonomic alterations detected by postural and Valsalvas'tests. All patients were submitted to a test by i.v injection of 0.04 mg/Kg atropine followed 3 minutes later by i.v injection of 0.02 mg/Kg propranolol. During these tests, HR y BP were continuosly recorded. Subsequently, 30 patients were treated with 100 mg/day of a gangliosides mixture i.m. during 15 days followed by 40 mg/day during 75 days. Other 30 patients were continuosly treated during 1 year. The remaining 30 patients were controls. Before treatment and at 3 months and 1, circulating anti-GM1 titers were determined (also in 30 healthy controls). Atropine and propranolol tests were performed at the controls of 3 months and 1 year. *Results*: 74 patients completed the study. HR increased slightly after atropine before any treatment. After 3 months of gangliosides treatment, a significant increase of the response to atropine was observed. After 1 year the response to atropine remained increased without differences between patientes treated during 3 months and those treated during 1 year. Control patients showed no modification of the response. Both groups treated with gangliosides showed an increased response to propranolol. The distribution of anti-GM1 titers was similar in healthy and chagasic patients. No patient had high anti-GM1 titers in basal conditions and no significant modifications were observed after treatment with gangliosides. *Conclusions*: 1) Chagasic cardiopathy was npt associated in this study with high anti-GM1 titers; 2) Chagasic patients showed a disminished HR response both to atropine and propranolol; 3) Treatment with gangliosides determined an increase of the HR response, particularly to atropine; 4) The increased response sustained up to one year of control, without differences between patients treated during 3 and 12 months; 5) no modification of anti-GM1 titers was observed during treatment with gangliosides.

IM-108
LONG-TERM EVALUATION AFTER THERAPY OF THE T-CELL MEDIATED IMMUNE RESPONSES IN CUTANEOUS OR MUCOSAL AMERICAN LEISHMANIASIS PATIENTS

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Previous results from our group have shown that in ATL patients a preferential induction of blood $CD4^+$ *L. braziliensis* (Lb)-reactive T-cell and a mixed type 1 (IFN-g) and a type 2 (IL-4 and IL-5) cytokine pattern is associated with active cutaneous (CL) or mucosal (ML) lesions. In CL patients the healing process is associated with an equilibrium in the percentages of the *in vitro* $CD4^+$and $CD8^+$ Lb-reactive cells. On the other hand, ML patients mantain a $CD4^+/CD8^+$ profile similar to that of active disease, displaying higher proportions $CD4^+$ than $CD8^+$ Lb-reactive T-cells even up to 6 months after the end of therapy. Our present objective is to investigate in a long-term follow-up the Lb reactive T-cell patterns in ATL patients after the healing of lesions. Patients suffering from CL and ML were studied before therapy (BT), at the end of therapy (ET) and two to five year (long-term) after healing (Lt-AT). Assays of lymphocyte proliferative responses of PBMC induced *in vitro* by *L. braziliensis* antigens and mitogen were performed. After five days in cultures Lb reactive T-cells were separeted in a Percoll gradient and phenotypically analysed by flow cytometry (B, T, $CD4^+$ and $CD8^+$). The supernatants were harvested for future type 1 and type 2 cytokine quantification. Preliminary results show that in CL patients the mean percentages of Lb-reactive $CD4^+$ cells were: BT=58.3±12.6%, ET=37.6±9.3% and Lt-AT=32±10.8%, while the percentages of $CD8^+$ reactive T-cells were BT=22.8±5%, ET=50±11.3% and Lt-AT=11.2±1.9%. The $CD4^+/CD8^+$ ratios were BT=4±2.2, ET=0.9±0.3 and Lt-AT=4.0±1.9. With respect to ML patients the mean percentages of Lb-reactive $CD4^+$ cells were BT=56.8±7.0%, ET=39.5±0.5% and Lt-AT=21.3±5.9%, while the percentages of $CD8^+$ Lb-reactive T-cells were: BT=18.7±7.0%, ET=14.4±3.6% and Lt-AT=29.9±2,7%.The $CD4^+/CD8^+$ ratios were BT=6.1±2.8, ET=3.1?0.9 and Lt-PT=0.8±0.3. In CL patients the proportion of $CD4^+$ Lb-reactive cells decreases after therapy (cure) while $CD8^+$ increases. A switch in the $CD4^+/CD8^+$ ratios were observed (BT=4±2, ET= 0.9±0.3). Two to five year after cure the proportions of $CD4^+$ and $CD8^+$ Lb-reactive T-cells became similar to that observed during the active disease. On the other hand, in ML patients a slow decrease in the percentages of the Lb-reactive $CD4^+$ cells was observed during the whole period. A switch in the $CD4^+/CD8^+$ ratio was observed only two to five years after cure ($CD4^+/CD8^+$ ratio: Lt-AT=0.8±0.3). ML patiens should be evaluated during a much longer period to detect a possible delayed return to a positive $CD4^+/CD8^+$ ratio.

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IM-109
MACROPHAGE ADHESION TO CONNECTIVE MATRIX IN LEISHMANIASIS

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Infection with different species of *Leishmania* are associated with the development of lesions in skin, mucosa and/or internal organs. In these lesions, parasites of all the species live inside macrophages. A message has, therefore, to be sent to the surface of these cells, to make them remain in a specific site (skin, mucosa or internal organ). In this work we examined the interactions between monocyte/macrophage and connective matrix in order to see if infection with *Leishmania* changes the adhesion of these cells to the connective tissue. Groups of BALB/c mice were infected with *L. amazonensis* and *L. braziliensis* and the connective matrix in the site was studied of infection using special staining for colagenous components. Adhesion assays of macrophages to purified connective matrix components and skin sections were also performed. Little connective matrix was formed in the areas of lesion in leishmaniasis. The macrophage infiltrate was associated with a loose mesh of collagenous fibrils distributed irregularly around each infected cell. There was infiltration and dissociation of collagenous bundles of tendons by infected cells indicating matrix degradation. Mice peritoneal macrophages and J774 macrophage cell line presented higher levels of adhesion to fibronectin than to collagen or laminin *in vitro*. The expression of the fibronectin receptors VLA-4 and VLA-5 were confirmed on J774 cells, through immunocytochemistry. The levels of J774 adhesion to collagen or fibronectin remained stable between 2 and 6 hours and increased after 12 hours of incubation on the connective matrix components. Infection of J774 cells with *L. chagasi* or *L. amazonensis* reduced the levels of adhesion to fibronectin and to sections of inflamed skin (Stamper-Woodrof assay). This change in adherence to fibronectin after infection was not observed with mice peritoneal macrophages. This data suggest that complex mechanisms of enzyme activation (matrix degradation) and modulation of adhesion are induced in macrophage during infection by *Leishmania*. Understanding these mechanisms may offer some clues to the genesis of lesions of leishmaniasis in different tissue and organs.

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IM-110
MODULATION OF HUMAN B CELL ACTIVATION BY GLYCOINOSITOL-PHOSPHOLIPIDS (GIPLS) PURIFIED FROM *TRYPANOSOMA CRUZI*

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We have previously observed that GIPL purified from *T. cruzi* (G strain) is a potent *in vitro* murine B cell activator (Bento et al., *J. Immunol*, 157: 4996, 1996). This GIPL was shown to have a inositolphosphosphingolipid moiety linked to an oligosaccharide. Both G and Y *T. cruzi* strains GIPL have related structures where the oligosaccharide moiety has a tetramannose core that is substituted by either a phosphorylated group (G strain) or a galactofuranose (Y strain) in the third mannose distal to the inositol (Carreira et al, *Glycoconjugate J.*, 13: 955, 1996). In the present study we investigated the effect of the GIPL purified from different strains of *T. cruzi* on human B cells. Our preliminary studies showed that B cells purified from about 50% of the individuals secrete immunoglobulin (Ig) when activated by the G strain *T. cruzi* GIPL. The effect of the GIPL on activated B lymphocytes was also investigated. B cells were activated by ligation to surface immunoglobulin by SAC (*Staphylococcus aureus*, Cowan I strain) in the presence of recombinant IL-2, a standard human B cell activation protocol. We observed that immunoglobulin secretion induced by SAC is increased by both the GIPL purified from the G and Y strains of *T. cruzi*. However, we observed a deep inhibition of immunoglobulin secretion when B cells were stimulated by SAC in the presence of the GIPL purified from the Colombiana strain. The structure of the *T. cruzi* Colombiana strain GIPL is currently under investigation. The *T cruzi* Colombiana strain is more virulent than either the G or Y strains. It is possible to suggest that this difference in infectivity may be related to modifications in the GIPL structure of distinct strains. Further studies, using purified GIPL and the GIPL-derived oligosaccharide moiety will corroborate this hypothesis.

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IM-111
MODULATION OF IMMUNE RESPONSE BY CYTOKINES AND CYTOKINE ANTAGONISTS IN SUBJECTS CURED OF VISCERAL LEISHMANIASIS

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American visceral leishmaniasis (AVL) is associated with a marked depression of T cell responses, which has been characterized by absence of lymphoproliferative response, IFN-γ and IL-12 production upon *in vitro* stimulation with leishmania Ag. Treatment and cure of AVL is associated with restoration of these T cell functions. In this study we evaluated the role of cytokines suppressors of immune response such as IL-10, IL-4 and TGF-β in modulate the immune response in subjects cured of AVL. We also evaluated if mAb α-IL-12 could down-regulate the T cell responses in these subjects.

PBMC were stimulated with leishmania Ag in the presence or not of IL-10, IL-4, TGF-b (20ng/ml), and mAb a-IL-12 (10mg/ml) and the lymphoproliferative response and IFN-g production was evaluated. IL-4 and TGF-b have no effect in these functions (SI, *L. chagasi* Ag = 181 ± 83, +IL-4 = 147 ± 22, +TGF-b = 194 ± 12). IFN-g production, after stimulation with *L. chagasi* Ag was 874 ± 400pg/ml. In the presence of IL-4 was 837 ± 244pg/ml and in cultures with TGF-b, 758 ± 523pg/ml). In contrast, addition of IL-10 suppressed the lymphoproliferative response and IFN-g production by almost 100% (SI, *L. chagasi* Ag = 181 ± 83, + IL-10 = 2 ± 0.3, IFN-g *L. chagasi* = 874 ± 400pg/ml, + IL-10 = 0). The addition of mAb a-IL-12 also suppressed the lymphoproliferative response from 210 ± 114 to 1 ± 0.5, isotype control 190 ± 69 and decreased IFN-g production (*L. chagasi* Ag = 2792 ± 402pg/ml, + mAb a-IL-12, 407 ± 449 isotype control, 2882 ± 616pg/ml).

We had previously shown that in patients with AVL there was a strong expression for mRNA of IL-10 and that IL-12 and monoclonal antibody anti-IL-10 were able to restore T cell responses in these patients. The present data using cells from patients cured of AVL supports that IL-12 and IL-10 are the main cytokines involved in modulation of T cell responses in AVL.

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IM-112
MORPHOLOGICAL AND IMMUNOLOGICAL CHARACTERIZATION OF CELLS OBTAINED BY FINE NEEDLE ASPIRATION OF LYMPH NODES IN CUTANEOUS LEISHMANIASIS PATIENTS

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In cutaneous leishmaniasis, prior to ulcer development an important enlargement of lymph nodes is observed in the majority of the patients. Lymph nodes may be important in containing disease spreading. To better characterize morphologically and phenotipically the cells present in the lymph nodes, we performed fine needle aspirations of enlarged lymph nodes in 45 patients (41 M / 4 F) with cutaneous leishmaniasis. Age ranged from 8 to 46 years old. In 80% of the patients were documented lymphadenopathy prior or simultaneous to ulcer development. Duration of disease varied from 7 to 90 days. The number of cells obtained varied from $1{,}8x10^6$ to $18{,}9x10^6$ cells. Cytospin and stain of samples for *Leishmania* visualization was positive in 40% of the patients and all of samples were positive by culture. Differential cell analysis by optical microscopy, cytochemical tests and by FACS using monoclonal antibodies revealed the following data: by microscopy the percentage of cells ranged of 29 to 100% of lymphocytes, 1.3 to 19% of monocytes, 0 to 13% of granulocytes, 0 to 1.9% of plasma cells and 0 to 3.4% for eosinophils. By immunohystochemical assay we found 2.9 $\pm$ 1,8 % of CD1-a cells, 72.15 $\pm$ 15 of CD3-cells and 54.6 $\pm$ 12,7 of HLA-DR. By FACS we found 31.56 $\pm$ 11.2 of positivity for CD4 cells and 12.31 $\pm$ 5.13 of positivity for CD8 cells.

Our results revealed a predominance of activated T-lymphocytes reinforcing the existence of an initial T-cell response in lymph nodes. Further characterization of cell subpopulation and cytokine production in lymph nodes is necessary for better understanding this aspect of the human immune response to *Leishmania.*

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IM-113
MSP-3 AND GLURP, TWO *PLASMODIUM FALCIPARUM* ANTIGENS TARGETS OF PROTECTIVE ANTIBODIES, ARE IMMUNOGENIC TO THE SQUIRREL MONKEY *SAIMIRI SCIUREUS*

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Passive transfer experiments have shown that IgG antibodies from malaria immune individuals are able to cause a striking decrease in parasitemia in non-immune *P. falciparum*-infected individuals. Purified antibodies to the Merozoite Surface Protein 3 (MSP-3) or to the Glutamate-Rich Protein (GLURP) of *P. falciparum* mediate growth inhibition of *P. falciparum* in antibody-dependent cell inhibition assays (ADCI), an *in vitro* assay shown to correlate with *in vivo* immunity to *P. falciparum*. We studied the immunogenicity of MSP-3 and GLURP in the New World monkey *Saimiri sciureus*, an essential step towards the development of a malaria vaccine. Groups of four to six *S. sciureus* monkeys were immunized with either: *i*) a recombinant MSP-3 protein, named DG210, in Ribi adjuvant (group 1); *ii)* a recombinant protein (R0) representing a non-repetitive portion derived from GLURP, in Al(OH)3

(Alum adjuvant) (group 2). Three immunization shots (100mg each) were given to each animal, subcutaneously, in intervals of 21 to 28 days. Three to 7 months after, a new series of boost shots were given: group 1 received either DG210 or MSP3b in Incomplete Freund's Adjuvant (IFA) and group 2 received R0-alum again. Blood was withdrawn before each shot and at given time intervals after that, and plasma was frozen. ELISA and immunofluorescence (IFAT) assays were performed to evaluate the antibody response. Challenge was done 21 days after the last boost shot, with $25x10^6$ *P. falciparum* parasites (FUP-Palo Alto adapted strain). Parasitemia was daily determined by examination of thin blood smears; treatment was given when parasitemia reached 6-10%. The two groups of monkeys showed high titres of antibodies directed to MSP-3b or R0, in ELISA, especially after boost shots, and low titres in IFAT. These data show that the two recombinant proteins - DG210 and R0 - are immunogenic to *Saimiri* monkeys, and antibodies raised can recognize the native protein on the parasite. Results of challenge experiments are currently being analyzed.

IM-114
NITRIC OXIDE-MEDIATED KILLING ACTIVITY IN THE ACUTE PHASE OF *TRYPANOSOMA CRUZI* INFECTION: THE ROLE OF NK1.1+ CELLS

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Multiple components of the immune system are simultaneously required for protection during the acute phase of *Trypanosoma cruzi* infection. The production of nitric oxide (NO) has been shown to be important in the control of *T. cruzi* infection. NO can be blocked by L-arginine analogs, that inhibit the induced nitric oxide synthase (iNOS) pathway. There upon, we have investigated the role of NO in vivo. Normal C57BL/6 mice were given different NO inhibitors. Four different groups of normal mice were treated intraperitoneally with: PBS, Nitro-L-arginine (Nitro), Aminoguanidine (AG) or L-NAME. Their T-cell subpopulations from thymus, spleen, lymph nodes and liver were examined by flow cytometry (FACS). These uninfected mice never died or decreased their body weight when treated with PBS, Nitro or AG, administered continuously for a total period of 3 weeks. Mice which received the above treatments have not presented alterated numbers of T cell subpopulations. Therefore, Nitro was not detrimental when cronically-administered to normal animals, and for this reason it was chosen for further studies. Young C57Bl/6 mice are extremely susceptible to the infection with the Tulahuen strain of *T. cruzi*, whereas aged C57Bl/6 mice are resistant. Inhibition of NO production by Nitro treatment increased mortality and parasitemia in young as well as aged infected mice. Measurements of NO2/NO3 and IFN-gamma serum levels at various time-points along the acute infection showed an augmentation of these mediators in aged infected mice seven days after initial infection, thereafter no differences between aged and young infected mice could be observed. In addition, TNF-alpha could only be detected in the sera of aged infected mice. Therefore, we treated *T. cruzi*-infected mice with an anti-NK1.1 mAb to prevent the initial production of IFN-gamma (Cardillo et al., Infect. Immunity, 64:128, 1996), since IFN-gamma and TNF-alpha induce NO synthase activity and thus indirectly stimulate the production of NO. Anti-NK1.1 mAb-treated infected mice (both young and aged) had high mortality rates and higher parasitemia, despite the elevated NO2/NO3 levels in their sera when compared to non-treated infected controls. The latter observation suggests at least three possibilities: 1-) The Tulahuen strain is selected towards resistance to NO-mediated killing along the infection. 2-) NK1.1+ cells might be fundamental for the production of a mediator other than NO, acting in a synergic manner with NO to kill the parasite (i.e. peroxynitrite). 3-) Depletion of NK1.1+ cells suppress the NO-mediated killing activity by an unknow mechanism. These hypothesis are not mutually exclusive and are under investigation.

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IM-115
NK CELLS EXPRESSION IN NK-DEPLETED MICE INFECTED WITH *LEISHMANIA*

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NK cells has been considered as an important source of IFN-gin the initial phase of *Leishmania* infection with the potential to trigger the TH-1 type of immune response in cutaneous leishmaniasis. Since it is known that IFN-g significantly inhibits synthesis of C3 by inflammatory cells (Volanakis, Annu.Rev.Immunol.13:277-305,1995) we have been studying the role of NK cells in conjunction with complement that is important for the evasion of the parasites (Laurenti,et al.-Int.J.Exp. Pathol.77:15-24,1996). In the previous study, in NK-depleted and *Leishmania (Leishmania) amazonensis*-infected mice we observed data suggestive of link between NK depletion, decrease in

IFN-γ production and consequent increase in complement activity that could be responsable for higher number of parasites found in the skin. By the other side, we observed unexpectedly higher amount of IFN-γ and IL-12 at 24 hours and 7 days of infection in the NK-depleted than in the respective non-depleted control mice.

Now, we studied NK cell populations in the spleen cryosection of these animals by immunohistochemistry using two monoclonal antibodies NK5E6 and NK1.1 to search for the source of IFN-γ. We observed an increase in the NK1.1 expression and a slight decrease in the NK5E6 expression on NK-depleted mice of C57BL/6 and BALB/c strains.

The data suggest that NK depletion using ^{90}Sr affects differently the subpopulations of NK cells, the cytotoxic function and cytokine production by these cells. The influence on the fate of *Leishmania* infection upon NK cell depletion can be a consequense of the decrease in the cytotoxic activity. In addition it can be a consequence of the complement level affected by the changes in the level of IFN-γ systemically and in the lesion considering that the complement is an importante evading factor for *Leishmania*.

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IM-116
ORAL IMMUNIZATION WITH LEISHMANIAL ANTIGENS ENCAPSULATED IN MICROSPHERES PREFERENTIALLY INDUCE IFN-γ IN *LEISHMANIA AMAZONENSIS*-INFECTED BALB/C MICE

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Attempts to induce protection against many infectious diseases by oral immunization are hampered by the tolerization of the immune system against the antigens. Encapsulation of antigens in polymer microspheres is a good means of enhancing antibody responses, but its effect on cellular responses after oral immunization is still unclear.

TH1-type cytokines, particularly IFN-γ, are normally associated with protective cutaneous leishmaniasis in BALB/c mice. In this work we investigate the effect of oral immunization with both free and encapsulated *Leishmania amazonensis* antigens on the IL-2, IFN-γ(TH1) and IL-10 (TH2) responses in comparison to subcutaneous immunization with the same antigen preparations.

Thus, prior to infection with promastigotes in the footpad, BALB/c mice received two intragastric doses of 130 mg of free or poly-lactide-co-glycolide-encapsulated total promastigote proteins. Alternatively, the animals received two sc doses of 25 mg antigen in the free form or emulsified in Freund adjuvant (CFA). Controls received PBS, microspheres or CFA alone. On day 35 of infection the draining lymph node cells were restimulated in vitro with ConA and the production of cytokines in the supernatants were measured by ELISA.

We observed that oral immunization with free Ag increased the production of all cytokines, and that Ag encapsulation promoted a threefold enhancement in IFN-γ production in relation to free Ag. We observed no significant changes in the CK production after sc immunization, except an increase in IL-2 when Ag was given with CFA. Irrespective of the route used, encapsulation of Ag in microspheres significantly reduced the capacity of the cells to produce IL-2, when compared to free antigen.

These preliminary results indicate that unlike sc immunization, oral immunization with a low dose of leishmanial antigen stimulate cytokine responses in infected mice. We observed no preferential induction of typical TH1 or TH2 cytokine patterns after oral immunization, but it was of interest that encapsulation of the Ag in microspheres of poly-lactide-co-glycolide polymer further enhances the capacity of cells to produce IFN-γ during infection, which may have implications in the prophylaxis of the disease.

IM-117
PERFORIN KNOCK OUT MICE ARE MORE SUSCEPTIBLE TO *TRYPANOSOMA CRUZI* INFECTION

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T lymphocytes have been claimed to play an important role in the control of different aspects of *T.cruzi* infection, such as control of parasitaemia and development of tissue destruction. This role could be performed by two basic functions: cytotoxic and/or secretory activity. In this work we used perforin knock out (-/-) mice to evaluate the precise participation of the perforin-based cytotoxic pathway, while the other cellular activities are still present.

Perforin (-/-) and (+/+) mice were infected with a highly (Y strain) or moderate (Dm28c and CL/Brener clones) virulent parasite samples of *T.cruzi*, the parasitaemia was conveniently scored and the plasma was collected after 8 and 15 days for serological studies. For cardiac inflammatory response analyses, both groups of mice were infected with 10^4 parasites of *T.cruzi* Y strain and the hearts collected after 8, 15 and 22 days of infection for rotine HE stain. The circulating parasitic load was equivalent in both groups of mice in different conditions, despite the virulence of the strain used for infection, but the mortality rate with the high virulent Y strain was much higher in the perforin (-/-) group. There were no relevant differences between the two groups in terms of splenomegaly, $CD3^+$ T cells and global or specific humoral response. In contrast, perforin (-/-) mice showed higher levels of plasma IFN-g, cardiac inflammatory infiltrates and tissue damage. These results indicate that perforin plays a pivotal role in the resistance of mice during the course of experimental Chagas'disease.

IM-118
PHAGOCYTIC AND MICROBICIDE CAPACITY OF PHAGOCYTES FROM SWISS AND BALB/C MICE ON *LEISHMANIA (LEISHMANIA) AMAZONENSIS* AND *LEISHMANIA (VIANNIA) BRAZILIENSIS*

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The protozoans of the *Leishmania* genus are intracellular parasites of the macrophages cells, where they can survive and multiply. The host's antigenic recognition is its first line of defense that occurs during the phagocytosis. To analyse the phagocytic and microbicide capacity of the peritoneal exudate cells from Swiss and BALB/c mice, were used *Leishmania (Viannia) braziliensis* (MHOM/BR/87/M11272) promastigotes forms (PM) and *Leishmania (Leishmania) amazonensis* (MHOM/BR/89/M112766) promastigotes and amastigotes forms (AM). The *Leishmania* were incubated with phagocytes previously adhered on coverslips, for 30, 60, 120 and 180 minutes. The phagocytic index (the average index of phagocyted parasites in 150 cells) was determinated after the staining with hematoxylin - eosin. The microbicide capacity (average index of alive, intermediate and dead parasites in 100 cells), was evaluated after the staining with acridine orange and crystal violet. The indexes were compared by Student's t – Test and the significant differences were only concidered when $p<0,05$. Using *L.(V.)braziliensis* PM and AM it was not observed significant differences in phagocytic index. Using *L.(L.)amazonensis* PM was significantly different in phagocytic index at 120 and 180 minutes, when compared BALB/c and Swiss. Microbicide capacity of Swiss phagocytes was significantly different at 60 minutes for intermediate forms of *L.(L.)amazonensis* PM and AM. Microbicide capacity of BALB/c phagocytes was significantly different at 180 minutes for alive, intermediate and dead forms and at 30 minutes for intermediate and dead forms of *L.(L.)amazonensis* PM. These results suggest that: BALB/c macrophages has a better phagocytic capacity and BALB/c phagocytes has a smaller microbicide capacity and this can explain its susceptibility to *L.(L.) amazonensis.*

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IM-119
PHENOTYPE AND Vb TCR REPERTOIRE OF LYMPHOCYTES FROM LYMPH NODES OF PATIENTS WITH CUTANEOUS LEISHMANIASIS

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Lymphatic involvement is present as an early, and sometimes the only clinical manifestation of cutaneous leishmaniasis. *Leishmania braziliensis* can be recovered from the enlarged lymph nodes of patients with no other sign of disease, what suggests that lymph nodes can constitute the first site of immunological reaction against the parasites. Lymphocyte populations present in the lymph nodes of infected individuals are therefore being investigated, in an attempt to correlate their phenotype and Vb TCR repertoire with the development of a protective or deleterious response. Lymph node aspiration samples from patients with cutaneous leishmaniasis were analyzed using flow cytometry as to the percentages of $CD3^-/CD19^+$ (B lymphocytes), $CD3^+/CD4^+$ (T helper), $CD3^+/CD8^+$ (T cytotoxic), $CD3^-/CD56^+$ (NK lymphocytes) , $CD3^+/CD45RA^+$ (naïve cells) and $CD3^+/CD45RO^+$ (memory cells). T CD4 and CD8 lymphocytes were assessed as to the percentages of Vb2, Vb3.1, Vb5, Vb8, Vb14 and Vb17 positive cells, to identify putative *in vivo* responding subsets. Preliminary data show a predominance of T lymphocytes in the lymph nodes of cutaneous leishmaniasis patients (50.86 ± 11.27), followed by B lymphocytes (41.21 ± 10.06), but low percentages of NK cells (0.87±0.44). The majority (8/13) of patients exhibited higher proportions of

$CD45RA^+$ T cells, although high percentages of $CD45RO^+$ T cells were found in some patients (2/13). Among the six patients which had their repertoire analyzed, the predominant phenotypes were: $CD4^+$Vb5 and $CD8^+$Vb3.1. Further analysis are being carried out to confirm those data and to associate the cell subsets observed with a protective response against *L. braziliensis*.

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IM-120
PLATELET ACTIVATING FACTOR (PAF) INDUCES NITRIC OXIDE SYNTHESIS IN *TRYPANOSOMA CRUZI* INFECTED MACROPHAGES AND MEDIATES RESISTANCE TO PARASITE INFECTION IN MICE

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Trypanosoma cruzi replicates in nucleated cells and is suscetible to be killed by IFN-g-activated macrophages through a mechanism dependent upon NO biosynthesis. In the present study, the role of PAF in the induction of NO synthesis and in the activation of the trypanocidal function of macrophages was investigated. *In vitro*, PAF induced NO secretion by *T. cruzi*-infected macrophages and the secreted NO inhibited intracellular parasite growth. The addition of a PAF antagonist, WEB 2170, inhibited both NO biosynthesis and trypanocidal activity. Trypanocidal activity was mediated by the iNOS/L-arginine pathway, since it was inhibited by treatment with L-NMMA, an L-arginine analog. PAF-mediated NO production in infected macrophages appears to be dependent on TNF-a production since addition of neutralizing anti-TNF-a mAb inhibited NO synthesis. In order to test the role of PAF in mediating resistance or suscetibility to *T. cruzi* infection, we treated infected mice with a PAF-antagonist, WEB 2170. These animals had increased parasitemia and earlier mortality than vehicle-treated mice. Altogether the results presented here suggest that PAF belongs to a group of mediators that coordinate the mechanisms of resistance to infections with intracellular parasites.

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IM-121
POLYMERASE CHAIN REACTION: AN ALTERNATIVE OR A COMPLEMENT TO THE QUESTIONNAIRE FOR SCREENING *PLASMODIUM VIVAX* INFECTED INDIVIDUALS AMONG BLOOD DONORS?

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In Brazil, no study has been done concerning the detection of malaria parasites by polymerase chain reaction (PCR) related to the diagnosis of *Plasmodium vivax* malaria. Thus we standardized a PCR method based on amplification of the cistein proteinase gene detected by single ethidium bromide staining. For this purpose we tested blood samples from 35 *P.vivax* malaria patients with different levels of parasitemia as well as blood samples from 12 clinical healthy individuals. After successfully standardization, we compared PCR methodology with parasitological as well as with the standard questionnaire, to evaluate the best approach for screening blood donors in endemic areas. Blood samples were collected in a blood bank in the Brazilian Amazon (HEMOAM - Manaus, Amazonas) from 301 blood donors and from 38 individuals excluded from blood donation by the questionnaire. All individuals presented negative parasitological examination and one suitable blood donor had positive nested PCR. Despite the subjectivity of standard questionnaire methodology that eliminated 38 individuals to blood donation, the PCR and microscopic examination results would eliminate one or no individual, respectively. However, when a high number of individuals must be tested the parasitological examination is not the method of choice. Therefore a PCR could be used in addition to the questionnaire to safely rescue blood candidates considered unsuitable on the basis of malaria history.

Notwithstanding we had already standardized a nested PCR for *P.falciparum* malaria, these first results point to the need of developing a PCR methodology specific to the *Plasmodium* genus in order to enable laboratory detection of malaria infection caused by any *Plasmodium* species.

IM-122
PRESENCE OF HSP70 ON THE SURFACE OF *LEISHMANIA DONOVANI* PROMASTIGOTES

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In this study, we demonstrate the presence of heat shock protein 70 (HSP70) on the surface of *L.donovani* promastigotes, using the direct agglutination test (DAT). The recombinant protein S7, composed of 230 aminoacids of the *L.chagasi* HSP70 C - terminal moiety, has been cloned and expressed in the *E.coli* DH5a. Total lysates of the transformed bacteria were used to prepare SDS-poliacrylamide gels, followed by the electroelution of the 45kD band containing the fusion protein. Immunoblots, using kala-azar serum samples, have been used to demonstrate that the electroeluted protein retained its antigenic properties. This semi-purified recombinant protein was then used to prepare policlonal anti-HSP70 rabbit sera (3 samples). Specific antibodies against S7 were already detected 8-10 days after the first injection with 100ug of recombinant protein. The second injection, with the same dosis, yielded a more intense response, as revealed by ELISA using the S7 fusion protein to coat the microtiter plates. The presence of HSP70 on the surface of *Leishmania* promastigotes was evaluated by testing the hiperimmune sera in the DAT. Intact, formalin-fixed and Coomassie blue-stained *L.donovani* promastigotes were mixed with serial dilutions of the rabbit sera (1:25 until 1:800), containing 2 - mercaptoethanol in the serum diluent. All 3 samples were positive at a dilution of 1:400, while a negative control serum showed no agglutination at all. Hence, the anti-HSP70 policlonal rabbit sera contained immunoglobulin molecules that recognized the native HSP70 of *L.donovani* distributed on the parasite surface. HSP70 have been found in several compartments of the cell, but their presence on the cell surface is uncommon.

IM-123
PRESENT STATUS OF *TRYPANOSOMA CRUZI* INFECTION AMONG RESIDENTS IN A RURAL AREA OF PERNAMBUCO, NORTHEAST BRAZIL

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Since January 1998 a survey has been carried out among rural residents in two villages of Alto Pajeú, Sertão of Pernambuco. A total of 695 inhabitants have been registered, with an average age of 25.9 years and a sex ratio of 1.13. The indirect immunofluorescence test was used to screen the chagasic infection, with a dilution of 1:20. Positive and inconclusive cases were controlled by indirect haemagglutination, at 1:32 dilution, and by serial dilutions up to 1:320 in immunofluorescence. Blood was collected from volunteers older than 2, in filter paper. Results are presented below as positive/total ratio.

	Cajueiro	Caatingueira
2 to 9 ys.	0/17	0/25
10 to 19 ys.	0/25	2/58
20 to 39 ys.	1/21	7/35
40 to 59 ys.	9/24	5/32
> 60 ys.	2/11	7/16
Total	12/98	21/166

A total of 65% of the population was tested. Positive cases have been informed and are now under medical supervision. Triatomine bugs, specially *T. braziliensis*, were frequently found in animal shelters near the houses and very rarely indoors.

The youngest case is 12 ys.old. The high general prevalence rate of 12%, increasing with age, demonstrates the importance of Chagas' disease in this area and suggests a decreasing transmission, although present until recently.

IM-124
PREVALENCE OF EB200-SPECIFIC ANTIBODIES IN NATURAL HIPOENDEMIC MALARIA AREA

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The clinical malaria symptoms have been attributed to blood asexual stages of *P. falciparum*. Cytophilic IgG1 and IgG3 specific antibodies to parasite blood asexual stages antigens seem to be important for the acquired immune resistance controlling the infection. In the present study we have evaluated the reactivities of antibodies developed by individuals living in low malaria endemicity areas, exposed to natural *Plasmodium* infection, to the purified proteins EB200, R23.1, R23.2 and to synthetic peptides AARP. These are all antigens expressed on membrane of P. falciparum-infected erythrocytes. Serum samples were collected in Portochuelo during a cohort study. First, spe-

cific IgG to P.falciparum extract antigen (Pf) was measured in samples collected from 175 individuals presenting with *P. falciparum* or *P. vivax* blood parasites and from nonparasitemic individuals. One hundred and forty-four individuals (82,3%) were positive for Pf. Among these, sixty individuals (34,8%) presented specific IgG antibodies to EB200, with titres varying between 1/85 to 1/6912. No reactivity were found for tested sera to either the R23.1, R23.2 proteins or AARP peptides. These results showed the development of specific IgG anti-EB200 in natural *Plasmodium*-infected individuals living in areas where malaria is hipoendemic. Both the EB200-specific IgG1 and IgG3 levels and the affinity of EB200-specific antibodies produced are under investigation in our laboratory.

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IM-125
PREVALENCE OF *TOXOPLASMA GONDII* INFECTION IN PIGS FROM BRAZIL AND PERU, DETECTED BY SEROLOGICAL ASSAYS

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Toxoplasmosis, a high prevalent zoonotic infection, had worldwide distribution and is acquired by ingestion of food contaminated with oocysts from cats stools, the definitive host, or by raw or undercooked meat of warm blood animals, intermediate hosts. Generally asymptomatic, this infection could cause eye involvement, or more severe disease, with deaths or abortions in fetus or immune-compromised patients. This infection had a great importance with the HIV epidemic, affecting 20% of AIDS patients with toxoplasmic encephalitis, a disabling and lethal disease. The sources of the infection, specially those attributed to animal products, was limited research, generally related to the ability of the animal to carry the infection, with few reports dealing with the prevalence of those infection on animals used for human nutrition. To evaluate the seroprevalence of this infection in pigs, we analyze 397 sera from 5 months old pigs of trading abattoirs from São Paulo, Brazil(300) and Lima, Peru(97). We detect specific antibodies by indirect hemagglutination, specific anti-*T.gondii* IgG by ELISA and Western Blotting, with some experiments of antibody avidity with urea as chaotropic reagent. Control sera was obtained from an experimentally infected pig, with week blood collection for antibody titers and avidity assays. This infection evokes a clear specific antibody response, with time increment in antibody avidity. Enzymatic assays, ELISA and WB, provides a high prevalence of specific antibodies in pigs both from Peru(34%) and in Brazil(9%), but a high proportion of animals had very low positive titers, most clearly seen in pigs from São Paulo(36%), that could be explained by maternal transmission of antibodies during delivery. The avidity assays showed no correlation with antibody titers, with most positive animals also with a high avidity index. The hemagglutination assays were less efficient in the definition of infection, with both false negative and false positive sera, despite its feasibility. We cannot avoid that other coccidian swine infection could present crossed reaction in this assay. These data demonstrate that pigs could be considered a significant source of human *Toxoplasma gondii* infection, with care and education in the cooking of pork-containing foods. Some serological improvements are needed in the diagnosis of this infection. A.J. Galisteo Jr. is a fellow of FAPESP(98/1681-0). RM Hiramoto is a fellow of CNPq. This study was a part of the thesis of F.Suárez FAPESP (96/5875-8) and LIMHCFMUSP-49 supported this work.

IM-126
PRIMARY *IN VITRO* STIMULATION OF SPLENIC CBA MOUSE CELLS WITH *LEISHMANIA MAJOR* OR *LEISHMANIA AMAZONENSIS*

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CBA mice are resistant to infection with *L. major* (*Lm*) but are susceptible to infection with *L. amazonensis* (*La*). *In vivo* studies showed that in this model resistance is associated with a Th1 type of cell-mediated immune response, whereas susceptibility is associated with Th2 type. These observation points to the possible role of factors of the parasite in the modulation of immunoregulatory mechanisms of the host response. Several studies have demonstrated that early events, occuring in the first week after infection, determine the type of the immune response mounted by the host. The primary *in vitro* stimulation (PIV) assay has been proposed to dissect the early events involved in the establishment of the immune response in leishmaniasis and a good correlation with the *in vivo* response have been described. Thereby, we used this system to search for differences in the model of CBA infection with *Lm* or *La*. CBA splenocytes were primed *in vitro* with *Lm* or *La* and supernants of the cultures from the first to the seventh days were assayed by ELISA for the presence of IFN-g, IL-4, IL-5 and IL-10. Additionally, NO production was measured by Griess reaction. Our results show that the amounts of IFN-g, IL-10 and NO increased in function of the time of stimulation, the number of parasites used and the concentration of spleen cells added in the

assays. Different from *in vivo* observations, PIV with *La* produced 3.5 times more IFN-g and 2.7 more IL-10 than PIV with *Lm*. On the seventh day, the ratio IFN-g/IL-10 was 5.0 in PIV with *La* and 1.8 in PIV with *Lm*. At the same time, the levels of NO were 2.9 higher in *La* than in *Lm*. IL-4 and IL-5 were undetectable at any time point. Similar results were obtained when lymph node cells were used. Our results show that *La* induces predominantly a Th1 response in PIV. This phenomenon has not been observed during *in vivo La* infection of CBA mice and may be due to the lack of factors or cells in the PIV system which are required to generate a Th2 type response.

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IM-127
PRODUCTION OF A MONOCLONAL ANTIBODY AGAINST A *LEISHMANIA (LEISHMANIA) AMAZONENSIS* PROMASTIGOTE SURFACE GLYCOPROTEIN WHICH REGULATES THE COMPLEMENT SYSTEM

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Recently, we have described that promastigotes of the subgenus *Leishmania* at late logarithmic phase of *in vitro* culture display a marked resistance to complement damage. In contrast, promastigotes of subgenus *Viannia* are consistently susceptible to complement lysis (Noronha et al., *Acta Tropica* 69: 17-29, 1998). This inhibitory activity is associated with a promastigote membrane glycoprotein. This protein blocks the lytic activity of complement by interfering with the cascade, in a fashion similar to human CD59 (Nunes et al. *Parasitology* 115: 601-609, 1997). Now, having purified and partially characterized this inhibitory protein, we decided to raise monoclonal antibodies against this molecule. *Leishmania (Leishmania) amazonensis* promastigotes were cultured in Schneider's medium/ 10% FCS and harvested at day 5 of culture. Parasite membranes were extracted by freezing/ thawing, solubilized with CHAPS and purified by FPLC using Q-Sepharose, Superdex-200 and Phenyl-Superose. The purified fraction was shown to contain a major component of 46 kDa and a minor contaminant of 36 kDa, as analyzed by SDS-PAGE. To prepare monoclonal antibodies against this molecule we have used 4-week old Balb/c mice which had been grafted with a syngeneic neonate spleen on their ears. The animals were immunized with 2 injections of 10mg of the FPLC-purified material in 50ml of PBS directly into the transplanted spleen. The antibody response was monitored by an ELISA assay using 96-well plates sensitized with 10mg/100ml per well of whole disrupted promastigotes. When a high level of anti-leishmania IgG was detected, the animals were challenged with a further 10mg of antigen. Three days later, mice were killed and the adult spleen removed. Spleen cells were collected and fused with SP20Ag14 myeloma cells using PEG1500. Hybridomas were selected with appropriate media, expanded in RPMI 1640/ 20% FCS and productive cells were selected and cloned. We obtained 5 clones monospecific to the 46 kDa complement inhibitory protein. We are now characterizing the isotype secreted by these clones and the monoclonal antibodies were used to follow the expression of the complement regulatory molecule on the surface of *L. amazonensis* promastigotes.

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IM-128
PRODUCTION OF INTERFERON BY A VACCINE AGAINST CANINE VISCERAL LEISHMANIASIS

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Control of human and canine visceral leishmaniasis (CVL) in Brazil had been done by treatment of all human cases, elimination of infected dogs and application of insecticide to the walls of dwellings and peridomestic buildings. However these measures are not enough to eradicate this disease. Immunoprophilaxis seems to an alternative for control measures. In order to evaluate the immunogenicity of a vaccine produced in our laboratory, twelve one 4 month-old laboratory-reared mongrel dogs of both sexes were tested for g-IFN. The *Leishmania* vaccine was composed of merthyolated sound-disrupted promastigotes of *L. braziliensis*; strain MCAN/BR/72/C348 according previously described (Mayrink et al., 1996 *Mem. Inst. Oswaldo Cruz, 91*:695-697). Two groups of dogs were studied: vaccinated group (10 dogs received vaccine plus BCG) and control group (10 dogs received BCG). Three doses of vaccine (600µg protein/dose) mixed with BCG (400µg/dose) were given intradermally at 21-day intervals. Blood collections were taken before and after the vaccination for interferon bioassay. Supernatants of mononuclear cells, collected at 24, 48 and 72 hours, were quantitatively assayed for the presence of IFN. The supernatants were tested for their ability to inhibit the cytopathic effect of the Vesicular Stomatitis Virus (VSV) over Madin-Darby

canine kidney cells (MDCK) according to STEWART (1979). MDCK were cultivated in D-MEM supplemented with 10% fetal calf serum in 96-well plates at an initial population density of $3.5x10^4$ cells/well. After 18 hours of incubation with the supernatants of the mononuclear cell cultures the MDCK cells were infected by VSV at a volume of 100µl viral suspension/well. IFN activity was expressed in standard units/ml, representing the inverse of the maximum dilution capable of protecting 50% of the infected cells, by visual estimation. Before the vaccination neither of the dogs produced IFN. After the vaccination 70% of the vaccine plus BCG group produced significant levels of IFN. Among the BCG group 40% of the animals produced IFN. These results show that BCG plus vaccine against CVL have important role in the stimulation of the TH1 response.

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IM-129
PROTECTION AGAINST TOXOPLASMOSIS IN MICE IMMUNIZED WITH DIFFERENT ANTIGENS OF *TOXOPLASMA GONDII* INCORPORATED INTO LIPOSOMES

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Different toxoplasma antigens were intrapped within liposomes and evaluated, in this form, for the ability to protect swiss mice against toxoplasma infection: soluble tachyzoite antigen (L/TAg), tissue cyst (L/CAg), tachyzoite plus tissue cyst (L/TCAg) or purified antigen of tachyzoite (L/pTAg).The protein used in L/pTAg was purified from tachyzoites using a stage specific monoclonal antibody which reacted at molecular weight 32 kD in SDS PAGE and silver stain using reduced condition. To compare the immuno adjuvant action of liposomes and of Freund's Complete Adjuvant (FCA), another group of mice was immunized with soluble tachyzoite antigen(S/TAg) emulsified in FCA. Control groups were inoculated with (S/TAg) alone, phosphate-buffered saline (PBS) and with empty liposomes (L/PBS). Mice were inoculated subcutaneously with these antigens 8, 4 and 2 weeks before challenge with 80 tissue cysts of the P strain of *T. gondii* by the oral route. All mice immunized with or without adjuvant showed humoral response, as measured by ELISA, however, no correlation was found between antibody titer and protection against challenge. All mice immunized with L/pTAg or L/TCAg survived (100%), whereas 80% of mice from groups which received PBS and empty liposome died. All mice immunized with antigens intrapped within liposomes (L/TAg, L/CAg, L/TCAg and L/pTAg) showed low number of intracerebral cysts.

IM-130
REGULATION OF IL-12 AND IFN-γ SYNTHESIS IN *TRYPANOSOMA CRUZI* INFECTION

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Previous investigations have confirmed that IL-12 stimulates IFN-γ synthesis by NK and T cells, mainly at the initial phase of the immune response, thus creating a cytokine milieu favoring differentiation of Th0 to Th1 cells. IFN-γ is a potent activator of macrophage microbicidal function and the synthesis of high levels of this cytokine during *T.cruzi* infection has been described. We investigated the production of IL-12 and IFN-γ as well as the regulatory mechanisms involved in their synthesis during the course of *T.cruzi* infection. Cultures of spleen cells obtained from infected C57Bl/6 mice were stimulated *in vitro* with trypomastigotes antigen and treated with neutralizing mAbs anti: IL-12 (C17.8), $CD4^+$ (GK 1.5), $CD8^+$ (TIB 105), TGF-b (Genzyme, anti TGF-b1, b2, b3), IL-10 (2A5) or IL-4 (11B11). N^G-metil-L-arginine was also used as an inhibitor of nitric oxide (NO) synthesis. IL-12 and IFN-γ production was measured in culture supernatants by ELISA. Our results show that IL-12 and IFN-γ production increased in the first week of infection (days 3, 5 and 7), declined during the second week (days 9 and 12) and augments again by the end of the third week maintaining their secretion rates until day 40 of infection. Production of IFN-γ was found to be essentially (60 to 80%) dependent of endogenously secreted IL-12 at all analyzed time points of infection with the exception of day 12 when IL-12 was undetectable. Neutralizing IL-10 during the first, third and fourth week of infection significantly increased both IL-12 and IFN-γ production in the cultures but only moderately increased their production in cultures from two-weeks-infected mice (days 9 and 12). In contrast, blocking NO synthesis in the cultures of the second week of infection increased IFN-γ production by 100 %. IFN-γ synthesis was predominantly $CD4^+$-activation dependent and blocking of $CD8^+$-activation pathway did not affect these cytokine production levels. Preliminary experiments showed that neutralization of IL-4 or TGF-b in cultures from the second week of infection did not modify IFN-γ production. Our results suggest that IL-12 has in important role stimulating IFN-γ production in the acute and also in the sub acute phase of *T.cruzi* infection. IL-10 and secondarily NO appear as the most important modulators of IL-12 and IFN-γ synthesis during infection.

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IM-131
REGULATION OF IL-12 SYNTHESIS IN PBMC CULTURES FROM CHAGAS' DISEASE PATIENTS

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In vitro Il-12 treatment (Immunol. Letters 57:39,1997) and IL-10 or Indomethacin treatments (unpublished results) significantly increased *T.cruzi*-antigen (T-Ag)-specific proliferation by PBMC from Chagas' disease patients. Interleukin-12 production in the presence of disrupted tissue culture trypomastigotes Ag (T-Ag) by PBMC from chronic chagasic patients (cardiac, indeterminate and cardiac + digestive forms) was analyzed by ELISA in cultures treated with: a) neutralizing monoclonal antibodies (mAb) to IL-10, IFN-g, IL-13 and IL-4; b) indomethacin as a COX inhibitor of prostaglandin (PG) production; N^G-methyl-L-arginine (NMLA) as a competitive inhibitor of nitric oxide (NO) synthesis and d) glutathione-peroxidase as inhibitor of reactive oxygen intermediates (ROI). The *in vitro* synthesis of IL-10 and PG were also investigated. Although T-Ag stimulation by itself did not augment IL-12 synthesis, treatments with Indomethacin, NMLA, anti-IL-4, anti-IL-13 and anti-IL-10 enhanced IL-12 production. Among the different groups of patients, those with the cardiac form had higher IL-12 production levels than those in the indeterminate phase of the disease. Patients with the cardiac form also had increased PG production by T-Ag-stimulated PBMC. IL-10 levels in unstimulated cultures from these patients were higher than those in cultures from indeterminate group patients. Prostaglandins and IL-10 predominantly exerted negative regulation on IL-12 production in cardiac patients as shown by the marked increased observed in the synthesis of this cytokine in cultures treated with indomethacin or anti-IL-10 mAb. These results indicate that IL-12 production by PBMC in chronic chagasic patients is under negative regulation by IL-4, IL-13, IL-10, PG and NO and suggest that endogenous IL-10 and PG exert significantly stronger control on IL-12 synthesis in cardiac patients. The higher levels of IL-10 and PG found in cultures from cardiac group patients agree with these data. The dual control of IL-12 could be important to balance Il-12-mediated effector mechanisms activated by the higher levels of this cytokine produced by PBMC from cardiac patients.

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IM-132
REQUIREMENT OF P38-MITOGEN ACTIVATING PROTEIN KINASE ACTIVATION FOR INDUCTION OF MONOKINE SYNTHESIS BY MURINE INFLAMMATORY MACROPHAGES EXPOSED TO GLYCOSYLPHOSPHATIDYLINOSITOL ANCHORS PURIFIED FROM *TRYPANOSOMA CRUZI* TRYPOMASTIGOTES

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The present study reports our efforts in defining the involvement of MAP kinase pathway(s) on induction of monokine synthesis by macrophages exposed to glycosylphosphatidylinositol (GPI) anchors purified from *Trypanosoma cruzi* trypomastigotes. As positive control we used LPS derived from *Escherichia coli*, known as potent inducer of different MAP kinase pathways. Among different inhibitors for protein kinases we found that SB203580, a pyridinil-imidazole compound and specific inhibitor for p38 MAP kinase, is a potent inhibitor of TNF-a and in a less extend of IL-12(p40) synthesis by inflammatory macrophages exposed to either GPI-anchored mucin like glycoproteins (tGPI-mucins) or purified GPI (tGPI) anchors isolated from *T. cruzi* trypomastigotes. In contrast, PD098059 and inhibitor of MAPKAP-K1 (MAPK-1 pathway) had no inhibitory effect on IL-12(p40) or TNF-a synthesis by macrophages expose to different microbial stimuli. We also measured the activity of downstream targets of p38 MAP kinase and MAPKAP-1 before and after macrophage stimulation with either tGPI-mucins or tGPI. Our results show that tGPI activates both MAPKAP-K1 and MAPKAP-K2. However only the MAPKAP-k2 activity was inhibited by the compound SB203580. The peak of MAPKAP-K2 activity induced by tGPI was at 15 min post macrophage simulation. We also studied the ability of different cytokines (i.e. IFN-gand TNF-a which are known to potentiate monokine synthesis by macrophages exposed to different microbial products, on their capacity to induce p38 MAP kinase activation. Despite of previous studies showing that TNF-a activates the p38 MAP kinase, in our system TNF-a had no effect in inducing or potentiating MAPKAP-K2 activity on macrophage lysates. In contrast, IFN-g a potent enhancer of IL-12(p40) and TNF-a synthesis by macrophages stimulated with microbial products was shown to significantly augment the activity of MAPKAP-K2 induced by either tGPI or LPS. Consistent with these findings, we observed that lysates obtained from macrophages derived of *Interferon Consen-*

sus Supressor Binding Protein knockout mice, known to be defective in their IFN-γ priming for IL-12(p40) synthesis, express less MAPKAP-K2 upon stimulation with tGPI or LPS in the presence of IFN-γ. Finally, we studied the ability of different compounds (i.e. cAMP analogues, dexametazone and IL-10) known to be potent inhibitors of IL-12(p40) and TNF-α synthesis in their ability to inhibit MAPKAP-K2 activation induced by tGPI or LPS in the presence or absence of IFN-γ. Our results show that cAMP analogues and IL-10 had no modulatory effect on MAPKAP-K2 activity present in lysates from macrophages stimulated with tGPI/LPS and/or IFN-γ. In contrast, dexametazone had a partial but consistent inhibitory effect on MAPKAP-K2 activity elicited by the different microbial stimuli. These studies indicate the involvement of p38 MAP kinase pathway on the induction of TNF-α and IL-12 synthesis by macrophages exposed to GPI anchors derived from *T. cruzi* trypomastigotes.

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IM-133
rK39 ANTIBODY KINETICS IN VISCERAL LEISHMANIASIS

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The diagnosis of visceral leishmaniasis, a disease that affects thousands of people around the world, remains problematic. Current methods include visualization of the parasite in bone marrow or splenic aspirates, the most accurate method but invasive for the patient. Serologic tests are nonspecific and clinical data collected overlaps with other diseases. The recombinant antigen K39, a kinesin superfamily-related protein, presenting a 39-amino acid repeat conserved between *L. chagasi* and *L. donovani* (1,2), has been shown to be a valuable antigen in detecting anti *Leishmania* antibody in serum of patients with visceral leishmaniasis. Anti-rK39 antibodies are virtually absent, in cutaneous and mucosal leishmaniasis, Chagas' disease, and self-healing infected patients (3). In this study, we confirmed that asymptomatic individuals (n=250) who are serologically reactive to crude antigen preparations of *L. chagasi*, but without any clinical symptoms for visceral leishmaniasis, were nonreactive to rK39 antigen. In our study, eigthy seven percent of patients hospitalized with visceral leishmaniasis diagnosed either by visualization of parasite in bone marrow aspirates, serology or clinical presentation (n=69), presented anti-K39 antibody. Eighty percent of patients continued to present anti-K39 antibody one year after treatment. Of a subset of patients followed up to seven years after treatment (n=34), 95% became seronegative within 13 months to 7 years. This data confirms the validity of antigen K39 as a tool in the diagnosis of active visceral leishmaniasis. 1-Burns et al. 1993. Proc. Natl. Acad. Sci. USA. 90:775-779.2-Jing-Qi Qu et al. 1994. Trans. R. Soc. Trop. Med. Hyg. 88:543-545.3-Badaró et al. 1996. J. Inf. Dis. 173:758-61.

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IM-134
ROLE OF CTLA-4/ B7.1 INTERACTIONS IN THE EARLY AND LATE T-CELL RESPONSES TO MURINE VISCERAL LEISHMANIASIS

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Pathogenic mechanisms leading to defective Th1 T-cell activity in the course of visceral leishmaniasis (VL) are unknown. In this work, we investigated the functional status of T-cell accessory cell interactions after infection of mice with *Leishmania chagasi*. We observed an expansion *in vivo* of activated CD4, compared to control mice. However, stimulation *in vitro* of explanted $CD4^+$ T cells from infected mice with anti-CD3 antibody resulted in deficient proliferation, which could not be attributed to activation-induced cell death. Anti-CD3 stimulation in the presence of the antagonist anti-CTLA-4 or anti-B7.1 mAbs, but not anti-B7.2 nor agonist anti-CD28 mAb, restored deficient T-cell responses up to control levels. Response to *L.chagasi* recombinant antigen LcR1 was also depressed in cultures from infected mice. However, anti-CTLA-4 addition induced vigorous proliferation to LcR1 and potentiated LcR1-specific IL2 and IL4 responses, without any effect in control cells. Furthermore, CTLA-4 blockade induced almost complete elimination of parasite burden in splenocyte cultures activated with anti-CD3 or LcR1. Together, these data indicate that CTLA-4 engagement by B7.1 is responsible for late T-cell unresponsiveness in chronic VL. We are currently investigating the role of CTLA-4/B7.1 interactions in the early response to *L.chagasi* using a priming *in vitro* (PIV) system.

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IM-135
ROLE OF IgG-ISOTYPES IN THE PROTECTION AGAINST ERYTHOCYTIC STAGES OF *PLASMODIUM CHABAUDI CHABAUDI*

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The mechanisms of antibody-mediated protection to malaria parasites are still unknown. The fact that in humans and monkeys only IgG preparations containing opsonic antibodies are able to passive transfer protection to *P. falciparum* indicates that, in those cases, antibody-dependent phagocytosis and/or cytotoxicity are probably involved. In this work, we analyzed the protective role of IgG-isotypes in the murine experimental model of malaria caused by a virulent *P. c. chabaudi* AJ (clone IP-Pc1). To obtain serum containing increased levels of IgG2a (cytophilic) or IgG1 (non-cytophilic) antibodies, BALB/c mice were immunized with a parasite extract diluted in incomplete (PE-IFA) or complete (PE-CFA) Freund'adjuvant. PE-CFA immunized mice presented high serum levels of parasite-specific IgG2a antibodies, while IgG1 was the major isotype in the PE-IFA group. When these mice were challenged with infected erythrocytes (50 days after immunization), PE-CFA immunized mice controlled the parasite growth more efficiently. After parasite challenge, spleen cells from these animals but not those from the PE-IFA immunized mice secreted high levels of IFN-g Interestingly, serum samples from PE-CFA immunized animals were also more efficient to passive transfer protection to naive animals. Taken together, these results suggest that cytophilic antibodies through Fc mediated cellular mechanisms play a major role in the control of erythrocytic stages of *P. c. chabaudi.*

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IM-136
SERODIAGNOSTIC SURVEY OF *ENDOTRYPANUM* AND *LEISHMANIA* INFECTIONS IN TWO AND THREE-TOED SLOTHS USING A MICRO-ELISA ASSAY AS A SCREENING TEST

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Parasitic protozoa of the genus *Endotrypanum* (Kinetoplastida: Trypanosomatidae) are biologically diverse group of microorganisms in that infection appears to be restricted to edentates, principally of forest-dwelling two-toed sloths of the genus *Choloepus*, but rarer infections with these flagellates seems to occur in three-toed sloths (genus *Bradypus*). Other distinct groups of trypanosomatids, such as *Leishmania* and *Trypanosoma*, are also found in sloths. It is important for epidemiological studies of leishmaniasis in the Amazon Basin to be able to discriminate *Leishmania* from *Endotrypanum* infection in sloths captured in this region (Shaw 1992, *Ciência e Cultura 44*: 107). Parasitological methods are not always successful in detecting parasitic infection in the hosts. Noninvasive diagnostic procedures such as the enzyme-linked immunosorbent assay, ELISA (once the results are comparable favorably with direct parasite detection) could be an alternative tool for detecting *Leishmania* or *Endotrypanum* infections in sylvatic animals. In this study, an enzyme-linked immunosorbent assay (micro-ELISA) was developed for detecting *Leishmania* or *Endotrypanum* antibodies in sera from sloths captured in sylvan areas of the Neotropics. To evaluated antibody responses, at first immunoglobulin (Igs) preparations were purified from the sera of sloths (from both genera, *Choloepus* and *Bradypus*), then used to generated specific rabbit antibodies to apply in ELISA-based serology. The specificities of the antisera produced (rabbit antibodies anti-sloth Igs) were tested (by immunodiffusion and ELISA assays, and Western-blot analysis), using a large number of sera colected from different mammals species. The micro-ELISA assay was defined testing selected parasite antigens (as defined characterizing soluble *Leishmania*, *Endotrypanum* or *Trypanosoma* antigen preparations using Western-blot analysis) with serum, antiserum and conjugate preparations in different concentrations/dilutions. Different levels of either specific *Leishmania* or *Endotrypanum* antibodies were found and the cut-off with normal sera was determined. The sensibility and specificity of the micro-ELISA assay results were compared with data obtained using other diagnostic procedures (direct demonstration of the parasite and/or PCR analyses). Work is now in progress to determine the seroprevalence of *Endotrypanum* and *Leishmania* infections in sloths captured in distinct sylvan areas of the Neotropics.

IM-137
SICKLE CELL CRISIS: A STUDY OF IL-8 LEVELS IN BRAZILIAN PATIENTS FROM SALVADOR-BAHIA-BRAZIL

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A number of observations indicated an elevated levels of IL-8 and others cytokines in sickle cell crisis. In this study we measured serum levels of this neutrophil chemokin in sickle cell patients during sickle cell crisis, in order to confirm the role of this in the development of pathogenesis of vasooclusive process. 16 patients during sickle cell crisis (5 females , 11 males) with an age range of 7 months to 53 years. 11 patients had their blood collected to the study of IL-8 with a mean time of crisis evolution less than 48hours and 5 after 48hours. The IL-8 levels were measured in non-symptomatic and healthy controls.

Sera sample and clinical data were collected from 9 patients with bone crisis; 3 with priapism crisis and 3 with acute chest crisis. A very high levels of IL-8 was observed between 17 patients studied. Some of them had a very high levels of IL-8 and two of then had very low levels. One was more than 48 hours in crisis and the other one took analgesic medicine to the pain and Il-8 was not detected. Mean of IL-8 in 16 patients was 963 pg/ml and two of then and the non-symptomatic and healthy controls had IL-8 levels less than 15 pg/ml.

These results confirm a role of IL-8 at sickle cell crisis. The find of a patient with more than 48 hours of crisis with low level of IL-8 showed that it could be detected at high levels only at the initiation of the crisis. The other result found here, showed that some factors, like use of pain medicine, can interfere with levels of this cytokine even during crisis, showing that studies of IL-8 levels in more number of sickle cell patients with several times of crisis and taking the most common medicine used in these crisis time to really know if some of them could modify this patient answer.

IM-138
SIMILARITY OF A RECOMBINANT ANTIGEN TO THE 30 KDA PROTEIN FROM *LEISHMANIA (L.) AMAZONENSIS* AMASTIGOTES

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An antigen of 30 kDa (p30) from amastigote forms of *L. (L.) amazonensis* was found to induce lymphoproliferative reponses in BALB/c mice mediated by $CD4^+$ Th1. The antigen was characterized as a cysteine proteinase and conferred partial protection against homologous infection (Beyrodt et al., 1997, Infect. Immun. 65, 2052). In order to clone and sequence the gene encoding the p30, a fragment of 500 bp was amplified by PCR using genomic DNA of *L. (L.) amazonensis* amastigotes and a pair of primers derived from evolutionary conserved active sites of *Dictyostelium discoideum* cysteine proteinase. Nucleotide sequence analysis of the amplified fragment showed a high degree of homology to sequences of cysteine proteinase genes from. After cloning and amplification the 500 bp fragment was subcloned in the pGEX expression vector in phase with glutathione *S*-transferase gene, resulting in a fusion protein of 43 kDa (p43). Two approaches were chosen to examine the relationship between the recombinant antigen and native p30.

1) The ability of the recombinant antigen to induce lymphoproliferative responses in BALB/c mice was studied. Significant proliferation responses were elicited by p43 in lymphocytes from animals previously immunized with either *L. (L.) amazonensis* amastigotes, p43 or native p30, with the production of hight levels of IFN-g.

2) A monospecific mouse serum was obtained against the recombinant protein. This serum was used in immunoprecipitation assays with *L. (L.) amazonensis* amastigotes extracts and the immune complexes were separated by low-voltage electrophoresis under nonreducting conditions on acrylamide gels containing gelatin. The proteolytic activity of the recombinant antigen migrated as a 30 kDa band. An identical electrophoretic pattern of proteolytic activity was obtained when *L. (L.) amazonensis* extracts were immunoprecipitated with the monoclonal antibody directed to the native p30.

These data support the similarity of the recombinant antigen and native p30. Experiments are in progress in order to compare the protection conferred by these antigens in BALB/c mice against *L. (L.) amazonensis* challenge.

IM-139
SIMULTANEOUS INTERACTIONS OF *TRYPANOSOMA CRUZI* AND APOPTOTIC CELLS WITH MACROPHAGES RESULT IN PARASITE ESCAPE

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Cell death by apoptosis is followed by phagocytosis of apoptotic bodies by macrophages and no inflammation ensues. Surface receptors on macrophages, including the vitronectin receptor (VNR) are involved in recognition of modified apoptotic cells. By using in vitro and in vivo models, we investigated the impact of apoptotic cells in the macrophage capability of dealing with *Trypanosoma cruzi*. *T. cruzi* infected macrophages were allowed to interact with apoptotic (heat-treated or irradiated) or necrotic (freezing-thawing or paraformaldehyde treated) T cells, and free trypomastigote forms were counted in supernatants from cultures one week later. *T. cruzi* replicated more in

macrophages treated with apoptotic cells than in those which received necrotic bodies or no treatment. Similar results were obtained with *in vivo*-infected macrophages explanted from the peritoneal cavity of *T. cruzi* infected mice. Apoptotic cells increaseed parasite growth even in IFN-g treated macrophages. Anti-VNR treatment also increased parasite replication, suggesting a role for VNR in mediating the effects of apoptotic cells. Finally, BALB/c mice infected with *T. cruzi* and injected with apoptotic T cells had increased parasitemia compared to untreated mice or to those injected with necrotic cells. Based on these results, we conclude that phagocytosis of apoptotic cells plays an active role in breaking macrophage-parasite equilibrium and results in *T. cruzi* escape from macrophage microbicidal mechanisms.

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IM-140
SOLUBLE *TRYPANOSOMA CRUZI* TRANS-SIALIDASE DOES NOT BIND TO SIALIC ACID ON MAMMALIAN CELL MEMBRANES

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It is has been proposed that the *Trypanosoma cruzi* trans-sialidase (TS) acts as a parasite ligand for sialic acid on host cells, since it is present on the parasite surface and has necessarily some affinity for sialic acid. Sialic acid has been shown to be one of the molecules involved in *T. cruzi* penetration into cells. Indeed, it has been proposed that soluble TS inhibits the infectivity of trypomastigotes in cell cultures by competing with parasite-bound TS for sialylated cell ligands (Mol. Biochem. Parasitol. 58:243, 1993). Alternatively, TS could theoretically inhibit penetration by desialylating host cells, as has been described for *Vibrio cholera* sialidase: The possible binding of soluble TS to normal and desialylated mammal cells was investigated herein, aiming at discriminating between the two possibilities described above. Fixed monolayers of a rat myoblastoid (L6) cell line were incubated with supernatants obtained from four *T. cruzi* strains, cultivated with bovine serum albumin-supplemented medium in LLC-MK2 cells. Detection of TS was done by an enzymatic assay based on the transfer of sialic acid residues to ^{14}C-lactose. Only a small percentage (less than 0.01%) of TS, from all *T. cruzi* strains tested, bound to cells. The binding was not affected by desialylation of cells and of supernatants by bacterial sialidase, or by the addition of sialyllactose to the binding assay. It was virtually abrogated, however, by depletion of amphiphilic molecules from parasite supernatants by precipitation with Triton-X114. The binding was inhibited by the addition of heat-inactivated fetal calf serum, and this inhibition was also reduced in serum depleted of amphiphilic molecules. The data described herein do not support the hypothesis that TS is a parasite ligand for host cells, since only a very small proportion of TS molecules, associated with or displaying hydrophobic structures, bound to host cells *in vitro*. This binding, however, could theoretically sensitise cells to the activity of immunological effector mechanisms *in vivo*. The present results also disallow using the observation that soluble TS inhibits the penetration of *T. cruzi* into host cells as evidence for TS being a cell-surface ligand for sialic acid.

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IM-141
SOME CLINICAL AND HISTOPATHOLOGICAL ASPECTS OF DOGS NATURALLY INFECTED WITH *LEISHMANIA (LEISHMANIA) CHAGASI* IN BELO HORIZONTE, MG, BRAZIL

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American visceral leishmaniasis is a zoonosis and dogs represent the main domestic reservoir. In the last decades, a spreading of this disease has been associated with urbanization of large cities, such as Belo Horizonte, Brazil.

In this study we have tried to make correlations among clinical, parasitological and histopathologycal features of the disease in dogs in attempt to understand the disease in these animals. Fifteen mongrel dogs naturally infected with *Leishmania chagasi* obtained from the City Hall of Belo Horizonte were previously classified as asymptomatic, oligosymptomatic and symptomatic (weakness, cutaneous lesions, alopecia, and clinical anemia were the most common symptoms). The dogs were killed with an overdose of Thionembutal (33%, 5ml/Kg dose, by i.v.) and samples of blood obtained for serological studies (indirect immunofluorescence test-IFAT) and liver function testing (TGO, TGP, Alkaline Phosphatase). During necropsy, livers were weighed and samples collected for Giemsa-smears (“inprints”). The slides (smears) were observed under a light microscope using a 100x objective to deter-

mine the liver parasite burden (L.D.U.). Other samples obtained from the same livers were fixed in formalin (10%, pH 7.0) for routine histophatologycal studies. Microscopically, intralobular granulomas were the main lesion observed in all infected dogs. The granulomas were rarely confluent and found mainly in the sinusoid lumen. Macrophages, parasitized or not, were the main cell type but there were also some epithelioid cells, a small number of lymphocytes and rare neutrophils.

Morphometrical analysis of hepatic granulomas was carried out using a Zeiss Imaging Processing Software (KS300). The average granuloma diameter was only significantly different when asymptomatic and oligosymptomatic dogs were compared (student's t test: pØ 0.05). Moreover, the L.D.U. and IFAT titers did not correlate with the number or size of granulomas and the clinical aspects. Liver function tests were normal in all animals. This study suggests that clinical aspects of canine visceral leishmaniasis are not adequate to indicate absence of lesions or disease. Future studies will be aimed at comparing clinical and immupathological features of the disease using immunohistochemistry and immunological assays.

IM-142
SPLICEOSOMAL FACTORS ARE RECOGNIZED BY CHAGASIC SERA

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Several studies have demonstrated autoantibodies in Chagas' disease, both in animal models and humans. Here we investigated the possibility that Chagas' patients develop an autoimmune response to human UsnRNPs or Sm epitopes. Using purified human UsnRNPs we detected anti-human UsnRNPs antibodies in sera from patients with Chagas' disease. The antibodies were also detected using peptide-ELISAs containing the Sm-motif 1 domain. The latter technique showed that 61% (31/51) of the Chagas sera contained antibodies against the Sm-motif 1. Preliminary results obtained by immunoprecipitation using Chagas cronically infected Balb/C sera and HeLa nuclear extracts showed that some autoantibodies could also be found during the course of the disease. Group of 20 mice (one-month-old) were infected i.p. with Y strain 30 bloodstream trypomastigotes and killed at 60 days post-infection and sera were used for immunoprecipitation analysis as mentioned. These antibodies cross-reacted with U2 snRNP in HeLa nuclear extract and revealed many different bands in *T. cruzi* nuclear extract. These results suggest that the autoantibodies may have a role in the pathogenesis of the disease that still remains unclear.

IM-143
STIMULATORY EFFECT OF THE GLYCOINOSITOLPHOSPHOLIPID PURIFIED FROM *TRYPANOSOMA CRUZI* ON B LYMPHOCYTE AND NATURAL KILLER CELL ACTIVITY

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Glycoinositolphospholipids (GIPLs) are one of the major glycolipids of the *T. cruzi* surface and several evidences suggest that its expression may be related to virulence. In the present study we investigated: a) if the *T. cruzi* GIPL (isolated from G strain) has a direct effect on murine B cells, and b) if natural killer (NK) cells would be one of the targets of the GIPL effect. B cells purified from mice deficient on both T and NK cells (CD3e transgenic mice) secreted immunoglobulin in response to the GIPL. This response was increased by the addition of a NK cell line to the culture. The *T. cruzi* GIPL also increased the interleukin 2-induced proliferative response of a NK cell line, without modifying interferon-g secretion. Taking together, our data indicate that the *T. cruzi* GIPL has direct stimulatory effect on both NK cells and B lymphocytes and suggest that *T. cruzi*-derived molecules may be one of the stimulators of the NK cells activation observed during infection.

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IM-144
STUDY OF APOPTOSIS PROFILE IN *PLASMODIUM FALCIPARUM* AND *P. VIVAX* ACUTE MALARIA

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Recent work have shown that individuals with *P. falciparum* acute infection present high percentages of spontaneous apoptosis in peripheral lymphocytes *in vitro*, compared with healthy individuals. Moreover, early in malaria

infection, drastic changes induced by *P. falciparum* in several different parameters of immune functions have been reported such as a decreased number of circulating T lymphocytes, *in vitro* depression of the proliferative response of pheripheral blood mononuclear cells (PBMC) to malaria antigens, decreased immune response to a plasmodial antigens (usually vaccines) and high levels of circulating soluble plasma interleukin-2 receptor (sIL-2R).Thus, the aim of this work is to study the apoptosis process in *P. falciparum* or *P. vivax* infections, in order to investigate a possible relationship with lymphocytes alterations observed during the infection. For this purpose, 100 blood samples from patients with *P. falciparum* (n=40) or *P. Vivax* (n=60) acute malaria were collected at Instituto de Medicina Tropical do Amazonas. We also collected 50 samples from individuals living in the same region, without a past malaria history. The percentage of apoptotic cells and their subsets are currently been analysed by cytometry flow after 24 hours of culture with antigenic (S20/87 *P. falciparum* strain), mitogenic (PHA) stimuli and just after a monoclonal anti-TCR/CD3 induction. The DNA fragmentation are been performed by eletrophoretic analysis. In order to evaluate a possible correlation of apoptotic nuclei percentages with antibody levels in these patients, plasma samples are being tested by ELISA with S20/87 *P. falciparum* strain antigen. Our preliminary results show that apoptosis mean in malaria patients was 19% and 22% to *P. falciparum* (n=12) and *P. vivax* (n=16), respectively, while 11% were found in controls (n=13). Concerning T-cell subsets we detected 18% and 15% of apoptosis for CD4+ and CD8+, respectively, contrasting with control individuals, where we observed only 8% for CD4+ and 12% for CD8+ T-cells. These data suggest a spontaneous apoptosis increase in malaria patients specially in CD4+ T-cells subsets.

IM-145
STUDY OF IMMUNE RESPONSE DURING *LEISHMANIA AMAZONENSIS* INFECTION IN STRAINS OF MICE SELECTED FOR SUSCEPTIBILITY AND RESISTANCE FOR ORAL TOLERANCE

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Selective breeding of genetically heterogeneous mice for quantitative antibody production has been developed by Biozzi et al. (1979). High (H) and Low (L) responder mice, resultant of selection experiments to various antigens were produced. These strains are useful tools in the analysis of the relative importance of humoral and cellular factors in resistance to infections and in the study of non-specific immunity. Our results are an evaluation of the importance of genetic control of immune function in the capacity of resistance to *Leishmania amazonensis* infection by using strains of mice selected for extreme phenotypes of susceptibility (TS) and resistance (TR) to peripheral immunological tolerance. Our concern is the observation of the existing correlation pattern between the aspects modified by selective process and the infection resistance to different patogenic agents. TS and TR mice were evaluated: (a) observations on footpad lesion increase by inject 1x 10^7 promastigote: TR mice showed higher increase of footpad thickness than TS mice. F1 animals (TR x TS) showed more resistance than original strains (TR=21$\pm$8.07; TS=9.6$\pm$4.4; F1=3.7$\pm$2.8 x10^{-1} mm). This suggests complementation between resistance-associated genes from two parental strains. (b) Mice more sensitive to infection, TR strain, showed more intense DTH response during infection course. The third week values are TR=3.15$\pm$1.19 e TS=1.12$\pm$0.73 x 10^{-1} mm. As a consequence of reverse correlation DTH x resistance we evaluated the non-immune macrophage activity of these strains. Special attention should be given to the macrophages for their potential participation in establishing the general capacity of TS mice in repressing the *L. amazonensis*. IgG levels also were higher in the more infection sensitive strain. The histopathological examination of infected footpads is in development. These preliminary data confirm the importance of selected strains for immunological tolerance as models for the study of the *Leishmania amazonensis* resistance.

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IM-146
STUDY OF THE IMMUNE RESPONSE TO *PLASMODIUM FALCIPARUM* PROTEINS IN A MALARIA ENDEMIC AREA IN BRAZIL (RONDÔNIA STATE)

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Several studies have been shown that antibodies have an important role in the acquisition of protective immunity against *P. falciparum*. Therefore, in the present work we eveluated the humoral immune response in autochthonous and migrant individuals living in a Brazilian endemic area (Porto Velho, Rondonia State) against malaria

vaccine candidate molecules. For this purpose, antibody frequencies against GLURP (R0 and R2), LSA-1, NANP (MAP), Nt47, SPf70, EBA and MSP3, as well as clinical and epidemiological data, were analyzed in individuals with different degree of exposure to malaria infection: *Group I*: autochthonous frequently exposed (RB, n=101); *Group II:* migrants frequently exposed (CL, n=86) and; *Group III*: individuals rarely exposed, living in an urban area (PV=120).

We observed that the group RB showed a frequency significantly higher of responders ($p < 0.05$) for the great majority of the antigens tested (6/8) regardless immunoglobulin types (IgG or IgM) and IgG isotypes: LSA-1, NANP, Nt47, GLURP R0, GLURP R2 and MSP3. It is worthy to point out that this group showed also parasitemia levels significantly lower than the group CL ($p < 0.05$). Isotype profile analysis of IgG antibodies against Nt47 revealed that the serum of these individuals with higher levels of cytophilic antibodies (IgG1 + IgG3 / IgG2 + IgG4) presented lower levels of parasitemia ($p < 0.05$). These data suggest that the antibodies directed to the antigens assayed in this study may contribute in the process of acquisition of anti-*P. falciparum* immunity.

IM-147
STUDY ON THE *IN VIVO* AJUVANT ACTIVITY OF COMPONENTS FROM MEMBRANES OF TACHYZOITE STAGE OF *TOXOPLASMA GONDII*

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The intracellular protozoa is a potent stimulator of cell mediated immunity, which is characterized by TCD+CD8- and TCD4-CD8+ lymphocytes, which produce high levels of IFN-g/IL-2 and IFN-g, respectively. In addition, the Ig isotype profile of antibodies anti-*Toxoplasma* antigens, induced during infection with this parasite in mice, is characterized by high levels of IgG2a and low levels of IgG1. It has been suggested that the early activation of the cellular compartment of the innate immune system is necessary for the development of a polarized Th1 response. One of our major interest is to identify *T. gondii* molecules that have the ability to initiate and/or potentiate the development of cell mediated acquired immunity. For this purpose we used a method previously employed to fractionate *T. cruzi* membrane components (Almeida, et al. J. Biochemistry 304:793, 1994). Tachyzoites (1 x 10E10) were obtained from tissue culture fibroblasts or peritoneal exudated from mice, washed several times with PBS, dried in a lyophilizer and submitted to a chloroform, methanol and water (C:M:W, 5:10:4). Aqueous and organic phase were obtained after centrifugation at 5000 x g. The organic phase was then dried in a speed vacuum and them ressuspended in a buthanol:water (2:1) mixture. The organic and aqueous phase were termed F1 and F2,respectively. The aqueous phase from C:M:W extraction was also dried in a speed vacuum and then re-extracted with 9% buthanol and centrifuged at 5000 x g. The supernatant and pellet were termed F3 and F4, respectively. All fractions (F1 to F4) were dried, resuspended into 1 ml of PBS, and the protein content measured using the Bradford method. Fractions F1 to F4 were tested for their ability to induce cytokine synthesis as well as nitric oxide (NO) release by murine inflammatory macrophages primed or not with 100 units of IFN-g. These results showed that the F1 presented the highest activity in terms of NO and IL-12 by IFN-g primed macrophages. Next we decide to further fractionate F1. A dried pellet of F1 received a sequential fractionation using different concentrations of Chloroform, Methanol and Water resulting in three new Fractions from more to less hydrophobic were termed F1a, F1b and F1c. These fractions were tested again in IFN-g primed macrophages and the F1c showed the highest activity in inducing NO and IL-12 synthesis. Therefore, we decide to test the adjuvant activity of F1c *in vivo*. BALB/c mice were immunized with ovalbumin (OVA) encapsulated into liposomes in the presence or absence of F1c derived from *T. gondii* tachyzoites. As controls we use animals vaccinated with liposome alone or liposome plus F1c but in absence of OVA. Animals were vaccinated subcutaneously, every 15 days for three times. Sera from individual mice were collected 1 day before each vaccine dose and used to measure the levels of OVA specific antibodies. Animals were then sacrificed at 4 and 6 weeks after the last immunization for measuring the antigen specific T cell responses as measured by proliferation and cytokine synthesis. Interestingly, we found that the group which received liposome plus OVA plus F1c presented the highest antibody and cellular responses to OVA. We are presently characterizing the cytokine and isotype profile of anti-OVA immune response, from animals belonging to the different groups.

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IM-148
T CELL SUBSETS IN PATIENTS WITH CUTANEOUS LEISHMANIASIS PRE AND POST CULTIVATION *IN VITRO*. ANALYSIS OF $CD8^+$ LYMPHOCYTE SUBSETS

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The proportion of T lymphocyte populations present in PBMC of patients with cutaneous leishmaniasis (CL) was analysed before and after *in vitro* cultivation in presence or absence of *Leishmania* antigen. Diagnosis of CL was confirmed by positive histopathology and leishmania culture. The lymphocyte populations were asssessed using anti-CD3, CD4, CD7, CD8, CD19, CD56, CD57, CD45RA or CD45RO monoclonals antibodies and phenotype analysis was performed by cytofluorometry. Cells were mantained in culture, 37^0C, 5% CO2 for 5 days in absence (Unst) or presence of 5 mg /ml of Soluble *Leishmania* antigen (SLA).

T lymphocytes present in PBMC of CL			
Lymphocyte Subset	Ex-vivo	Unst.	SLA
$CD4^+$	36 ± 8%	59 ± 6%	51 ± 8%
$CD8^+$	22 ± 5%	24 ± 5%	23 ± 4%
$CD19^+$	6 ± 3%	5 ± 3%	5 ± 3%
$CD56^+$	15 ± 7%	11 ± 4%	15 ± 4%
Memory markers present in $CD8^+$ lymphocytes			
Lymphocyte subset	Ex-vivo	Unst.	SLA
$CD8^+/45RA^+$	23 ± 2%	23 ± 4%	24 ± 4%
$CD8^+/45RO^+$	9 ± 5%	5 ± 3%	5 ± 2%
$CD8^+/28^-$	2 ± 2%	4 ± 2%	3 ± 2%
$CD8^+/57^-$	2 ± 0%	1 ± 0%	1 ± 1%
$CD8^+/57^+$	11 ± 6%	9 ± 4%	10 ± 3%

Based on these preliminary results we concluded that there is no change in the lymphocyte subsets in response to *Leishmania* antigen *in vitro.* Among the $CD8^+$ lymphocytes, the majority are naive cells ($CD45RA^+$). The proportion of cytotoxic cells observed by the presence of $CD57^+$ is approximately 10% of total of $CD8^+$ cells and do not increase after exposure to *Leishmania* Ag *in vitro.*

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IM-149
TRYPANOSOMA CRUZI TRYPOMASTIGOTE MUCINS INDUCE PROLIFERATION OF PERIPHERAL BLOOD MONONUCLEAR CELLS FROM NORMAL AND CHAGASIC INDIVIDUALS

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The pathogenesis of Chronic Chagas' disease cardiomyopathy (CCC) is still the subject of intense debate. Mucin-like glycoconjugates from *T. cruzi* are potent stimulators of IL-12 production by monocytes, and may be implicated in the shift to T1 profile observed in chronically *T. cruzi*-infected patients. Recent reports from our group showed that activated heart infiltrating T cell lines from Chagasic patients potentiated the production of IL-12 in the presence of *T. cruzi* mucins In an attempt to futher study role of mucins in the pathogenesis of Chagas disease, we analysed the PBMC proliferation from CCC (n=16) asymptomatic Chagas'disease patients (ASY n=3) and normal (N n=12) individuals in the presence of mucins derived from trypomastigotes and epimastigotes. The results showed that PBMC proliferative response to mucins derived form trypomastigotes were more frequent and intense (63%,with average SI =14.51 in CCC; 100% with average SI = 8.10 in ASY; 67% with average SI= 4.58 in N, respectively) an in the presence of mucins derived from epimastigotes (6%, with average SI = 1.29 in CCC; 0%, with average SI = 1.02 in ASY; 25%, with average SI = 1.65 in N respectively in the three groups tested. T cell proliferation was almost the same for N, CCC and ASY patients and the proliferative responses were almost completely abolished when adherent cells were depleted. This could indicate that a population contained in normal or *T. cruzi*-infected human PBMC is capable to proliferate in response to *T. cruzi* mucins the proliferating population may be plastic-adherent per se (eg monocytes) or dependent of adherent cells. The proliferative response verified in N individuals might indicate a T cell response to a crossreactive antigen, like that observed with *T. cruzi* B13 protein or the non-immune activation of another cell type. The nature of the proliferating cell type is currently being investigated, and this might be another mechanism relevant for the pathogenesis of *T. cruzi* infection.

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IM-150
TRYPANOSOMA CRUZI GLYCOINOSITOLPHOPHOLIPID AND ITS CERAMIDE DOMAIN INDUCES APOPTOSIS AND REGULATES THE EXPRESSION OF MHC CLASS II IN HUMAN MACROPHAGES

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It has been shown the effects of *Trypanosoma cruzi* – GIPL and its isolated carbohydrate and lipid moieties on the modulation of some functions of different cells from the immune system. Freire de Lima et al. (1997) showed that purified GIPL induced increased spreading and proliferation on the J774 cell line. The isolated lipid moiety when added together with the cytokine rINF-g lead to cell death due to apoptosis. We have investigated the effects of GIPL and its isolated domains on human monocytes/ macrophages. GIPL, CHO or ceramide at different concentrations in combination with rINF-g were added to 5 days cultured macrophages for 72 hours, and analyzed by flow cytometry for the expression of HLA-DR,DQ,DP. In order to investigate if GIPL and its moieties also induce apoptosis in human macrophages, we stained the cells to epifluorescence microscopy, with Acridine Orange or Hoescht. Our results showed that the GIPL and its ceramide portion in association with IFN-g also induced macrophage apoptosis, but the kinetics of induction was delayed, only seen in 72h of incubation. The CHO did not show any effect over macrophages. Our data suggests that this parasite molecule may mediate scape from immune response generated by Th1 cells, leading to suppression observed in early phases of infection. We are evaluating the early phases of apoptosis and the expression of several activation markers, and the intracellular route of the molecule.

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IM-151
THE ACTION OF PENTOXIFYLLINE (POF) IN EXPERIMENTAL CHAGAS'DISEASE

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During the acute phase of infection by *T. cruzi*, tumor necrosis factor (TNF) is present in sera of susceptible C3H.He/N mice, in contrast to its nearly complete absence in more resistant C57BL/6 strain. The decrease of TNF levels in susceptible mice by in vivo injection of Pentoxifylline (POF), a fosphodiesterase inhibitor, attenuated cachexia, decreasing mouse wasting and changed parasitaemia and mortality. The possible role for TNF during Chagas'disease was explored by Santos Lima and cols. (1998) using C57Bl/6 transgenic mice expressing in blood high levels of a soluble TNFR1-FcIgG3 fusion protein, that neutralizes the effect of TNF *in vivo*. The transgenic mice showed high susceptibility to *T. cruzi* infection, with higher than 80% mortality with sublethal dose of *T. cruzi*. Starobinas and cols.(1991) showed that, in acute Chagas'disease, TNF is produced in high levels in susceptible mice in contrast to in resistant mice. Liew and his group, working with CBA and BALB/c mice, strains naturally resistant and susceptible to *Leishmania major* infection, observed that both mice presented an enhancement of lesions when mice were treated by anti-TNF monoclonal antibodies. In addition, they observed that CBA infected mice treated with TNF-a intra-lesionally become more resistant to *Leishmania*. Cupolilo (1998) manipulated the immune response of different mice strains to cutaneous leishmaniasis by *L. amazonensis* using POF, and observed that TNF plays an important role in the earlier infection stages, inducing a favorable Th1 cytokine profile. Zabel and cols.(1993), in his work with POF, showed that this drug was capable of inhibit TNF production by elevating intracellular cAMP. Wenisch and cols.(1998), in a placebo-controlled randomized study, treated adults with severe *Plasmodium falciparum* malaria with standard anti-malarial treatment associated with POF (low or high doses) for three days. Their results showed that the high dose (40mg/Kg/day) of POF reduced TNF plasma levels. In previous experiments, we investigated the susceptibility and resistance of different inbred mice strains (A/J, C3H.He/N, C3H.He/J, BALB/c, C57Bl/6, C57Bl/10; female, 6 to 8 weeks old) to the CL and Y *T. cruzi* strains. We choose the C3H.He/N as susceptible and C57Bl/6 as resistant mice strains for the present data. Mice were infected subcutaneously with 10^4 blood form trypomastigotes obtained from OF1 mice in which the strains had been routinely maintained. The acute phase was determined by following the parasitaemia as described by Pizzi and Prager (1952). Mortality rate was followed by daily observation of each experimental group. Groups of 15 mice of either C3H.He/N or C57Bl/6 strains were infected with CL or Y strains. Two schedules were performed: one group received POF only at the first three days of infection; the other group received POF every day, for 20 days. In both groups mice received POF intraperitoneally, at doses of 10(low) or 50(high) mg/Kg. Susceptible C3H.He/N mice treated with POF in either low or high doses showed an altered course of the parasitaemia by comparison with infected control group. In group treated with POF daily, parasitaemia was lower and occurred earlier than control. The mortality of this group started earlier than control, although their duration had been the same.

Studies investigating the kinetics of TNF are being carried out.

IM-152
THE b CHEMOKINE JE/MCP-1 MEDIATES IN VIVO CHEMOTAXIS OF *TRYPANOSOMA CRUZI* AMASTIGOTE FORMS

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Infection with the protozoa *T. cruzi* leads to an acute infection characterized by circulating parasites in the bloodstream and a strong inflammatory responses in multiple tissues followed by a chronic stage in which almost no circulating parasites can be found. Since its first observation, it was noticed that different *T. cruzi* strains behaves differently according to the most parasitized tissue or tissue-tropism. However, several mechanisms were proposed to explain these differences without a complete understanding of this process. In the present study, we demonstrated that circulating parasites respond to the chemotactic stimuli provided by injection of the b chemokine JE/MCP-1 into the air pouch in the back of infected BALB/c mice. We found a strong amastigote migration to the site of injection, which was time- and dose-dependent. The addition of antibodies against JE/MCP-1 blocked this effect, suggesting a specific effect. The high counting of amastigotes into the air pouch led us to suppose that the migration could be inducing the morphogenesis from circulating trypomastigote forms to tissue-adapted amastigote forms. Based on this hypothesis we tested the direct effect of the addition of JE/MCP-1 to trypomastigote forms, and surprinsingly, we observed time- and dose-dependent amastigote forms morphogenesis. Moreover, the amastigote morphogenesis could be blocked by antibodies anti-JE/MCP-1, suggesting a direct effect of JE/MCP-1 in this phenomena. Further, this event could also be blocked in the presence of Pertussis toxin, evidentiating that the parasite receptor of JE/MCP-1 is a G protein-coupled receptor. We performed binding assays in Nitrocellulose imobilized parasites in which we can observe an specific binding of JE/MCP-1 to the parasites. Altogether, the results presented here provides evidence of a functional JE/MCP-1, G protein-coupled receptor on the surface of *Trypanosoma cruzi* parasites. These results may provide helpful data on the comprehension of the pathogenesis of Chagas' disease, as well as to understand the specific tropism also observed during infections with other protozoa.

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IM-153
THE DETECTION OF ANTIBODIES IN HUMAN CASES OF CUTANEOUS LEISHMANIASIS BY THE ELISA TEST USING DIFFERENT SPECIES OF AMAZONIAN *LEISHMANIA* AS ANTIGEN

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In the Lower Amazon region 6 named species of *Leishmania* are known to infect man and others have been isolated but not yet described. The present study was undertaken to compare antibody titres using homologous and heterologous antigens to some of these parasites. Plasma samples from 48 patients suffering from cutaneous leishmaniasis and 30 from individuals with no known history of the disease were examined by the ELISA test. Of the patients 26 were infected with *Leishmania* (*Leishmania*) *amazonensis*, 9 with *L.* (*Viannia*) *braziliensis* and 13 with *L.* (*V.*) *shawi*. Thirty eight had uncomplicated cutaneous infections (CL), 8 suffered from anergic diffuse cutaneous leishmaniasis (ADCL) and 2 had mucosal lesions (MCL). Antigens were made from *L. (L.) amazonensis*, *L. (V.) braziliensis* and *L. (V.) shawi* promastigotes by 10 cycles of freezing (-182ºC) and thawing (37ºC). Tests were read in a spectrophotometer at 492nm. A peroxidase labelled human anti-IgG conjugate was used in conjunction with O-phenylenediamine dihydrochloride to visualize the positive reactions. It was found that the sensitivity of the test varied according to the species of parasite used to prepare the antigen. The overall sensitivity for the cases of uncomplicated cutaneous leishmaniasis was 89.5% for the *L. (L.) amazonensis* antigen, 76.3% for the *L. (V.) braziliensis* antigen and 94,7% for the *L. (V.) shawi* antigen. The sensitivity of all 3 antigens was 100% with the plasma from the DCL cases. For normal individuals the specificity was 73,3% for the *L. (L.) amazonensis* and *L. (V.) shawi* antigens and 70% for that of *L. (V.) braziliensis* . The ELISA tests results indicated that both *L.(V.) shawi* and *L. (L.) amazonensis* antigens were more sensitive and more specific in detecting antibodies in cases of cutaneous leishmaniasis caused by some of the commoner Amazonian *Leishmania* species. *L. (V.) braziliensis* antigen was proportionally less specific and sensitive. The greater sensitivity of the *L. (V.) shawi* antigen suggests that it is the antigen of choice for detecting antibodies in cases of active cutaneous leishmaniasis in the Lower Amazon region. However these observations need to be confirmed by immnoblotting.

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IM-154
THE FUCOSE-MANNOSE LIGAND OF *LEISHMANIA (L.) DONOVANI* IN DIAGNOSIS AND PROGNOSIS OF CANINE VISCERAL LEISHMANIASIS

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São Gonçalo do Amaranto, is a district located in the State of Rio Grande do Norte, Brazil, endemic for both human and canine visceral leishmaniasis (CVL). The FML-ELISA assay previously showed 100% sensitivity and 96% specificity identifying human patients with Kala-azar and subclinical assymptomatic individuals that further developed overt Kala-azar. In the present study, the diagnostic and prognostic potential of the FML-ELISA assay was investigated on canine visceral leishmaniasis, in sera from naturally and experimentally infected dogs. The FML-ELISA using 2 mg/well and mean value + 3SD as cut-off discriminated dog sera with clinically, parasitologically and serologically confirmed Kala-azar from normal dog sera of a non-endemic area, showing 100% sensitivity and 100% specificity (tegumentar leishmaniasis, toxoplasmosis, demodesic boils, dirofilariasis, dipetalonema, canine distemper or from animals infected with *B. canis*, *E. canis*, *Leptospira sp.*, *Toxocara canis*, *Trichuris vulpis* and *Ancylostoma caninum*). The overall prevalence of anti-*Leishmania* antibodies in canine sera of the endemic area was 23% (79/343). The seroreactivity disclosed by an Immunofluorescent assay (IF) was much lower: 2,9% (10/343), and close related to the percent of kala-azar symptomatic dogs 2.6% (9/343). 21/21 assymptomatic, FML-seropositive animals death from kala-azar in a period ranging from 0-6 months after diagnosis, demonstrating its highly predictive value (100%). An ELISA assay with *L.(L.) mexicana* soluble antigen recognized 9/21 of these samples while *L.(L). mexicana* and *L.(L.) chagasi* IF assays disclosed only 5/21 making 33 and 24% of predictivity, respectively. In FML-QuilA saponin vaccinated dogs, seropositivity was disclosed 7 days after the first vaccine dose as seen by the FML-ELISA assay, after the second dose by the *L.(L.) mexicana* IF assay and only after the third dose by the *L.(L.) amazonensis* ELISA assay. In experimentally infected control dogs (10^8 *L.(L.) donovani* amastigotes), all the assays disclosed seropositivity 90-120 days after infection showing similar sensitivities: 20% for the FML-ELISA and 27% and 20% for the *L(L.)mexicana* IF and ELISA assays, respectively. Since the current strategy for control of CVL is based on detection and destruction of infected dogs, the highly predictive, sensitive and specific FML-ELISA assay represents an useful tool for field control of the disease.

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IM-155
THE *IN VIVO* CHEMOKINE GENE EXPRESSION IN INFLAMMATORY SITE FROM SUSCEPTIBLE BALB/C AND RESISTANT C57BL/6 MICE INFECTED WITH *LEISHMANIA MAJOR*

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Chemokines are a group of chemoattractant cytokines that play crucial role on especific cell migration to an inflammatory site. They have four structurally conserved cysteines residues and the first two may be separated from each other by any amino acid (CXC or a-chemokines) or be adjacent to each one (CC or b-chemokines). The a-chemokines attract neutrophils (e.g. MIP-2), NK as well as activated T lymphocytes (e.g. IP-10 and MIG). The b-chemokines are related to the attraction of monocytes, NK cells as well as activated T lymphocytes (e.g.RANTES, MIP-1a/b MCP-1/JE, MCP-5). In the present study we focused our attention on expression of above chemokine mRNAs during acute and chronic inflammation caused by infection with *L. major*. Resistant C57BL/6 and susceptible BALB/c mice were inoculated with 1 x 10E6 stationary phase of *L. major* in the right footpad and sacrificed at days 1, 2, 14, 42 and 77 post-infection and total RNA extracted from the footpad. The expression of chemokines mRNA was tested by RT-PCR. Our results show that expression of various chemokines in the footpad were already stimulated within 24h of infection and kept being expressed until later time-points. In BALB/c mice the chemokines MCP-5, IP-10 and RANTES were expressed in higher levels at 1 and 2 days post infection. Interestingly, expression of different chemokines in susceptible mice was modulated at 14 and 42 days after infection, when higher tissue parasitsm was observed. In contrast, in C57BL/6 mice, the MCP5, RANTES, IP-10 and MIG were poorly expressed at days 1, 2 and 14 and highly expressed at days 42 and 77 post-infection. Thus, our preliminary results suggest there is a distinct pattern on chemokines kinetics comparing BALB/c and C57BL/6 mice to *L. major* infection. It is possible that the chemokine genes transcription is influenced by either the type 1 and type 2 cytokine synthesis which are observed during infection with *L. major* in resistant and susptible mice, respectively.

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IM-156
THE MODULATORY EFFECT OF CAMP ON IL-12(P40) BUT NOT ON TNF-a SYNTHESIS BY INFLAMMATORY MACROPHAGES STIMULATED WITH MICROBIAL PRODUCTS IS DEPENDENT ON IL-10

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We have recently identified a glycosylphosphatidylinositol-anchored mucin-like glycoprotein (GPI-mucin) as a main membrane component responsible for induction of cytokines by macrophages exposed to *Trypanosoma cruzi* trypomastigotes. One of the main interest of our research is to identify the signaling pathway(s) involved in the induction or regulation of the pro-inflammatory cytokines which are elicited during infection with *T. cruzi*. In the present study, we used murine inflammatory macrophages primed with IFN-g (100 units/ml) stimulated with either GPI-mucin (1 pmol/ml) or LPS (100 ng/ml) in the presence or absence of different inhibitors of specific signaling pathways. Among the different inhibitors tested, we found that Cholera Toxin an activator of *adenylate cyclase* was a potent inhibitor of TNF-a and IL-12(p40) synthesis with an IC50 lower than 1 ng/ml. In order to confirm that the inhibitory effect of Cholera Toxin was due to the generation of cAMP we used two cAMP analogues which mimic the action of cAMP *in vivo*. Either 8-bromo cAMP (IC50 30 mM for both IL-12p40 and TNF-a) or dibutyryl cAMP (IC50 30 mM for IL-12p40 and 15 mM for TNF-a) were found to inhibit both TNF-a and IL-12(p40) synthesis by macrophages stimulated with GPI-mucins or LPS. Because prostaglandins of the E series (PGE) are physiological stimulators of cAMP generation, we also tested the ability of PGE2 for its ability to modulate TNF-a and IL-12 synthesis by macrophages exposed to microbial stimuli. Our results show that after stimulation with either LPS or GPI-mucins, PGE2 inhibited TNF-a and IL-12(40) synthesis with IC50s of 1×10^{-7} M and 1×10^{-6} M, respectively. Since high intracellular levels of cAMP have been shown to augment IL-10 expression in T lymphocytes and macrophages, we decided to investigate a possible role for IL-10 on the cAMP modulatory effect on IL-12(p40) and TNF-a synthesis. Our data demonstrate that IL-10 is a potent inhibitor of GPI-mucin induced IL-12(40) and TNF-a synthesis, even at concentration as low as 0.5 ng/ml. Our experiments also show a gradual increase of IL-10 synthesis by macrophages stimulated with microbial products cultured in the presence of increasing concentrations of either Cholera Toxin, 8 bromo cAMP or dibutyryl cAMP. Finally, in order to test the importance of endogenous IL-10, we used macrophages from IL-10 knockout mice. For our surprise, cAMP inhibited microbial stimuli-induced IL-12(p40) but not TNF-a synthesis in macrophages lacking a functional IL-10 gene. Consistent with these findings are the kinetics of cytokine synthesis by macrophages showing a relative early TNF-a (peaking at 4 h post stimulation) versus late synthesis of IL-12p40 (peaking at 24 h post-stimulation) upon stimulation with either GPI-mucins or LPS. The understanding of the mechanisms controlling of macrophage stimulation by microbial products may suggest novel ways of controlling parasite-induced immunopathology.

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IM-157
THE T CELL SUPPRESSION INDUCED BY *LEISHMANIA AMAZONENSIS* ANTIGENS MAY BE DUE TO APOPTOSIS

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We have systematically observed that *L. amazonensis* antigens suppress murine lymphocyte proliferation in vitro in a dose-dependent manner. As an attempt to identify the suppressive substance(s), promastigote lysate was fractionated in water and MeOH/CH3Cl. We found that the active substance(s) is water-soluble and that removal of lysate lipids with MeOH/CH3Cl does not affect its capacity to inhibit mitogenic responses of lymphonode cells by Concanavalin A. The suppressive activity is not triggered during the preparation of the lysate, as the same level of anergy is achieved with live parasites.

Addition of IL-2 (50 U/ml), IL-4 (100 U/ml), IL-12 (5 ng/ml), IFN-g (50 U/ml) or Indometacin (125 mg/ml) alone or in combination did not restore the capacity of lymphonode cells from infected mice to respond to parasite antigens, suggesting that the anergic state induced by the lysate is not due to lack of those cytokines or to the presence of PGE2. Pre-incubation of cells in the presence of the lysate for 48h followed by its removal permanently impairs their mitogenic response.

To determine whether the anergy was associated with apoptosis, lymphonode cells from normal or infected BALB/c mice were incubated for 17h in the presence of varying concentrations of the lysate, and their isolated DNA analysed for fragmentation in agarose gel. We observed that the lysate induced a very strong DNA fragmentation in both cell cultures, particularly in the cells from infected animals.

Altogether, these results suggest that T cell anergy induced by *L. amazonensis* lysate in vitro envolves an apoptotic process.

IM-158
TISSUE EXPRESSION OF INDUCIBLE NITRIC OXIDE SYNTHASE IN MYOCARDIAL TISSUE OF MICE EXPERIMENTALLY INFECTED WITH *TRYPANOSOMA CRUZI*

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Inducible nitric oxide synthase (iNOS), the enzyme that catalyzes the conversion of L-arginine and molecular oxygen to L-citrulline and NO was shown to be expressed in a variety of cells, including cardiac myocytes. An increasing number of studies demonstrate that NO is related to protection to many different pathogens. On the other hand, it has also been demonstrated that NO can have a deleterious effect due to its cytotoxicity, resulting in tissue damage. Infection with *Trypanosoma cruzi*, the etiologic agent of Chagas disease, can induce severe destruction of the heart.

We have previously shown that BALB/c, C3H and C57BL/10 mice infected with the Colombian strain of *T. cruzi*, present high serum levels of nitrate as compared to other strains of the same H-2 haplotype (DBA, CBA and C57BL/6 respectively). Levels of serum nitrate were found to parallel the parasitemia. Here, we have examined the tissue expression of iNOS in hearts of these mice infected with *T. cruzi*. Immunohistochemical staining with an iNOS-specific antiserum (Transduction Labs, USA) revealed that the intensity of iNOS expression in cardiac tissue correlates to the serum levels found in these mice: BALB/c, C3H and C57BL/10 show more iNOS staining than the other strains of the same haplotype. The iNOS expression was found to be mostly near inflammatory foci, often surrounding parasite nests. C57BL/6, CBA and BALB/c mice are able to survive the acute infection and develop a chronic disease. The expression of iNOS persisted in chronic cardiac lesions (300 days of infection) in the last two mice strains. These results suggest that NO is likely to have a role in the tissue damage of *T. cruzi* infection.

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IM-159
TNF-a AS A MARKER OF THERAPEUTIC RESPONSE IN CUTANEOUS LEISHMANIASIS

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Increased levels of TNF-a have been documented in sera, lymphocyte supernatants and tissue of patients with cutaneous leishmaniasis. The major aim of this study was to evaluate if TNF-a could be a marker of therapeutic response in cutaneous leishmaniasis. Participants of the study included 65 patients with cutaneous leishmaniasis ulcer with less than 1 month of illness duration, and ulcer size ranging from 10 to 30mm. Mononuclear cells were stimulated with leishmania antigen and TNF-a and IFN-g levels measured by ELISA before and after therapy (60 days). Patients were treated with intravenous pentavalent antimonial (20mg/kg/weight) four 20 days and the end point for analyze the role of TNF-a as a marker was 60 days. Total cure was considered when there was a scar after 60 days of therapy and absence of recurrence or appearance of a new lesion one year after therapy. Of the 65 patients 45 had a partial cure and 20 a total cure after 60 days of therapy. There was no difference when the levels of TNF-a before therapy in the patients with total cure (627 ± 701pg/ml) were compared to the patients with partial cure 421 ± 369pg/ml. TNF-a levels decreased significantly ($p<0.01$) in the group with TC (88 ± 101pg/ml) while in patients with partial cure there was only a slight reduction (323 ± 316pg/ml) ($p<0.05$). These data indicate that decreasing in TNF-a levels is a marker of therapeutic response in patients with cutaneous leishmaniasis.

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IM-160
TOXOPLASMA GONDII INHIBITS NITRIC OXIDE PRODUCTION BY ACTIVATED MOUSE MACROPHAGES DERIVED FROM BLOOD MONOCYTES

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It has been shown that mouse macrophages derived from blood monocytes (Møno) treated with interferon-? (INF-?) and lipopolysaccharide (LPS) cannot kill trypomastigotes of *Trypanosoma cruzi* and produce nitric oxide (DaMatta *et al*., *Men. Inst. Oswaldo Cruz, Suppl*. I, 92: 230, 1997). Trying to understand the biology of nitric oxide production by Møno, different culture conditions were used and interactions with *Toxoplasma gondii* and *T. cruzi* performed. Møno were obtained by blood monocytes purification on a Percoll cushion and cultivated for 6 days in Dulbecco's Modified Eagle's Medium (DMEM) containing 5 % inactivated fetal bovine serum (FBS) with or without macrophage colony-stimulating factor (MCSF - 10% of L929 conditioned medium). Peritoneal macrophages were cultured for 24 hours in DMEM containing 5 % FBS. One day before the interaction, macrophages were activated with INF-g and LPS. Bloodstream trypomastigotes of the Y strain of *T. cruzi* were purified from blood harvested from mice on the seventh day post infection. Tachyzoites of the RH strain, were obtained by peritoneal washes of infected mice. Before the interaction, macrophages were washed with DMEM and infected with the parasites in a 10 or 100 to 1 parasite/macrophage ratio. After 2 hours of interaction macrophages were washed with

DMEM and new medium containing FCS, supplemented or not with INF-g LPS and MCSF, was added. After 24 and 48 hours the supernatants of the cultures were collected and assayed with the Griess reagent for the presence of $NO2^-$. Mouse blood monocytes cultured with MCSF resulted in a macrophage that, when activated, was able to produce high levels of nitric oxide (NO). NO production by Møno was inhibited by interactions with Tachyzoites of *T. gondii*. However, high levels of NO could be produced with *T. cruzi*. To determine if *T. gondii* could inhibit NO production by activated peritoneal macrophages, interactions were also performed. Interactions with 100 to 1 parasite/macrophage ratio showed a 30 % NO production inhibition when compared to activated macrophages alone. These results suggest that *T. gondii* owned a deactivating macrophage mechanism.

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IM-161
TRYPANOSOMA CRUZI INFECTION WITH LOW NUMBER OF PARASITES TRIGGERS INTENSE MYOCARDITIS IN MICE HIPERIMMUNIZED WITH SYNGENEIC HEART ANTIGENS

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Infection with *T. cruzi* elicits inflammatory cell accumulation in the heart and often leads to severe myocarditis. The pathogenesis of heart damage is controversial and autoimmune phenomena have been implicated in the etiology of the chronic cardiopathy in Chagas' disease. In this study we have investigated the effect of immunization with crude heart extracts on the development of myocarditis upon infection with *T. cruzi*. DBA/2 or C57Bl/6 mice were repeatedly immunized with heart extract/Freund's adjuvant (Group A) or saline/Freund's adjuvant (Group B) for 2 months. After a complete immunization protocol, the animals were bled and sacrificed for histopathological analysis. No pathological changes were observed in myocardium of either group. *In vitro* proliferative response of spleen cells to myocardial antigens was undetectable in both experimental groups in spite of detection of anti-heart antibodies in group A (ELISA). Mice were challenged i.p. with 10^2 blood trypomastigotes (Y strain). In this model, low numbers of parasites were detectable in the bloodstream up to day 30^{th} after infection and no mortality was observed. After 60 days of infection, group A mice infected with *T. cruzi* developed intense myocarditis as judged by gross alterations (cardiac enlargement plus apical aneurysm) and histopathologic changes (intense multifocal myocarditis). Infected animals from group B showed only mild inflammatory changes of heart muscle. No significant inflammatory alterations were observed in the skeletal muscle of mice from both groups. These results suggest that immunization with heart extract leads to expansion of heart-specific self-reactive lymphocytes that promote myocarditis albeit heart fibers are damaged by *T. cruzi* infection.

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IM-162
USE OF RECOMBINANT PEPTIDE ANTIGENS OF *TRYPANOSOMA CRUZI* IN A DIAGNOSTIC TEST FOR CHAGAS' DISEASE

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In recent years, the use of *T. cruzi* recombinant peptide antigens (RA) has provided a new approach to the accurate immunologic diagnosis of Chagas' disease. Several *T. cruzi* genes have been cloned and some of them have been assayed for their use in serodiagnosis. The results presented in this report describe the use of 3 RA peptide, which have been shown to increase sensitivity and specificity of Chagas' disease. To evaluate of the sensitivity and specificity of specific peptides antigens in the serodiagnostic of Chagas' disease in blood donors centers from Salvador, Bahia, Brazil. We carried out a prospective cross-sectional study, in 2 blood banks in Salvador, BA. The blood donors were submitted to xenodiagnosis and blood collected to perform *T. cruzi* culture and serology. ELISA was used to assay the 3 specific reagents (tripeptide, tetrapeptide and *Tc*F) antigens originated from the *Tc*D epitope cloned by screening of *T. cruzi* genomic library with sera from chronic Chagas' disease patients. There were expressed or synthesized at Corixa Corporation, Seattle, WA and made available to this study. From May 1997-July 1998 we screened 86 blood donors: 58 were positive for tetrapeptide, 52 for both tripeptide and *Tc*F. From 78 of 86 donors, which have already tested with xenodiagnosis 8 were positive and all were also positive with the 3 tests, and only one from the 8 was negative with the tripeptide. By using the clinical and/or epidemiological criteria for *T. cruzi* infection, 48 were identified. Again, all were positive with the peptides. However, 1 was negative with the tripeptide, another with the tetrapeptide and the other with the *Tc*F. So, sensitivity was 100% for the peptides antigens. In order to test the specificity for the ELISA peptides 41 individual with no exposure in endemic areas and

with negative Indirect Immunofluorescence Test, and classical ELISA for *T. cruzi* infection did not react with these peptides recombinant. Interesting, 20 blood donors positive with either hemaglutination test or Classical ELISA also were negative with the peptide recombinant antigens and xenodiagnosis. Specific *T. cruzi* peptides combinations are well defined and highly sensitive and specific antigens for antibody detection of *T. cruzi* infection. Cross reactions, batch variations of crude antigens, and instability of automated methods no longer will exist with these new tool for the serodiagnostic of *T. cruzi* infection.

IM-163
VACCINATION OF RHESUS MACAQUES AGAINST CUTANEOUS LEISHMANIASIS (CL) USING KILLED *LEISHMANIA* (*L.*) *MAJOR* VACCINE

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Leishmaniasis is one of the major parasitic diseases target by the World Health Organization (WHO 1990 Tech Rep Ser 793). The solid immunity observed following convalescence to leishmaniasis has suggested that vaccination may prove to be the most cost-effective intervention method for the prevention and control of the various leishmanial infection at a population level. Both cellular and humoral immune responses to leishmanial antigens and resistance to reinfection usually develop in subjects vaccinated against CL using living *L.*(*L.*) *major* promastigotes. However, large scale human trials have clearly indicated that protection requires prior controlled induction of disease with a virulent parasite (reviewed in Grimaldi JrG 1995, *Mem Ist Oswaldo Cruz 90*: 553-556).

Prophylatic immunization using killed promastigote vaccine is currently only in experimental stages. In this project, we are evaluating the usefulness of autoclaved *L.* (*L.*) *major* vaccine in the Asian rhesus macaques (*Macaca mulatta*), an experimental model for study of CL (Amaral et al. 1996, *Exp Parasitol 82*: 34-44). The healthy rhesus macaques (N=14) received a triple vaccination, either with (*i*) a total dose of 3 mg autoclaved *L. major* promastigotes, mixed with BCG (injected intradermally) [group A] or (*ii*) BCG alone [group B]. The immunologic features after vaccination were assessed. The results of this trial demonstrated that the combined vaccine [group A] results in the stimulation of an specific immune response in a high percentage of vaccinees (>90%), manifested by lymphocyte proliferation *in vitro* to leishmanial antigen. This was evident after the first dose of vaccine for lymphoyte proliferation and was maintained for at least a month after three doses of vaccines. In comparison to PPD-stimulated cultures, lower proliferative responses of T lymphocytes to parasite antigen was detected in Group B (which received BCG alone). Antigen-stimulated cells were harvested for CD4 and CD8 phenotype analysis and the levels of gamma interferon (IFN-g), interleukin 2 (IL-2) and IL-4 produced will also be determined. However, no skin test conversion was recorded in these experiments (the skin-test reactivities to parasite antigen and PPD were reassessed at 4-10- and 14-17 week follow-ups). Further studies are required to determine whether the presence of proliferative (and/or IL-2, IFN-g responses) in the absence of a skin test response are sufficient indicators of potential vaccine success. Currently we are testing if these responses elicit substancial resistance to a subsequent challenge with virulent *L. major.*

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IM-164
VACCINATION AGAINST CANINE KALA-AZAR WITH THE FML ANTIGEN AND QUIL-A SAPONIN IN AN ENDEMIC AREA (SÃO GONÇALO DO AMARANTE, RN)

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The American visceral leishmaniasis is a canine zoonosis. Canine vaccination against the disease should protect dogs reducing the reservoir of *L. donovani* infection.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%, $p<0.001$) and the Swiss albino (85%, $p<0.01$) models. The *Quillaia saponaria* saponin: Quil-A is an adjuvant that showed to be effective in experimental vaccination against *T. cruzi*, *Babesia canis*, *Cytomegalovirus*, HIV, etc. In this work we report the results of vaccination with FML+saponin in dogs of a Kala-azar endemic area, São Gonçalo do Amarante, RN. Only domiciliary dogs were included in this trial. A first serological screening was performed with the FML-ELISA assay. The seronegative individuals were divided into two groups: one treated with saline and the second with three subcutaneous doses of FML (1.5mg) and saponin (1mg). A fourth dose of vaccine was given 8 months after. Two and seven months after the last immunization, the seroreactivity to FML and DTH response to *L. (L.) donovani* promastigote lysate was recorded. A significant increase of the FML-seropositive animals was observed in the vaccinated group in relation to saline control in the three screening (%): 46/100 after 2 months and 32/96 after

7 months. Seropositivity along the time was highly correlated to FML vaccination (c2= 14.382 and 21,717, p<0.005, respectively). The IDR (24h was also significantly increased in the vaccinated group over the saline control (%): 50/100 and 32/100 (p<0.005). IDR positive reaction along the time was also highly correlated to FML vaccination (c^2=21.284; 13.444, p<0.005). The IDR measured after 48 and 72h was also significantly higher in vaccinated animals since 2 months after vaccination. The increase in FML-seroreactivity and IDR reactions in dogs of the saline treated group is the result of recent infections (after complete vaccination). The protective effect of the FML+saponin vaccine is strongly supported by the detection of 1 obits due to kala-azar in dogs of the saline control group while no death was detected in the group of FML+saponin vaccinated animals. Protection against canine Kala-azar due to FML- vaccine is also probably related to the reduction of human disease in this area. Indeed, São Gonçalo district represented until 1996 the origin of 6% of total human Kala-azar cases in Rio Grande do Norte. This dog vaccination trial started at December 1996. The number of human cases in this area decreased from 15 cases in 1996 to 6 cases until July 1997 and to zero until May 1998. The total number of kala-azar patients in 1998 was 40 (in Giselda Trigueiro and Varela Santiago Hospitals) with 6 obits but none of these cases was came from São Gonçalo.

Support PCDEN, PNUD, FNS, Finep, CNPQ Capes, MCT, Pronex, Rhae, FUJB, UFRJ, Faperj, CEPG.

IM-165
VACCINATION AGAINST CANINE KALA-AZAR WITH THE FML ANTIGEN AND RIEDEL DE HAEN SAPONIN IN AN ENDEMIC AREA (SÃO GONÇALO DO AMARANTE, RN)

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Prevention of canine zoonotic visceral leishmaniasis appears to be the best approach for interrupting the domestic cycle of the disease, reducing the human cases of Kala-azar. In previous work, using the FML antigen of *Leishmania donovani* and the Riedel De Haën saponin, we were able to achieve an 87.7% (p<0.01), 84% (p<0.001) 85% (p< 0.01) average protection against Kala-azar, in the isogenic CB hamster and Balb/c and Swiss albino mouse models. In this work we report the results of vaccination with FML+saponin (Riedel de Haën) in dogs of a Kala-azar endemic area, São Gonçalo do Amarante, RN. Only domiciliary dogs were included in this trial. A first serological screening was performed with the FML-ELISA assay. The seronegative individuals were divided into two groups: one treated with saline and the second with three subcutaneous doses of FML (1.5mg) and saponin (0.5mg). 12 months after vaccination a fourth dose was injected. Forty five days, 7 and 12 months after the last immunization, the seroreactivity to FML and DTH response to *L. (L.) donovani* promastigote lysate was recorded. A significant increase of the FML-seropositive animals was observed in the vaccinated group in relation to saline control in the three screening (%): 14/62 after 45 days, 45/97 after 7 months and 40/97 after 13 months. Seropositivity along the time was highly correlated to FML vaccination (c2= 10.802, 23,252 and 27,420, p<0.005, respectively). The IDR (24h was also significantly increased in the vaccinated group over the saline control (%): 15/58; 37/97 and 25/94. IDR positie reaction along the time was also highly correlated to FML vaccination (c^2= 20.529; 12.798; 33.430, p<0.005). The IDR measured after 48 and 72h was also significantly higher in vaccinated animals since 7 months after vaccination. The increase in FML-seroreactivity and IDR reactions in dogs of the saline treated group is the result of recent infections (after complete vaccination). The protective effect of the FML+saponin vaccine is strongly supported by the detection of 4 obits due to kala-azar in dogs of the saline control group while no death was detected in the group of FML+saponin vaccinated animals. Protection against canine Kala-azar due to FML- vaccine is also probably related to the reduction of human disease in this area. Indeed, São Gonçalo district represented until 1996 the origin of 6% of total human Kala-azar cases in Rio Grande do Norte. This dog vaccination trial started at December 1996. The number of human cases in this area decreased from 15 cases in 1996 to 6 cases until July 1997 and to zero until May 1998. The total number of kala-azar patients in 1998 was 40 (in Giselda Trigueiro and Varela Santiago Hospitals) with 6 obits but none of these cases was came from São Gonçalo.

Support PCDEN, PNUD, FNS, Finep, CNPQ, Capes, MCT, Pronex, Rhae, FUJB, UFRJ, Faperj, CEPG.

IM-166
VACCINATION AGAINST EXPERIMENTAL CANINE KALA-AZAR WITH THE FML ANTIGEN OF *LEISHMANIA DONOVANI* AND THE QUIL-A SAPONIN

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The American visceral leishmaniasis is a canine zoonosis. Vaccination against the disease should protect dogs reducing the reservoir of *L.(L.) donovani* infection. In this work we immunized mongrel dogs with three doses of

1.5mg of FML, either with 1mg or 0.5mg of QuilA through the sc route. Control animals received only saline. One month after immunization, animals were challenged with 10^8 amastigotes through the iv route. The protective response was monitored upto 540 days after infection, monthly by titration of anti-FML antibodies by the FML-ELISA assay, IDR to promastigote lysate, analysis of: the proliferative *in vitro* response of blood mononuclear cells to promastigote lysate, FML and GP36 antigens, hematocrit and clinical signs. Statistic analysis of the IDR response and clinical signs was performed by the x^2 test and for the rest of the variables by the ANOVA analysis-SPSS program. The anti-FML antibodies increased in both vaccinated groups: 1mg (0.371 ± 0.032) and 0.5mg QuilA (0.350 ± 0.046) over the saline control (0.086 ± 0.046) 7 days after the first vaccination dosis. Both vaccinated groups attained a "plateau" after the complete vaccination maintaining high titers (>1.200) up to 540 days after infection. The saline group started to develop an antibody response only 90 days after the infection. The anti-FML response was significantly higher in both vaccinated group (time and treatment) than in saline control upto 540 days after infection ($p< 0.001$). The IDR response was positive and significantly higher than saline control, only in the group vaccinated with FML+1mg QuilA ($p<0.01$). While the saline treated animals showed IDR + between 90-120days after infection and loose the response after 210 days, the vaccinated group maintained IDR+ since the infection and along the whole period. The differences between the *in vitro* proliferative response (GP36 antigen) were signifficant along treatment and time ($p<0.001$). The group treated with 0.5mg QuilA was different both from the one treated with 1mg and saline control. Although differences in hematocrit percents were no signifficant, probably due to the heterogeneity of the animal model, platelets count were signifficantly lower in saline control than in vaccinated animals ($p<0.01$). Clinical signs of disease were more evident in saline control than in vacinated animals ($p<0.001$). One dog from the saline control and two dogs from the 1mg QuilA treated group, died from kala-azar, at 297, 376 and 503 days after infection, respectively. These animals belong to the same brood, indicating that even in this highly susceptible family, vaccination with FML induced protection.

Support PCDEN, PNUD, FNS, Pronex, CNPQ, Rhae, Finep, Capes, FUJB, CEPG, UFRJ, Faperj.

IM-167
VACCINATION OF *SWISS ALBINO* MICE AGAINST EXPERIMENTAL VISCERAL LEISHMANIASIS WITH THE FML ANTIGEN OF *LEISHMANIA (L.) DONOVANI*

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An 87.7% ($p<0.01$) and 84% ($p<0.001$) specific average protection against Kala-azar was achieved in the isogenic CB hamster and Balb/c mouse models, respectively, with saponin combined to the fucose mannose ligand (FML) antigen of *Leishmania (L.) donovani*. In this investigation, we analysed the protective potential of the FML antigen on experimental visceral leishmaniasis, in the Swiss Albino outbred mice model. We compared the adjuvant potential of saponin, aluminum hydroxide (Al(OH)3) and Freund's adjuvant, in vaccine formulations administrated either through the intraperitoneal (ip) or subcutaneous (sc) routes. Protection criteria included: the increase of the anti-FML specific antibody response, both before and after infection with $2x10^7$ amastigotes by the intravenous route, and the reduction of liver parasitic load, 15 days after challenge. The humoral response was significantly higher in the groups treated with FML+saponin or FML+ Al(OH)3, than in controls, both before and after the infection. Animals immunized by the ip route gave higher antibody titres while animals that received FIA, either with FML or not, showed a very low response, unrespectively of the immunization route. A signifficant reduction of parasitic load in relation to saline control was seen in the groups treated with FML+saponin, either by the ip (85 %, $p<0.01$) or the sc route (89 %, $p<0.005$). However, protection was specific only in the ip group ($p<0.025$, in relation to saponin control). 88% of reduction in parasitic load was achieved by the FML+Al(OH)3 sc group, but the Al(OH)3 treatment itself accounts for 68% of this protection. The anti-FML subtypes of antibodies specifically increased after vaccination were: IgG1, IgG2a and IgG2b for FML saponin ip group (coincidently with the reduction in liver parasitic load), IgG2b for FML saponin sc and IgG1 and IgG2b for FML Al(OH)3 ip and sc groups. In our conditions, vaccination with FML and saponin ip showed the best performance with no toxic effect due to saponin contribution.

Support PCDEN, PNUD, FNS, Finep, CNPQ Capes, MCT, Pronex, Rhae, FUJB,UFRJ Faperj, CEPG.

IM-168
VACCINATION WITH A GENETICALLY ATTENUATED *LEISHMANIA* VACCINE LINE AGAINST CUTANEOUS LEISHMANIASIS IN THE ASIAN RHESUS MACAQUES

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The usefullness of a live avirulent *Leishmania* line [capable of inducing protective immunity in mouse models, *PNAS 92*:10267, 1995] for vaccination against cutaneous leishmaniasis is currently being evaluated in *Macaca mulatta*, the Asian rhesus macaques. Experiments are designed (*i*) to test the inability of a homozygous dhfr-ts$^-$ *L.*(*L.*) *major* cell line (*Exp Parasitol 78*: 425, 1994) to cause disease in primates; (*ii*) to study the immune response of knockout-vaccinated monkeys using a variety of parameters [with emphasis to those required for the evaluation of T-cell responder phenotypes to parasite antigen]; and (*iii*) to examine if immunization is able to induce protection to challenge infection with virulent *L.* (*L.*) *major*, and how the immunologic parameters correlate with acquired immunity. These studies should provide the basis for a vaccine against human leishmaniasis.

Preliminary data show specific cellular immune responses in healthy rhesus macaques following infection with 10^8 promastigotes (s.c. route) of the dhfr-ts- *L.* (*L.*) *major*. T-cell responsiveness is detected in a high percentage of vaccinees (>90%), manifested by lymphocyte proliferation *in vitro* to leishmanial antigen, but no skin test conversion was recorded in these experiments (the DTH responses to parasite antigen were assessed at 4, 10, 14 and 17 week follow-ups). The results (including from PCR assays) also indicate that the thymidine auxothrophic organisms persisted in infected animals for up to 2-3 months. Nonetheless, the null mutant parasites were incapable of causing disease in susceptible rhesus monkeys. Further studies are required to determine whether the presence of proliferative (and/or IL-2, interferon-γ responses) in the absence of a skin test response are sufficient indicators of potential vaccine success. Currently we are testing if these responses elicit substancial resistance to a subsequent challenge with virulent *L. major*.

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IM-169
WESTERN BLOTTING (WB) TECHNIQUE IN AMERICAN CUTANEOUS LEISHMANIASIS (ACL) DIAGNOSIS

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Tripanosomatid protozoa of genus Leishmania cause a spectrum of human diseases, collectively know as leishmaniasis. American mucocutaneous leishmaniasis is the most chronic and caused by protozoans of the *Leishmania brasiliensis* complex. The serologic methods more extensively used in leishmaniasis are Indirect Imunofluorescence and ELISA (enzime-linked immunosorbent assay). Acording to FARGEAS C. et al (1996), a problem of serologic assays with Leishmania crude antigens is the existence of cross-reactivity with other microorganisms. The possibility to establishing a proteic recognition pattern by human sera in Western Blottin (WB) technique, able to confirm American Cutaneous Leishmaniasis (ACL) diagnosis, gave us rise to try better caracterization of total antigens of *Leishmania brasiliensis* through this technique. Serum samples were obtained from 108 patients with cutaneous leishmaniasis (Group I), 23 from chagasic patients (Group II) and 78 health individuals (Group III). Protein bands with apparent molecular weights of 110, 72, 66, 63, 58, 45, 42, 38 and 30kDa were the most recognized among Group I sera giving frequencies of 38,89%, 34,25%, 27,78%, 63,89%, 39,81%, 27,78%, 35,18%, 28,70% and 30,55%, respectively. In the 23 serum samples from Group II the bands with molecular weights of 14, 28, 30, 33, 38, 46, 50, 63, 66, 68, 72, 90 and 105 KDa were recognized in more than 30% of the serum samples analyzed, giving frequencies of 47,83%, 30,43%, 78,26%, 47,83%, 73,91%, 39,13%, 69,59%, 30,43%, 43,48%, 60,86%, 73,91%, 69,56% and 69,56%, respectively. Serum samples from Group III, recognized only few antigens and with low intensity of reaction. The bands 11kDa (14,10%), 84kDa (16,66%), 120kDa (23,07%) and 160kDa (16,66%) were the most frequently recognized. Apparently, the protein bands with 110, 72, 66, 63, 58, 45, 42, 38 and 30kDa were the antigens more specific to leishmaniasis. The profiles of proteic recognition obtained in WB technique were complex, at least in part due to manual measurement of the molecular proteins weight,. A zone of the poliacrilamide gel centralized in 63kDa protein and with superior and inferior limits respectivly in 65 and 60kDa was eluted and after a second SDS-PAGE it was incubated with 26 positive sera to ACL and a negative control by WB technique. The results showed that the proteic band primarialy measured as 63kDa , in fact seems to be a compound constituted by three protein with close molecular weights and that the sera were able to recognize each protein separatly.

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IM-170
DIFFERENTIAL IMMUNOLOGIC RESPONSES FROM LEISHMANIASIS PATIENTS DIRECTED AGAINST LEISHMANIA ANTIGENS

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This study was designed to analyze the cellular immune response to both soluble promastigote leishmania antigen (SLA) and an important recombinant antigen from *Leishmania* p24/LACK. A better understanding of the dynamics of the patient's immune response to this defined antigen and SLA through the analysis of cytokine production (profiles), T cell repertoire and sub-population responses have been attained. Data showed here were obtained with patients with cutaneous clinical form of disease from two endemic areas for leishmaniasis. One of the areas in Minas Gerais and the other in the state of Bahia. By characterizing the cellular and molecular response to LACK and SLA in patients we are gaining information concerning what types of responses are dominant with these antigens, and gain insights into ways in which these responses could be manipulated, if deemed advantageous, through vaccine approaches. In particular, we determined the proliferative response, lymphocyte profiles (types and activation state), cytokine profiles (which cytokines and which cell types are producing them), as well as, CD4 and CD8 TCR Vb region usage before and after the stimulation with the above mentionated antigens. This approach allows us to compare each individual's response to a panel of stimuli, as well as compare between clinical forms of the disease in future (currently under way). In summary we have found: 1) about 72% of the individuals responded to recombinant LACK, 90% responded to SLA and 100% responded to the SEB; 2) differences in the TCR repertoire when comparing the patients from Minas Gerais with those from Bahia; 3) a possible bias in TCR usage from cutaneous patients; 4) the presence of an activated CD4+ T cell phenotype, *ex vivo*; 5) an apparent increase in IL-10 production by large cells from cultures stimulated for 5 days with LACK as compared with SLA stimulation; 6) a general increase in IFN-??production by CD4+ and CD8+ T lymphocytes in responses to SLA, but not to LACK; 7) an increase of TNF-??production by CD14+ cells after stimulation with SLA and LACK and, finally, non-detectable levels of IL-4 and IL-5 using FACS analysis. These data indicate an intricate system of immunoregulation in these individuals, with each antigen or combination of antigens playing a role in the overall immune response. Supported by: UNDP/World Bank WHO Special Program for Research and Training in Tropical Diseases, CNPq/PRONEX, and DNAX Research Institute, Palo Alto, CA.

IM-171
PURIFICATION AND CHARACTERIZATION OF ANTIGENS PRESENTS IN VACCINE AGAINST LEISHMANIOSE - LEISHVACIN^{0}

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Leishmaniasis constitute a spectrum of human protozoan diseases caused by intracellular parasites from the genus *Leishmania*. The tegumentary form of the disease is endemic in tropical regions constituting a relevant public health problem. Several studies have been developed with the purpose of obtaining and improving prophylactic or therapeutic methods which may benefit the infected population and prevent the risk of infection. The identification of several leishmania antigens able to induce a protective response and, consequently serve as potential candidates for vaccines have been reported by different groups. The isolation and characterization of antigens with the purpose of diagnosing leishmaniasis have also been described. Among several vaccines produced so far, Leishvacin^{0} was developed by the department of Parasitology, Biochemistry and Immunology of "UFMG" and by "Biobras do Brasil". This vaccine has been considered as one of the most effective. It is a mixture of dead, sonicated leishmania from various strains. However, its antigenic composition has not been molecularly characterized yet.

Aiming to identify and characterize antigens that constitute Leishvacin^{0} samples of the vaccine were centrifugated at 70,000 rpm at 10°C during 1:30. The supernatant was submitted to protein quantification (Bradford, 1976) and to fractionation by FPLC chromatography in a column of filtration gel (Superose 12 HR), previolusly equilibrated with Na^{++} elution buffer (PBS 0,05M). Several runs were performed using 200ml of the vaccine. The fractions obtained were combined into 12 pools and the respective volumes concentrated in a speed-vac. Each pooled fraction was dosed and the reactivity with sera from human and canine leishmaniasis positive individuals was performed using ELISA. Analysis of the pools reveiled that one had a retention period characteristic of material with high moleclar weight (pool 1), and several with a molecular weight lower than 15,000. We observed that around 90% of the total proteins of Leishvacin^{0} are found in pools II - IX and XII. However, the reactivity of sera to these pools was very reduced, representing only 10% of the total reactivity. Pools I and X, which contained only 10% of the protein, had a high level of reactivity to sera from human and canine by ELISA. The results obtained suggested that they are molecules contained a small quanty of protein, along with other as yet undefined molecules. Preliminary results recently obtained in our laboratory suggest the participation of carbohydrates as an important antigenic component of leishvacin^{0}.

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IM-172
IMMUNOSELECTION FOR *LEISHMANIA (VIANNIA) BRAZILIENSIS* ANTIGENS

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Comparative enzyme-linked immunosorbent assays (ELISAs) and selective immunobloting procedures were used in attempts to identify differential serological indicators of: a) *Leishmania braziliensis*-complex (*Viania*) infection; b) *Leishmania braziliensis* species infection; and c) Therapeutic cure of localized or mucocutaneous leishmaniasis (LCL or MCL). Mean ELISA absorbance values could not be used as a reliable indicator of the clinical form of disease. Immunoblotting profiles were similar with sera from MCL and LCL, although there were differences in the frequencies that antigens suggested that recognition of antigens in the size regiosn 56 kDa, 60 kDa, 66 kDa, 72 kDa, 88 kDa and 110 kDa, might be specific to the subgenus *Viannia*. Two colour, sequential, dual ELISA immunoblotting was unable to detect antigens only recognized b with sera from MCL patients. After glucantime therapy immunoblotting profiles detected; a 104 kDa antigen was newly detected with post-treatment LCL sera. Overall these results showed the value of immunoselection strategies, supported the close relationship between species of the genus *Viannia* and were unable to identify a prognostic antigen for mucocutaneous leishmaniasis (MCL).

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IM-173
INDUCTION OF INDOLEAMINE 2,3-DIOXYGENASE SEEMS TO BE INVOLVED IN THE TRYPANOCIDAL ACTIVITY OF HUMAN MACROPHAGES STIMULATED WITH RIFN-g AND/OR RTNF-a

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Pfefferkorn (1984) observed that human fibroblasts stimulated with IFN-g blocked the growth of the intracellular parasite *Toxoplasma gondii*. This inhibition occurred due to the depletion of the essential amino acid tryptophan, via induction of the host indoleamine 2,3-dioxygenase (INDO), the initial enzyme of tryptophan catabolism. Cerávolo e cols. (1996) reported that *T. cruzi* replication was not altered by stimulation of human fibroblasts with rIFN-g and/or rTNF-a In contrast, it was shown by other authors that *T. cruzi* is killed by human macrophages activated with rIFN-g and to a lesser extent with rTNF-a. In this study we have evaluated the ability of human macrophages in killing *T. cruzi in vitro*. Our results showed that the rIFN-g induced trypanocidal activity in human macrophages. A significant synergistic trypanocidal effect was also observed when the macrophages were stimulated by rIFN-g + rTNF-a. To verify if INDO is involved in some extent in the trypanocidal activity of the human macrophages activated with rIFN-g and/or rTNF-a, the inhibitor of INDO, norharmane was tested. It was observed that norharmane was able to revert part of the *T. cruzi* intracellular growth in rIFN-g stimulated human macrophages. The expression of INDO mRNA was confirmed by RT-PCR on human macrophages stimulated with rIFN-g and/or rTNF-a. The rTNF-a alone also induced a small but significant amount of INDO mRNA. INDO activity determined by high-performance liquid chromatography (HPLC), showed high activity in stimulated macrophages and a very low or almost absent activity in non stimulated cells. The major tryptophan catabolites added to the culture exerted no toxic effect on *T. cruzi* development in macrophages. In conclusion, the data suggest that at least part of the development of *T. cruzi* in human macrophages stimulated by rIFN-g/rTNF-a is regulated by the tryptophan depletion caused by INDO.

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