IM-1 – MURINE VISCERAL LEISHMANIASIS: CTLA-4 MEDIATED CD4$^+$ T CELL SUPPRESSION IS EFFECTED THROUGH RELEASE OF TRANSFORMING GROWTH FACTOR-β

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CTLA-4 engagement induces TGF-β production by CD4$^+$ T cells, and TGF-β production accounts for some of the suppressive effects of CTLA-4 engagement. Important roles were demonstrated both for CTLA-4 engagement in T cells, and for TGF-β production in the immunopathogenesis of murine visceral leishmaniasis (VL), but a functional link between these two pathways is unknown. Here, we investigated a direct link between CTLA-4 downregulation of CD4$^+$ T cell responses and TGF-β secretion in murine VL.

Activation of CD4$^+$ T cells from VL leads to intense CTLA-4 mediated TGF-β production, as assessed either by CTLA-4 blockade, or by direct CTLA-4 crosslinkage. Intracellular growth of *Leishmania chagasi* in cocultured splenic macrophages was dependent on both CTLA-4 function and TGF-β secretion. Crosslinkage of CTLA-4 markedly increased *L. chagasi* replication and this effect could be completely blocked with neutralizing anti-TGF-β. Addition of rTGF-β1 restored parasite growth in cultures protected from parasitism by CTLA-4 blockade. Anti-TGF-β failed to increase IL-2 production, but markedly increased IFN-γ production by CD4$^+$ T cells. Our findings indicate that parasite persistence in murine VL depends on CTLA-4 mediated TGF-β production by CD4$^+$ T cells.

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IM-2 – EVALUATION OF IMMUNE RESPONSES OF SUBCLINIC INDIVIDUALS INFECTED BY *L. BRAZILIENSIS,* LIVING AN ENDEMIC AREA OF CUTANEOUS LEISHMANIASIS

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Resistance to leishmania infection is mediated by cellular immune response, characterized by IFN-γ activated macrophage killing of leishmania. Different from visceral leishmaniasis (VL), patients with tegumentary leishmaniasis usually have a strong T cell response to Leishmania antigen. Such response is caracterized by delayed-type hipersensitivity, IFN-γ and IL-2 production *in vitro* by antigen stimulated peripheral blood mononuclear cells (PBMC). Tegumentary leishmaniasis have a spectrum of diseases, including anergic diffuse cutaneous leishmaniasis (DCL), and the responsives forms of mucosal leishmaniasis (MCL), disseminated cutaneous leishmaniasis and the localized cutaneous leishmaniasis (CL) (GRIMALDI et al., 1993).

The aim of the present study was to characterize the immune responses of individuals with subclinical form of tegumentary leishmaniasis, characterized by positive intradermal skin test and no sign of disease or previous scar. The Mean ± SE of stimulation indices of proliferative responses in PBMC stimulated by leishmania antigen (2mg) of these subclinical was 12,6 ± 3,7. The levels of IFN-γ and TNF-α in antigen stimulated PBMC from individuals with subclinical form of tegumentary leishmaniasis was significantly lower than the ones from CL patients (296,0 ± 119,6 pg/ml versus 1549,5 ± 283,9 pg/ml and 55,0 ± 20,4 pg/ml versus 258,9 ± 65,1 pg/ml for IFN-γ and TNF-α, respectively; $p < 0,001$, Mann Whitney). IL-5 levels in antigen stimulated PBMC from individuals with subclinical form of tegumentary leishmaniasis were higher than the ones from CL patients (105,7 ± 31,6 pg/ml versus 31,8 ± 9,7 pg/ml, respectively; $p = 0.1392$, Mann Whitney). Previous publications have shown that IFN-γ is important in control parasite multiplication. However, an excess of IFN-γ production could up regulate TNF-α production, leading to the tissue damage and development of the lesion seen in tegumentary leishmaniasis patients (JESUS et al., 1997). A comparison by western blot of antigens recognized by sera from four subclinical individuals with sera from CL and MCL patients showed that the subclinical individuals recognize bands of 16 KDa and 25 KDa, not recognized by sera from CL and MCL patients, suggesting a specific antigenic pattern recognition that might be involved in imune protective response in human leishmaniasis.

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IM-3 – IL-4 INHIBITED TRYPTOPHAN CATABOLISM AND CONTROL OF *TOXOPLASMA GONDII* REPLICATION INDUCED BY IFN-γ IN NON-PROFESSIONAL PHAGOCYTIC CELLS

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The ability of different cytokines to induce or regulate the activity of indoleamine 2,3-dioxygenase (INDO) and control the *Toxoplasma gondii* replication in a human fibrosarcoma cell line (2C4) was evaluated. The cells were cultured in the presence of up-regulatory (i.e. rIFN-γ, rIFN-β and rTNF-α) and/or down-regulatory cytokines (i.e. IL-4 and IL-10) for 12-24h, following evaluation of INDO mRNA expression, tryptophan degradation and intracellular tachyzoite replication. 2C4 incubation with rIFN-γ (100 UI/ml), rIFN-β (1,000 UI/ml) and rTNF-α (60 UI/ml) resulted in 59.1, 12.8 and 29.6% increase of INDO activity assessed by reduction in tryptophan and increase in L-kynurenine concentration in the culture media. We also observed an additive effect showing a total of 65 and 72.9% in reduction of tryptophan concentration, when host cells were incubated with rIFN-γ plus rIFN-β or rIFN-γ plus rTNF-α, respectively. Our results showed that increase of INDO activity induced by different cytokines was directly correlated with expression of INDO mRNA. The parasite replication inside 2C4 cells was inversely correlated with INDO activity induced by rIFN-γ, rIFN-β and/or rTNF-α. In regard to the down-regulatory cytokines we observed that IL-4 (10 ng/ml), but not IL-10 (10 ng/ml), antagonized the IFN-γ activity in 2C4 cell line. Thus, IL-4 induced 50, 30, and 70% inhibition of IFN-γ induced INDO mRNA expression, tryptophan catabolism and control of parasite replication, respectively. No effect of IL-4 was observed when the cells were stimulated with either rIFN-β or rTNF-α. The results of inhibition of INDO activity by rIL-4 were confirmed by using radioactive tryptophan. In contrast, to IL-4, IL-10 did not affect any of the IFN-γ induced activity in human fibrosarcoma cell lines. Consistent with these findings we were able to detect by RT-PCR the expression of different chains of IL-4 receptor (IL-4Rα, IL-13Rα1 and IL-13Rα2) but not of IL-10 receptor (IL-10R) in the 2C4 cell line. Together our results indicate that IL-4, but not IL-10, is a major regulator of IFN-γ induced anti-*Toxoplasma* activity in human cells from fibroblast lineage.

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IM-4 – PROTECTIVE ROLE OF ALPHA-2-MACROGLOBULIN IN EXPERIMENTAL *T. CRUZI* INFECTION

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Trypanosoma cruzi proteinases are very likely involved in host cell invasion. Alpha-2-Macroglobulins (A2M) are physiological proteinase inhibitors with important roles in inflammation and immunomodulation that behave as acute-phase proteins in many animals. Asymptomatic Bolivian children acutely infected by *T. cruzi* have higher levels of A2M compared to age-matched symptomatic patients, suggesting a protective role for this protein in Chagas disease. Increased levels of A2M was found in tissues and in the plasma of *T. cruzi*-infected mice, and human A2M was able to inhibit in vitro cell invasion by *T. cruzi.* Reaction of A2M with proteinases alters the conformation of native A2M such that its electrophoretic mobility in native PAGE is increased from a "slow" (N-A2M) to a "fast" (F-A2M) form. Both N-A2M and F-A2M are able to bind to *T. cruzi* surface: N-A2M binds to parasite proteinases and F-A2M binds to a receptor-like expressed on parasite surface. Hepatic synthesis of the tetrameric A2M increased during the first second week post infection of C57bl6 (B6) mice with *T. cruzi* (Y strain) and the synthesis of the monomeric AM murinoglobulin (MUG) still high for a longer periodo, during the second and third weeks, as indicated by north ern blot mRNA analysis. A2M and MUG plasma determinations using rocket immunoelectrophoresis confirmed this increase at the protein level. To further study the role of A2M in experimental infection, we investigated the susceptibility of A2M knock-out (KOAM) mice to *T. cruzi* infection in vivo, as compared to wild type controls (WT, B6). We found that parasitaemia recorded in KOAM was lower than in WT mice all over the period studied, but that KOAM presented more amastigote nests and inflammatory infiltrates than B6, suggesting a protective role for A2M at the acute phase of murine *T. cruzi* infection. Since A2M accumulates in sites of the inflamed myocardium, associated with parasite antigens, N-A2M that leak through increased vascular permeability very likely complex to parasite or damaged-cell derived proteases, to form F-A2M. N-A2M is a TGFβ neutralizer that helps to sustain IFNγ-mediated macrophage activation. Besides, F-A2M can modulate the phagocytic and microbicidal activity of resident macrophages. Thus, the knock-out of the A2M gene is consistent with data showing increased tissular parasitism and mononuclear infiltration. In vitro effect of F-A2M as a possible modulator of *T. cruzi* invasion in cardiomyocytes was then investigated, showing that: (a) it alters trypomastigote morphology and motility in a dose dependent way and (b) it impairs *T. cruzi* ability to invade cardiomyocytes. All together, these *in vivo* and *in vitro* findings indicate that A2M contribute to mice resistance to acute myocarditis induced by *T. cruzi* infection.

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IM-5 – IN VITRO PRIMING OF HUMAN LYMPHOCYTES AGAINST *LEISHMANIA AMAZONENSIS*

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Understanding the mechanisms during the first encounter of parasite and its host is of utmost importance in design immunoprophylactic approaches. Human initial response to *Leishmania* is not possible to be studied but for their emulation in *in vitro* systems. Herein we report on a system of sensitizing human lymphocytes with *Leishmania amazonensis*-infected autologous macrophages. Thirty-nine volunteers, from non-endemic areas for leishmaniasis, with age ranging from 25 to 45 years, without previous history of leishmaniasis, and a negative serology and a negative delayed-type hypersensitivity reactions for leishmania were evaluated. We have performed two cycles of *in vitro* cell stimulation, using *L.amazonensis*-infected macrophages. Lymphocyte priming and response were evaluating by measuring cytokines (FNγ, TNFα, IL-10, IL-5) in culture supernatants and by phenotyping cell populations (CD4, CD8, CD19 and CD56) and evaluating the presence of activation (CD25) and memory markers (CD45RO). Upon sensitization there is a statistically significant expansion of CD4+ cells (1.4 X increase), with reduction of CD8+ and CD56+ cells, while B cells remained unchanged. Despite the reduction of total CD8+ cells there was an expansion in the subset of CD25+ (activated) CD8+ cells. Primed cells exhibited a significant increase in IFNγ production (54.7 vs. 109.2 pg/10^6 cells in naive and primed cells, respectively), as well in IL-5 production. Production of IL-10 and TNFα did not show important changes upon infection. Our data demonstrate that the *in vitro* system used here generates memory cells and lead to significant cytokine production, allowing for the study of the first steps of human immune response against leishmania. Investigation of the operating mechanisms and the role of specific antigens may be helpful in designing new strategies in vaccination.

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IM-6 – IN SITU CHARACTERIZATION OF THE MYOCARDIAL INFLAMMATORY INFILTRATE IN DIFFERENT MOUSE INBRED STRAINS INFECTED WITH *TRYPANOSOMA CRUZI*

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Using three inbred mouse strains – C57BL/6, BALB/c and CBA- we have investigated the kinetics of the myocardial inflammation after infection with 50 trypomastigotes of the Trypanosoma cruzi, Colombian strain. Commercial rat anti-mouse monoclonal antibodies (Pharmigen) that recognize the CD3, CD4 and CD8 surface molecules, as well as anti-Thy1.1, GK1.5 and H35 were tested.

Tissue parasitism, parasitemia and myocarditis reached maximum levels between days 30 and 50 after infection. The inflammatory infiltrate occupied about 1/4 of the tissue section in all mice. CD8+ T-cells were predominant in all strains (50.6±4.6 in C57BL/6, 40.1±5 in BALB/c and 25.5±13.2 in CBA mice). The average of the ratio CD4/CD8 was 0.8, 0.6 and 0.7 at days 21, 30 and 50 respectively. Slides stained with the moAb anti-CD3 and H35 always showed about 15% less cells than those stained with anti-Thy1 and anti-CD8 respectively.

60% of the animals were able to reach the chronic phase. Histopathological examination showed an intense inflammatory infiltrate, which was clearly more severe in CBA mice, occupying 32.8%±6.1 of the tissue section, often associated with necrosis. BALB/c mice showed small but multiple inflammatory foci, while C57BL/6 showed few foci, only occasionally associated with necrosis. CD8+ T cells were predominant in C57BL/6 (34.8±3.7 vs 25.6±15.5 CD4+) and CBA mice (42.1±6.9 vs 23.6±5.3 CD4+), but not in BALB/c mice (34.7±8 vs 44±5 CD4).

The results suggest that these mouse strains present a similar pattern of acute myocarditis, but differ in the chronic infection, expressing different T-cell phenotypes, which functional activity remains to be defined.

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IM-7 – GALECTIN-3 MAY INFLUENCE INTRATHYMIC MIGRATION IN *TRYPANOSOMA CRUZI* INFECTED MICE

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Following *Trypanosoma cruzi* infection, the thymus presents an intense atrophy with loss of cellularity. In addition, an escape of immature cells from the thymus to the periphery occurs. The process of migration involves ligands and receptors of extracellular matrix. In infected mice, there is an increase of the extracellular matrix molecules expression, like laminin, fibronectin and type-IV collagen by thymic cells. Galectin-3 is an endogen lectin involved in processes of apoptosis, cellular proliferation and de-adhesion. In the thymus, galectin-3 modulates thymocyte release by thymic nurse complexes (TNC) as well as the adhesion between thymic epithelial cells and thymocytes. Considering these data, we decided to study the expression of galectin-3 in the thymus of *T.cruzi* infected mice and its role in the intrathymic migration and differentiation in Chagas' disease.

Firstly, we studied the expression of galectin-3 in the thymic microenvironment by immunofluorescence and confocal laser scanning microscopy analysis. Frozen sections of thymus from infected animals showed an enhanced expression of galectin-3 as compared to control. Thymic nurse cells isolated from infected mice also had an increased expression of galectin-3 in relation to control. The detection of galectin-3 in IT-76M1, a murine thymic epithelial cell line, and in TNC infected *in vitro* was higher then in non-infected cultures. In addition, we studied the colocalization of galectin-3 and laminin in the thymus. Our results showed a possible colocalization of galectin-3 and laminin in the cortex and preferencially in the medulla, which suggest an interaction between these molecules.

The increased expression of galectin-3 in *Trypanosoma cruzi* infected animals and in *in vitro* systems indicates that this lectin may influence the process of migration favoring the escape of the immature T cells from the thymus to the periphery.

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IM-8 – BENZNIDAZOLE TREATMENT FAILS TO CURE IFN-G KNOCKOUT MICE INFECTED WITH *TRYPANOSOMA CRUZI*

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It is well documented that treatment with nitroheterocyclic derivatives is more effective during the acute phase of human and experimental Chagas' disease. During this stage of disease, it is observed a strong activation of the cellular compartment of the immune system and release of high levels of IL-12, TNF-α, IFN-γ and nitric oxide (NO), all important mediators of resistance to *T. cruzi*. In fact, our recent studies demonstrate that treatment with benznidazole (BZ) enhances phagocytosis, parasite destruction and cytokine (i.e. IL-12 and TNF-α) as well as NO release during *in vitro* infection of mouse macrophages with the drug susceptible Y strain of *T. cruzi* (Murta et al. 1999, *Parasite Immunol. 21: in press*). Therefore, we proposed a cooperative effect of immune system and nitroheterocyclic derivatives and trypanosomicidal effects. Our early study indicates that simultaneous treatment with rIL-12 overcomes resistance of Colombiana strain of *T. cruzi* to treatment with BZ (Michailowsky et al. 1998, *Antimicrob. Agents Chemoth. 42:2549*). In the present study we compared the ability of drug resistant and drug susceptible Y strains to induce IFN-γ and NO synthesis after *in vivo* treatment with BZ. Our results show that one day after initiation of BZ treatment the drug-susceptible, but not the drug resistant parasites, presented a remarkable capacity to elicit IFN-γ and NO synthesis in the day preceding parasite clearance. In order to test the importance of IFN-γ and NO pathway in the efficacy of chemotherapy, we infected the IFNγ-KO, TNFα-KO and iNOS-KO mice with the Y strain of *T. cruzi* (5,000 blood trypomastigotes/mouse i.p.) and treated then with BZ (100 mg/Kg/day during 20 consecutive days). All infected knockout (KO) mice untreated died at day 15 post-infection or earlier, whereas most of the C57BL/6 wild type (WT) animals survived up to 70 days post infection. Consistent with the mortality the parasitemia was elevated in IFNγ-KO, TNFα-KO and iNOS-KO untreated mice as compared to WT animals. In addition, the parasitemia that was subpatent during BZ treatment in IFNγ-KO mice infected, became patent after stopping treatment. 100 % mortality of IFNg-KO was observed at the 40th day after the end of BZ therapy. The TNFα-KO and iNOS-KO mice showed intermediary susceptibility, showing 30 and 60% of mortality respectively after stopping BZ. No mortality was observed in WT mice infected with *T. cruzi* and treated with BZ. The IFNγ-KO, TNFα-KO, iNOS-KO and WT mice presented 0, 56, 71 and 100% of parasitological cure, respectively. Together our results indicate a major role of endogenous IFN-γ, followed by TNF-α and NO, on the efficacy of BZ treatment against *T. cruzi*.

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IM-9 – EFFECTS OF THE STEROL BIOSYNTHESIS INHIBITORS SCH 56592 AND DO870 ON MICE CHRONICALLY INFECTED WITH *TRYPANOSOMA CRUZI*

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The effect of treatment with the sterol biosynthesis inhibitors (SBI), DO870 (Zeneca Pharmaceuticals) and SCH56592 (Schering-Plough) on the survival and cure of *T. cruzi* chronically infected mice was evaluated. The mice were infected either with a benznidazole (BZ) susceptible (CL), a partially resistant (Y) and a resistant (Colombiana) *T. cruzi* strain. The effects of both drugs were compared to BZ. The animals treated with DO870 (20 mg/kg.d) and SCH (5, 10 and 20 mg/kg.d) showed a survival respectively equal and higher than those treated with BZ (100mg/kg.d). The percentage of cure was dose dependent for SCH, being 10 and 20 mg/kg.d equally effective, however more effective than BZ 100 mg/kg.d. For DO870 nearly the same percentage of cure was obtained when using 20 mg/kg daily or every other days. DO870 at 20 mg/kg.d.d was as effective as BZ 100 mg/kg.d, whereas SCH at 10 mg/kg.d was more effective than either drug. The survival and cure rates were drug dependent and *T. cruzi* strain independent. The effectiveness of DO870 and SCH56592 could probably be associated to the higher affinity of these triazole derivatives to its biochemical target, citocrome P-450- dependent C 14 sterol demethylase. The present results, added to others previously published, support the proposal that fourth-generation azoles derivatives should be considered for clinical trials in human Chagas' Disease.

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IM-10 – EXPONENTIAL FUNCTION IN THE PROGNOSIS OF SEROLOGICAL CURE IN BENZNIDAZOLE-TREATED CHRONIC CHAGASIC PATIENTS BY USING CHEMILUMINESCENT-ELISA WITH *TRYPANOSOMA CRUZI* TRYPOMASTIGOTE MUCINS

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The effectiveness of chemotherapy in chronic adult patients with Chagas' disease has not been adequately evaluated because, in the absence of ostensive clinical evolution, the available conventional serological tests do not reflect in proper time the parasitological cure of treated individuals. Here, we have used a chemiluminescent ELISA test (A&T CL-ELISA), as previously described (Almeida *et al.*, Transfusion 37:850-7, 1997), which monitors the immunological response to a specific trypomastigote glycosylphosphatidylinositol (GPI)-anchored mucin (Almeida *et al.*, Biochem. J. 304:793-802, 1994), clearly confirming the successful treatment by negative seroconversion in a significant number of patients. Fifty-six adult patients with Chagas' disease, diagnosed by conventional methods, were treated with benznidazole for 60 days. At the onset of treatment and up to 10 years afterwards, 3-10 serum samples per patient were titrated by A&T CL-ELISA. Successfully treated patients showed decreasing serum titers for trypomastigote mucins according with an exponential funtion: $Y=Yo \times 10^{-kt}$, where Y is the titer in relative luminescent units (RLU) ; Yo is the serum titer before treatment, according with the exponential function; k is the constant for each patient's curve; and t is the time in years. For calculation of the necessary time for negative seroconversion the Y value in the formula above should correspond to the cutoff titer. Three groups according with their correlation coeficients (r) in the titration curves were recognized. The results are shown in the table bellow:

Group	r	n	%	$Yo_{mean} \pm SD$	$k_{mean} \pm SD$	Sera with Negative A&T CL-ELISA[a]	Average time (years) for negative seroconversion	Sera with positive A&T CL-ELISA[a]
1	0.801–1.0	31	55.4	17271 ± 10604	0.099 ± 0.043	14 (25%)	4.1 ± 2.0	17 (30.4%)
2	0.601–0.8	13	23.2	17980 ± 12100	0.077 ± 0.053	4 (7.1%)	9.7 ± 6.1	9 (16.1%)
3[b]	0.001–0.6	12	21.4	15838 ± 6354	0.034 ± 0.033	0	—	12 (21.4%)

[a] At the end of the follow-up; [b] Three patients in this group did not respond to the treatment.

Although 95% of chronic patients in this sample showed decreasing titers after treatment, only Group 1 curves with a high r value can be used for a reliable prognosis of negative seroconversion, in agreement with the exponential function. The time for seroconversion is dependent on the titer at the beginning of treatment (Yo) and the k value for each curve. For the 17 patients of Group 1 with still positive A&T CL-ELISA results, the average time for negative seroconversion is projected to 8.2 ± 3.3 years. This is the first report on a serological assay for Chagas' diasease that can both evaluate the response to treatment and predict the time of serological cure (negative seroconversion).

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IM-11 – ACUTE CHAGAS´ DISEASE: IMMUNOHISTOCHEMICAL CHARACTERIZATION OF THE T CELL INFLAMMATORY AND ITS RELATIONSHIP WITH THE PARASITE ANTIGENS

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Although Chagas´ disease is being controled in many countries of Latin America, there was recently in outbreak Chagas´ disease in Venezuela. This work deals with the pathological findings in endomyocardial biosies (EMB) performed in patients presenting with acute Chagas´ disease, in a period of 9 years from 1989 to 1998 in the state of Barinas, Venezuela, in order to better evaluate the inflamatory infiltrate and its relantionship with the parasite antigens.

Materials and methods: Although there was 58 patients with acute Chagas´ disease in that peiod, only 13 patients had EMB with at least 5 high power fieds (x400) to be analysed. These 13 patients constitute the group of study. Their age´s range from 12 to 51 years, and 7 of them were males. All patients received specific therapy anti T-cruzi after the diagnosis.

The histopathological study was finished at Pathology Department, INCOR. Identification of T cruzi antigens and lymphocyte subsets was performed using the avidin-biotin peroxidase complex technique, the primary antibodies used were policlonal serum anti T cruzi and monoclonal antibodies anti CD4+ (helper) and anti-CD8 (cytotoxic/supressor) T cells. Positive T cells for each marker were counted in all fields of each EBM at a magnification of 400. The presence and intensity of the lymphocytic myocarditis was evaluated acording to the Dallas criteria, and parasitic antigens were recorded as present or absent, acording to the inmunoperoxidase reaction.

Results: The grade of celular lesion obtained was: interstitial reaction (7,7 %), mild myocarditis (38,5%), myocarditis moderate (23,1%) and myocarditis severe (30,6%). There was no significant difference between the counts of CD4 and CD8 T cells. The technique showed parasites in 7 cases (53,8%).We observed direct correlation between amount of T cruzi antigens and severity of myocarditis and presence of vasculitis. There were interstitial fibrosis in 30,76% and vasculitis in 46,15%.

CONCLUSION: The myocardial involvement varies greatly among the patients with acute Chagas´ disease and it is directly related with the presence of parasitic antigens as previously reported in the chronic phase. Both CD4+ and CD8+ T cells are participating in the acute lymphocytic myocarditis with the same intensity of cellular infiltration. The follow up of these patients may show if patients who presented more severe myocarditis and parasitic antigens in the acute phase will be those that will develop chronic heart disease.

IM-12 – T CELL RECOGNITION AND CYTOKINE PROFILE TO *T. CRUZI* ANTIGENS CY-HSP 70 AND GRP78, TIP11, FCABP, CRUZIPAIN AND ITS PEPTIDES IN CHAGASIC PATIENTS AND N INDIVIDUALS

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We have previously shown that B13 recombinant protein was recognized by peripheral blood mononuclear cells (PBMC) from Chagas'disease cardiomyopathy (CCC) and asymptomatic (ASY) patients with similar intensity and frequency producing a T1 cytokine profile. The objective of this study was to evaluate the proliferative and cytokine profile in the presence to another antigens: cruzipain and peptides (P442-453; P291-302; P261-272), recombinant antigens of *T. cruzi* obtained from amastigote library*:* heat shock proteins cy-HSP 70 and GRP78, TIP11, FcaBP. The results showed that 9/17 of CCC patients presented proliferative response to cruzipain, showing a preferential recognition to P291-302 (12/17). Among ASY patients, 5/14 presenting proliferative response to cruzipain and 3/14, 6/14 and 3/14 to P442-453, P291-302 and P261-272 peptides respectively. Only 2/12 of N individuals showed proliferative response to cruzipain, 4/12 P291-302, 2/12 P261-272 and none to P442-453.Curiously, P291-302 presents 74% homology over a 5-residue stretch with cardiac myosin (AAVPY x AAAPY). *T. cruzi* heat shock proteins, cy-HSP70 and GRP78 were recognized by 6/11 and 8/11 CCC patients, 7/11 and 8/11 ASY patients and 2/5 N individuals, respectively. TIP 11 and FCaBP antigens were recognized by PBMC from 7/11, 10/11 CCC patients; 7/11, 10/11 ASY patients and 3/5 N individuals. IFN-γ production was generally higher among Chagasic patients than N individuals, with low or undetectable levels of IL-4. Significant differences were observed in the production of IFN-γ towards the following antigens: cruzipain (P=0.01, CCC x ASY); P291-302 (P=0.024, ASY x N) TIP 11 (P=0.014, CCC x N), FcaBP (P=0.038, CH x N). These results are in line with out previous findings of a dominant T1 with a suppressed T2 cytokine profile of Chagasic patients against B13 recombinant antigen or PHA.

Supported by FAPESP, CNPq.

IM-13 – GLYCOINOSITOLPHOSPHOLIPIDS ISOLATED FROM *TRYPANOSOMA CRUZI* STRAINS AND FROM NON-PATHOGENIC TRYPANOSOMATIDS: UPTAKE BY HUMAN ANTIGEN-PRESENTING CELLS

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Glycoinositolphospholipids that are not linked to either protein or polisaccharide are the major cell surface glycolipids in all trypanosomatids investigated to date. In previous studies, the molecular structure of GIPLs from different strains of *Trypanosoma cruzi* was characterized, and classified into two series based on the substituent (ethanolamine phoshate or beta-galactofuranose) on the third mannose residue (Man3) distal to inositol (Carreira et al., 1996. *Glycoconjugate* J 13:955-966). Here we investigate the endocytic pathways of GIPLs from G, Y, CL, Tulahuen, and Colombiana *T. cruzi* strains, plus *L. samuelli* and *P. serpens* GIPLs, by human monocytes, immature dendritic cells (iDC) and macrophages. Molecules were directly coupled to FITC and the internalization analyzed by flow cytometry after 1h of incubation at 4 and 37°C. Monocytes efficiently internalized GIPL from G>Y>CL, while iDC internalized all three glycoconjugates with similar efficiency. Macrophages clearly showed an increased uptake of Y over G and CL, and even more over Tulahuen GIPL. Interestingly, only a small percentage of monocytes and iDC was capable of internalizing GIPLs from Tulahuen and *L. samuelli*. We performed blockage assays with the CHO portion of G strain, and competition assays with the unlabeled molecule. Our results strongly suggest that endocytic cells express receptors that regognize the CHO portion of GIPLs. We are currently investigating the role of pattern recognition receptors (PRRs) and the class Ib antigen presenting molecule CD1 on the binding and uptake of GIPL by these cells.

Supported by FENORTE, CNPq and PRONEX

IM-14 – IFN-γ INDUCES EXPRESSION OF FAS-L IN *T. CRUZI* SPECIFIC CD8+ T LYMPHOCYTES WHICH ARE ABLE TO KILL INTRACELLULAR PARASITES

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Trypanosoma cruzi is the causative agent of Chagas´ Disease, an important disease in South and Central America. CD4+ and CD8+ T cells play a crucial role in mediating resistance to infection by this parasite. CD8+ T lymphocytes kill target cells through two effector pathways; one involving exocytosis of preformed secretory granules, and another through expression of CD95 ligand (FasL) on the cell membrane, which then cross-links CD95 (Fas) on target cells. We have previously shown that Fas and Fas-L expressions are enhanced during the acute phase of experimental *T. cruzi* infection, and Fas-deficient mice are more susceptible to the acute infection. Also, when stimulated with rMu IL-2, spleen cells from chronically infected mice are able to kill *T. cruzi*-infected macrophages. Herein, we show that these effector cells enhanced Fas-L expression after culture with IL-2, live trypomastigotes or with a parasite lysate (TcLy). Splenocytes from chronically infected mice cultured with trypomastigotes or TcLy, showed enhanced Fas-L expression after 48, and reached maximum levels by 72 to 96 hours, whereas cells cultured with IL-2 exhibited increased expression of CD95L after 96 hours. Neutralization of IFN-γ, or addition of rMu TGF-β led to a significant inhibition of Fas-L expression induced by TcLy or live trypomastigotes. The cytotoxic activity of these cells after 96 hours in culture in the presence of IL-2 was exerted mainly by the CD8+ T cells, since depletion of CD8+, but not of CD4+ T cells, led to a significant reduction in this activity. The CD8+ T cell-mediated citotoxicity is Fas-L-dependent, since the addition of mAb anti-Fas-L inhibited the cytotoxic activity in more than 60%. These results suggest an important role to the Fas-Fas-L system in the modulation of immune response in *T. cruzi* infected mice, by limiting the parasite replication and maybe promoting the establishment of a chronic disease.

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IM-15 – INFECTION WITH *TRYPANOSOMA CRUZI* MODULATES NITRIC OXIDE PRODUCTION AND FAS/FAS-L EXPRESSION IN CULTURED MURINE CARDIAC AND SKELETAL MYOCYTES

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Following infection with *T. cruzi*, the parasites are able to survive and replicate in a variety of nucleated cells, including nonactivated macrophages, cardiac myocytes and skeletal muscle cells. The infection of cardiac myocytes or other nucleated cells into the heart tissue may trigger an inflammatory reaction, which may result in severe consequences, either in the acute and chronic phases of infection. We have recently raised the hypothesis that cardiac myocytes effectively participate in modulating the inflammatory reaction in heart of *T. cruzi*-infected animals, since our data showed that these cells express mRNA to several CC and CXC chemokines. In addition, they could be involved in the inhibition of parasite growth, since infected cardiac myocytes stimulated with IFN-γ, TNF-α and IL-1β produced enough nitric oxide (NO) to kill intracellular parasites. In the present work, we have investigated the cytokine mRNA expression and the NOS activation in cultured murine cardiac myocytes. Moreover, since NO modulates Fas and Fas-L expression, we also compared the expression of these molecules and the levels of NO production between skeletal and cardiac myocytes cells. We have found that cultured infected cardiac myocytes produce IL-1β and TNF-α that, in addition to exogenous IFN-γ, leads to trypanocidal activity as a consequence of iNOS and cNOS enzymes activation. Similarly, cultured murine skeletal myocytes stimulated with the cytokines and infected with *T. cruzi* also produce high levels of NO and control intracellular parasite replication. NO production was mediated by NOS/L-arginine pathway, since it was inhibited by treatment with L-NMMA. Moreover, we found that *T. cruzi* infection in skeletal myocytes leads to TNF-α and IL-1β production, that potentiates the IFN-γ-induced NOS activation. FACs and immunohistochemistry assessed the expression of Fas and Fas-L in normal and infected myocytes. Uninfected cells expressed Fas-L but not Fas. However, the addition of trypomastigote forms of *T. cruzi* induced Fas and decreased Fas-L expression in cardiac myocytes. Differently, Fas-L expression decreases in uninfected skeletal myocytes and increased in infected cells. Taken together, these results suggest that cardiac and skeletal myocytes are not only passively involved in inflammatory reaction that happens in infected mice. On the contrary, they might be integrated in the inflammatory phenomena by modulating Fas and Fas-L expression and secreting cytokines and chemokines, which could attract leukocytes to the inflammatory site, and promote the control of intracellular parasite replication.

Financial support: FAPESP and CNPq.

IM-16 – T CELL RECOGNITION AND CYTOKINE PROFILE TO *T. CRUZI* ANTIGENS CY-HSP 70 AND GRP78, TIP11, FCABP, CRUZIPAIN AND ITS PEPTIDES IN CHAGASIC PATIENTS AND N INDIVIDUALS

[1]Abel LCJ, [2]Engman D, [3]Giordanengo L, [3]Gea S , [1]Ianni B, [1]Mady C, [1]Kalil J and [1]Cunha-Neto E.
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We have previously shown that B13 recombinant protein was recognized by peripheral blood mononuclear cells (PBMC) from Chagas'disease cardiomyopathy (CCC) and asymptomatic (ASY) patients with similar intensity and frequency producing a T1 cytokine profile. The objective of this study was to evaluate the proliferative and cytokine profile in the presence to another antigens: cruzipain and peptides (P442-453; P291-302; P261-272), recombinant antigens of *T. cruzi* obtained from amastigote library*:* heat shock proteins cy-HSP 70 and GRP78, TIP11, FcaBP. The results showed that 9/17 of CCC patients presented proliferative response to cruzipain, showing a preferential recognition to P291-302 (12/17). Among ASY patients, 5/14 presenting proliferative response to cruzipain and 3/14, 6/14 and 3/14 to P442-453, P291-302 and P261-272 peptides respectively. Only 2/12 of N individuals showed proliferative response to cruzipain, 4/12 P291-302, 2/12 P261-272 and none to P442-453.Curiously, P291-302 presents 74% homology over a 5-residue stretch with cardiac myosin (AAVPY x AAAPY). *T. cruzi* heat shock proteins, cy-HSP70 and GRP78 were recognized by 6/11 and 8/11 CCC patients, 7/11 and 8/11 ASY patients and 2/5 N individuals, respectively. TIP 11 and FCaBP antigens were recognized by PBMC from 7/11, 10/11 CCC patients; 7/11, 10/11 ASY patients and 3/5 N individuals. IFN-g production was generally higher among Chagasic patients than N individuals, with low or undetectable levels of IL-4. Significant differences were observed in the production of IFN-g towards the following antigens: cruzipain (P=0.01, CCC x ASY); P291-302 (P=0.024, ASY x N) TIP 11 (P=0.014, CCC x N), FcaBP (P=0.038, CH x N). These results are in line with out previous findings of a dominant T1 with a suppressed T2 cytokine profile of Chagasic patients against B13 recombinant antigen or PHA.

Supported by FAPESP, CNPq.

IM-17 – POSSIBLE CHECKPOINTS FOR THE DEVELOPMENT OF CHRONIC CHAGAS' CARDIOMYOPATHY: DIFFERENTIAL ANTI-B13 T CELL REPERTOIRE AND INCREASED FREQUENCY OF IFN-G PRODUCING CELLS AMONG CCC PATIENTS

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The mechanisms underlying the pathology associated with Chagas'disase cardiomyopathy (CCC) have been subjects of polemic discussion. Susceptibility factors leading 30% of infected patients to undergo such transition are largely unknown, but immunological, genetic, environmental, and parasite-related factors may play a role. Recently we showed that cardiac myosin-crossreactive recombinant B13 protein from *T. cruzi* is recognized in by PBMC from Chagasic patients and controls in an MHC restricted manner. Furthermore, B13 stimulates high levels of IFN-γ among Chagasic patients and either IL-4 or low levels of IFN-γ among normal individuals. In order to assess recognition of variant epitopes of tandemly repetitive B13 protein, we synthesized and tested 10 peptides 15-mer in proliferative assays with PBMC from CCC, ASY and N individuals. Peptides S15.4 and S15.8 were recognized by 55% CCC patients and 9% ASY (P=0.05) and S15.1 and S 15.3 by 11% CCC patients and 27% ASY (P=0.374). N individuals showed individual-specific but diverse recognition. Only Chagasic patients (CCC and ASY) recognized S15.9 and S15.10. The frequency of IFN-γ producing cells assayed by ELISPOT in PBMC in the presence of PHA were 7910+ 3405; 4096+3083 and 2530+1738 SFC/10^6 cells in PBMC from CCC, ASY and N individuals, respectively, with significant difference among CCC x ASY (P= 0.05) and CCC x N (P= 0.005). These results suggest that the distinct repertoire among CCC and ASY patients and the high frequency of IFN-γ producing cells in CCC patients could be important for the development of CCC.

Supported by FAPESP, CNPq.

IM-18 – INFLUENCE OF OVARIECTOMY IN FEMALE C57BL/6J MICEINFECTED WITH THE Y STRAIN OF *TRYPANOSOMA CRUZI*. I - CYTOKINE PATTERN CHANGES AND PARASITEMIA LEVELS DURING THE ACUTE PHASE OF INFECTION

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It is well known that the different susceptibility between males and females to several parasitic infections is controled by a cascate of sophisticated events where both endocrinological and immunological interactions appear to be hormonally regulated. Nearly all inflamatory processes result in activation of immune cells that trigger the production of several cytokines. During *T.cruzi* infection, the resistance and susceptibility of the host depends of many factors and it is not completely understood. The objectives of this work is to study the behaviour of some cytokines on ovariectomized mice, during the acute phase of experimental Chagas disease. A group of female C57Bl/6J weighing 22 to 25g and aged one month were submitted to ovariectomy. At the same time other two groups of the same age and weight were separated in Sham Operated and Control groups. One month after the surgerall animals were i.p. inoculated with 4000 trypomastigotes of the Y strain of *T,cruzi*. Blood was colected and TNF, IL-2 and IL-10 were analyzed through Eliza Method from serum, on 5th, 9th and 25th day after the inocule. In the initial phase of the infection all tested cytokines did not show important differences. In the peak of parasitemia, ovariectomized group showed a significant drop in the levels of IL-2, IL-10 and TNF. These low levels persisted until 25th day after inocule. TNF (7.0pg/ml against 25pg/ml of the control group and 20pg/ml of the sham group), IL-2 (0.15pg/ml against 0.33pg/ml of the control and sham groups) and IL-10 (100.0pg/ml against 590.0pg/ml of control and sham groups respectively). The fact that ovariectomized C57Bl/6J display a lower parasitemia when compared to control and sham groups (4,0 x 10^6 against 2,3 x 10^6) can be explained through our results. In the peak of parasitemia as well as in the late phase, there is a significant drop in the levels of all cytokines tested. It has been previously published that some cytokines like TNF and IL-10 render to the host a higher susceptibility. So a lesser parasitemic level showed by ovariectomized group could be linked to lower levels of cytokine, specially TNF and IL-10. These results demonstrate that the steroid hormones play a significant role in the process of the immune response against *T.cruzi*. Further studies are being conducted in order to found out the role of these interleukins and cell populations in ovariectomized animals submitted to female and male steroid reposition.

IM-19 – PROGRESSION OF CHAGASIC CARDIOPATHY TO END-STAGE CORRELATES WITH INFILTRATED T-LYMPHOCYTES, MACROPHAGES, MAST CELLS AND INTRAMYOCARDIAL MICROVESSELS

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Human chronic chagasic cardiopathy (CCC) is produced through a pathogenic process with an immunopathologic component, according to several evidences. In severe cases of CCC we have previously found infiltration by T-lymphocytes, which produce glycoprotein substances; a rise in intracardiac mast cells and neoformation of microvessels with high endothelium(HRA Cabral, ITC Novak, GB Robert. *Rev Argent Cardiol*, 61: 463, 1993; HRA Cabral, ITC Novak, *Mem Inst Oswaldo Cruz*, 90, Suppl I, p195, 1995; HRA Cabral, ITC Novak, GB Robert. *Pren Med Argent*, 85, 525, 1998).

In order to determine the respective occurrence of the above mentioned facts, as well as of other immunologic cells, into the myocardium of chagasic patients* who died of irreversible cardiac failure at different ages, we studied here 10 cases of CCC dead at a mean age of 63 years (group 1) and 6 cases of CCC dead at a mean age of 42 years (group 2). All patients had several positive reactions for Chagas disease. Their electrocardiograms showed several pathologic changes typical of chagasic heart disease with severe arrhythmia. The study was performed with monoclonal antibodies for T-lymphocytes, B-lymphocytes, monocytes-macrophages, respectively, and also by classical and histochemical methods (PAS, Feulgen). The results showed that the number of foci of intramyocardium-infiltrated cells were higher in the hearts of group 2 as well as their total cell population number. Qualitatively, the cell type composition was similar in both groups: T-lymphocytes predominate throughout, being 63 % in group 2 and 60 % in group 1. Monocyte-macrophages were ≥ 25 % in both groups. B-lymphocytes were less than 2 % in both groups. Mast cells were found — in the myocardium— in quantities of 0.7 per high power field at 400x, in group 1, and of 1.55 % in group 2, being both values higher than those of normal hearts. Neoformation of microvessels both with high endothelium or with a normal one, was found in both groups, but was more intense in group 2 (+ and +++, respectively). *Trypanosoma cruzi* parasites were not found in any case. T-lymphocytes and monocyte-macrophages as well as mast cells were found contacting and surrounding cardiomyocytes with signs of cell damage. These findings suggest a predominancy of such immunocytes in the pathogeny of hearts from chagasic patients which developed severe heart failure, and were more intense in the patients who suffered early cardiac death.

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IM-20 – IGG SUBCLASS AND FINE EPITOPE SPECIFICITY OF ANTI-B13 ANTIBODIES IN LATIN AMERICA CHAGASIC SERA

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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 chagasic sera from nine countries of Central and South America showed variation in the O.D. mean values (MD) of B13 ELISA according to the analysed country: (*eg* Argentina, MD=1.79; Brazil, MD=1.51; Honduras, MD=0.91) (Umezawa et al., 1999). Previous studies indicated that the immunodominant epitope core of B13 protein is the hexapeptide AAAGDK (Cunha-Neto et al., 1995). Chagasic sera from Argentina, Brazil, Bolivia, Colombia, El Salvador, Honduras and Venezuela were investigated concerning the IgG subclass reactive to B13 and the B13 epitope recognized by these sera. IgG subclass analysis was performed in 224 sera by direct ELISA with recombinant protein B13 in the solid phase. Results indicate that IgG1 is the major subclass reacting with B13 in all Latin America chagasic sera. Analysis of the B13 epitope specificity was performed in 147 sera by competitive ELISA with B13 in the solid phase and three peptides - pB13 (GDKPSLFGQAAAGDKPSPLF), S4 (FGQAAAGDK) and S5 (QAAAGDKPS), derived from B13 amino acid sequence. Data indicate a very similar behaviour of the sera in respect to the inhibition promoted by the peptides. The mean percent inhibition was 83%-93% for pB13; 78%-88% for S4; and 63%-75% for S5. It is concluded that although chagasic sera from countries of Latin America have different reactivities to B13 (mean values of B13 ELISA) there is no variation in the IgG subclass induced by the B13 antigen and in the immunodominat B13 epitope recognized by these sera.

Supported by FAPESP and CNPq

IM-21 – EXPERIMENTAL *T. CRUZI* INFECTION: II. ROLE OF TNF-A, INF-γ AND IL-10 IN THE PROTECTION OF MICE IMMUNIZED WITH HEMOCYTES FROM UNINFECTED *T. INFESTANS*

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Hemocytes (HC) from uninfected triatomines were shown to interact with chagasic sera. Moreover in immunized mice with HC from uninfected *T. infestans* followed by a challenge of trypomastigotes of "Y" strain, a partial protection was observed with low parasitemia and mortality. However, sera from these mice before the challenge failed to recognize both trypomatigotes as well as epimastigotes. Since the humoral immune response was not detected, the involvement of cellular response was here studied. Female A/Sn mice were immunized using alumen's adjuvant with HC from uninfected 5^{th} instar *T. infestans* nymphs, three times with 10-15 day intervals. The group I of mice received successively 2, 3 and 4 x 10^6 HC/mL. The group II received 188-200mg protein of hemolymph (HL) free of HC and the group III (control) received only alumen's adjuvant (0.5mg/mL). All groups of mice were challenged with 5 x10^2 bloodstream trypomastigotes 15 days after immunization. Sera were collected before *T. cruzi* infection, before the peak of parasitemia, shortly after the peak and in the chronic phase. TNF-α, INF-γ and IL-10 were assayed by ELISA (Biosource). HL-mice presented higher level of INF-γ before the challenge than the other groups, followed by a significant decrease and kept low values until the end of the experiment. HC-mice, however, showed higher value than the control group before the challenge. Then, after a slight fall before the peak (like the control group) the level increased at the peak and in the chronic phase, in contrast to the control group. In respect to the TNF-α, HL-mice showed a similar profile to the control group, whereas in the HC-mice, there was a remarkable increase towards the peak, decreasing slightly thereafter. The IL-10 in turn was high in the control group, low in HL-mice and significant lower in HC mice. The data demonstrate that the partial immunoprotection is provided by HC cells rather than HL, through the mechanisms of cellular immunity.

IM-22 – ASSOCIATION OF MEGAESOPHAGUS WITH BLOOD AND TISSUE PARASITEMIA IN CHRONIC CHAGASIC PATIENTS USING PCR, HEMOCULTURE AND XENODIAGNOSIS

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The mechanisms involved in the development of lesions in Chagas disease are not well understood. However, the persistence and density of parasites in tissues appear to be associated with the evolution of different clinical forms. Here we evaluate blood and tissue parasitemia in 50 chagasic patients with megaesophagus who underwent therapeutic surgery. Esophageal fragments from three individuals with idiopathic megaesophagus were used as controls. The following tests were performed: hemoculture, xenodiagnosis and PCR using S35 and S36 primers to amplify the 330-bp region of *Trypanosoma cruzi* kDNA. In blood, parasite DNA was detected in 76% of patients using xenodiagnosis or hemoculture and in 81.6% using PCR. In tissue fragments, the presence of parasite DNA was observed in 50% of samples and DNA extraction was random, that is, not associated with an inflammatory focus. Of the patients that had positive PCR results in tissues, 84% had positive parasitologic and PCR results in blood. Among patients that had negative PCR results in tissues, 79.16% had positive PCR results in blood while the parasite was detected in 68% of them using xenodiagnosis or hemoculture. *T. cruzi* is easily detected by PCR in blood of patients with both positive and negative PCR in tissues, with no correlation observed between the presence of the parasite in tissue and in blood. The high parasitemia observed in subjects suggests some type of immunosuppression related to this clinical form or could reflect an intrinsic characteristic of *T. cruzi* populations associated with megaesophagus. The presence of *T. cruzi* DNA in esophageal tissue illustrates the importance of the parasite in the evolution of chagasic megaesophagus.

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IM-23 – PATHOLOGY OF CHAGAS' DISEASE ASSOCIATED TO SYSTEMIC ARTERIAL HYPERTENSION

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Chronic Chagasic Myocarditis is characterized by a diffuse or focal inflammatory process with predominance of lymphocytes, destruction gradual of cardiac fibers and interstitial fibrosis. Progressing the disease occur a heart failure and cardiomegaly. Individuals infected by *T. cruzi* in the indeterminate phase present positive serology without any signal of disease. Most of those individuals (80-85%) do not evolve to the cardiac chronic form of disease. Some individuals with positive serology without myocarditis develop systemic arterial hypertension. It is believed that patients with chronic chagasic myocarditis can not sustain high levels of blood pressure. We studied 54 necropsied cases of chronic chagasic myocarditis from 1974 to 1998. Five of them (10%) presented also systemic arterial hypertension. Histopathological study did not show differences about the inflammatory process but the cases of Chagas' disease associated to systemic arterial hypertension presented more intense hypertrophy of fibers and interstitial fibrosis.

IM-24 – SERA OF INDIVIDUALS INFECTED BY *T.CRUZI* IN ACUTE FORM, INDETERMINATED FORM AND CHRONIC CARDIAC FORM BUT NOT SERA OF NORMAL INDIVIDUALS BLOCK ATP RECEPTORS OF THE PARASITE

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Trypanosoma cruzi has a plasma membrane ATP transport system that may consist of an exterior receptor domain (ATP-R). Trypomastigote$[ATP]_o \leftrightarrow ATP\text{-}R \rightarrow ATP]_i$ transport was [ATP]o-dependent and saturable at100uM. $[ATP]_o \leftrightarrow ATP\text{-}R \rightarrow [ATP]_i$ transport was abrogated by the tyrosine kinase inhibitors, genistein and lavendustin A. $[ATP]_o \leftrightarrow ATP\text{-}R \rightarrow [ATP]_i$ transport was also inhibited by the serine/threonine/kinase inhibitor, staurosporin. Suramin, the antagonist of P2x and P2y purinergic receptors, was also a very effective competitive inhibitor of the trypomastigote ATP transport system(Sadigursky & Santos-Buch,1997). It is very well known that cells of different organs of mammalians have ATP receptors. We do not know weather ATP receptors of *Trypanosoma cruzi* are different of the ATP receptors of human host cells and weather the receptor is antigenic capable to induce the production of specific antibodies by the host. In the present study we tested the capacity of sera from individuals infected by *T. cruzi* block the attachment and transport of ATP in a Peruvian strain of *T. cruzi*. To detected this we used a radio assay with a radiolabeled ATP (^{3}H-ATP). All the sera from infected individuals (4 acute form, 18 chronic indeterminate form and 23 chronic cardiac form) reacted with the ATP receptor and blocked the ATP transport in a range of 11,1 to 76,5%. Five sera from normal American individuals did not inhibited the ATP transport. We conclude that the ATP receptor in *T. cruzi* is antigenically different of the human host ATP receptor and this knowledge can be used to development of drugs against this parasite.

IM-25 – SEROPREVALENCE OF ANTIBODIES TO *TRYPANOSOMA CRUZI* IN BLOOD DONORS IN LONDRINA, PARANÁ, BRAZIL

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Chagas's disease caused by *Trypanosoma cruzi*, affects millions of people in Latin America. The disease is often lethal and virtually incurable. Risk of infections is related to exposure to insects harboring *Trypanosoma cruzi* or to the transfusion of blood from an infected donor. Serological diagnosis of *T. cruzi* infection is still the method of choice for routine analysis and screening in blood banks and clinical laboratories. Early investigations at blood banks in the town of Londrina, Brazil, demonstrated that the seroprevalence of anti-*Trypanosoma cruzi* antibodies among blood donors was approximately 7.0% in the fifties (Brofman, 1958, *Arq. Bras. Cardiol.*, 11: 209-210; Queiroz & Pascual, 1958, *Rev. Med. Paraná*, 27: 27-30). Further studies demonstrated pratically the same seroprevalence until the eighties (Baldy *et al*., 1978, *Rev. Saúde Públ.* (São Paulo), 12: 409-416; Pontello *et al*., 1985, Semina (Londrina) 6: 87-92). More recently, was found a serumprevalence rate of 1.4 % (Reiche *et al*., 1996, *Rev. Inst. Med. Trop. S. Paulo*, 38 (3): 233-240) and 1.3% (Bonametti *et al*., 1998, *Rev. Saúde Pública*, 32 (6): 566-71) for positive serum findings for *T. cruzi* infection on blood donors in the region. In the present work, the seropositivity for Chagas disease was evaluated in 165,179 serum samples from voluntary blood donors of Instituto de Hematologia de Londrina (IHEL) from January 1995 to December 1998. The serum samples were studied by the indirect hemagglutination assay (IHA, using kit commercially obtained from Biolab-Mérieux) and commercial ELISA Kit (Chaganostica, Organon Teknika). The results demonstrate that 1,273 serum samples were positive in both assay corresponding to a seroprevalence of 0.77% , i.e., a significant decrease in anti-*Trypanosoma cruzi* antibodies in the region in comparison with the previously mentioned rates. Data correlating sex and age of seropositive blood donors are presented, as well as the possible factors that may have contributed to the results observed.

Supported by IHEL and UEL

IM-26 – NITRIC OXIDE AND IL-10 ARE THE MOST IMPORTANT NEGATIVE REGULATORS OF IL-12 AND IFN-γ SYNTHESIS IN EXPERIMENTAL *TRYPANOSOMA CRUZI* INFECTION

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IFN-γ is a potent macrophage activator to a trypanocidal effect and participates in the resistance to *T. cruzi* infection. Previous results from our lab have shown that trypomastigote-Ag (T-Ag)-stimulated spleen cell cultures from infected C57BL/6 mice produce increasingly higher levels of IFN-γ and IL-12 from days 3 to 7 after infection, followed by a marked reduction during the 2nd week (days 9 and 12) and a subsequent increase by the end of the 3rd week that is maintained until the 4th week infection.

In order to identify the molecules that exert the down-regulation of IFN-γ synthesis, we treated the cultures with anti-IL-4, anti-IL-10, anti-IFN-α,β or anti-TGF-β neutralizing antibodies, or with the inhibitor of NO synthesis, NMMA. Increase of IFN-γ and of IL-12 synthesis was observed following anti-IL-10 treatment, whereas NMMA treatment only increased IFN-γ production. Combined treatment with both anti-IL-10 and NMMA, had an additive effect on both IFN-γ and IL-12 synthesis resulting in 3-fold and 2-5 fold higher levels respectively. No effect was observed for anti-IL-4, anti-TGF- α,β or anti-IFN- α,β treatments on IFN-γ or IL-12 synthesis at the tested times. Anti-TGF- β + NMMA resulted in only a moderate increase of IFN-γ synthesis. Neutralization of IL-12 in anti-IL-10 treated cultures abolished the increase of IFN-γ promoted by anti-IL-10.

Taken together, these results indicate that IL-10 and NO are the most important molecules exerting negative regulation of IFN-γ synthesis in experimental *T.cruzi* infection. IL-10 inhibits IFN-γ production by inhibiting IL-12 synthesis. NO-mediated inhibition is possibly related to its suppressive effects on T cell proliferation and activation.

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IM-27 – NITRIC OXIDE PRODUCED BY MACROPHAGES AND 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 are highly susceptible to *T.cruzi* infection. Host resistance is dependent on innate and acquired immune responses. This work aims to investigate the production of nitric oxide and cytokines by macrophages at the initial stages of infection and the possible regulation of resistance. Macrophages were obtained from mice inoculated by the intraperitoneal route with 70,000 and 5,000 trypomastigotes Y strain. We investigated the production of NO, IFNγ, and IL-12 and the expression of mRNA for TGF-β, iNOS, TNFα, IFNα and IFNβ by intraperitoneal macrophages. NO production was measured 12h, 48h and 72h in macrophage culture supernatants from mice 6 hours after infection. B6 produced higher levels of NO than BALB/c. However, production of NO was inhibited in both strains of mice when infected with 70,000 trypomastigotes. Both BALB/C and B6 produced IL-12. Comparison of mRNA levels for the different cytokines was done by a competitive, semi-quantitative RT-PCR assay. As early as six hours after infection, we detected similar levels of TNFα mRNA in BALB/c and B6 mice. Message for IFNα and for IFNβ was detected only in macrophages from B6 mice. TGFβ mRNA wasn't detected in either strain..

These results suggest the importance of very early production of NO in determination of resistance. In addition, this resistance could be modulated by IFNα/β production.

Supported by FAPESP, CNPq and CAPES.

IM-28 – DNA VACCINATION WITH A GENE THAT ENCODES THE GLYCOPROTEIN OF 82 KDA (GP82) OF METACYCLIC TRYPOMASTIGOTES OF *TRYPANOSOMA CRUZI*

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Metacyclic forms of *Trypanosoma cruzi* express a surface glycoprotein of 82 kDa (gp82) that is involved in the penetration of parasites into host cells. Immunization with the recombinant protein protects mice against the challenge with metacyclic forms (Santori et al., 1996, *Infect Immun* 64:1093). To determine whether similar protective immune response could be achieved by immunization with DNA, the ORF of gp82 plus the virus haemagglutinin signal peptide was subcloned in the vector pcDNA3 (InvitroGen) or pCI (Promega), generating plasmids pc-gp82+SP and pCI-gp82+SP, respectively. Groups of BALB/c mice were immunized with plamids pc-gp82+SP, pCI-gp82+SP, pcDNA3 or pCI. Each mouse received four doses of 100μg of DNA intramuscularly in the *tibialis anterioris*, at 3 week intervals. Significant differences in anti-gp82 antibody titers were observed in pc-gp82+PS or pCI-gp82+PS groups when compared with controls immunized with non recombinant plasmids. However, antibody titers of pCI-gp82+SP group were significantly higher than the titers obtained for pc-gp82+SP group. These antibodies specifically reacted with a gp82 protein on the surface of metacyclic trypomastigotes. Spleen lymphocytes of mice immunized with plasmids pc-gp82+SP or pCI-gp82+SP were able to proliferate in the presence of the recombinant gp82 protein. Each group of mice were subsequently challenged intraperitonealy with 10^5 metacyclic forms of *T. cruzi* CL strain. Our results suggest that a partial protection against the accute phase infection can be obtained in mice immunized with plasmid pCI-gp82+SP.

Supported by FAPESP, CNPq/PADCT.

IM-29 – QUANTITATIVE ANALYSIS OF $CD4^+$ AND $CD8^+$ CELLS IN EXPERIMENTAL ACUTE CHAGASIC MYOCARDITIS IN DOGS

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We have previously shown that lymphocytes are the predominant cell type in the inflammatory infiltrate of acute chagasic myocarditis induced by Berenice 78 (Be78) and Berenice 62 (Be62) *Trypanosoma cruzi* strains in dogs (Caliari et al., 1995, *Int. J. Exp. Pathol.* 76:299-307; Caliari et al., 1995, *Rev. Soc. Bras. Med. Trop.* 28:13-17). Like in other experimental models of Chagas disease, the identification and quantification of the cells in the inflammatory infiltrate is essential for a complete understanding of the natural history of the disease. The present study aims to quantify the number of infiltrating $CD4^+$ and $CD8^+$ cells in acute chagasic myocarditis in dogs. Eight dogs aged approximately 60 days were inoculated via the conjunctiva route with 2,000/kg metacyclic trypomastigotes of the Be78 strain. Animals were sacrificed 35 days later, necropsied and fragments of the right atria, spleen, lymphnodes (submandibular, intertracheobronchial and inguinal) were obtained. Some of these fragments were stored at - 70°C and some fixed in bouin, processed for paraffin and stained with hematoxylin/eosin and Gomori's Trichrome for histopathology. The frozen fragments were fixed in acetone PA 100% -20°C overnight, infiltrated and included in catalyzed acrylic monomer (JB-4 Kit; Polysciences, Inc., Warrington, USA) at 4°C. Four µm thick cuts were obtained and processed for immunohistochemistry using rat anti-canine CD4 and CD8 antibodies (1:10 dilution, Serotec Ltd., Oxford, UK). Positive cells were identified using a biotinylated goat anti-rat IgG (1:50, Zymed Laboratories Inc., San Francisco, USA) and streptavidine (1:100, Zymed Laboratories Inc.). Color was detected using diaminobenzidine 0,05% in H_2O_2 0,2% buffer as substrate and slides were counterstained with hematoxylin and eosin. Substitution of PBS for the primary antibodies were used as negative controls. Under light microscopy (40X objective), tissue images were digitalized (15 images/cell marker, $8x10^5$ mm^2 of tissue area) and positive cells counted using the KS300 software in a Kontron Elektronik/Carl Zeiss image analysis system. Histopathological analysis demonstrated a moderate to intense acute myocarditis which was predominantly composed of lymphocytes and a large number of amastigotes nests. In the resin sections, the mean ± SD (range) of $CD4^+$ and $CD8^+$ cells in heart sections were 1029,63 ± 375,25 (688 to 1798) and 1120,62 ± 290,25 (768 to 1576), respectively. These differences were not statistically significant as assessed by Student's *t* test ($t = 0,4$; $p>0,05$). This quantitative analysis agrees with the impression gained when examining stained tissue sections. This is the first time CD4 and CD8 immunophenotyping is carried out in the heart of chagasic dogs. It will be important to evaluate the expression of these surface markers in the chronic model to compare our results to the findings in chagasic humans.

Supported by FAPEMIG and PRPq/UFMG.

IM-30 – LEUKOCYTE PHENOTYPES IN MYOCARDITIS INDUCED BY TWO DIFFERENT *TRYPANOSOMA CRUZI* POPULATIONS IN RATS

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Rat susceptibility to *Trypanosoma cruzi* depends on the parasite population. The inoculation of 10,000 trypomastigotes of JG strain induces low parasitemia, discrete to moderate acute myocarditis and no mortality. The inocula of 10,000 and 1,000 trypomastigotes of the CL-Brener clone provoke higher parasitemic values, severe myocarditis with death of 90% (10,000) or 74% (1,000) of the animals. Only the CL-Brener strain (both inocula) was able to cause severe cardiac sympathetic denervation. Now we aim at verifying the leukocyte phenotypes during acute myocarditis induced by these two *T. cruzi* populations. Holtzman rats aged 27-29 days were inoculated with 10,000 (JG strain) or 1,000 (CL-Brener clone) trypomastigotes ip. For the immunostaining, monoclonal antibodies (ED1, ED2, CD8, CD4, CD45RA and NKR/CD161 from SEROTEC) and peroxidase labeled secondary antibodies (PHARMINGEN) were used. Besides heart tissues from non-infected animals, the immunoreaction was controlled by omitting the primary antibodies (negative control) and by using the full procedure in lymph nodes (positive control). Regardless the *T. cruzi* population, the acute myocarditis was characterized by predominance of $ED1^+$ and $ED2^+$ macrophages. The $ED1^+$ macrophages were found dispersed or accumulated in the inflammatory foci. In contrast, the $ED2^+$ macrophages were always diffusely distributed. The $CD8^+$ and NKR+ cells occurred in small number with focal distribution. The $CD4^+$ and $CD45RA^+$ cells were rarely observed. The leukocyte phenotypes were also studied in animals inoculated with 300,000 trypomastigotes of the JG strain at both acute and chronic phase. With this higher inoculum the parasitemia remains low with no mortality. The acute myocarditis was more severe and sympathetic denervation was observed only at the end of the phase (37 days post inoculation). Inflammatory processes were found till day 130 of infection. At day 37, the immunostaining showed the same results described above. At day 130 of infection, the $ED1^+$ cells were still elevated but less numerous in comparison with the acute phase. In contrast the amount $ED2^+$ cells remained about the same. The amount of the other leukocytes varied greatly among the animals with tendency toward decrease. Studies of cytokines levels are in progress to further investigate mechanisms involved in nerve terminal damage (CL-Brener) and chronic myocarditis (JG strain).

PRONEX-1996, CNPq, FAPEMIG

IM-31 – PCR REACTION, PARASITOLOGICAL AND SEROLOGICAL DATA DURING THE CHRONIC PHASE OF EXPERIMENTAL CHAGAS´DISEASE IN DOGS.

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Fourteen dogs experimentally infected with *Trypanosoma cruzi* (7 with Be-62 strain, 5 with Be-78 strain and 2 with Colombiana strain) 4 -15 years were evaluated at a regular interval of 3 months , during 2 years in parallel with control animals (8 dogs). Parasitemia was evaluated by hemoculture, xenoculture and the serology by ELISA, indirect immunofluorescence test (IIFT) and analysis of anti-live trypomastigote antibodies - ALTA) by Flow Citometry (FACScan).

Parasitemia was detected in 57.1 and 21.4 % of dogs by hemoculture and xenoculture respectively and 71,4% when both results were considered. The conventional serology (ELISA and IIFT) and FACScan/ALTA were reactive in 95,7% of the animals. The PCR reaction processed in 2 samples collected in 2 periods of the infection (2 years of interval) detected k-DNA of the parasite in 78,5% of the dogs. Taking into account each collection the rates of positivity of PCR reaction ranged from 50 to 64 %. However this percentage reaches 100% when more samples of each animal collected in different periods of the infection are analysed. In control animals all tests (hemoculture, xenoculture, ELISA, IIFT and Flow Citometry) were negative.

These findings validate the dog as a good experimental model for the study of Chagas´disease showing the presence of the parasite in 100% of the infected animals similar than in human infections.

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IM-32 – ACUTE PHASE OF CHAGAS DISEASE IN BOLIVIAN CHILDREN: A BENIGN PHASE OF *TRYPANOSOMA CRUZI* INFECTION THAT CAN BE TRACED BY HUMORAL SEROLOGICAL MARKERS AND ACUTE PHASE PROTEINS

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Acute phase of Chagas disease was studied in children acutely infected by *T. cruzi* in the department of Cochabamba, an important endemic area in Bolivia. Cases (aged 2 to 15 years-old) came from seroepidemiological surveys in three rural villages (Pasorapa, Sipe-Sipe and Aiquile) and in one suburban area in the city of Cochabamba; acute patients undertaking hospital medical care at UMSS were also studied. All the seropositive cases found in the epidemiological study were asymptomatic, and the frequency of ECG alterations found in this group was similar to that found in seronegative age-matched controls from the same region. Through blood type determinations we confirmed that genetic background of this Bolivian population did not vary so much: 90 % were blood typed O, 8 % A, 1.2 % B and 0.8 % AB, most Rh+. We then used a combination of three serologic markers and showed that it can trace the approximate timing of infection, classifying acute Chagas disease as early, intermediate, and late infection based on the levels of anti-Gal $a_{1,3}$Gal IgG (Gal) and specific IgM (M) and IgG (G) anti-*T. cruzi* reactivity. While early phase was M+G-Gal-; intermediate was M+G-Gal+ or M+G+Gal- or M+G+Gal+; and late was M-G+Gal+. This sequence of stages fitted well with studies on some acute phase proteins (APP): A2M, CRP and fibronectin. However, those APP could not be used as direct markers of recent infection, since they did not increase in all the seropositive cases, and a percentage of seronegative cases did always show high APP levels, varying with the APP, probably due to other infections. Using PCR analysis for parasite DNA in samples of children living in Cochabamba, Bolivia, we observed a significant correlation (90.8%) between acute combined serodiagnosis by ELISA and PCR positivity. PCR+ occurred in samples where only Gal was increased, suggesting a very early *T. cruzi* infection, when specific antibodies were not yet present. Anti-Gal IgG can thus indicate presence of the parasite and of an active infection. The non applicability of indirect hemoagglutination assay (IHA) as a diagnostic test in acute cases, and the low correlation between PCR and IHA (58%), indicated that IHA has a worse performance than ELISA. Only one seronegative child (out of 65 studied) was PCR+, indicating a false negative serological result. The high heterogeneity in APP was antecipated for samples collected at different times after natural infection, different inocula, and even different numbers of possible re-infections. The most precocious APP were CRP and Fn, which increased significantly in early and intermediate acute stages. A2M increased in some early cases but with a higher frequency in late acute stages. A2M levels were significantly higher in asymptomatic, as compared to symptomatic acute cases. By associating anti-Gal IgG with specific serology, early *T. cruzi* infection could then be detected with greater precision, helping on detection of recent *T. cruzi* infections, at least in endemic areas where diseases caused by other trypanosomatids do not overlap.

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IM-33 – IMMATURE T LYMPHOCYTES IN PERIPHERAL LYMPHOID ORGANS OF *TRYPANOSOMA CRUZI*-INFECTED MICE

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Previous studies in our laboratory demonstrated that acute *Trypanosoma cruzi* infection promotes several alterations in the thymus. Recently, we evidenced an increase in the kinetics of thymocyte release from thymic nurse cells of infected mice, concomitantly to an enhancement in the production of extracellular matrix (ECM) proteins in these cells. In addition, expression of ECM ligands and receptors is augmented in the thymus of these mice. The finding of immature CD4+CD8+ T lymphocytes in the peripheral lymphoid organs of infected mice, lead us to postulate the existence of a disturbance in intrathymic T cell migration following acute *T. cruzi* infection. We observed that these immature cells from infected mice express higher levels of VLA4, VLA5 and VLA6 molecules in comparison to the same subset from control mice. Herein we also investigated the contribution of the thymus to the presence of those cells in the periphery. We observed that thymectomy prior to the infection lead to a significant decrease in the number of those immature T cells in the periphery. Moreover, analyses of some TCR Vbeta elements showed increased numbers of immature T cells carrying Vbeta 5 and Vbeta 12 segments, which are virtually absent from the periphery in BALB/c strain, and normally present in the thymus of these mice only prior to the selective events in this organ. These data suggest that the premature exit of thymocytes from infected mice can lead to changes in the T cell repertoire, and most importantly, allowing the escape of cells carrying « prohibited » Vbetas. Hypothetically, such cells may represent clues for the generation of an autoimmune response.

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IM-34 – CYTOKINES IN THE PATHOGENESIS OF MEGABLADDER IN EXPERIMENTAL CHAGAS DISEASE

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We have been studied the histopathological changes of the bladder in experimental Chagas Disease by Haematoxylin-Eosin (H&E) and Masson stainings. Balb/c mice infected with 50 forms of peripheral blood trypomastigotes by intraperitoneal route showed that the bladder involvement is characterized by dilatation and fibrosis in muscular layers after two months of infection. The inflammatory process seen in acute phase is substituted by collagen bundles mixed up with muscular cells with the evolution of the disease (Scremin,L.H.G et al. - Rev. Hosp. Clín. Fac. Med. S. Paulo 54 (2): 43-46, 1999). Some studies suggest the direct relationship between the host protection and the trypanosomicidal macrophage activity through NO production induced by IFN-γ and TNF-α (Muñoz-Fernandez,M.A. et al. - Eur. J. Immunol. 22: 301-307, 1992), while others, related the parasite proliferation with IL-4. However, there are no descriptions about the cytokines involvement in the megabladder pathogenesis during chagasic infection.

In order to study some immunopathological mechanisms of the disease and their relationship to histopathological features, we inoculated Balb/c mice with 20 forms of blood trypomastigotes (Y strain) and evaluate the chronicity of the disease by serology after six months of the infection. Frozen sections of mice bladders in chronic phase of the experimental infection were used for immunohistochemistry using monoclonal antibodies for cytokines and H&E stain method for histopathology.

Thirty per cent of the animals infected showed positive reaction for specific IgG in the sera by direct immunofluorescence method. These IgG-positive animals showed positive reaction for IFN-γ and TNF-α besides to negative reaction for IL-6 and IL-4 in chronic megabladder frozen sections. Compared with previously histopathological results, there was regression of the bladder inflammatory infiltrate in chronic phase and IFN-γ and TNF-α are still present in the lesion.

Our preliminary data suggest that the regression of the inflammation in the megabladder can be related to the presence IFN-γ and TNF-α, cytokines involved on host protection and trypanosomicidal activity.

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IM-35 – CIRCULATING AUTOANTIBODIES AGAINST MYELIN BASIC PROTEIN DURING EXPERIMENTAL CHAGAS' INFECTION: INVOLVEMENT IN PATHOGENESIS OR PROTECTIVE AUTOREACTIVITY?

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During Chagas' infection damage in the central nervous system (CNS) is more severe in children under two years of age and immunosuppressed patients such as AIDS and transplanted patients. Rare amastigote forms of the *T. cruzi* and inflammatory infiltrates with variable intensity and irregular distribution are localized within the CNS during the acute phase of the disease. Lymphocytes and circulating antibodies specific for neurons and myelin basic protein (MBP) have been detected in experimental models suggesting the participation of the immune system in the CNS lesions. However, some questions regarding the participation of antibodies against the CNS components in the nature and extention of the CNS damage during Chagas' disease remains unsolved. To shed light on these questions experimental Chagas' infection was induced in C3H/He (susceptible) and C57BL/6 (resistant) mice using the Colombian strain of *T. cruzi*. Both C3H/He and C57BL/6 strains exhibited peak of parasitemia at 42 days post infection (dpi) and trypomastigotes were rarely found in the blood at 63dpi, characterizing the onset of the chronic phase. Around 30% of the infected mice died during the acute infection.The C3H/He mice showed several pathological alterations in the CNS such as edema, enlargement of perivascular spaces and intense inflammatory infiltrate mainly composed of $CD8^+$ T cells and macrophages during the acute phase. These inflammatory lesions were not related to the presence of the *T. cruzi* antigens that were immunohistochemically characterized as isolated positive cells scattered throughout the parenchyma (Silva *et al*, 1999, *Clin. Immunol.*, 92: 56-66). Moreover, antibodies that recognize MBP and its encephalitogenic fragment comprising the 4-14 amino acid sequence were detected 28 dpi, contrasting with low levels of antibodies against *T. cruzi* and the in the absence of polyclonal activation. When these sera were incubated with normal brain sections the cerebellum white matter was specifically recognized. During the late acute and chronic phase of the infection, the level of anti-MBP antibodies persisted high and paralleled the levels of total IgG and antibodies against *T. cruzi*. The C57BL/6 mice did not present the histopathological alterations in the CNS observed in C3H/He mice. They presented rare inflammatory infiltrates restricted to areas of incomplete blood-brain barrier. Antibodies recognizing MBP were not found in these mice during the acute phase and low levels were detected during chronic infection.

In order to approach the biological role of these anti-MBP antibodies, sera of *T. cruzi*-infected C3H/He mice were absorbed with parasite antigens. The immunoreactivity against MBP was partially abrogated, suggesting the existence of a cross reactivity (or molecular mimicry) between *T. cruzi* and MBP. This idea was reinforced by the results showing that the reactivity against MBP from sera of MBP-immunized mice is also inhibited by previous incubation with *T. cruzi* antigens. Considering the proposed hypothesis that self-reactivity lies at the basis of immune reactivity (Sercaz & Mitchison, 1999, *The Immunologist*, 7: 49-51), i*n vivo* experiments aiming to investigate a possible protective effect of these autoreactive anti-MBP antibodies during Chagas' infection are currently in progess.

Sponsored by: CNPq, CAPES, PAPES-Fiocruz

IM-36 – INFLAMMATORY PROCESSES IN THE CENTRAL NERVOUS SYSTEM DURING EXPERIMENTAL *TRYPANOSOMA CRUZI* INFECTION: PARTICIPATION OF $CD8^+$ T CELLS, FIBRONECTIN AND VLA-4 INTEGRIN

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Trypanosoma cruzi-infected patients rarely show neurological alterations. Damage in the central nervous system is more severe in children under two years of age and immunosuppressed individuals as AIDS or transplanted patients. During the acute phase, rare amastigote forms of the *T. cruzi* were localized within glial cells, macrophages and neurons. However, inflammatory foci are frequently found within the CNS. In spite of the various studies in human and experimental models, some questions regarding the nature and extention of damage in the CNS during Chagas' infection remain unsolved. To shed light on these questions C3H/He mice were infected with the Colombian strain of *T. cruzi*. Peak of parasitemia was observed at 42 days post infection (dpi) and trypomastigotes were rarely found in the blood at 63dpi, characterizing the onset of the chronic phase. Around 70% of the infected mice survived and developed the chronic infection. During the acute phase, several pathological alterations were observed in the CNS such as edema, enlargement of perivascular spaces and intense inflammatory infiltrates mainly composed of $CD8^+$ T cells and macrophages. These inflammatory lesions were not related to the presence of the *T. cruzi* antigens that were immunohistochemically characterized as isolated positive cells scattered throughout the parenchyma (Silva *et al*, 1999, *Clin. Immunol.*, 92: 56-66). It has been shown that fibronectin (FN) and VLA-4, its receptor to integrin play a role in the migration of inflammatory cells into the CNS in demyelinating diseases (Shin *et al*., 1995, *J. Neuroimmunol*., 56: 171-177; Yednock *et al*., 1992, *Nature*, 356 (5): 63-66). Also, it was demonstrated that antibodies against VLA-4 inhibit the binding of T cells to FN, and that VLA-4 engagement increases the anti-CD3-induced T cell adherence to FN and proliferation (Shimizu *et al*., 1990, *J. Immunol*., 145: 59-67). These data raise the possibility that FN and VLA-4 could be involved in the migration/retention of inflammatory cells into the CNS during chagasic infection. The immunohistochemical assay revealed an increased expression of FN in the meninges, leptomeninges, choroid plexus and basal lamina of blood vessels during the acute and chronic infection. Moreover, perivascular spaces presenting a FN-containing filamentous network filled with $a4^+$ cells were observed. The cytofluorimetric analyses showed that all $CD4^+$ and $CD8^+$ T-cells isolated from blood of infected mice during the acute and chronic infection are VLA-4^+. Interestingly, most of the $CD8^+$ T-cells express high density of VLA-4 whereas the $CD4^+$ T-cells are VLA-4^{low}. Taken together, our results suggest that FN and its receptor the VLA-4 integrin in association with chemoattractant factors (see Abstract by Roffê *et al*. in this issue) might be involved in the preferential entrance, migration and retention of $CD8^+$ inflammatory cells into the CNS during chagasic infection.

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IM-37 – PARTIAL PURIFICATION AND CHARACTERIZATION OF A PROTEIN COMPLEX FROM *T. CRUZI* EPIMASTIGOTES FOR USE IN SERODIAGNOSIS OF CHAGAS' DISEASE

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Chagas' disease is a public health problem which affects more than 18 million people in Latin America. Although there are relatively sensitive and specific serologic tests which detect the infection during the chronic and acute phase, the absence of a "gold standard" still stimulates the development and improvement of methodologies and techniques. In earlier studies (Teixeira et al 1990) , identified highly immunodominant bands between 30-35 kDa by Western blot (WB) analysis, using serum samples of individuals from an area endemic for Chagas' disease. In this study, we partially purified this complex of bands from epimastigote extract (Y strain), using preparative electrophoresis (Prep Cell) and analysed the fractions by ELISA and Western blot tests. Three protein complexes were purified (28-30 kDa, 32-33 kDa and 34-35 kDa) and were tested by ELISA with 84 serum samples from four Brazilian endemic areas and 10 sera from patients with leishmaniasis. The results were compared to six serological tests: an Indirect Immunofluorescence test, three commercial ELISAs, an In-House ELISA, and a Line Immunoassay (LIA) test. These proteins fractions were also tested by WB using 18 sera samples from this panel and 4 sera samples from patients with leishmaniasis and compared with WB using total extract of epimastigotes (Y strain) as antigen. The ELISAs showed excellent performance: the 28-30 kDa complex showed 100% of sensitivity and specificity; the 32-33 kDa complex, 94,59% of sensitivity and 98% of specificity; the 34-35 kDa complex, 100% of sensitivity and specificity, and the mixed complexes of proteins showed 100% of sensitivity and 98% of specificity. All positive samples were reactive for the three complexes analysed and none samples positive for leishmaniasis showed reactivity. These results corroborate earlier studies and indicate that these antigens are promissory in the serodiagnosis for Chagas' disease.

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IM-38 – ORGAN DISTRIBUTION OF $CD8^{LOW}CD3^{LOW}$ T CELLS IN *TRYPANOSOMA CRUZI*-INFECTED CHRONIC MICE

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During the course of murine chronic infection with *T. cruzi* (Y strain), $CD8^+$ cells accumulate in the spleen. Moreover, more than half of these cells have downregulated expression of TCRab, CD3, CD8 and CD45 receptor and correceptor molecules, a phenotype found in tolerant states. Here, we determined by three color FACS analysis the phenotype of $CD8^+$ cells at the peritoneum (infection site), blood, inguinal lymph nodes and liver (a presumed graveyard of damaged lymphocytes and a site of parasite colonization and blood trypomastigote clearance) of chronic and control mice. While $CD8^+CD45RC^{LOW}$ cells in the spleen of chronic mice (60% of $CD8^+$ cells) were uniformly downregulated for CD3 and CD8 molecules, in the peritoneal cavity and liver, these cells (92,7% and 85% of $CD8^+$ cells, respectively) comprised both $CD8^{LOW}CD3^{LOW}$ and $CD8^{HIGH}CD3^{HIGH}$ lymphocytes. Among peripheral blood cells of chronic mice, 94,2 % of $CD8^+$ cells expressed low levels of CD8, CD3 and CD45RC molecules, a phenotype similar to that observed in the spleen. In the inguinal lymph nodes, however, $CD8^+$ cells displayed a phenotype similar to that found in control mice, with very few $CD8^+CD45RC^{LOW}$ cells. In non-infected animals, CD8LOW cells were always a minor population, except in the liver, where they represented up to 60% of $CD8^+$ cells.

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IM-39 – SERA FROM CHRONIC CHAGASIC PATIENTS REDUCE GAP JUNCTION COMMUNICATION IN CARDIAC MYOCYTE CULTURES

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The etiopathogeny of cardiac conduction disturbances in chronic chagasic patients is still poorly understood, but several reports indicate that autoimmune mechanisms might be involved. We have previously suggested a direct effect of anti-*T. cruzi* antibodies from chronic chagasic patients in the electrogenesis and conduction in isolated rabbit hearts perfused by the Langendorff technique (Masuda et al., FASEB J. 12, 1998). We now investigated the effects of these sera on the intercellular coupling in primary cultures of mouse neonatal cardiac myocytes. Primary cultures were obtained as previously described (Meirelles et al., 1986) and 48 hours after plating they were incubated with sera from normal and chagasic patients diluted 1:10 (vol:vol). No significant alteration in dye coupling was observed at early hours after incubation in any of the cultures (85% of the cells were well coupled – coupling degree > 4 cells). However, 24 hours after incubation with sera from a group of chagasic patients a marked reduction in functional coupling was detected by the reduced extent of Lucifer yellow transfer (90% of cell were poorly coupled – coupling degree < 2 cells). In contrast sera from another group of chagasic patients and also those from normal donors did not affect dye coupling when compared to age-matched duplicate control cultures and also with those incubated with sera from normal patients. Most of the effective sera tested induced adrenergic-like effects in the isolated whole rabbit heart, suggesting that their action on coupling might be, in an as yet unknown mechanism, related to b adrenergic receptor recognition.

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IM-40 – ANTIBODIES TO THE CARDIAC MUSCARINIC RECEPTOR RECOGNIZE *T. CRUZI* BUT NOT *LEISHMANIA* DERIVED P-RIBOSOMAL PEPTIDES

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We have previously reported that IgG of sera from chronic chagasic patients (Cha) is able to depress cardiac electrogenesis and conduction in isolated rabbit hearts (Circulation 96(6): 2031-2037, 1997). At least, some of the antibodies present in these sera are generated against P-ribosomal proteins of *T. cruzi* and interact with the second extracellular loop of the cardiac muscarinic receptor (FASEB J. 12:1551-1558, 1998).

To further map the functionally relevant P-ribosomal protein epitopes and their specificity in generating antibodies capable of interacting with the cardiac muscarinic receptor, we synthesized peptides corresponding to the carboxyl terminus of *T. cruzi*, *Leishmania* and human P-ribosomal protein. These peptides differ by only one amino-acid in their 13 residues and were tested in competition experiments in which whole rabbit hearts studied by the Langendorff technique were perfused with chagasic patient's sera (or IgG) incubated with excess of the peptides. All sera (or IgG) used were previously tested in the same preparation and shown to interact with the cardiac muscarinic receptor, blocking AV conduction and/or reducing beat rate.

While the *T. cruzi* derived peptide (R13) was effective in abolishing the sera effects in isolated hearts, preliminary experiments with the *Leishmania* derived peptide (A13) indicate that it is unable to block these effects. Experiments using the human derived peptide (H13) indicated that this 13mer partially blocks the Cha sera effects (abolishes the AV block but does not inhibit the decrease in beat rate)

These results suggest that the cross-reactive antibodies generated against *T. cruzi* ribosomal proteins are parasite specific and that the human protein may further contribute to a cross-reactive response targeting to the cardiac muscarinic receptor.

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IM-41 – COMPARATIVE IMMUNOLOGICAL STUDIES BETWEEN A LACTOSYLCERAMIDE FROM *T. CRUZI* DM 28C AND A NEOGLYCOLIPID (LAC-PTDETN(NAC))

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Chagas'disease is caused by the protozoa *Trypanosoma cruzi* and represents a public health problem in many developing countries. Neutral glycosphingolipids are located primarily in the outer leaflet of plasma membranes. Glycosphingolipids are effective antigen and immunogens, and have shown to function as cell type-specific and developmental stage-specific antigens as well as isogeneic or heterophile antigens (*i.e* histo-blood group antigens).

In our previous studies (Villas Bôas *et al*., 1997, Memórias do Instituto Oswaldo Cruz, Suppl., Vol. 92, pg. 204) we have compared the antigenicity of neutral glycosphingolipids derived from the epimastigote *T. cruzi* Y strain and Dm 28c. Rabbit antiserum against epimastigote *T. cruzi* Y strain was used. Ceramide monohexoside from Y strain showed higher reactivity than Dm 28c strain , whereas ceramide dihexoside showed similar reactivity for both strains.

In this work in order to know the role of the ceramide moiety in the antibody recognition a lactose derived neoglycolipid (Lac-PtdEtn(NAc)), carrying a phospholipid instead of ceramide as a lipid anchor was used. A very weak reactivity was observed with the ceramide-free glycolipid showing that the dominant epitope recognised by the antibodies contains galβ(1→4)-glc-linked to ceramide. These results suggest that the antibodies recognise a particular conformation or organisation of the sugar structure of the glycosphingolipids which arise on interaction with the ceramide moiety.

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IM-42 – THYMUS ATROPHY IN EXPERIMENTAL *T. CRUZI* INFECTION: A POSSIBLE INVOLVEMENT OF $P2X_7$ RECEPTORS

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One of the most remarkable properties of the immune system is the ability to distinguish self from non-self antigens in normal conditions. This discrimination is essentially performed by T lymphocytes and requires a strictly controlled selective process that takes place in the thymus. Thymus is the lymphoid organ that provides the microenvironment for thymocytes differentiation and maturation, besides the elimination of self reactive cells. It is a very dynamic organ, displaying dramatic changes in structure and cellular loss according to some physiological conditions such as aging, stress and pregnancy and a number of pathological situations. In *T. cruzi* infection, the thymus drastically atrophies in the acute phase and restores its normal weight in the chronic phase. In spite of its primary importance, the molecular mechanisms involved in this thymic atrophy are virtually unknown, with no clear consequences to the positive/negative selection and cardiac inflammation in chagasic myocarditis. Recently, it was shown that ATP and analogues may induce cell death in many cell types. This effect can be mediated by $P2X_1$, $P2Y_2$ and $P2X_7$ receptors, members of the P2 purinoceptors family. All three receptors were already described in thymocytes. $P2X_7$ is often associated with the formation of a large pore, non selective to molecules up to 900Da, and may also induce rapid cell death (necrosis).

In this work we used C57Bl/6 mice intraperitoneally injected with either 10^4 trypomastigote forms of *T. cruzi* Y strain or saline to investigate the possible modulation of the purinoceptor $P2X_7$ in thymocytes. $P2X_7$ expression and activity was measured by immunohistochemistry and permeabilization assays. The permeabilization assays enables to measure the $P2X_7$-associated pore through the entry of fluorochromes with molecular weight below 900Da. The thymocytes were collected at different time points of the infection and incubated at 3_7°C for 10min with 2,5mM of Ethidium Bromide in the presence or absence of ATP, and then analyzed by flow cytometry. We found no thymic atrophy at dpi 8, correlating to undetectable immunohistochemical labelling for $P2X_7$ receptor and negligible levels of permeabilization induced by exogenous ATP. In contrast, at dpi 11, 13 and 15 we observed a severe atrophy of the thymus and high levels of permeabilization. In addition, we observed an overexpression of $P2X_7$ receptor at dpi 15. In the chronic phase, the thymus reestablished normal values of cellularity and susceptibility to ATP, demonstrating an interesting association between thymus atrophy and ATP-induced permeabilization through $P2X_7$ receptors.

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IM-43 – EXPERIMENTAL *T. CRUZI* INFECTION IN *GLD* AND PERFORIN KNOCK-OUT MICE

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Similar to the human infection, experimental murine infection with *T. cruzi* induces an acute phase with parasites circulating freely in blood as well as intracellular proliferating forms, and a chronic phase with parasite persistence at subpatent levels. The infection also induces serious immunological disturbs, such as immune depression, T and B cell polyclonal activation and cardiac autoimmune inflammation. Concerning to the cardiomyopathology, the inflammatory infiltrates are mostly composed by T cells, correlating to myofiber damage, but the mechanisms and the putative cytotoxic cells and molecules that are effectors for cardiomyocyte destruction are still unclear. T lymphocytes induce cell death through two major vias, dependent on perforin ($CD8^+$ cells) or Fas/Fas-L interaction ($CD4^+$ cells). The former produces colloid osmotic lysis by non selective pores and apoptosis triggered by granzime B. On the other hand, the Fas-based cytotoxic pathway induces apoptotic cell death stimulated by trimerization of the Fas surface molecule and activation of caspases cascade. In this work we studied the course of the *T. cruzi* infection in C57Bl/6 and Balb/c syngeneic mice lacking the perforin- (perforin knock out mice-KO) or Fas- (*gld*) based cytotoxic vias, infected with virulent (Y) or avirulent (Dm28c) stocks of *T. cruzi* respectively. The absence of perforin does not alter the number of circulating and cardiac intracellular parasites, the parasitemia curves, creatine kinase (CK-MB) activity as a myocardial damage marker and Th1 driven immune response. In contrast, perforin knock out mice (KO) showed higher mortality rates and greatly stronger cardiac inflammation in the acute phase, with more infiltrates per mm^2 and more inflammatory cells per infiltrate. The *gld* infected mice showed more dramatic differences when compared to infected Balb/c mice, with higher and more prolonged parasitemia curves, higher mortality rates, Th2 driven immune response and increased cardiac inflammatory response in the chronic phase. Taken together, these data indicate that although the cardiac inflammatory infiltrates are rich in $CD8^+$ T cells, perforin does not seem to be responsible for the cardiomyocytolysis, but somehow regulating the inflammatory response. In addition, the Fas/Fas-L pathway appears to lead the host cytokine immune response to a protective Th1-type, once *gld* mice have high levels of IL-4 and IL-10 and are clearly more susceptible. Our results suggest that the control of the cardiomyopathy depends on both cytotoxic vias, and this requirement is decisive in different stages of the infection.

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IM-44 – IMMUNITY PRODUCED BY *TRYPANOSOMA CRUZI* CLONE CL-14: MULTIPLE MECHANISMS PROTECT MICE AGAINST VIRULENT CHALLENGE

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Efficient immunity against *T.cruzi* has long been searched. In this regard, we have previously reported that vaccination with live CL-14 trypomastigotes does not induce pathology, but generates functional humoral and cellular responses against infective challenge. Herein, we unravelled other aspects of this protection, concerning the relevance of immune mechanisms in vivo: (1) vaccination with live trypomastigotes protected only a portion of $\beta 2m^-$-mice against infective challenge, but induced efficient protective immunity (in terms of control patent parasitemia / survival) in perforin-knockout mice; (2) cellular invasion was not required to induce protective immunity, as fixed CL-14 trypomastigotes successfully vaccinated normal mice against infective challenge, but viability lowered requirements of doses and time intervals; (3) depletion of CD4 or CD8 subsets after vaccination with live CL-14 trypomastigotes does not affect established protective immunity, but alters isotype immunoglobulin profile (towards IgG2a); (4) 600 rad irradiation rendered normal controls susceptible to infection with low parasite doses, while vaccinated mice develop low (but persistent) parasitemia late after challenge. Our data point to activation of multiple immune mechanisms as a reason to the success of CL-14 vaccination.

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IM-45 – IMUNE RESPONSE OF BALB/C MICE TO CLONE CL-BRENER

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CL-Brener, a clone isolated from blood of mice infected with the CL strain of *Trypanosoma cruzi* (Brener,Z. and Pereira, M.E.S.), was selected as the reference organism for all studies related to the *Trypanosoma cruzi* Genome Project (DeGrave et al., Mem. Inst. Oswaldo Cruz 92:859, 1997) The biological parameters and molecular markers of this clone are very similar to the parental strain: it is highly infective both in vitro and in vivo, and succumbs to the administration of chemotherapeutic agents (Zingales et al., Mem. Inst. Oswaldo Cruz 92:811, 1997). However, the immune response to this clone was not investigated. Considering the fundamental role of soluble mediators in *T.cruzi* infection (dos Reis et al., 1997; Tarleton et al., 1996), we studied the secretion of cytokines in mice infected with CL-Brener. Groups of BALB/c mice inoculated i.p. with 10^2 bloodstream trypomastigotes were sacrificed at various intervals post-infection. Splenocytes were stimulated *in vitro* with ConA or a-CD3 and cytokine levels on the supernatants were evaluated by ELISA. The results obtained indicated that TH1 profile to clone CL-Brener and the parental CL-strain infection is very similar: supression of IL-2 and high production of IFN-γ. Our results also indicate that compared to CL-strain, the production of TH2 cytokines (IL-4 and IL-10) induced by CL-Brener infection is either decreased or delayed. We are currently evaluating the effect of these alterations on other parameters of cellular and humoral response

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IM-46 – MODULATION *IN VITRO* OF *T. CRUZI*-INFECTED MICE B CELL RESPONSE BY THE HIDROALCOOLIC EXTRACT OF THE *CISSAMPELOS SIMPODIALIS* EICHL

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Infection with *T. cruzi* is associated with altered function of different lymphoid cells. One important abnormality is the polyclonal B cell activation that is associated to increased circulating immunoglobulin (Ig) levels. This response is detected early during infection and can be observed throughout the chronic phase. Polyclonal B cell activation was shown to be T cell-dependent and it was suggested that this response may be associated to the phatological events observed during infection. In order to pharmacologically modulate this response, we tested the effect of the hydroalcoolic extract isolated from *Cissampelos sympodialis* (HAE) in both B cell proliferation and Ig secretion. This extract was show to have anti-inflammatory and immunomodulatory effects (Piuvezam *et al*, 1999, *J. Ethnopharmacology.* In press)

Methods and Results: B cells were obtained from normal or *T. cruzi* (Dm28c) infected mice. Those cells were stimulated with LPS (10 mg/ml) either in the absence or in the presence of different doses HAE, proliferation and Ig production was analyzed. *Cissampelos sympodialis* HAE inhibited proliferation and IgM production by both low- and high-density B cells but low density cells were inhibited only when high doses of the extract was added. The Ig secretory response of B cells from *T. cruzi* infected mice were more sensitive to inhibition by the HAE when compared to the response of cells obtained from normal mice. This extract induced a 20% inhibition on the Ig secretion by normal B cells compared to a 70% inhibition on the response of cells from infected animals. Also, the *Cissampelos sympodialis* HAE inhibited B cell proliferation but the response was less affected by this extract. Despite of the inhibitory effect, the *Cissampelos sympodialis* did not have any toxic effect on B cells obtained from either normal or *T.cruzi* infected animals. Those results suggest that B cells obtained from infected mice posses a different profile of activation and can be completely modulated by substances that have only slight effect on normal B cells.

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IM-47 – TRANS-SIALIDASE INHIBITION ASSAY (TIA) AS A HIGHLY SENSITIVE SEROLOGICAL TECHNIQUE FOR CHAGAS' DISEASE DIAGNOSIS

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The *trans*-sialidase inhibition assay (TIA) is based on the detection of neutralising antibodies of the *trans*-sialidase activity, a virulence factor of *Trypanosoma cruzi*. The enzyme is absent in other protozoan parasites, such as *T. rangeli*, *Leishmania* sp. and *Plasmodium* sp., that are frequently found in geographical regions where *T. cruzi* is present. The neutralising antibodies are present in sera from chronic and acute *T. cruzi* human infections (J. Infect. Dis. 1994(170):1570-4). TIA employs a recombinant *trans*-sialidase, that is preincubated with the serum to be tested, and the remnant ability to transfer the sialyl residues from sialylactose to [^{14}C]lactose is evaluated. Pooled normal human sera is employed as negative control and the value of inhibition obtained is taken as 0% (Infect. Immun. 1994(62):3441-6). At present 90 samples from chronic chagasic patients were tested and 100% were found reactive by this assay.

Since TIA seems to be a highly sensitive diagnosis technique, it was employed to analyze a panel of sera showing different degrees of diagnostic difficulty. Sera obtained from a population of Amerindians of Paraguay at very high risk of infection but parasitologically and serologically negative for conventional assays, together with borderline serum samples and sera from idiopathic megaviscera and cardiomyophaty cases from Brazil and Paraguay were analysed.

From 32 sera obtained of Amerindians, 59% were found to be TIA positive. When they were analysed by dot spot against other *T. cruzi* recombinant antigens (SAPA, Ag1, Ag2, Ag30), only 4 were simultaneously positive by dot spot and TIA. The samples that were TIA negative were also found to be unreactive by the dot spot assay.

Then we analysed borderline cases for *T.cruzi* infection obtained from Paraguay (n=36). TIA was able to solve 25 (70 %) of the cases as infected individuals.

Other sera panel employed was from patients with idiopathic megaviscera (n=24) or cardiomyophaty (n=4) obtained from Brazil and Paraguay. Two serum samples, one from each Country, coming from endemic regions were TIA positive.

The ability of TIA to discriminate between *Leishmania sp*. and *T. cruzi* infection was also tested. Serum samples (n=29) obtained from patients with cutaneous leishmaniasis, parasitologically diagnosed, were all TIA negative. However, those infected with *T. cruzi* and *Leishmania sp.* were positive both by TIA and dot spot assays.

We conclude that TIA is a highly sensitive technique able to detect positive samples that might be missed by other available serologic assays.

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IM-48 – *TRYPANOSOMA CRUZI* IN DIDELPHID MARSUPIALS: CELLULAR IMMUNE RESPONSE IN PATENT AND SUBPATENT PHASES OF INFECTION

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One of the most interesting aspects of the *Didelphis marsupialis* and *Trypanosoma cruzi* interaction is the early control of Y strain characterized as Zimodeme 2 (Miles et al, 1977, Deane et al, 1984) and lineage 1 by RAPD analysis (Fernandes et al, 1999) wich is associated to domiciliar cycle of transmission. The fact that lineage 1 parasites could be associated to sylvan reservoirs (*Leontophitecus rosalia* and *Philander frenata* (Pinho et al, 1997 ; Lisboa et al, 1997) changed the idea about Chagas' Disease epidemiology, since the animals infected by lineage 1 are probably the wild reservoirs of *T. cruzi* and could represent the establishing potential of a domiciliar cycle. The comprehension about mechanisms of parasite selection and control of infection by reservoirs, will be important to elucidate for what reason some parasites subpopulations are associated with an especific animal species. Since the humoral immune response do not explain the differences in the course of Y (Z2) and sylvan (Z1) strains we undertook a study to compare the Delayed Type Hypersensitivity (DTH) in two distinct species of didelphids infected by *T. cruzi*: *Philander frenata* and *Didelphis marsupialis*. For this purpose we inoculated 1000 trypomastigotes metacyclics of Y (Z2) and G 645 (Z1) strain subcutaneously in laboratory reared and born opossums and injected the antigen (50-400 ug) in one or two months post infection by intradermic and subcutaneous route, in skin in the ventral area. The DTH was measured in the local of aplication and the material was colected to histopatological analysis. The stronger response was seen in *D. marsupialis* inoculated with 50 ug of *T. cruzi* antigen by intradermic route in the subpatent phase of infection (in natural and experimental infections). The histopatological analysis showed a significant difference in Y and G645 infections: a characteristic DTH inflamatory infiltrate was observed only in *D. marsupialis* infected by Y strain, wich could explain the selection of this *T. cruzi* subpopulation. Further experiments (lymphocyte proliferation, Nitric oxid mesuring) will be performed to clarify these questions about this fascinating well balanced host-parasite interaction.

Supported by CNPq, FIOCRUZ, FAPERJ, PAPES

IM-49 – GLICOINOSITOLPHOSPHOLIPID (GIPL) FROM *TRYPANOSOMA CRUZI* INHIBITS HUMAN MACROPHAGE FUNCTION

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GIPLs are present in the glycocalix of both *Leishmania* and *T. cruzi* and seem to be important to parasite survival. GIPLs have been implicated in modulating murine macrophage response. Little is known of the effect of GIPLs from *T. cruzi* on human cells. We have investigated the effect of GIPL from the Colombiana strain of *T. cruzi* (GIPLcol) on human peripheral blood mononuclear cell-derived macrophages. GIPLcol has been added (1 to 20 ug/ml) to LPS (500pg/ml)-stimulated human macrophages and TNF-α production has been determined 48 hours later. Addition of GIPLcol (20 ug/ml) 3 hours before LPS stimulation led to approximately 90% reduction of TNF-α production. Such an inhibition was dose-dependent. Similar results were obtained when GIPLcol was added simultaneously to LPS. Virtually no effect is seen when GIPLcol is added 3 hours after LPS stimulation. Such an inhibition was not due to cell toxicity since cells remained viable even when cultured for 5 days with GIPLcol (10 ug/ml). Addition of GIPLcol to LPS-stimulated cultures were also able to induce a 30% reduction in the number of CD54 (ICAM)-positive cells. An even higher reduction of CD54 expression was obtained when cells where stimulated with LPS and IFNγ. We have also tested whether GIPLcol was able to influence IFN-γ production by anti-CD3-stimulated lymphocytes. PBMC cultures stimulated with soluble anti-CD3 (10ug/ml) produced 5400 pg/ml of IFNγ, and addition of GIPLcol did not change such production. Taken together our results suggest that GIPLcol has an inhibitory effect on human macrophage activation, but this effect does not extend to lymphocytes. Further evaluation of macrophage function and molecule expression is necessary to further delineate the action of *T. cruzi* GIPL on human macrophages.

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IM-50 – TREATMENT OF EXPERIMENTAL CUTANEOUS LEISHMANIASIS WITH OIL/WATER (O/W) EMULSIONS CONTAINING PAROMOMYCIN

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Ointments containing paromomycin (PA) at 15% in association with penetration enhancers, such as methylbenzethonium chloride (MBCL - 12%) or urea (10%), were shown to be an alternative therapy for cutaneous lesions caused by Leishmania major. Important drawbacks are, however, associated with this formulation. MBCL is quite irritant, a common complain of patients and what has also prevented its use in some cases (Bryceson, 1987; Elon et al., 1992). Ointments containing only paromomycin are not as efficient as its association with skin penetration enhancers. This limitation could be attributed to a low penetration of paromomycin into the skin from ointments, hydrophobic formulations, which would difficult the release of the necessary drug concentration in tissues. We investigated if the incorporation of PA in o/w emulsions could enhance the PA release into the skin and also its in vivo efficacy. Groups of eight BALB/c mice were infected with 1 x 106 promastigotes of either L major or L. amazonensis and, after lesions have reached 6mm in average, were treated with one of the following formulations: 5% PA in o/w emulsions or ointments (soft white parafin) with or without the addition of 10% urea. Lesions were measured weekly using a calipter and for the presence of ulcers, nodules, scars and hair growth. No significant differences in the rate of cure of lesions caused by L. major were observed between o/w emulsions and ointments. Both formulations, were equally effective, promoting 100% of healing, in spite of the addition of urea. In contrast, in the animals infected with L. amazonensis, which is less susceptible to PA, a significant (50%) reduction in lesion size and a cure rate of 50% compared to control mice and to animals that received ointments were observed. In contrast with previous reports, in this model, no significant differences were observed by the addition of urea in the o/w emulsions or in ointments. Although in vitro percutaneous absorption experiments showed that o/w emulsions would be the best preparation for topical delivery of PA, we were only able to observe significant differences between the two formulations in the L. amazonensis model. This could be attributed to differences in the susceptibility to paromomycin between the two Leishmania species evaluated. Previous work have shown that, among the Leishmania species, L. major is the most susceptible to PA.

Supported by CAPES, FAPEMIG, PRONEX

IM-51 – THE ADJUNCT EFFECT OF IL-12 IN THE TOPICAL TREATMENT WITH PAROMOMYCIN OF BALB/C MICE INFECTED WITH *LEISHMANIA MAJOR*

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Experimental studies have shown that topical treatment of cutaneous leishmaniasis with formulations containing paromomycin sulfate associated to gentamicin or to skin penetration enhancers can promote local healing of lesions caused by *Leishmania major* in BALB/c mice. We have observed, however, that 70 days post-treatment, lesions progressively return, even after a complete healing and hair growth at the site of infection. We have investigated if the relapse of lesions in this model could be prevented by the association of paromomycin in oil/water emulsions and IL-12. Groups of eight BALB/c mice were infected with 1 x 10^6 promastigotes of *L. major* and, after lesion development, treated with either topical applications of 5% PA in O/W emulsions, subcutaneous injections of IL-12 (a gift from the Genetics Institute, Inc.) or the association of them. Control mice received only O/W emulsions. Complete healing of lesions (100%) was obtaining only in the groups that received 5% of paromomycin. Treatment with IL-12 alone did not promote healing or even reduction in lesion size. Seventy days post-treatment, lesions relapsed in 100% of the animals treated with only PA and in 50% of those that received simultaneously the IL-12. Nodules or ulcers were completely absent in the remaining 50% of the PA + IL-12 treated mice, 120 days after treatment. An impressive reduction of parasite loads and increased levels IgG2a antibodies were detected in these animals. In addition, no IL-4 mRNAs were detected by RT-PCR at the site of infection. In contrast, positive levels of IL-4 were observed in mice that received PA or IL-12 only. Our results indicate that the association of IL-12 in the topical chemotherapy for leishmaniasis results in an increased cure rate as well as a lower incidence of relapsing.

Supported by CAPES, CNPq, PADCT and FAPEMIG

IM-52 – REACTIVITY OF THE AMASTIGOTE-STAGE-SPECIFIC (A2) RECOMBINANT PROTEIN OF *LEISHMANIA DONOVANI* WITH SERA FROM BRAZILIAN PATIENTS AND DOGS WITH VISCERAL LEISHMANIASIS

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A2 antigens comprise a family of proteins preferentially expressed in the amastigote stage of *Leishmania donovani.* The genes coding for A2 are also present in *L. mexicana* strains, but not in *L.major* or *L. braziliensis.* A2 is composed predominantly by a repetitive element, which makes it an attractive antigen for diagnosis. The fusion protein A2-GST has been previously evaluated in ELISA assays with sera of Indian and Sudanese patients with kalazar and being reactive with 60 and 82% of the tested sera, respectively (GHEDIN et al., 1997). In this study the reactivity of A2 was evaluated with of a large panel of canine kalazar sera (previously tested by IFA) and also of patients with visceral or tegumentar leishmaniasis, tuberculosis and hanseniasis. A2 was expressed in *Escherichia coli* after cloning of its coding region in a histidin tag expression system. ELISA was performed with either A2 or total extracts of promastigotes of *L. chagasi* as antigens. Anti-A2 antibodies were detected by ELISA in 88% of canine sera tested, in 40% of the sera of patients with visceral leishmaniasis and only in 15% of patients with the tegumentar disease. Using the total extract as antigen we found 95%, 60%, 55% of reactivity, respectively. The reactivity of A2 with sera of *Mycobacterium* infected patients was 10%. Similar results were obtained by Western bloting analysis of selected sera. Our findings suggest that A2 may be an useful antigen to improve the serodiagnosis of visceral leishmaniasis.

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IM-53 – T-CELL RESPONSES IN HUMAN AMERICAN TEGUMENTARY LEISHMANIASIS: LONG-TERM EVALUATION AFTER THERAPY

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Previous publications have shown that cellular immune responses play a pivotal role in the outcome of American tegumentary leishmaniasis (ATL). Studies from our laboratory have demonstrated that cutaneous leishmaniasis (CL) patients present a preferential induction of CD4+ *L. braziliensis* (Lb)-reactive T-cells and a mixed type 1 (IFN-γ) and type 2 (IL-4 and IL-5) cytokine production as verified by peripheral blood mononuclear cells (PBMC) cultures stimulated with leishmanial antigens. Our present aim is to compare the *in vitro* T-cell responses of PBMC from CL and mucosal leishmaniasis (ML) patients during active disease and long-term after healing of lesions. A total of 47 patients were studied: 31 before therapy (BT), 22 at the end of therapy (ET) and 25 long-term (one to thirteen years) after therapy (Lt-AT). All patients had healed lesions at the end of therapy. 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 harvested and separated in a Percoll gradient for phenotypic analysis by flow cytometry (B, T, CD4+ and CD8+). The culture supernatants were examined for type 1 and type 2 cytokines using an ELISA test. Results (mean ± S.E.) showed that the percentages of Lb-reactive CD4+ cells in CL patients were: BT=54.9±7.3%, ET=41.5±6% and Lt-AT=30.1±5.6%. The percentages of CD8+ reactive T-cells were BT=23.9±4%, ET=49.3±7.7% and Lt-AT=30.7±5.7%. Thus, CD4+ Lb-reactive cells decreased after therapy while CD8+ increased; however one to thirteen years after cure the proportions of CD4+ and CD8+ Lb-reactive T-cells became similar to that observed during the active disease. In ML patients, the percentages of Lb-reactive CD4+ cells were BT=53.7±7.3%, ET=45.5±7.1% and Lt-AT=20.9±3.3%, while the percentages of CD8+ Lb-reactive T-cells were: BT=20.1±6.5%, ET=11.6±2.9% and Lt-AT=22.6±3.3%. It was shown a delayed decreased in the percentages of the Lb-reactive CD4+ cells in ML patients after therapy; nevertheless, a switch in the CD4+/CD8+ ratio was observed later on, after one year follow-up. Our results showed that active CL and ML patients presented a preferential induction Lb-reactive CD4+ T-cells as compared to CD8+, as well as production of type 1 and type 2 cytokines. In healed ML patients a similar induction of CD4+ and CD8+ T-cells and a preferential type 1 response were detected, although, different from CL patients, this pattern was only observed long-term after therapy. As an extension of our previous results it was concluded that in ATL the healing process is associated with an equilibrium of CD4+/CD8+ T-cells. However, higher percentages of CD4+ T-cells were again observed in CL patients when a long-term evaluation was performed. Those parameters could be relevant for the evaluation of the immunoprotection induced by a vaccine.

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IM-54 – *LEISHMANIA*-SPECIFIC T-CELL RESPONSES OF ASYMPTOMATIC INDIVIDUALS FROM ENDEMIC AREAS OF HUMAN LEISHMANIASIS

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American tegumentary leishmaniasis (ATL) produces a spectrum of clinical manifestations ranging from benign cutaneous leishmaniasis (CL) that may heal spontaneously to the chronic and disfiguring lesions of mucosal leishmaniasis. Field studies have shown that some individuals are able to control *Leishmania* infection without any clinical symptoms. Immunological studies from our group have shown that cured CL patients as well as individuals vaccinated against leishmaniasis present an equilibrium between the proportions of CD4+ and CD8+ Lb-reactive T-cells, and type I cytokine production. However one year after cure CL patients have a preferential induction of CD4+ T-cells as compared to CD8+ lymphocytes, although they maintained a predominant type 1 cytokines response. Our goal was to characterize the T-cell responses associated with resistance to *Leishmania* infection by studying asymptomatic individuals living in endemic areas of leishmaniasis. Twelve healthy subjects from Rio de Janeiro and Paraty were studied, all of them without any lesion or scar suggestive of leishmaniasis. These individuals have never been submitted to the Montenegro's skin test (MST) before. Indirect immunofluoresce antibody test for leishmaniasis was negative (IgG and IgM classes). Assays of lymphocyte proliferative responses (LPR) of peripheral blood mononuclear cells (PBMC) induced *in vitro* by *L. braziliensis* antigens (Lb-Ag) and mitogen (concanavalin-A) were performed. After five days in culture Lb-reactive T-cells were harvested and separated in Percoll's gradient for phenotypic analyzed (CD3+, CD4+ and CD8+) by flow cytometry. The T-cell culture supernatants were investigated for type 1 (IFN-γ) and type 2 (IL-4, IL-5) cytokines production using an ELISA test. MST (performed following blood collection during the present study) was positive in all cases. All individuals presented a positive LPR (stimulation indices – SI ≥ 2.5 over the background cultures) (mean±s.e.=5.3±1.1). The majority of Lb proliferating cells (mean±s.e.) were T lymphocytes (CD3+=61±4.4%). The percentages of CD4+ Lb-reactive T-cells (X=26.9±2.5%) was significantly higher than CD8+ (X=19.4±2.1%) (p=0.02). However, culture from one individual presented similar induction of CD4+ (23.3%) and CD8+ (22.4%) Lb-reactive T-cells. Another subject presented higher proportions of CD8+ (35.6%) than CD4+ (11.2%) Lb-reactive T-cells. The IFN-γ production was detected in the culture supernatants from seven out of 12 subjects (mean±s.e.=2.475±1396 pg/ml). The positive leishmanial antigens responses induced *in vivo* (MST) and *in vitro* (T-cell proliferation and/or IFN-γ production) indicate that those individuals have already been infected. These preliminary results show that the Lb-reactive T-cells pattern observed in asymptomatic subjects is similar to that occurring in CL patients long term after healing. However the demonstration of two individuals presenting a CD4/CD8 profile similar to that occurring in ATL patients early after cure suggest that this immunological pattern could be triggered by re-exposition to the *Leishmania* parasite. This hypothesis is still under investigation in our laboratory.

Supports: CNPq/PIBIC, Instituto Oswaldo Cruz and Economic European Community.

IM-55 – IMMUNE RESPONSE TO MONTENEGRO SKIN TEST ANTIGEN WITH DIFFERENT PRESERVATIVES IN A NON- ENDEMIC AREA FOR TEGUMENTARY LEISHMANIASIS

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Montenegro skin test is used routinely in diagnostic and epidemiological surveys for leishmaniasis around the world. However, there are several doubts about the antigen and its preservative standardization. Thiomersal (Merthiolate), the most preservative used, is a known allergen, inducing delayed-type and immediate hypersensitivity, and the skin response for this compound maybe act as a confounder for a response to the Leishmania antigen in vaccination studies (Marzochi et al, 1998, Mem. Inst. Oswaldo Cruz, 93: 205-212). In order to evaluate the skin response to a merthiolated or a non-merthiolated Montenegro antigen, a randomized double blind study was developed, with normal male volunteers conscripted in Santa Maria- Rio Grande do Sul, Brazil. The 400 volunteers were placed in four groups, each receiving an intradermal test with one of the following substances: I: FIOCRUZ® antigen, in 1:10000 merthiolated saline; II: FIOCRUZ® antigen in 0,4 % phenolated saline; III: 1:10000 merthiolated saline alone; IV: 0,4 % phenolated saline alone. After 48 hours, the local reactions ≥ 5 mm were considered positive responses to the test. Considering non Leishmania transmission in the study area, and no ancient leishmaniasis in the conscripts, frequency of positive responses in group I was expected to be similar to the group III, and in group II to group IV. One week after the first test, all positive volunteers in groups I and II were tested again, only with the saline corresponding to the received antigen, in an identical manner of the first test, to attest a true positive response to the antigen (a positive reaction in the first test and a negative response in the second) or a allergic response (positivity in the two tests).

In group I, 42,48 % (42/102) volunteers were positive in the first test, and in group III, 9,2% (9/97). In groups II and IV the frequency of positive response was, respectively, 35,6% (36/101) and 0,0 % (0/100). The cutaneous reactions to merthiolated saline alone were morphologically identical to the antigen reactions, and ranged from 5 to 24 mm in diameter . After the second test, the prevalence of skin response was calculated for each reactive, being as follows: merthiolated saline, 12,6 %; phenolated saline, 0,9 %; FIOCRUZ® merthiolated antigen, 28,4 % and FIOCRUZ® phenolated antigen, 34,0 % (x^2= 0,66, p> 0,05, Fischer exact test, no difference between the two antigens). Considering the high frequency of positive reactions to Merthiolate in this study, and the characteristics of these reactions, we suggest the substitution of this compound in all Montenegro antigens. Moreover, interpretation of this kind of test may take into account the possibility of a false positive response, associated with the preservative, particularly in vaccination studies, when using thimerosal-containing vaccines and thimerosal-containing antigens for analysis of protection. Studies are in progress trying to explain the high frequency of positive response to the antigens without association with allergy, disease and epidemiological conditions of the volunteers, since there are no human notificated cases of leishmaniasis in the study region.

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IM-56 – DISTINCT NITRIC OXIDE PRODUCTION BY MACROPHAGES INFECTED WITH DIFFERENT *LEISHMANIA* SPECIES

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Nitric oxide (NO) is involved in a variety of biological functions, including anti-microbial activity against *Leishmania*. The various *Leishmania* species cause different clinical manifestation, these differences are tought to be mainly due to parasite characteristics and to interaction with the host. In this abstract we describe the levels of nitrites produced by macrophages activated *in vitro* with interferon (IFN)-γ and lipopolysaccharide (LPS), and infected with different *Leishmania* species. BALB/c mice were injected with thioglycolate and the peritoneal macrophages were cultured on glass coverslips, macrophages were infected with promastigotes and than stimulated (simultaneously or 4 hours after the infection) with IFN-γ (50UI/ml) plus LPS (50ng/ml) for 48h. The nitrites concentration in the culture supernatants was determined by Greiss reagent. When the macrophages were stimulated 4 hours after *L. (L.) amazonensis* or *L. (L.) major* infection the production of NO was significantly inhibited. On the other hand, no significant inhibition in NO synthesis was detected with *L.(V.) braziliensis.* Also no significant variation in the NO production was observed when the macrophages were stimulated simultaneously with the parasite infection.

	Concentration of Nitrites (μM) ± SD. IFN-γ/LPS	
	after infection	simultaneously with nfection
Macrophage	39.3±11.8	55.9±10.9
Macrophage + *L. (L.) amazonensis*	11.2±3.4*	35.6±7.6
Macrophage + *L. (L.) major*	12.5±3.3*	54.1±12.2
Macrophage + *L. (V.) braziliensis*	21.7±5.0	57.1±5.9

* P<0.01

Considering that BALB/c mice are susceptible to *L. (L.) amazonensis* and *L. (L.) major*, and resistant to *L. (V.) braziliensis*, these results strongly suggest that different *Leishmania* are able to modulate the macrophage NO production in a suitable way, thus reflecting in different interactions between *Leishmania* and host cells.

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IM-57 – IL-12 AND NO PRODUCTION BY *LEISHMANIA (L.) AMAZONENSIS* INFECTED CELLS

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Effective immunity against *Leishmania* infection is mediated by parasite-specific Th1 cells and IL-12 production is of fundamental importance for the development of this immune response (reviewed by Bogdan, 1996, *Curr.Op.Immunol.*8: 517-525). There are evidences that NO regulates Th1 response through the inhibition of IL-12 synthesis by macrophages (Huang et al., 1998, *Eur.J.Immunol.*28: 4062-4070). The purpose of this study was to analyze the correlation between LPS-induced IL-12 and NO production in different macrophage populations after infection with *L.(L.) amazonensis* promastigotes. IL-12 (p40) levels in culture supernatants were evaluated by ELISA and NO production by Griess reaction. M-CSF derived bone marrow macrophages upon LPS-stimulation *in vitro* (48 h) produced 2.1 ng/ml of IL-12 and 45 μM of NO. However, previous infection (4h) of these cells with *L. (L.) amazonensis* (10 parasites:cell) followed by LPS activation resulted in a 63% reduction in IL-12 production. Despite IL-12 inhibition, NO synthesis in these cells was similar to the production of LPS-stimulated cells. LPS activation (48 h) of BALB/c resident peritoneal cells (RPC) lead to a production of low concentrations of IL-12 (0.606 ng/ml) and 12 μM of NO. Undetectable IL-12 and total NO reduction were observed when these cells were stimulated with LPS by 2h and infected with 10 parasites:cell. Infection with a 2 parasites:cell ratio lead to inhibited NO production but sustained IL-12 production. LPS-stimulated J774-G8 or RPC (48 h) produced similar concentrations of IL-12 and NO. Infection (10 parasites:cell) and LPS stimulation caused in J774-G8 cells a 100% decrease in IL-12 production and 90,3% of NO inhibition. Infection of these cells with 2 parasites:cell and LPS stimulation caused a lower IL-12 (32.2%) and NO (41.9%) production. These results suggest that infection with high number of *L.(L.) amazonensis* promastigotes leads to IL-12 inhibition and this inhibition is not always associated with NO reduction.

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IM-58 – DIFFERENTIAL PRODUCTION OF NITRIC OXIDE BY MURINE MACROPHAGES FROM *LEISHMANIA* RESISTANT AND SUSCEPTIBLE MICE STRAINS

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Nitric oxide (NO) produced by activated macrophages is an important molecule for the elimination of intracellular parasites. In macrophages, the enzyme responsible for NO synthesis, inducible nitric oxide synthase (iNOS), is expressed upon stimuli by cytokines and pro-inflammatory agents such as IFN-γ, TNF-a and LPS. In studying the role of NO in the elimination of *Leishmania amazonensis*, we have previously observed that peritoneal macrophages from C57BL/6 mice, resistant to *Leishmania,* consistently produced more NO than cells from BALB/c mice, susceptible to the infection, when stimulated with IFN-γ plus LPS. In the present work, we study in more detail the differential sensitivity of these macrophages, defining the time and the combination of doses of IFN-γ plus LPS as well as IFN-γ and TNF-αγ in which the maximal difference in NO production is expressed. We show that maximal difference occurs 72 hours after stimulation with IFN-γ co-stimulated with either LPS or TNF-α. We define that the difference in the production of NO is evident only when small doses of either IFN-γ or LPS or TNF-a are used. With higher doses of these stimuli, the production of NO by BALB/c macrophages is as efficient as in C57BL/6 cells. We also demonstrate that the expression of iNOS in macrophages from BALB/c mice is much lower than that of C57BL/6 mice, as shown by Western blotting using anti-iNOS, for either LPS or TNF-α as co-stimulators. These results indicate that the lower levels in NO in macrophages from BALB/c mice is probably due to the low levels of the iNOS produced in these cells. It is possible that the differential sensitivity to IFN-γ and LPS or TNF-α contribute for the manifestation of resistant and susceptible phenotypes of C57BL/6 and BALB/c mice, respectively.

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IM-59 – SUPPRESSED PRODUCTION OF CYTOKINES AND T CELL PROLIFERATION MAY BE DUE TO APOPTOSIS INDUCED BY *LEISHMANIA AMAZONENSIS* ANTIGENS

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We have observed that *L. amazonensis* antigens suppress normal and infected murine lymphocyte proliferation in vitro in a dose-dependent manner involving an apoptotic process. To evaluate the sensitivity of lymphonode cells to antigen -induced apoptosis during the course of infection, cells were taken from inguinal nodes at days 0, 7, 14 and 21 of infection and incubated with different concentrations of parasite lysate in vitro for 17 h. We observed that from day 7 of infection on the cells remained unresponsive to antigen induced proliferation. Induction of apoptosis by the antigen was quantitatively measured by the TUNEL method in the lymphonode cells obtained from BALB/c mice at day 7 of infection. The cells were incubated for 17 h in the presence of varying concentrations of the lysate and then fixed, permeabilized and incubated with TUNEL reactives to mark the fragmented DNA. We counted the marked cells using an inverted fluorescence microscope. We also withdrawed 50 µl of the culture supernatants to quantify the lactate dehydrogenase (LDH) released by lysed cells. We observed that in cultures stimulated with the lysate of 4 x 10^6 promastigotes/ml, 27% of cells were dead by necrosis and 62% were dead by apoptosis. Whereas the percentage of necrotic cells remained the same in the presence of antigen, there was an increase of 105% of apoptotic cells in relation to unstimulated cells. The remaining viable cells were in an irreversible anergic state, as they did not respond to mitogenic stimulation after antigen removal.

We also investigated the cytokine (IFN-γ, IL-2, IL-4, IL-10) production during BALB/c infection infection with *L. amazonensis.* Cells were collected at different days of infection and stimulated in vitro with Concanavalin A or leishmanial lysate. In the supernatant from cultures stimulated with antigen all cytokines were undetectable, except for IL-2, which was present at low levels. In the supernatant from the cells stimulated by Con A we observed that the levels of IL-10 decreased during the infection whereas high levels of IFN-γ was produced in early infection (PID=7), decreasing subsequently. To investigate if the impairment of proliferation was associated with scanty IL-2, we added exogenous rIL-2 in different concentrations to cells from normal and infected mice but that did not revert the suppressive effect of the antigen. Taken together, these results suggest that the strong suppression of T cell responses by parasite antigens is probably due to induced cell death with consequent reduced expression of cytokines like IL-2, IL-10, IFN-γ and IL-4.

Finantial support: CNPq

IM-60 – EVALUATION OF ANTIGENS FROM VARIOUS *LEISHMANIA* SPP IN THE WESTERN BLOT FOR DIAGNOSIS OF AMERICAN TEGUMENTARY LEISHMANIASIS

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The flagellate protozoa belonging to the Trypanosomatidae family comprises important ethiological agents human parasites, mainly *Trypanosoma cruzi,* of Chagas' disease and various species of *Leishmania* wich cause a spectrum of human disease such as mucocutaneous and visceral leishmaniasis. The american tegumentary leishmaniasis (ATL) are transmitid by the sandfly vector, Phlebotominae of the genus *Lutzomia,* to a suitable host where *Leishmania* invades macrophages starting a localized chronic gtanulomatous lesion at the sandfly bite site. The diagnosis of leishmaniasis mainly rests on the clinical picture and the isolation of the parasite from skin or bucopharingeal lesions (Palma *et al*., 1991, *Clin Lab Med,* 11:909-922.). The parasitological methods have been considered the first choice procedures for the diagnosis of leishmaniasis due to their high specificity (100%) although present variable sensitivities (Weigle *et al, Am J Med* Hyg, 36:36-42). However , in some instances it is very difficult to demonstrate the parasites (Cuba-Cuba *et al*., 1986, *Trans Royal Soc Trop Med Hyg*,80:346) and immunodiagnosis becomes an important tool for the diagnosis of the disease. Several techniques have been developed for the serologic diagnosis of the leishmaniasis and the methods used so far are indirect immunofluorescence (IFA), enzyme-linked immunosorbent assay (ELISA) and Western Blot (WB) (Walton *et.al.*, 1972, *Am J Med* Hyg, 21:296-299). We report the Western Blot method, using antigens from culture promastigote forms of *Leishmania (Viannia) braziliensis, L. (Leishmania) amazonensis, L.(Leishmania) tropica* and a trypanosomatid (268T strain) isolated from naturally infected tomatoes evaluated for laboratory diagnosis of american tegumentary leishmaniasis (ATL). Serum samples were obtained from 108 mucocutaneous leishmaniasis patients (Group I), 23 chagasic patients (Group II), 32 patients with other diseases (Group III) and 78 healthy individuals (Group IV). The overall analysis showed a sensitivity of 76.9%, 90.4%, 78.5%, and 87.9%, a specificity of 100.0%, 93.8%, 87.8% and 77.1%, a predictive positive value of 100.0%, 94.0%, 89.5% and 84.0%, a negative predictive value of 75.7%, 90.0%, 75.4%, and 90.2% and concordance coefficient Kappa was 0.7358, 0.8400, 0.6491, and 0.6287 for *L.(V.) braziliensis, L.(L.) amazonensis, L.(L.) tropica* and 268T strain antigens, respectively. The antigenic profile recognized by serum samples from patients with leishmaniasis and with Chagas' disease, permits serologic distinction between these infections.

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IM-61 – THE ROLE OF *L. AMAZONENSIS* ARGINASE IN MACROPHAGE INFECTION

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The production of superoxide anions (O_2^-, H_2O_2) and nitric oxide (NO) by macrophages is an important microbicidal mechanisms against infection of *Leishmania spp.* To survive inside macrophages, *Leishmania* need to get rid of NO and superoxide radicals (1, 2). It was shown that LPG can inhibit the oxidative burst in many species of *Leishmania*, and that GIPL and phosphoglycan can inhibit iNOS activity in *L. major* (2). The production of NO by citokine-inducible nitric oxide synthase (iNOS) in macrophages requires L-arginine as substrate (1). L- arginine is also the substrate of arginase (3), what lead us to think that *Leishmania* arginase may play an important role in the survival of this protozoa inside macrophages, reducing the availability of substrate to iNOS and, in consequence, decreasing NO generation.

To investigate this hypothesis, we did two preliminar experiments. Initially, *L. amazonensis* was grown in the presence of 2,3 mM or 5 mM of L-arginine for four days. Total RNA from those cells was fractionated and a Northern blot was hybridized to an arginase-coding fragment of *L. amazonensis*. No differences were observed in the level of hybridization, indicating that L-arginine does not induce the expression of arginase RNA. In a second experiment, *L. amazonensis* was grown in the presence of 25 mM, 50 mM and 75 mM of L-lysine (an inhibitor of arginase of *Bacillus brevis* (4)) and used to infect J774 macrophages activated with IFN-γ. After 12 hours, the cultures were washed and reincubated with normal culture medium for 24 h and 48 h. The number of infected macrophages and the number of *Leismania* per infected macrophage were determined and no differences were observed. As the macrophages also synthesize their own arginase, the use of L-lysine in the incubation medium of the macrophage cultures was precluded because it would increase availability of L-arginine by blocking macrophage arginase and thus increase NO synthesis by the macrophage. Because of this, *Leishmania* arginase was not continuously inhibited during the intracellular phase of the experiment and possibly is the reason why we were not able to distinguish significant differences in the fate of arginase-blocked *Leishmania*.

Therefore, obtaining an arginase knocked out *Leishmania* is the alternative approach to verify the role of parasite arginase in the macrophage infection process. This mutant *Leishmania* is in under construction.

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IM-62 – INVOLVEMENT OF $CD4^+$ AND $CD8^+$ T CELLS IN THE PATHOGENESIS OF RENAL LESIONS IN CANINE VISCERAL LEISHMANIASIS

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Renal involvement in canine visceral leishmaniasis (VL) is frequent but the pathogenesis is not clearly elucidated. In several diseases T cells, mainly $CD4^+$ cells, have been observed in renal lesions. Since in nephropathy of VL this aspect has not been studied, here we searched for the involvement of $CD4^+$ and $CD8^+$ T cells in the renal lesions in naturally infected dogs with *Leishmania (L.) chagasi* from Terezina city in the State of Piauí. Thirty five dogs with positive serology for VL and positive culture of *Leishmania* from bone marrow, spleen and/or popliteal lymph nodes were studied. Parafin embedded-kidney samples were processed by specific and sensitive immunohistochemistry catalyzed signal amplification (CSA) system and *Leishmania* antigen, $CD4^+$ cells and $CD8^+$ cells were detected using mouse polyclonal anti-*Leishmania (L.) amazonensis*, monoclonal anti-dog CD4 (VMRD) or monoclonal anti-dog CD8 (VMRD) antibodies, respectively. Histopathological studies have shown the presence of both glomerular and interstitial changes in most of 35 animals: only 2 did not present any lesion, 4 presented only glomerular alterations and 3, only interstitial inflammation and/or tubular changes. The glomerular alterations comprised mesangial proliferative (N=8), membranoproliferative (N=4), crescentic (N=2) and chronic glomerulonephritis (N=1) and focal segmental glomeruloesclerosis (N=12). In 82.8% of the samples *Leishmania* antigen as characteristic diffuse dark brown peroxidase staining was detected in the phagocytic cells of glomeruli and in the interstitial mononuclear cell infiltrate. The presence of T cells both in glomeruli and in interstitium was expressive, mainly constituted by $CD4^+$ cells and there was no clear correlation with histopathological patterns. In the majority of samples $CD4^+$ cells were present except in 2 cases with histologically normal kidney, in one with chronic and in 2 with focal segmental glomerulonephritis. In 50% of cases where $CD4^+$ cells were present, $CD8^+$ cells were also expressed and there was no clear correlation with histopathological patterns. The presence of $CD4^+$ and/or $CD8^+$ cells were related to the presence of *Leishmania* antigen; even in those cases where no glomerular alteration was seen, $CD4^+$ cells were present when the *Leishmania* antigen was present.

The present results show an expressive presence of T cells mainly $CD4^+$ cells in the kidney in canine VL suggesting their involvement in the mechanism of renal injury.

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IM-63 – CHEMOKINE EXPRESSION IN LESIONS CAUSED BY EXPERIMENTAL INFECTION WITH *LEISHMANIA MAJOR*

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Chemokines are cytokines with chemotactic activity, which role in cell migration, activation and modulation of the immune response have been extensively investigated. However, to date, little is known as to the role of chemokines in cutaneous leishmaniasis. The aim of this study was to determine which chemokines are present in the course of infection of susceptible (BALB/c) and resistant (C57BL/6) strains of mice with *Leishmania major.* Mice were infected in the hind footpad with 10^6 stationary phase procyclic culture forms of the parasite. Groups of mice were sacrificed at 1, 2, 12, 42 and 77 days of infection. Lesions were analyzed by histopathology and for the expression of chemokines by RT-PCR. We observed a higher expression of MCP-1 (chemotactic for macrophages) in BALB/c mice as compared to C57BL/6 mice. This higher expression of MCP-1 correlated with an infiltrate richer in macrophages in the former strain of mice. The expression of MCP-1 was significantly above the background levels in BALB/c mice from day one of infection and was kept in high levels throughout infection. MCP-5, on the other hand, was expressed at higher levels in C57BL/6 mice from the second week of infection, and stayed at high levels up to week 11. Balb/c mice presented no significant increase of MCP-5 expression. In addition, RANTES (chemotactic for lymphocytes) was expressed at high levels in C57BL/6 mice after 42 days of infection, which is coincident with the high numbers of lymphocytes in lesions. In order to investigate if the expression of RANTES had a role in the resolution of lesions, we treated C57BL/6 mice with MetRANTES, which is an antagonist for RANTES to the receptors CCR1, 3, 4 and 9, but not to CCR2, which also binds MCP-1 and MCP-5. MetRANTES was injected i.p. daily, at 10μg/mouse, from the third week of infection, for 6 weeks. Controls were injected with the same volume of PBS. We found that the group treated with MetRANTES had larger lesions than controls. Histopathology of the lesions revealed that the group treated with MetRANTES had a more intense cellular infiltrate, and macrophages with light and elongated nuclei, suggesting these cells were more activated than in the control group. Our studies suggest that lesions from mice susceptible to *L. major* express more MCP-1 and less RANTES, as opposed to lesions from resistant mice. Accordingly, RANTES has a role in the resolution of lesions in resistant mouse strains.

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IM-64 – TNF RECEPTOR 1 (TNFRP55) MEDIATES DTH AND THE CONTROL OF THE CELLULAR INFILTRATE IN LESIONS CAUSED BY INFECTION WITH *LEISHMANIA MAJOR*

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Tumor necrosis factor-a (TNF-α) has an essential role in the activation of infected macrophages to kill *Leishmania major* after activation with IFN-γ. Although TNF-α has two receptors on the surface of most cells, the induction of nitric oxide (NO) by TNF-α was believed to be mediated by the receptor 1 (TNFRp55). However, mice in which this receptor was deleted by homologous recombination (TNFRp55-/-) resolved parasitism in the footpad when infected with *L. major*, albeit more slowly than heterozygous and the C57BL/6 wild strain. More interestingly, even after the levels of parasites at the site of infection were undetectable, TNFRp55-/- did not resolve lesions, and an intense inflammatory infiltrate was present after 25 weeks of infection (Vieira *et al.*, 1996, *J. Immunol.* 157: 827-835). The aim of this work is to investigate the reason for the permanence of the cellular infiltrate in lesions from TNFRp55-/- mice infected with *L. major*. Thus, we determined the expression of chemokines by RT-PCR at the site of infection in C57BL/6 mice (bearing the TNFRp55), TNFp55-/- and the heterozygous TNFp55+/- mice, which express half of the receptors present in wildtype mice. Wildtype C57BL/6 mice expressed high levels of the chemokines CRG-2, KC and MIG at 6 and 11 weeks after infection, while TNFRp55-/- mice expressed lower levels of these chemokines at these time points. TNFRp55+/- presented levels of MIG similar to the C57BL/6 wildtype, CRG-2 levels similar to the TNFRp55-/- and no KC expression at 6 and 11 weeks. MCP-5 and RANTES expression was upregulated in C57BL/6 wildtype and TNFRp55-/- mice. However, levels of these chemokines were downregulated at 11 weeks of infection in C57BL/6 mice, while there was still a high level of expression of both chemokines in lesions from TNFRp55-/- at this time point. TNFRp55+/- mice did not express MCP-5 at significant levels at these time points, and the kinetics of expression of RANTES was similar to that found in wildtype mice, although levels were lower. In order to investigate the role or the TNFRp55 on the delayed-type hypersensibility (DTH) in response to formalin-treated *L. major,* we injected 10^7 dead parasites in the right footpad of mice infected for 2, 4 and 11 weeks in the left footpad. The size of the response was measured at 24, 48, 72 hours. Surprisingly, TNFRp55-/- presented no detectable DTH in response to formalin-treated *L. major.* C57BL/6 mice presented a positive DTH from week 4, while TNFRp55+/- showed a positive reaction 11 weeks post-infection. Cellular infiltrate at the site of injection of antigens was rich in mononuclear and polimorphonuclear cells in both wildtype and TNFRp55+/- mice, while TNFRp55-/- mice presented a discreet infiltrate where mononuclear cells predominate. We conclude that the inflammatory response to live parasites in the absence of the TNFRp55 is characterized by an infiltrate that does not receed and by the prolonged expression of MCP-5 and RANTES, chemotactic for macrophages and lymphocytes, respectively. In contrast, inflammation in response to a trigger with dead parasites during an active infection does not follow the same pattern, and TNFRp55-/- do not build a significant response, in contrast to wildtype mice.

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IM-65 – USE OF *CORYNEBACTERIUM PARVUM* AND IL-12 PLUS AL(OH)$_3$ AS ADJUVANTS IN IMMUNIZATION SCHEDULES AGAINST *L. (L.) CHAGASI* INFECTION

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Corynebacterium parvum has been used in immunization protocols against *Leishmania* species implicated in cutaneous as well as in visceral leishmaniasis with a significant degree of protection (Scott et al., 1987, J. Immunol., 139:221-227; Jaffe et al., 1990, J. Immunol. 144: 699-706). IL-12 has been used as a vaccine adjuvant and normally susceptible mice was protected from *Leishmania (L.) major* challenge when previously immunized with soluble leishmanial antigen and IL-12 (Afonso et al., 1994, Science 263: 235-237; Mougneau et al., 1995, Science 268: 563-566). In addition, adsorption to aluminum hydroxide [Al(OH)$_3$] promotes the adjuvant activity of IL-12 leading to a more stable Th1 phenotype (Jankovic et al., 1997, J. Immunol. 159: 2409-2417).

Our studies focused on antigens from *L. (L.) chagasi* amastigotes showed a 30 kDa one (p30) implicated in lymphoproliferative responses mediated by $CD4^+$ Th1 and able to induce a partial protection against challenge with *L. (L.) chagasi* in BALB/c mice.

The purpose of the present work was to compare the protective ability of *L. (L.) chagasi* p30 by use of either *C. parvum* or IL-12 adsorpted to Al(OH)$_3$ as adjuvants.Two different preparations of *L. (L.) chagasi* p30 were also tested, p30 purified by immunoaffinity chromatography (p30col) and by electroelution (p30ee).

BALB/c mice received two doses with 14 days interval of 10 µg of either p30col or p30ee plus 100 µg *C.* parvum by intraperitoneal route. Control animals received PBS plus 100 µg *C. parvum*. Another group of animals received two doses with 14 days interval of 10 µg of either p30col or p30ee plus 200 µg Al(OH)$_3$ previously incubated with 0.5 µg IL-12 injected subcutaneously in the nape of the neck. Control animals received either 200 µg Al(OH)$_3$ plus 0.5 µg IL-12 or 10 µg p30ee plus 200 µg Al(OH)$_3$. On day 14 all animals were challenged with $1x10^7$ *L. (L.) chagasi* amastigotes by endovenous route. Animals from each group were sacrified three months after challenge. The parasite burden was evaluated by amastigote counts in Giemsa-stained imprints of spleens (Stauber et al., 1958, J. Protozool. 5: 269-273).

Animals immunized with either p30ee or p30col plus *C. parvum* presented a parasite load reduction of 59 and 78%, respectively, when compared to controls. The animals which received either p30ee or p30col plus Al(OH)$_3$ + IL-12 showed a parasite load reduction of 41 and 60%, respectively, when compared to animals who received Al(OH)$_3$ + IL-12.

Thus, these data allow us to conclude that p30col was more efficient than p30ee as a protective antigen and *C. parvum* administred with either p30ee or p30col was more suitable than Al(OH)$_3$ + IL-12 as adjuvant in immunization schedule against *L. (L.) chagasi* infection in a murine model.

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IM-66 – INTRACELLULAR PROTEIN DEGRADATION DURING *IN VITRO LEISHMANIA AMAZONENSES* DIFFERENTIATION

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Constitutive or stage regulated cellular proteins are constantly transformed or eliminated by cells but in Trypanosomatides as *Leishmania* sp there is little information about this intracellular protein catabolism. In this work the intracellular turnover of *L. amazonensis* proteins was investigated during the differentiation of promastigote to amastigote. Washed promastigote cells were metabolically labeled with -[^{125}S] metionine and incubated at 34^0C in Schneider's medium supplemented with 20% FCS. After SDS-PAGE separation and quantification it was observed that the level of bands radioactivity was constant during the first five hours of cultivation in BHI, at 28^0C. On the other hand, the radioactivity level decreased along the differentiation process, being 10% in promastigotes and 50%, after five hours, in amastigotes. These results with immunocitochemical and immunobloting assays suggest that the enzymes like-metallo proteinases, serine proteinases and cathepsin D presents a maximal expression at the first moments of differentiation with a vesicular and flagellar pocket localization. In the other hand, polypeptides like-cathepsin B present a maximal expression after the morphological parasite transformation, in spite of being at the same cellular localization. These preliminary results suggest the existence of a synergistic effect between the four proteases class.

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IM-67 – THE ROLE OF COMPLEMENT ON THE SURVIVAL OF CUTANEOUS STRAIN OF *LEISHMANIA* IN THE SKIN

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Leishmaniasis develops when after inoculation the *Leishmania* parasites survive the attack of innate immune elements such as phagocytic cells and the complement system, and disseminate to target organs evading the specific immune elements. It is knwon that most of promastigotes from cutaneous strain of Leishmania are susceptible to lysis by normal fresh sera (Mosser, D.M. et al. - Exp. Parasitol. 62: 394-404, 1986), and amastigotes of the cutaneous strains are more susceptible to lysis by complement than of the visceral strains (Hoover, D.L. et al. - J. Immunol. 132: 893-7, 1984). We have previously shown that complement is an important factor for the escape of parasite and its dissemination to liver and spleen in hamsters infected subcutaneously with promastigotes of *L. (L.) chagasi* (Laurenti, M.D. et al. - Int. J. Exp. Pathol. 77: 15-24, 1996). However, when mouse peritoneal macrophages were infected with promastigotes of *L. (L.) amazonensis* the infection index were lower when the parasites were opsonized by normal mouse fresh sera compared with parasites opsonized by heat-inactivated normal sera (Ura, D.M. et al. - Mem. Inst. Oswaldo Cruz (Suppl. II): 136, 1998). This datum suggests that, contrary to the effect of complement on visceral strains where it contributes for the escape of parasite, complement has an important role on the control of parasite on cutaneous strains.

In order to study the role of complement on the early phase of cutaneous leishmaniasis *in vivo* we evaluated the parasite burden at inoculation site after 7 and 30 days of infection by histophatology and by limiting dilution assay in susceptible (BALB/c) and resistant (C57BL/6) mice, depleted and non-depleted in complement, infected subcutaneously with promastigotes of *L. (L.) amazonensis*.

We observed in the skin lesion at 7 days of infection a mild inflammatory infiltrate predominantly constituted by mononuclear cells and few polimorphonuclear neutrophils in both mouse strains, depleted and non-depleted in complement, but more parasites in complement depleted mice from both strains. This observation was confirmed quantifying the parasites in whole lesion by limiting dilution assay when we found higher number of parasites in complement depleted mice at 7 and 30 days of infection, specially in BALB/c mice ($p<0.05$).

Our data show that the complement has divergent role on cutaneous and visceral strains of *Leishmania* and that has an important role at the beginning of cutaneous leishmaniasis, exerting a control on the growth of parasites in the lesion.

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IM-68 – NATURAL MODELS OF LEISHMANIA MAJOR INFECTION REVEAL SURPRISING OUTCOMES IN IMMUNE DEFICIENT MICE

Yasmine Belkaid, Rosalia Lira, Susana Mendez and David Sacks

We previously established a model of infection combining two main features of the natural transmission, inoculation of a low number of metacyclic promastigotes into the mice dermis. This approach allows us to dissect and carefully monitor the dynamic of events occurring at the site of the inoculation and in the compartments connected to it, the epidermis and the draining node. The inoculation of a hundred parasites into the dermis of a genetically resistant C57BL/6 leads to the development of a small lesion which resolves spontaneously. Different observations indicate that we can dissociate the evolution of this infectious process in two phases, one favors the multiplication of the parasite at the site and the second drives the killing of the parasite prior to the development of the pathology. This fist phase seems to be independent of the presence of IL-12 or IFN-gamma as shown by the results obtain on Knock-out mice. An analysis of the epidermal cytokine production by flow cytometry in the wild type indicates a consistent production of IL-4 during the first 5 weeks followed by the production of IL-12 and a significantly increased IFN-g around the 6th week. In order to understand the mechanisms underling those two phases we followed the progression of the infection at the levels of the epidermis, dermis and draining node in CD40, IFN-gamma, NO,IL-4, MHC Class II,and SCID mice. Our result show a complete dissociation between the parasitical charge and the pathology as well as surprising outcomes in terms of modification of the inflammatory site, cytokines production and the nature of the cells infected. The model also revealed a requirement for CD8+ T cells in the control of Leishmania infection in the skin.

IM-69 – 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. The vaccination with FML+saponin (Riedel de Haën) in dogs of a Kala-azar endemic area, São Gonçalo do Amarante, RN, let to a significant increase of the FML-seropositivity in vaccinated animals in relation to saline control (%): 62/14, 45 days after vaccination, 97/45 after 7 months and 97/40 after 13 months. Seropositivity and IDR along the time were highly correlated to FML vaccination ($p<0.005$). The IDR (24h was also significantly increased in the vaccinated group over the saline control (%): 58/15; 97/37 and 100/25. The protective effect of the FML+saponin vaccine was suggested by the detection of 4 obits due to kala-azar in saline control group while no death was detected in vaccinated animals. Also, a reduction of human disease in this area was concomitant to canine vaccination (15 to 0, 1996-1998). A final evaluation of protective effect was performed 24 months after vaccination, on surviving dogs. Symptomatic dogs were removed from the area, analyzed by FMLELISA assay (cut-off=0.435), IDR (>5mm), PCR for *L. donovani* of peripheral blood and for the presence of amastigotes in spleen or liver smears, after necropsy.

Group	Dog	FML-ELISA	IDR /mm	Symptoms	PCR	Amastigotes
FML-R	43/09/227	1.618	+10	Long nails	+++	-
FML-R	50/04/267	1.760	+10	Anorexia	-	-
FML-R	50/01/272	1.177	+10	Allopecy	++	-
FML-R	51/31/290	0.659	+10	Long nails	++++	-
FML-R	52/09/301	1.439	+5	Alopecia	-	-
Salina-R	47/07/250	0.564	-	Skin lesions	+++	-
Salina-R	51/04/282	0.936	-	Long nails	++++	-
Salina-R	51/34/291	0.484	-	Isolation	+	-
Salina-R	51/44/293	0.386	+5	Alopecia	+++	-
Salina-R	52/09/302	0.766	-	Long nails	+++	-
Salina-R	59/16.1/346	1.088	5	Alopecia	++	-

As seen in experimental model, FML-ELISA and PCR accuse infection in symptomatic dogs even when parasite detection is difficult. These results confirmed the protective potential of the FML-Riedel vaccine on canine kala-azar in the field, since kala-azar cases in saline group reached 33% while only 8% of vaccinated dogs showed symptoms with no obit. This make 92% of protection in vaccinated group. These differences between vaccinated and saline treated animals are significant ($p<0.05$).

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IM-70 – THE FMLELISA ASSAY IN SERODIAGNOSIS OF CANINE VISCERAL LEISHMANIASIS OF A RECENT OUTBREAK IN ARAÇATUBA, SÃO PAULO

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The current strategy for control of Canine Visceral Leishmaniasis (CVL) is based on detection and destruction of infected dogs. Detection is performed by serodiagnosis with an immunofluorescent assay (IF). Seropositivy on IF usually correlates well with parasitological and clinical evidence of infection. Due to its low sensitivity, this method however, underestimates the true prevalence of canine infection. Also, asymptomatic dogs are reservoir of infection in endemic areas. The FML-ELISA assay showed 100% sensitivity and 100% specificity in diagnosis of canine visceral leishmaniasis (CVL) in sera from naturally infected dogs from São Gonçalo do Amaranto, RN, Brazil. The overall prevalence of anti-*Leishmania* antibodies in the endemic area was 23%. Seroreactivity detected by a *L. chagasi* immunofluorescent assay (IF) was much lower (2.9%), very similarly to the percent of dogs with kala-azar symptoms (2.6%). 21/21 asymptomatic, FML-seropositive animals death from kala-azar in a period ranging from 0-6 months after diagnosis. The predictive value was 100% for the FML-ELISA, 43% for the *L. mexicana* ELISA and 24% for the *L. mexicana* and *L. chagasi* IF assays, respectively. A canine and human kala-azar burst was recently reported in Araçatuba, São Paulo. 9085/45000 canine sera samples were already collected. Among them, 908 corresponded to IF positive animals that were sacrificed. In the present study we analysed samples of 19 IF positive dogs from Araçatuba both by cytological analysis and FML-ELISA assay. The fine needle aspirates of enlarged lymphnodes were smeared, air-dried, fixed, stained (hematocolor) and analysed by optical microscopy. 19/19 smears revealed amastigotes of *Leishmania*. In addition, sera were collected and analysed by the FMLELISA assay. 16 among 19 samples showed to be positive showing high absorbancy values ($m \pm SD = 1.076 \pm 0.438$). Since the FML-ELISA assay previously showed to be 100% specific in diagnosis of infection by *L.donovani-L.chagasi* and negative in cases of tegumentar leishmaniasis, our preliminary data point out the probable co-existence of different *Leishmania* species causing canine infection in Araçatuba.

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IM-71 – PROTECTIVE EFFECT OF THE FML + QUIL-A SAPONIN VACCINE AGAINST EXPERIMENTAL CANINE KALA-AZAR

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In previous report we showed the FML-VACCINE protective potential, in experimental mongrel dogs, immunized with 3 doses of 1.5mg of FML, either with 1mg or 0,5mg of Quil A Saponin, and further challenged with 10^8 *L. donovani* amastigotes. In the present investigation, clinical signs as well as parasitological confirmation of disease, were studied. The presence of *Leishmania* DNA in peripheral blood was assessed by PCR analysis in samples obtained between 90-210 days after infection. The hot start PCR was performed with a pair of oligonucleotides that anneal to the origin of replication of both strands of the mini circle molecules. 90 days after infection, we could demonstrate the presence of PCR products in only 1 dog from saline control and 1 dog treated with 0.5mg QuilA-FML. The group treated with 1mgQuilA_FML showed to be protected disclosing positivity only 120 days after infection. Between 120 to 210 days of infection all animals were positive. Our results indicate that in this experimental model, the sensitivity of FMLELISA assay and of PCR analysis of blood were equivalent. We considered as clinical signs of disease: alopecy, skin lesions, caquexy, long nails, asthenia, anorexia and diarrhea, that let us to characterize the animals as polysymptomatic, olygosymptomatic and asymptomatic cases. 5 dogs were polysymptomatic. 4 among them corresponded to the same brood indicating they belonged to a highly susceptible family. 3 of these animals died of kala-azar at 297, 376 and 503 after infection. The first was a saline control, while the second and third were vaccinated with FML-QuilA (1mg). This delay confirmed the protective effect of the vaccine even in this highly susceptible family. Dogs treated with saline, from two other families showed to be polysymptomatic or olygosymptomatic, while their vaccinated brothers remained asymptomatic until the end of experiment (647 days after infection). An inversion in the serum albumin/globulin ratio was detected in the highly susceptible family, throughout the experiment while the two other families reached normal levels at one or two points. At 647 days after infection, all surviving animals were sacrificed and the presence of *Leishmania* parasites assessed by optical microscopy in: bone marrow, spleen, liver, lymphnode smears and in haematoxilin-eosin stained hystologycal slides. Amastigotes were found in all samples of polysymptomatic dogs while no parasites were evident by this approach neither in olygosymptomatic nor in asymptomatic dogs. Our results confirm the protective potential of the FML-QuilA (1mg) vaccine on experimental canine kala-azar.

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IM-72 – OCCURRENCE OF *LEISHMANIA DONOVANI* DNA IN BONE MARROW OF FML-SEROREACTIVE BRAZILIAN BLOOD DONORS

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Human kala-azar transmitted upon blood transfusion was already described in individuals of non-endemic areas of United Kingdom, Belgium, France and India, either with history of foreign travel or not. The finding of congenital transmission of Kala-azar confirmed the blood-borne characteristic of the disease. In previous work we showed that the prevalence of anti-*Leishmania donovani* antibodies in the Kala-azar endemic area (Natal, Brazil) is: 100% in Kala-azar patients, 9% in blood donors and 37% in hemodyalisis polytransfused patients. Blood transfusion is a risk factor for seropositivity (χ^2= 8.567, p<0.005). Among healthy asymptomatic FML-seroreactive blood donors of Natal, 5 (24%) were also reactive in PCR analysis for *L. donovani* DNA and 9 (43%) on dot assay of blood samples, showing a significant correlation (χ^2= 14.24, p<0.01). In the present investigation, the screening for anti-*Leishmania* antibodies was done with the FML-ELISA assay (100% sensitivity, 96% specificity) in sera of 400 asymptomatic healthy blood donors of endemic area. Seropositive individuals returned for clinical survey and bone marrow analysis. Leishmanial DNA was assessed by PCR in bone marrow samples. The hot start PCR was performed with a pair of oligonucleotides that anneal to the origin of replication of both strands of the mini circle molecules which are one of the components of the genus *Leishmania* mitochondrial DNA (kDNA): Primer A: 5'(G/C)(G/C)(C/G)CC(A/C)CTAT(A/T)TTACACCAACCCC and primer B: 5'GGGGAGGGGCGTTCTGCGAA. 51/400 healthy blood donors showed anti-*Leishmania donovani* antibodies in the FML-ELISA assay. Bone marrow puncture was successfully performed in 32 of them. 28/32 individuals were reactive in PCR (87.5% sensitivity) showing a significant correlation between positivity in PCR and in the FML-ELISA assay (p= 0.028; SPSS). The FML-ELISA assay is useful in blood donor screening for Kala-azar control. PCR confirms the presence of *Leishmanial* DNA in bone marrow with higher sensitivity and correlation to serology than in blood indicating the presence of subclinical infection in asymptomatic blood donors of endemic areas.

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IM-73 – THE FML-VACCINE AGAINST MURINE EXPERIMENTAL VISCERAL LEISHMANIASIS. COMPARISON OF THE ADJUVANT EFFECT OF BCG AND QUIL-A SAPONIN

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In previous studies was demonstrated the immunoprotective potential of the FML+saponin vaccine in the CB hamster 87.7% ($p<0.01$), Balb/c 84% ($p<0.001$) and Swiss albino (85%, $p<0.01$) models. Saponin showed to be better than $Al(OH)_3$ and Freund's Adjuvant. In the present study, female inbred Balb/c mice (2-3-month-old) were immunized with three weekly doses of *L. donovani* FML antigen (150 µg) in combination with either BCG (Fundação Ataulpho de Paiva) (100 µg) or Quil-A saponin (Superfos) (100 µg), by the sc route. Control animals were treated with only saline or adjuvant. Fifteen days after immunization, animals were challenged by i.v. injection of 2×10^7 amastigotes of LD-1S. 15 days after infection, animals were sacrificed and the protective response assayed by measuring the: anti-FML antibody levels (total IgG and subtypes) both before and after infection (FML-ELISA), delayed type of hypersensitivity (DTH) against *L. donovani* f/t promastigote lysate antigen, specific lymphoproliferative *in vitro* response against the FML antigen (T cell blot) (MTT method), serum γIFN levels (ELISA assay) and liver parasitic load (LDU units of Stauber= number of amastigotes/1000 cell nuclei x mg liver weight). The humoral anti-FML response was higher in the groups vaccinated with FML+Quil-A saponin and FML+BCG, than in controls treated with only the adjuvant or saline. Animals immunized with FML+saponin gave the highest antibody response on IgG1, IgG2a and IgG2b subtypes, both before and after the parasite challenge (log2 titres= 21:20:19 and 19:18:18, respectively). Also, the DTH response was positive and significantly higher than saline and adjuvant controls, only in the FML+Quil-A vaccinated group, ($p<0.001$-$p<0.01$ at 24h and $p<0.02$-$p<0.05$, at 48h incubation, respectively). Additionally, only the FML+Quil-A group induced a significant and specific increase of proliferative *in vitro* response and an enhanced response of serum γIFN over saline control (709.5/461.0 pg/ml). Coincidently, with the above described results a significant and specific decrease in liver parasitic burden was detected only in FML+QuilA treated animals. Indeed, parasitic load was 32.2% lower than saline ($p<0.025$) and 42.5% lower than saponin controls ($p<0.05$). While no protective response was detected for BCG+FML group, the BCG treated animals showed even higher parasitic loads than saline control ($p<0.005$). These results suggest that FML+Quil-A saponin formulation, that already proved to be protective in dog model, is more effective than FML+BCG against experimental visceral leishmaniasis in Balb/c model. Furthermore, in our conditions, BCG adjuvant showed several undesirable effects.

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IM-74 –ANTIBODY PROFILES IN *CEBUS APELLA* EXPERIMENTLY INFECTED WITH *LEISHMANIA (LEISHMANIA) AMAZONENSIS* DETECTED BY TWO DIFFERENT ANTIGENS USING THE ELISA AND IMMUNOBLOTTING METHODS

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Although T lymphocytes are considered to be responsible for immune protection in cutaneous leishmaniasis circulating antibodies are usually present and there is evidence that they play a role in immune regulation. The antibody response of *L. (L.) amazonensis* during primary and secondary experimental infections in *Cebus apella* was followed with the ELISA and immunoblotting techniques using two different antigens. Stationary phase *L. (L.) amazonensis* promastigotes (IFLA/BR/67/PH8) were inoculated intradermally into the dorsal surface of the tails of 4 monkeys. Different parasite doses were used to induce infection in each animal (A: 0.5×10^6, B: 1.0×10^6, C: 2.0×10^6 e D: 5.0×10^6). After spontaneous cure the four monkeys were challenged with a single dose (2.0×10^6). A total of 10 plasma samples per monkey were collected before and during the primary and secondary infections. The antigens consisted of disrupted promastigotes of *L. (L.) amazonensis* (IFLA/BR/67/PH8) and *L. (V.) shawi* (MHOM/BR/84/M8408). In the primary infection the incubation period (IP) ranged from 20 to 30 days and all the animals developed lesion at the inoculation site. Lesions were bigger in C and D and persisted for 2 to 3 months. All animals developed lesions when challenged with homologous parasites. In the secondary infections both the latent period and the duration of the lesions were significantly ($p<0.05$) shorter and smaller than those of the primary infections. The ELISA IgG titres were low in the primary response with homologous antigen in animals A and B (£320), but higher in C and D (≤1280). After challenge all the animals had higher IgG titres (≤1280). With the heterologous antigen, titres were almost always negative during primary infection (≤80) and increased slightly in the secondary infection (≤640). The difference between the titres obtained with each antigen was only statistically significant at 42 and 60 days post challenge ($p<0.05$). Immunoblotting showed that the IgG antibodies in the plasma of infected animals recognized different polypeptides. With the *L. (L.) amazonensis* antigen similar banding patterns were observed in animals A and B: one strong 46kDa band during primary and secondary infections. Animal C recognized bands at 17, 46, 49, 90, 100, 120 e 200kDa during primary infection, contrasting with a total absence of reactivity for D. Nevertheless, in the secondary response both C and D presented bands of 46, 78 e 88kDa. Only C had 35 and 41kDa bands. When *L. (V.) shawi* antigen was used, there were no bands in animal A during primary and secondary infection, but IgG against fractions of 100kDa (primary infection) and 66kDa (secondary infection) were detected in B. More bands were detected in animal C (66, 84 e 91kDa) and D (66 e 91kDa) in both the primary and secondary infections (C: 46, 66kDa and D: 46, 66, 100kDa). Although the number of animals is small there is some evidence that the number of promastigotes may influence the development of IgG antibodies of primary and secondary responses. Higher titres (ELISA) and more bands (immunoblotting) were detected in the animals that received higher doses of promastigotes and had larger lesions. Different profiles of IgG detection were seen with each antigen by both methods. The *L. (L.) amazonensis* antigen was more sensitive than *L. (V.) shawi* for IgG detection in the plasma of monkeys infected with *L. (L.) amazonensis*. More studies are needed since in some recent studies we have found that *L. (V.) shawi* is highly sensitive in detecting anti-leishmanial IgG in humans, even when the inducing parasite was heterologous.

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IM-75 – TNFα PRODUCTION IN *L.(L.) CHAGASI* INFECTED HUMAN MACROPHAGES

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L.(L.) chagasi initially inoculated in the skin migrates inside host cells to spleen and liver causing severe and sometimes fatal visceral leishmaniasis. High serum TNFα levels have been reported in human visceral leishmaniasis and may be involved in the main aspects of the disease such as severe weight loss and fever. Since human macrophages (Mϕ) are the parasite host cells and the main source of TNFα, we explored the mechanisms of TNF-α production by these cells. Mϕ were grown in RPMI medium, 10 % normal human serum and 5 % CO_2 at 37^0C. Mf were infected at 10:1 parasite/cell ratio. Supernatants were collected at 48h, and cytokine levels determined by ELISA. Isolated human Mϕ did not produce TNFα in response to stationary phase promastigotes of *L.(L.) chagasi*, the vector inoculation form. Log phase promastigotes on the other hand induced TNFα production in human Mϕ. These cells secreted large amounts of TNFα (200-500pg/ml) in response to LPS (10ng/ml). Human polymorphonuclear cells were also unable to produce TNFα. Infection by *L.(L.) chagasi*, or treatment with lipophosphoglican (LPG, 1-100μg/ml), did not modulate LPS (10ng/ml)-induced TNFα production by Mϕ. IFNγ (10-100 U/ml) stimulated Mϕ were unable to produce TNFα in response to infection. Only when PMA (10ng/ml;12hs)- or ConA (10μg/ml;48hs)-stimulated and paraformaldehyde-fixed lymphocytes were added to the cultures of *L.(L.)chagasi*-infected Mf there was an important TNFα production. Activated lymphocytes are of utmost importance in driving TNF-α production by stationary-phase *L.(L.) chagasi*-infected human Mf.

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IM-76 – PARTICIPATION OF INOS AND TNFRP55 IN THE DEVELOPMENT OF A PROTECTIVE RESPONSE AGAINST CHALLENGE WITH *LEISHMANIA AMAZONENSIS*

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Control of infection by *Leishmania* has been shown to be dependent on the development of a sustained Th1 type response against the parasite. While control of lesion development has been demonstrated to be dependent on the activity of inducible Nitric Oxide Sintase (iNOS) and also on the presence of TNF receptor p55 (Diefenbach, A. *et al*., Immunity 8: 77-87, 1998; Vieira, L.Q. *et al*. J. Immunol. 157:827-835, 1996), no data is available on the role of these proteins on the induction of a Th1 response in this specific model. Vaccination against infection with *Leishmania amazonensis* can be achieved in C57BL/10 mice by a combination of *L. amazonensis* antigen plus *Corynebacterium parvum* as an adjuvant (Costa, C.A. *et al., Parassitologia* 34:45-51, 1986). Thus, we decided to investigate, whether immunization of iNOS- as well as TNFRp55-deficient mice could lead to the development of a Th1 type response and if this response was able to protect these animals against a challenge infection with *L. amazonensis*. Animals were immunized at the base of the tail with two doses of antigen + *C. parvum* (seven days apart) followed by a booster injection 4 weeks after the second dose. One week after the booster animals were challenged with 1 $x10^5$ stationary phase *L. amazonensis* PH8 strain in the left hind footpad. Lesion development was followed weekly by measurement of the thickness of the infected footpad. At several time points animals were sacrificed and spleen and lymphnode cells stimulated *in vitro* for cytokine production.

Our results show that similarly to C57BL/10 mice, vaccination of C57BL/6 mice (wild type control) also protected the animals against a challenge infection by *L. amazonensis*. In addition, TNFRp55 deficient mice were also able to be partially protected by the same vaccination protocol. TNFRp55 deficient control mice developed lesions that were larger than those from wild type control. Immunized TNFRp55 deficient mice developed lesions that were smaller than control mice but were similar in size to those from non-immunized C57BL/6 wild type control animals. These observations were associated with elevated levels of IFN-g and very low levels of IL-4 production by both spleen and lymphnode stimulated cells. These results confirm previous observations that TNFRp55 is not directly involved in the development of a Th1 response but is important in the control of the healing process. On the other hand, iNOS deficient mice were not able to control lesion development even after administration of antigen + *C. parvum*. No differences were detected throughout the experiment between control and immunized animals. Furthermore, cells from both spleen and lymphnode were not able to produce IFN-γ both during the immunization protocol and after infection. These results indicate that iNOS is involved not only in the elimination of the parasite but is apparently also involved in the differentiation of T cells.

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IM-77 – *LEISHMANIA AMAZONENSIS:* LIPOPHOSPHOGLYCAN EXPRESSION DURING AMASTIGOTE-PROMASTIGOTE TRANSFORMATION

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As part of its life cycle, *Leishmania* lives as amastigote forms inside hosts macrophages and as promastigotes in its insect vector; these forms can also be found in axenic cultures. Lipophosphoglycan (LPG) is the major glicoconjugate found at the promastigotes surface, and in amastigotes, its expression is down-regulated. This molecule plays a crucial role in parasite infection and survival mechanisms in both vertebrate and invertebrate hosts. However, little is known about LPG's dynamics of expression during amastigote-promastigote transformation. In the present work we studied LPG expression in *L. amazonensis* (Josefa strain) using anti-LPG monoclonal antibodies (MoAbs). *L. amazonensis* amastigotes were isolated from golden hamsters or Balb/C mice footpad lesions and cultured in Schneider's Insect medium with 10% fetal bovine serum at 26ºC. Aliquots were taken at each 4 hours until 48 hours, when the morphological promastigote transformation was completed. Fixed parasites were incubated with the MoAbs LuCa-D5 and 3A1-La, anti-*L. amazonensis* LPG (respectively IgG3 and IgG2b), and then revealed by anti-mouse IgG fluorescein labeled. LuCa-D5 MoAb staining was weak and irregular, being almost exclusively on the parasites surface in all time intervals analysed. It is interesting to note that around 50% of the amastigote population was labeled by LuCa-D5, and only by 12 hours more than 80% of the cells were stained. In contrast, staining with 3A1-La was both more delayed and selective. No staining was apparent on amastigotes with 3A1-La; internal labeling was observed on a small proportion of cells after 4 hours, reaching 80% after 12 hours. Surface staining was not aparent until 12 hours and only in 17% of the cells. Parasite cell membrane label increases gradually and by 36 hours, all the parasites showed strong surface staining.

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IM-78 – CHARACTERIZATION OF *LEISHMANIA SSP* ISOLATED FROM AMERICAN CUTANEOUS LEISHMANIASIS PATIENTS FROM AN ENDEMIC AREA OF PERNAMBUCO STATE, BRAZIL, USING MONOCLONAL ANTIBODIES

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American cutaneous leishmaniasis (ACL) presents two basic forms: cutaneous and mucocutaneous. The severity and pathogenicity of the disease depend on the infecting *Leishmania* species and probably on the intra-specific variant. Recently, several molecular and immunological tools for the taxonomic and diagnostic studies of *Leishmania* were developed such as those based on isoenzyme eletrophoresis, analysis of restriction enzyme digestion patterns and monoclonal antibodies. In the present communication, *Leishmania spp* isolates from patients living in the Amaraji Municipality, an endemic area of Pernambuco State, were characterized by immunofluorescence using a panel of species-specific monoclonal antibodies. The diagnosis was performed on the basis of clinical examination, epidemiological history and direct detection of the parasite in the lesion. Twenty seven isolates were obtained from biopsies of the lesions through cultivation in biphasic 4N (Difco) and Schneider's *Drosophila* (Sigma) media suplemmented with 10% inactivated fetal bovine serum, and innoculation in hamsters. The analysis of the monoclonal antibody reactivities showed that the 27 isolates were *L.(V.) braziliensis* pertaining to the serodema-1. The results suggest that *L. (V.) braziliensis* is the predominant causative agent of American cutaneous leishmaniasis in Zona da Mata, an endemic area of Pernambuco State.

Finacial support:FIOCRUZ, FAPESP.

IM-79 – SEARCHING T CELL INHIBITORS IN *LEISHMANIA AMAZONENSIS*

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The development of effective vaccines against protozoan infections is hampered by the antigenic complexicity of the parasites. A vaccine against cutaneous leishmaniasis currently under clinical trial in Brazil uses whole killed *Leishmania amazonensis* parasites, which may carry along molecules which decrease its efficacy. We have observed that the promastigote lysate from *L. amazonensis* suppresses the mitogenesis and induces apoptosis in lymphonode cells from both normal and infected animals. In this work we propose to identify the apoptosis-inducing substance(s) present in the lysate whose removal may improve the vaccine efficacy. The promastigote lysate in aqueous solution was centrifuged at 6000 x g/ 4^0C / 10 min The supernatant (soluble fraction) was liophylized and fractionated on a gel filtration column (BIOGEL P-10) yielding four sub-fractions (A,B,C and D). The precipitate (unsoluble fraction) was solubilized in 40% phenol / water and purified in a HPLC column (SUPERDEX 75) equilibrated with distilled water yielding three sub-fractions (E, F and G). The whole lysate, all fractions and sub-fractions were tested independently at 40 mg/ml on normal and Concanavalin A-stimulated lymphonode cells from BALB/c mice infected for 7 days with *L. amazonensis.* After 48h of culture the inhibition of proliferation was assessed by incorporation of ^{3}H-thymidine. The results showed that whereas the whole lysate inhibited 93%, the soluble and the unsoluble fractions inhibited 62% and 79% of spontaneous proliferation, respectively. The soluble sub-fractions A, B, C and D reduced the proliferation by 44%, 0 %, 41% and 27%, respectively whereas the unsoluble sub-fractions E, F and G inhibited the proliferation by 11%, 31% and 0%, respectively. As to the inhibition of mitogenesis, sub-fractions A, C and D were the most potent, producing 93%, 93% and 88% reduction in relation to medium alone. These preliminary results indicate that there are different inhibitors of cell proliferation in the L. amazonensis lysate and we are presently trying to chemically characterize those substances.

Finantial support: CNPq

IM-80 – ORAL IMMUNIZATION WITH LEISHMANIAL ANTIGENS DELAYS THE ONSET OF INFECTION WITH *L. AMAZONENSIS.*

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As mucosal administration of proteins has been controversely shown differentially affect TH1 and TH2 T cell responses, we decided to use the murine cutaneous leishmaniasis as a model to investigate the systemic effect of oral immunization with leishmanial antigens. We had previously observed that oral immunization with free leishmanial antigen indiscriminately increased the production of IFN-γ, IL-2 (TH1 cytokines) and IL-10 (TH2 cytokine), and that IFN-γ could be greatly increased when the antigen was encapsulated in biodegradable PLGA (poly-lactide-co-glycolide acid) microspheres.

In this work we investigate the effect of oral immunization with free and PLGA-encapsulated *Leishmania amazonensis* whole antigens during the course of infection as compared with s.c. immunization. Thus, BALB/c mice received two intragastric doses of 130 μg free/encapsulated antigens or two s.c. doses of 25 μg free/ Freund adjuvant (CFA)-emulsified antigens on days -11 and -4 of infection. Controls received PBS. Lesion development was monitored up to day +35 of infection.

The results indicate that unlike sc immunization, oral immunization with a relatively low dose of leishmanial antigen prevented the increase in the lesion sizes during the first two weeks of infection. As previously reported, s.c immunization exacerbated the disease, but it was of interest that encapsulation of the Ag in PLGA microspheres prevented the exacerbation of lesion. The specific proliferative response of inguinal lymphonode cells re-stimulated *in vitro* with the antigen was also evaluated in animals orally given 100 μg or 1 mg doses of free antigen on days -12 and -7. A third group received 100 μg on the same days plus additional 100 μg doses on days +7 and +11 of infection. The T cell response of all orally immunized animals was significantly higher than in the PBS controls, particularly in the animals receiving the four doses.

These results indicate oral immunization with whole leishmanial antigens activate systemic immune responses which induces a transient protection against leishmaniasis through still unknown mechanism.

IM-81 – CHEMOKINES IN LEISHMANIASIS: EVIDENCE FOR A ROLE OF *LEISHMANIA* SPECIES IN THE PROFILE OF CHEMOKINE GENE EXPRESSION IN CBA MICE

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Previous work from our Lab have shown that CBA mice are resistant to *L.major* and are highly susceptible to *L.amazonensis* infection. Resistance to *L.m* is related to a Th1 type -immune response, whereas susceptibility to *L.a* is associated with a Th2 type-immune response. The histological patterns of the lesion reflect the host immune status in this model of disease and are similar to those described in human leishmanaisis. Resistance is associated with a mixed mononuclear inflammatory cell infiltrate composed of few infected macrophages, granulomas and a progressive increase in the number of lymphocytes. Upon infection, susceptible animals show tissular reaction constituted by a monomorphic infiltrate of heavily parasitized macrophages and scanty lymphocytes. Given the role of chemokines in the recruitment of leukocytes, we decided to analyze whether differences in inflammatory infiltration observed in the CBA infected with *L.a* or *L.m* could be due to a differential expression of chemokine genes. We took advantage of the fact that the same isogenic strain of mice is susceptible to one specie of *Leishmania* and resistant to another to overcome possible differences related to differences in the host genetic background. Mice were infected in the footpad with 5 x 10^6 promastigotes of *La* or *Lm* and RT-PCR was used to measure the expression of chemokine mRNA such as KC, MIG, CRG-2, JE/MCP-1, MCP-5, RANTES and of cytokines TNF-α and IL-4. Our results show that the expression of CRG-2, JE/MCP-1 and TNF-α are slightly augmented by day 1 post-infection in mice infected with *Lm*, and that expression of MCP-5 was significantly increased in these mice by the same day. In a different way, mice infected with *La* showed inhibition of KC and MIG. On day 12 post-infection, a slight increase in the expression of CRG-2 and a significant increase in MIG were seen in *Lm*-infected mice. By the same time, *La*-infected mice had inhibition of KC expression. Forty days after infection the expression of MIG mRNA was high in *Lm*-infected. Taken together, results herein show that a differential expression of chemokine mRNA occurs in CBA mice infected with *Lm* as compared to those infected with *La*. These differences may be implicated in the diverging inflammatory cell infiltrate profile that correlates with resistance or susceptibility.

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IM-82 – EFFECT OF ZINC ON THE *IN VITRO* IMMUNE RESPONSE TOWARDS *L. AMAZONENSIS*

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In spite of the well-documented importance of zinc in an ongoing immune response, *in vitro* as well as *in vivo*, virtually no data exist on the possible role of zinc (Zn^{2+}) in leishmaniasis.

We have previously shown that zinc supplementation of human macrophages infected with *Leishmania spp. in vitro* resulted in a strong decrease in parasite burden. This leishmanicidal effect of zinc was maximal at 10 uM, i.e. normal serum zinc concentration, suggesting zinc deficiency as a possible factor involved in leishmaniasis pathogenesis.

In this study, we examined the effect of zinc (as $ZnCl_2$) on the *in vitro* and *ex vivo* immune response towards *L. amazonensis*, in healthy controls as well as patients with cutaneous leishmaniasis from the endemic area of Corte de Pedra (BA). Although zinc (at 10 to 30 uM) was able to decrease parasite load in monocytes or total mononuclear cells (MNC) at 48 h of culture *in vitro*, this did not result in increased lymphocyte proliferation, as measured by ^{3}H-thymidine incorporation at 72 or 96 h of culture. However, significant lymphocyte proliferation was induced by 100 uM of $ZnCl_2$, which synergistically increased in the presence of *L. amazonensis*. *In vitro* stimulation with *L. amazonensis* also induced IFN-γ and TNF-α production in healthy controls as well as in patients, which was slightly, but not significantly, diminished by 10 uM Zn. In an *ex vivo* whole-blood assay, lectin-stimulated lymphoproliferation and *L. amazonensis*-induced IFN-γ production were unchanged by low zinc (10 uM). Again, only supra-physiological concentrations (100 uM) of zinc induced lymphoproliferation and IFN-γ production, even in the absence of lectin or *L. amazonensis*.

In conclusion, low dose zinc does not seem to modify the *in vitro* or *ex vivo* immune response towards *L. amazonensis*, in spite of the significant decrease in parasite load.

Taken together, our results seem to suggest a potential beneficial effect of topical rather than systemic zinc supplementation.

Support : CNPq, PRONEX

IM-83 – DIFFERENTIAL EFFECT OF IFN-B AND TGF-B ON IL-12-DRIVEN ANTI-*LEISHMANIA* IMMUNE RESPONSE *IN VITRO*

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The murine model of experimental leishmaniasis has been instrumental for the development of the actual Th1/Th2 paradigm, with Th1 standing for protection and Th2 for progression of the disease. In human leishmaniasis, however, the situation is less clear. We have previously shown that both IFN-β and TGF-β are able to increase parasite burden in Leishmania-infected human macrophages and to antagonize the protective effect of IFN-γ, thereby classifying them as Th2-like. Aiming at a further characterization of the role of IFN-β and TGF-β in Th1/Th2 development, we studied their possible interaction with IL-12, the key Th1 cytokine. We found that IFN-β by itself induced only modest amounts of IFN-γ but was able to synergize with IL-12 for IFN-γ induction. TGF-β, on the other hand, had no effect by itself and significantly inhibited IL-12-induced IFN-γ secretion. These opposed effects of IFN-β and TGF-β were observed in healthy controls as well as cutaneous leishmaniasis patients. Stimulation with *L. amazonensis in vitro* induced IFN-γ production in both patients and controls, which was again synergistically increased by IL-12 or IL-12+IFN-β treatment and decreased by TGF-β. On the other hand, both IL-12 and L. amazonensis-induced lymphoproliferation, as assessed by ^{3}H-thymidine incorporation, were significantly inhibited by both IFN-β and TGF-β.

In conclusion, the ability of IFN-β to synergize with IL-12 for IFN-γ synthesis, suggesting a strong boost to Th1 development, in contrast to its antagonistic effect towards IFN-γ-induced leishmanicidal activity. However, this stimulatory effect of IFN-β does not counteract its antiproliferative effect on IL-12- or L. amazonensis-stimulated lymphocytes.

Support : CNPq, PRONEX

IM-84 – GLYCOLIPID ANTIGENS OF *LEISHMANIA VIANNIA* RECOGNIZED SPECIFICALLY BY MOAB SST-1

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A hybridoma cell line was established after immunization of BALB/c mice with promastigote membranes obtained after N_2 cavitation and ultracentrifugation. The monoclonal antibody (MoAb) secreted by this hybridoma was termed SST-1 (IgG3), and specifically recognizes the glycolipid fraction extracted from *L. (V.) braziliensis* promastigotes with mixtures of chloroform/methanol and/or isopropyl alcohol/hexane/water. Reactivity of the MoAb SST-1 with the glycolipids was analyzed by HPTLC immunostaining, using as solvent chloroform:methanol/$CaCl_2$ (60/40/9; v/v/v). In these conditions, all *L. (V.) braziliensis* serodeme[3] showed two slow migrating components reactive with SST-1. The same reactive components were detected in *L. (V.) guyanensis*[3] and *L. (V.) naiffi*[3]. Promastigotes of *L. (V.) panamensis*[3] presented only the upper reactive component. In *L. (V.) lainsoni*[3], a distinct glycolipid (with higher chromatography mobility) than those observed in other *L. Viannia* subgenus was reactive with SST-1. The parasite reactivity with SST-1 varied when tested by solid-phase radioimmunoassay or indirect immunofluorescence, suggesting that the different *Leishmania Viannia* coud be presenting the glycolipid antigens in different ways (antigen presentation/crypticity). No reactivity was found with *L. (L.) amazonensis*, *L. (L.) major* and *L. (L.) chagasi*. SST-1 reactivity was abolished after mild treatment of these parasites or glycolipids with sodium m-periodate, indicating that this MoAb recognizes carbohydrate epitope.

In order to analyze the localization of these glycolipids on the parasite, transmission immunoelectron microscopy (TEM) studies were carried out. Briefly, parasites were fixed in 4.0 % paraformaldehyde and 0.05% glutaraldehyde, incubated sequentially with MoAb SST-1, and gold-conjugated anti-mouse IgG. Labeled promastigotes were fixed in 2.0% glutaraldehyde and dehydrated with ethanol. The pellet was included in araldite and the slices analyzed by TEM . By this technique we have been able to clearly visualize gold particles in the external leaflet of parasites plasma membrane. Parasites were also processed for cryoelectron microscopy and grids were incubated with SST-1 and anti-mouse IgG immunogold conjugate, by this technique it was possible to observe a better parasite labeling, confirming the surface localization of glycolipid antigens. The structures of *L. (V.) braziliensis* glycolipid antigens and the possible biological role of these glycoconjugates are under investigation.

[3]Kindly provided by Dr. J.J.Shaw (IEC, Belém, PA)
Supported by : CAPES, CNPq, FAPESP and PRONEX

IM-85 – MONOCLONAL ANTIBODIES SPECIFIC TO *LEISHMANIA (VIANNIA) BRAZILIENSIS* PROMASTIGOTES

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Three monoclonal antibodies (MoAbs) highly specific to *L. (V.) braziliensis* promastigotes (serodeme 1) were produced. The MoAbs were named SST-2 (IgG1), SST-3 (IgG3) and SST-4 (IgG1). The MoAbs, when analyzed by immunofluorescence, solid-phase radioimmunoassay and Western blotting, did not show any cross-reactivity with *L. (L.) amazonensis*, *L. (L.) major*, *L. (L.) chagasi*, *L. (V.) panamensis*[3], *L. (V.) guyanensis*[3], *L. (V.) naiffi*[3] and *L. (V.) lainsoni*[3] promastigotes. All *L. (V.) braziliensis* serodeme[3] were reactive with SST-2, SST-3 and SST-4. It was observed an inhibition of macrophage invasion of 52% and 43% by Fab fragments of SST-3 and SST-4 , respectively. MoAbs SST-2 and SST-3 were reactive with *L. (V.) braziliensis* serodeme (1 to 8) by indirect immunofluorescence and solid-phase radioimmunoassay. By Western blotting it was observed that: i) MoAb SST-2 recognized a low molecular weight component (about 24 and 28 kDa), ii) MoAb SST-3 recognized glycoproteins (180 to 200 kDa), and iii) MoAb SST-4 recognized a component about 98kDa. The immunolocalization of the antigens recognized by these MoAbs was carried out by indirect immunofluorescence (IIF) and by transmission electron microscopy (TEM). The parasites were fixed in 4.0 % paraformaldehyde and 0.05% glutaraldehyde, incubated sequentially with the MoAbs and with gold-conjugated anti-mouse IgG. The parasites were included in araldite and the slices analyzed by TEM. Parasites were also processed for cryoelectron microscopy and grids were incubated with the MoAbs and gold-conjugated anti-mouse IgG. By cryoimmunoelectron microscopy it was observed a strong labeling with gold particles of the parasite surface when incubated with MoAb SST-2 , this data is in agreement with the intense fluorescence detected after IIF assay. MoAb SST-3, by IIF, reacted only with flagellae, this result was confirmed by TEM, where the immunogold labeling was predominant on flagellae surface. Regarding MoAb SST-4 no fluorescence was detected with the parasites, also by TEM reactivity of SST-4 was very weak. Our lab are now actively investigating if MoAbs SST-2, SST-3 and SST-4 recognize specific antigens of *L. (V.) braziliensis* or specific epitopes only expressed in *L. (V.) braziliensis* but localized in common antigens to all *Leishmania* .

[3]Kindly provided by Dr. J.J.Shaw (IEC, Belém, PA)
Supported by : CAPES, CNPq, FAPESP and PRONEX

IM-86 – *PLASMODIUM* TRAP AS A TARGET OF HUMORAL IMMUNITY AGAINST SPOROZOITES

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TRAP (SSP2) is a transmembrane protein found on the surface and in micronemes of malaria sporozoites, and is a member of a family of Apicomplexan proteins that include MIC2 of *Toxoplasma gondii*. The TRAP-null mutant showed that TRAP is required for gliding motility and infectivity. In addition to a region of repeats, the extra-cellular portion of TRAP contains a thrombospondin type 1 repeat, a conserved N-terminus of unknown function (designated N-10) and an A-domain. A-domains are found in proteins involved in adhesion to cells or matrix, e.g. integrins. A-domains are defined by a Metal Ion Dependent Adhesion Site (MIDAS), a motif which coordinates a divalent cation and is critical for ligand binding. We have recently shown by site-directed mutagenesis that disruption of the MIDAS of *P. berghei* TRAP significantly reduces sporozoite infectivity in vivo. Therefor, the crystal structure of the A-domain of CD11b was used to construct a 3-D model of the A-domain of *P. yoelii* TRAP. From this model, we chose 3 regions adjacent to the MIDAS that correspond to antibody blocking epitopes in homologous A-domains. These regions were incorporated into polyoxime and HBc- (Hepatitis B Virus core antigen) fusion protein vaccines. Mice actively immunized with these constructs were not protected from sporozoite challenge, however none displayed high titers against intact sporozoites by IFA. Despite pre-incubation with high concentrations of monoclonal and polyclonal antibodies made against these A-domain epitopes, as well as the repeats and N10, no inhibition of sporozoite infectivity was detected in vivo. Only a proportion of sporozoites have detectable amounts of surface TRAP, and large amounts of TRAP are probably only released from micronemes onto the parasite surface at the moment of target-cell contact, as is true of MIC2. This dense, instantaneous release of TRAP, in the immediate vicinity of the hepatocyte, may prevent antibodies from blocking receptor binding and cell invasion.

IM-87 – THE ISOTYPE DISTRIBUTION OF EB200-SPECIFIC ANTIBODIES IN INDIVIDUALS NATURALLY INFECTED BY PLASMODIUM

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There is increasing evidence that protection against blood stages of *P.falciparum* involves cooperation between cytophilic antibodies and monocytes, resulting in phagocitosis of parasitized erythrocytes or merozoites. Naturally acquired short-lived cytophilic antibodies anti-*P.falciparum* have been described as predominant during acute *P.falciparum* infections while an heterogeneous pattern in its isotype composition was identified in Brazilian unprotected subjects. However, most of these data were obtained using whole parasite extracts. The isotype distribution of cytophilic antibodies recognizing parasite purified proteins remains to be determined. Among the proteins of asexual blood stage of *Plasmodium*, the recombinant protein EB200 seems to have the ability to bind monkey antibodies involved in phagocitosis of parasitized red blood cells. The recombinant protein EB200 is a 135 aa-repetitive fragment, derived from PF332 (Mattei & Scherf, 1992. Gene, 110: 71), an antigen expressed on the membrane of the trophozoite- and schizont- infected erythrocyte. In the present study we have evaluated the isotype distribution of antibodies anti-EB200 in individuals living in Portochuelo, a cohort of low malaria endemicity, exposed to natural *Plasmodium* infection. First, specific IgG to purified protein EB200 were measured in samples collected from 175 individuals presenting *Plasmodium* blood parasites and from nonparasitemic individuals. Among these, sixty individuals (34,8%) presented specific IgG antibodies to EB200, with titers varying between 1/85 to 1/6912. The isotype analysis of these group were estimated by subclass-specific ELISA. The results showed that 48,3% and 15% of individuals presented IgG1 and IgG3 antibodies specific for this protein, respectively. Both isotypes were simultaneously presented by 16,7% of these individuals. Smaller proportions of individuals presented IgG2 (6,7%) or IgG4 (1,7%) antibodies anti-EB200. These results showed the development of specific cytophilic antibodies anti-EB200 in natural *Plasmodium*-infected individuals living in areas where malaria is hypoendemic. The EB200-specific IgG1 and IgG3 antibodies affinity is under investigation in our laboratory.

Supported by FAPESP.

IM-88 – DETECTION OF MALARIAL ANTIBODIES IN MAN BY ENZYME-LINKED IMMUNOSORBENT ASSAY USING *P.CHABAUDI* AS ANTIGEN

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Enzyme-linked immunosorbent assay (ELISA) was standardized and evaluated using *P. chabaudi* as an alternative antigen in the serological diagnosis of human malaria. Conventional ELISA using *P. falciparum* as antigen were used as reference test.

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. The antigen extract was utilized as antigen in ELISA in antimalarial IgG antibody detection. At the same time, *P. falciparum* was cultered *in vitro* for preparing antigen for ELISA development. ELISA sensibility, specificity and reproducibility employing *P. chabadui* were compared with ELISA using *P. falciparum* in detection of IgG plasmodial antibodies.

Sensitivity of ELISA with *P.chabaudi* antigen from individuals with past/present malaria was 86.98% while that of *P.falciparum* ELISA was 91.56%. Specificity was 99.72% and 100% with *P. chabaudi* and *P. falciparum* antigens respectively when ELISA was assayed in 62 sera from health blood donors of blood bank as well 76 sera from individuals with others diseases. Tests reproducibility study showed and agreement between serum titers, at the time, as in tests accomplished on different days.

On account of the low cost and similar results of ELISA with *P. chabaudi* antigen as compared to *P. falciparum* 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.

Financial Support: FUNAPE/UFG/GO, LIMHCFMUSP/SP, CAPES.

IM-89 – WHY IS IT DIFFICULT TO FIND CHICKEN MALARIA CAUSED BY *PLASMODIUM GALLINACEUM* IN NATURE BUT *P. JUXTANUCLEARE* IS COSMOPOLITAN?

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Over 150 species of malaria parasites have been described, four in man, two in the domestic fowl (*P.gallinaceum =Pg* and *P.juxtanucleare =Pjx*). *Pg* was first isolated by Brumpt, 1935, through sub-inoculation of blood from chickens with negative smears, surviving an epizooty in Colombo. The parasite seems restricted to the Orient (Sri Lanka, Indo China, India) not observed elsewhere. *Pjx* was first isolated in Belo Horizonte, Minas Gerais (MG) by Versiani & Gomes (1941, *Rev. Brasil. Biol*., 1: 231-233), in fowls from the market. Rather cosmopolitan, *Pjx* was found in Africa, Asia, South America, Oceania. The *Pg* model, in the laboratory, has helped clarifying many aspects of malaria cycle, vaccines, antimalarials, etc. A close relationship between *Pg* and *P.falciparum (Pf)* based on homologies of sporozoite DNA sequences (McCutchan *et al*., 1996, *PNAS*, 93: 11889-11894), has reintroduced *Pg* in modern research. There are few laboratory isolates of *Pg.* Ours was received from IOC-Fiocruz, originated from Brumpt's first isolate (Galvão, 1945, *Rev. Clín. S. Paulo,* 17: 137-210), being kept through: (i) weekly blood passages from acute infections; (ii) sporozoites injected by mosquito bites or syringe. *Pjx* is endemic among chickens raised outdoors, with prevalence of 10-40% thus strain isolation is easy in nature, unlike *Pg*. Fowls raised outdoors harbor circulating parasites including gametocytes detected in blood smears (Krettli, 1972, *J Parasitol*., 58(3): 630-631). Chickens inoculated with *Pjx* blood parasites, like those found naturally infected, have a sustained, long lasting parasitemia months later. Our present data show that *Pg* sporozoites in chickens result in a brief, self-controlled acute parasitemia followed by a long lasting chronic phase when no parasites are seen in blood smears. Six or more months later parasites are recovered through blood sub-inoculations from all chickens to originate then infections with high and lethal parasitemia. The same evolution of *Pg* malaria is expected in nature, based on the rare event of finding chickens with patent parasitemia. Such differences may explain why only *Pjx* is cosmopolitan.

Financial support: PRONEX, PAPES, CNPQ (fellowship to AUK)

IM-90 – DIFFERENTIAL IMMUNE CROSS-REACTIVITY TO *PLASMODIUM GALLINACEUM* SPOROZOITES BY SERA OF PATIENTS WITH MALARIA BY *P. FALCIPARUM* OR *P. VIVAX*

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Malaria, still endemic in Brazil, causes about half a million acute cases in the Amazon region. Three species are endemic here (*P. falciparum* - Pf, *P. vivax* - Pv and *P. malariae* - Pm) but the prevalence of the latter is underdiagnosed, a consequence of the method used (thick blood smears) as routine by the Health Services. The only species causing death is Pf therefore methods of differential diagnosis based on serology should help to predict epidemies and to detect areas with more intense transmission of Pf, a tool to intensify the disease prophilaxis. Based on the homologies demonstrated between Pf and *P. gallinaceum* (Pg) sporozoite genes we have undertaken studies to identify their possible cross-reactivity based on serology using indirect immunofluorescence (IIF) (MS Thesis, A.A.M. Fernandes, IOC, Fiocruz, 1998). Since Pf and Pv are transmitted concomitantly in the Amazon we used sera obtained in focal areas of Pv transmission only to investigate seropositivity of human malaria sera with P.g. sporozoites. Our results were: (i) all vivax malaria sera were non reactive with Pg sporozoites; (ii) in the areas in which transmission of Pf was more intense we observed 70% of positivity (P. Azevedo and Apiacas); (iii) as the epidemy decreased in Apiacas (Feb, 1999) the positive IIF decreased to 40%. Sera from areas of lower transmission were also less reactive to Pg sporozoites. The positive sera tested against total sporozoite extract (NP40 lysate) by Western blotting (WB) however were surprisingly low (25-30%). The number of positive sera by WB in the three areas tested were respectively 8/38 (P. Azevedo), 3/10 (Apiacas, 1997) and 5/14 (Apiacas, 1999). Normal sera and sera from Pv or Pf malaria patients recognized several antigens by WB but there was one distint epitope only recognized by the Pf sera. Our present goal is to clone and sequence the relevant protein to produce one recombinant antigen and/or a peptide sequence to be used for serological tests to differentiate Pf and Pv in areas of intense and superposed transmission.

Supported by CNPq through fellowships to ACN and AUK; by PAPES and PRONEX.

IM-91 – SUSCEPTIBILITY OF THE VERTEBRATE HOSTS WITH DIFFERENT AGES TO MALARIA SPOROZOITES INOCULATED BY MOSQUITO BITES OR SYRINGE

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The situation of malaria transmission in Brazil and Africa is overall different, but mortality seems lower among primo-infected subjects in Brazil - 10,000 death/year here, against 2-3 million (children) in Africa. Since the exposed population in Brazil is mostly adults, one assumes that age may be among the protective factors. In addition, extensive studies of the natural immune response to the circum-sporozoite (CS) protein show anti-CS antibodies among 30% of healthy, non-infected adults exposed to malaria in Brazil: (a) in a focus of *P.vivax* (*Pv*), outside the endemic area (Fontes *et al*., 1991, *Am. J. Trop. Med. Hyg*., 44(1): 28-33); and (b) in different localities in the Amazon region (Carvalho *et al*., 1997, *Parasite Immunol*., 19: 47-59). To investigate whether the host age is involved in natural resistance we used the model *P. gallinaceum (Pg)* sporozoites in chickens, their natural host. Sporozoites were injected in chickens from one-week (~40g) to 3-weeks-old (~200g) by mosquito bites or by syringe via subcutaneous (*sc*) or intravenous. All animals were followed by daily blood smears to determine the malaria pre-patent period (PPP) and course of infection. The older chickens were always less susceptible then the young animals, only part of them developed malaria, with increased PPP, lower parasitemia and mortality. After a second sporozoite inoculation, there was a transient low parasitemia in the animals negative at first. All chickens bitten by mosquitoes had a more severe malaria compared to groups inoculated by *sc* or *iv* routes. However, antibodies to sporozoites, detected by immunofluorescence, were better induced after *sc* inoculation of sporozoites, not surprisingly since antigens are better presented by *sc* route. The profile of sporozoite antigen recognition in the Western Blot shows that the chicken immune sera recognize CS and other sporozoites antigens of lower molecular weight. These results corroborate our hypothesis to interpret previous data in subjects exposed to malaria transmission, part of them with anti-sporozoites antibodies but no malaria parasites. Thus adults may be naturally resistant to malaria sporozoite and yet produce anti-CS antibodies.

Financial support: PRONEX, PAPES, CNPQ (fellowship to AUK)

IM-92 – EPITOPE MAPPING OF PROTECTIVE MONOCLONAL ANTIBODIES RAISED AGAINST MEROZOITE SURFACE PROTEIN 1 (MSP-1_{19}) OF *PLASMODIUM YOELII* BY SYNTHETIC PEPTIDES

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A panel of 6 monoclonal antibodies (mabs) directed against *P. yoelii* merozoite surface protein 1 (MSP-1) were mapped by using synthetic peptides. Based on the protein sequence of the C-terminal ~19kDa polypeptide (MSP-1), the peptides were manually synthesized using the solid phase t-boc strategy. Their recognition was evaluated by a dot-blot assay, the multiple antigen blot assay (MABA).

MSP-1 is the precursor protein, which suffer proteolytic cleavage to give four fragments of approximately 83, 30, 38 and a C-terminal fragment of 19kDa. At the time the invasion, secondary processing of the membrane-bound 42kDa fragment occurs to produce a N-terminus of 33kDa and C-terminal of 19kDa.

In a previous work, these mabs were characterized in detail: three (B6,D3, and F5) were effective in suppressing a lethal blood stage challenge infection with *P. yoelii*, two (B10 and G3) were partially effective but recognized an epitope within the N terminus of MSP-1_{33}. However, B6, B10, F5 and G3 mabs bound to MSP-1_{19}.

Interestingly, the mabs G3, B4 and D9 (non-protective) did not recognized synthetic peptides derived from MSP-1_{19} C-terminal fragment. This sequence is a cysteine-rich region and it is a dominant part of MSP-1. Otherwise, mabs F5 (protective) and B6 (partially protective) weakly recognized the peptide IMT-385 of 50 amino acids, which corresponds to the amino terminal region MSP-1_{19}. Mabs B10 (partially protective) weakly recognized the peptide IMT-387 (50 amino acid), which correspond to the central region of the same molecule. In conclusion, this study of epitope mapping by using synthetic peptides derived from MSP-1 sequence, has identified protective epitopes of this protein, that will be of interest in vaccine design.

IM-93 – ANTIBODY RESPONSE IN HUMANS NATURALLY PRIMED TO A *PLASMODIUM FALCIPARUM* VACCINE CANDIDATE ANTIGENS IN A BRAZILIAN ENDEMIC AREA

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The development of efficaccious synthetic malaria vaccine requires the identification relevant B and T cell epitopes for the target population . Therefore, in the present work we evaluated 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 prevalence against GLURP-R0, GLURP-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 (7/8) 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-94 – EVIDENCES FOR REACTIVITY OF IMUNOGLOBULINS FROM AUTOIMMUNE DISEASE PATIENTS WITH PLASMODIAL ANTIGENS

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The mechanisms of acquired immunity in malaria are still poorly understood. Autoantibodies (AAb) with specificities comparable to those associated to autoimmune diseases (AID) are observed in high frequency during the course of malaria infection. Although a correlation between exposure to malaria and low incidence of AID has been already demonstrated, suggesting a negative influence of malaria infection on the development of such pathologies, the opposite relationship i.e. a role for AAb in the protection against malaria has been considered but not proved. The objective of the present work is to search for reactivity of AAb with plasmodial antigens as a step in the study of a possible role of AAb in malaria immunity.

For this, sera from AID patients (80 with Systemic Lupus Erythematosus [SLE] plus fifty with other AID) and murine monoclonal autoantibodies (MAAbs) were tested against defined plasmodial antigens and against the parasite in ELISA, immunofluorescence (IFAT) and immunoblot. Sera from malaria-infected individuals from Amazonia and from health individuals were used as positive and negative controls, respectively. IFAT was performed using the following *P. falciparum* strains: FCR3, NF54, T996 and T23; and the antigens used for ELISA were: *P. falciparum* crude extract; synthetic peptides from proteins p126 (Nt47), Spf70, LSA3 -(NRI, NRII, RE, CT1), MSP-3 (MSP-3b) and RESA; and the recombinant proteins R0, R2 (GLURP), ICB10 (MSP-1), MSP-3 C-terminus and CS C-terminus.

Among the 80 SLE sera, forty-one reacted against at least one of the defined plasmodial antigens in ELISA, and twenty-six reacted in IFAT (in total, 54 sera were positive). Forty-five SLE sera were positive for antinuclear antibodies (ANA) by IFAT, and there was a strong association between reactivity against nuclear and parasite antigens by IFAT. The other 50 AID sera - against actin, nuclear antigens and cardiolipin, among others - as well as MAAbs showed strong reactivity against the parasite. These results show that patients with autoimmune processes may produce antibodies that recognize plasmodial antigens in absence of malarial infection and that, if AAb have a protective effect against malaria, the potential targett antigens necessary for such an effect are present on the parasite.

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IM-95 – IMMUNIZATION OF *SAIMIRI SCIUREUS* MONKEYS WITH MSP-3 AND GLURP, TWO *PLASMODIUM FALCIPARUM* ANTIGENS TARGETS OF PROTECTIVE ANTIBODIES

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Merozoite Surface Protein-3 (MSP-3) and Glutamate Rich Protein (GLURP) of *P. falciparum* are considered malaria vaccine candidates due to characteristics such as wide recognition by human immune sera and ability of antibodies against them to inhibit *P. falciparum* growth *in vitro* in Antibody Dependent Cellular Inhibition (ADCI) assays. The objective of this work is to perform pre-clinical evaluation of constructed antigens derived from MSP-3 and GLURP in combination with several adjuvants in the *Saimiri sciureus* monkey, one of the W.H.O.-recommended non-human primate models for studies on malaria.

Five antigen-adjuvant combinations have been used: a) MSP-3: DG210-Incomplet Freund's Adjuvant (IFA) and MSP-3 C-terminus in association with: Ribi, Montanide or SBAS2; b) GLURP: R0-alum [$Al(OH)_3$]. Each group received 4 or 5 shots in a time interval of 6 to 10 months. Immune response has been analyzed by ELISA, immunofluorescence (IFAT), immunoblotting and ADCI.

Results obtained so far have shown that all antigen-adjuvant combinations are able to elicit high serum titres of antibodies directed against the immunogen, as detected in ELISA. However, the same sera showed very poor or no recognition of the parasite in IFAT or immunoblot assays; in addition, they were not able to inhibit *P. falciparum* growth in ADCI, whereas IgG or anti-MSP3 antibodies purified from saimiri immune sera showed strong effect.

We currently perform deeper immunological analysis of sera from immunized animals (cytokine dosage, ADCI, IFAT, immunoblot); data will be compared with similar analysis performed with sera and purified antibodies from saimiris hyperimmunized by repeated infections, which may lead to the optimization of formulations to be used in further studies.

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IM-96 – EFFECTS OF IMMUNE SERUM, IGG1 AND IGG2A SPECIFIC ANTIBODIES IN THE PROTECTION INDUCED BY PASSIVE IMMUNIZATION FOR THE TREATMENT OF MURINE MALARIA

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The mechanisms of antibody-mediated protection to malaria are still unknown. In this work, we analyzed the protective role of immune sera, IgG1 and IgG2a in protection induced by passive immunization for the treatment of a lethal murine malaria caused by *Plasmodium chabaudi chabaudi* (strain AJ, clone IP-Pc1). Prior to these studies, we observed that when protected BALB/c mice were injected with high numbers of ring infected erythrocytes, parasitemias did not decline up to the moment of erythrocyte rupture, suggesting that rings, trophozoites and young schizonts are not susceptible to serum effector mechanisms. Confirming these findings, the treatment of infected BALB/c mice with immune sera resulted in inhibition of the generation of new ring forms, but it did not alter schizont blood numbers. When these mice were treated with IgG1 or IgG2 antibodies purified from immune serum, we observed that both isotypes conferred protection to *P. c. chabaudi* AJ, acting at moments close to re-invasion. IgG2a was more efficient than IgG1 at higher doses (30 μg/mouse), but at lower doses (3,7 μg/mouse), only IgG1 displayed protective activity. Protection by IgG1 or IgG2a was greatly reduced in recently infected mice, indicating that activation of the immune system is essential for parasite control. Other than protection, however, antibody-treated mice showed deposition of IgG on the surface of nearly all erythrocytes, accumulation of normal and infected erythrocytes in the spleen with increased adhesion to leukocytes and phagocytosis, death of splenic leukocytes and signs of systemic shock with death of some animals. In most cases, these immune reactions occurred in mice treated with both IgG isotypes but those injected with IgG1 showed stronger symptoms. In addition, the immune reactions described above were observed essentially when antibodies were administrated at the end of schizogony, indicating that recognition of parasite antigens is needed to trigger those processes. Moreover, the treatment with specific antibodies resulted in the accumulation of mononuclear and polymorphonuclear cells in the lungs, a place where the final maturation of infected erythrocytes is believed to occur. Taken together, our results suggest that, in this experimental malaria model, opsonization of circulating infected erythrocytes is not a major mechanisms for parasite control. Moreover, our data showed that IgG1 and IgG2a specific antibodies are both able to passively immunize *P. c. chabaudi* AJ infected BALB/c mice, acting over mature schizonts and/or merozoites at the moment of erythrocyte rupture and re-invasion.

Financial Support: FAPESP and CAPES.

IM-97 – ASYMPTOMATIC MALARIA IN A RIVERINE POPULATION IN RONDONIA (WESTERN AMAZON REGION)

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Different studies have shown that the incidence of malaria in migrant populations of Rondonia is higher in adults than in youngsters. In contrast, in the riverine population of Portuchuelo, rio Madeira, it was shown that youngsters under 16 years constituted the risk group for malaria. Moreover, in epidemiological transversal surveys at Portuchuelo, cases of positive slides for *Plasmodium* in absence of malaria symptoms could be detected, a situation which had never been reported among migrants (see Camargo et al., 1999, Acta Tropica 72:1-11). These observations led to the hypothesis of the existence of naturally acquired immunity to malaria in the riverine population.

To test this hypothesis, longitudinal surveys of the Portuchuelo population were carried out by: monitoring of symptoms, blood smears microscopic examination and PCR for rDNA amplification of *Plasmodium* spp. (Snounou et al. 1996, Meth. Mole. Biol. 50: 263-291).

Since September 1998 our medical team is visiting Portuchuelo 3 times a week. In the days between, the entire population (200 people) is under permanent surveillance of local health agents trained by us. Since September 1998, 70 cases of symptomatic malaria were registered. Preliminary results about symptomless cases of *P. vivax* infection were recently published (Camargo et al., 1999, The Lancet 353: 1415-1416). Here we report on the occurrence of asymptomatic infections by *P. falciparum* in the riverine population of Portuchuelo.

Sixteen individuals out of 183 people examined (92% of the total population) were found PCR-positive for *P. falciparum.*

Of these, 11 remained symptomless after a period of 6 months (4 with blood smears positive for gametocytes), 3 presented classical malaria symptoms and 2 left the community.

Contrary to patients of migrant populations, almost all of the adults of Portuchuelo were either born in the Amazonian region or have lived in Portuchuelo (or at its immediate vicinity) for at least 16 years.

The disclosed existence of acquired immunity to *Plasmodium* spp. among native Amazonians poses a serious problem for the control of malaria in the region since apparently healthy people may act as reservoirs of the parasite. Therefore, malaria control programs have to be prepared to uncover these reservoirs and even consider the treatment of asymptomatic individuals.

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IM-98 – STUDY OF CYTOKINES AND CHEMOATTRACTANT FACTORS INVOLVED IN THE GENESIS AND RESOLUTION OF LESIONS IN THE CENTRAL NERVOUS SYSTEM DURING EXPERIMENTAL *TRYPANOSOMA CRUZI* AND *TOXOPLASMA GONDII* INFECTION

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The central nervous system (CNS), considered an immunoprivileged site able to restrict the entrance of immune cells, is shown to be a target for infection of several viruses and intracellular parasites. During systemic inflammatory processes, such as parasitic infections, the blood-brain barrier is disrupted and inflammatory lesions are established into the CNS. In *Trypanosoma cruzi* infection encephalitis occurs during the acute phase, whereas in *Toxoplasma gondii* infection encephalitis is an asymptomatic chronic persisting process. In immunocompromised individuals such as AIDS patients, *T. cruzi* or *T. gondii* infection can lead to severe CNS damage resulting in reactivation of these infections. However, there are no data available regarding the molecules involved in the entrance of inflammatory cells into the CNS during parasitic encephalitis, neither regarding their role in the resolution or perpetuation of the inflammatory lesions in this organ. Chemokines are a group of cytokines produced by different cell types involved in recruitment, activation and effector functions of different populations of leukocytes to inflammatory sites. Some studies have related the participation of different chemokines in mechanical trauma or demyelinating diseases in the CNS, leading to establishment, progression or regression of inflammatory lesions. In this study, we investigated the presence of chemokines and the phenotype of the inflammatory cells present in the CNS during chagasic and toxoplasmic infections. C3H/He and C57BL/6 were infected with 100 blood trypomastigotes of *T. cruzi* (Colombiana) or 15 cysts of *T. gondii* (ME49), respectively. Edema, enlargement of perivascular spaces and irregular distribution of inflammatory infiltrates were observed during the acute phase of both infections. Inflammatory lesions persist only in *T. gondii*-infected C57BL/6, while in *T. cruzi*-infected C3H/He resolution of the inflammation is observed. The inflammatory infiltrates consisted mainly of macrophages and $CD8^+$ cells, although a few $CD4^+$ cells were also found. Also, the inflammatory infiltrates were not directly associated with *T. cruzi* antigens or *T. gondii* cysts. RT-PCR analyses show that in *T. cruzi*-infected C3H mice high levels of TNF-α in the presence of low levels of IFN-γ are observed during acute phase, whereas in *T. gondii*-infected C57BL/6 mice there is a predominance of IFN-γ mRNA. During the chronic phase low levels of TNF-α and IFN-γ are detected in the CNS of *T. cruzi*-infected C3H mice. In contrast, mRNAs coding these cytokines are still detected in the CNS during chronic infection of C57BL/6 mice infected with *T. gondii*. RANTES and the IFN-γ-induced chemokines (Mig and CRG-2) were expressed in high levels in the CNS of *T. cruzi*- and *T. gondii*-infected animals during the acute phase, reflecting a Th1 profile of the immune response in this tissue. During the chronic phase the expression of these chemokines was maintained in the *T. gondii*-infected mice, but not in the *T. cruzi*-infected mice. Interestingly, it was observed a predominance of MIP-1α during *T. cruzi* acute infection and MIP-1β in acutely *T. gondii*-infected mice. Altogether, these results suggest that the differential expression of cytokines and chemokines may have a crucial role in the immune response observed in the CNS during *T. cruzi* and *T. gondii* experimental infections attracting different cell populations, leading to the genesis and progression/resolution of the encephalitis observed during these parasitic infections.

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IM-99 – TOXOPLASMOSIS SEROLOGY IN PREGNANT AND BABIES OF THE NORTHWEST REGION OF THE STATE OF RIO GRANDE DO SUL, BRAZIL

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Toxoplasmosis, a worldwide distributed parasitic disease causing subclinic or inapparent infection in immunocompetent individuals, is known to cause severe complications in fetuses or newborns from mothers acutely infected during pregnancy. This fact brings important neurologic and systemic alterations in newborns, such as: hydrocephalus , convulsions, brain calcification and atrophies, hepatic and splenomegaly and slight alterations. Ocular and neurologic manifestations are most serious and frequent. The fetus damage can be attenuated or prevented through therapeutics, if there is early screening of pregnant women at risk (serum not reagent) and/or periodic serology, as well as the ones who present serologic profile of recent infection. Congenital *Toxoplasma* infection does not usually produce recognizable signs of infection at birth. We were concerned by the fact that most cases remain untreated because they are not detected by routine clinical examination. This study is being developed in order to analyze serological profile in pregnancy and newborns serum in the northwest of Rio Grande do Sul. During the period from January 1998 to January 1999, serum were obtained from 49 pregnant women and her (37) babies coming from sanitary units of the 11ª Regional Health in the region of northwest, Department the State of Rio Grande do Sul, in the South of Brazil. For dosage of IgG and IgM, the research group accomplished with IFI and ELISA; for IgA dosage MEIA was used and avidity of specific IgG antibody was measured with ELISA. From the 49 samples processed, we observed all pregnant with positive IgG and IgM, 27 (55%) women with IgA positive and only one (2%) presenting characteristic patterns of recent toxoplasmosis (avidity of IgG below 30%). All babies were followed, clinical and serologically, during the first year of their lives. They were all, apparently, healthy and serologically presenting serum negatives IgM and IgA. Further studies are been carried out to evaluate the occurrence of acute infection.

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IM-100 – DOWN REGULATION OF COSTIMULATORY B7, ADHESION CD62L, CD49 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 against toxoplasmosis and begins by interaction of T cell receptor with MHC proteins. The interaction of proteins present in T cell membrane, with costimulatory molecules exerts an additional signal to T cell effective activation. In this work we investigate the expression of some costimulatory and adhesion molecules in J774.G8 macrophages infected by RH strain of *Toxoplasma gondii*. The infected and control group of macrophages was stained with anti-B7, CD40, CD49b, CD54, CD62L, CD69, MHC class II and MHC class I antibodies and analyzed by flow cytometry. Our results shows a down regulation of 25% in expression of MHC class II and B7 costimulatory molecule in *Toxoplasma gondii* infected cells. The adhesion and costimulatory molecules CD62L and CD49b displayed a down regulation of 70 and 35% respectively. Analysis of mean fluorescence shows down regulation of 45 % in expression of class I MHC molecule. The CD54 and CD69 molecule 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.

Supported by FENORTE/ PRONEX.

IM-101 – CLUES OF MECHANISMS OTHER THAN ACTIVATION OF THE ALTERNATIVE COMPLEMENT PATHWAY CAN ALSO PARTICIPATE IN LYSIS OF *TRYPANOSOMA CRUZI, T. CONORHINI* AND *T. RANGELI* EPIMASTIGOTES BY FRESH NONIMMUNE SERA

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Lysis of *Trypanosoma cruzi* epimastigotes by fresh nonimmune mammal sera was first described by Muniz & Borriello (1945), and has been confirmed with different parasite strains in tests using cells from axenic cultures or triatomines. The phenomenon was explained by Nogueira *et al*. (1975, *J. Exp. Med.* 142: 224) as occurring by activation of the alternative complement pathway. Although as a variable phenomenon, lysis of *T. conorhini* and *T. rangeli* epimastigotes by those sera has also been reported (Carreira 1987 MSc thesis, UFMG; Sousa *et al.* 1994, *Mem. Inst. Oswaldo Cruz* 89, suppl. I: 54; Sousa *et al.* 1998, *Mem. Inst. Oswaldo Cruz* 93, suppl. II: 132). Lysis rates in *T. conorhini* varied according to the parasite strain, culture age and *in vitro* maintenance time; the Deane strain passed through *Triatoma rubrofasciata*-rat before the test became resistant to lysis (Sousa *et al.* 1994). Some *T. rangeli* strains were more susceptible to lysis than others and their lysis rates also varied during growth in culture (Carreira 1987; Sousa *et al.* 1998).

In the present work we carried out some experiments to search possible mechanisms involved in the epimastigote lysis in *T. conorhini* and *T. rangeli*, using *T. cruzi* for comparisons. The following sera were assayed: fresh (or -70ºC stored) nonimmune guinea-pig serum (GPS); GPS plus 10mM EDTA or EGTA, GPS plus 10mM EGTA/$MgCl_2$ or EGTA/$CaCl_2$; GPS heated at 50ºC with accurate temperature control; C4-deficient guinea-pig serum, and C2- or C3-depleted human sera (Sigma). In all experiments, 1-2 x 10^6 epimastigotes from axenic cultures of each species were incubated (37ºC, 30-40min) with 25% of serum. In some experiments, rabbit or sensitized sheep erythrocytes were assayed for activity control of the alternative and classical complement pathways. Our results suggest that lysis of *T. conorhini* and *T. cruzi* epimastigotes do not depend on factor B, can also require Ca^{++} and that, at least partially, can depend on C2 and C4 components. In two *T. rangeli* strains assayed, partial dependence on C2 was also observed (C4 not tested). Intriguingly, while C3-depleted serum abolished totally or partially the lysis of *T. rangeli*, in *T. cruzi* and *T. conorhini* it determined (respectively) similar or higher lysis rates than the normal serum. Our results evidence that lysis of epimastigotes by fresh sera in these species is a very complex phenomenon, its mechanisms deserving further investigation, mainly in C3-deficient serum.

IM-102 – PRELIMINARY STUDIES ON THE MICROBICIDAL ACTION OF A CHICKEN MACROPHAGE CELL LINE INFECTED BY *TOXOPLASMA GONDII*

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Nitric oxide has been shown to have diverse biological roles, including a significant cytotoxic effect in the killing of intracellular parasites, i.e. *Toxoplasma gondii*. Chicken macrophages (peritoneal and cell line) are able to produce nitric oxide when properly activated. In this study we have used a chicken macrophages cell line, MQ-NCSU, for the study of the interaction with *T. gondii*. This cell line is able to produced nitric oxide after stimulation by bacterial Lipopolysaccharide (LPS). The aim of this study is to analyze the action of nitric oxide produced by the MQ-NCSU upon *T. gondii*. MQ-NCSU was cultivated in DMEM with 10% fetal bovine serum in a 37°C, 5% CO_2 incubator. The macrophages were activated with LPS, 24 hours before or just after the interaction with *T. gondii*. Tachyzoites of the RH strain were obtained by peritoneal washes of infected mice. The macrophages were infected with the parasites in a 10 to 1 parasites/macrófago ratio. After 2 hours of interaction, macrophages were washed and cultivated. After 2, 24 and 48 hours cells attached to coverslips were stained with DAPI and the kinetics of the infection analyzed in a fluorescence microscope. After 24 and 48 hours the supernatants of the cultures were collected and assayed with the Greiss reagent for the presence of NO_2^-. After the interaction the MQ-NCSU showed percentage of infected macrophages and mean number of parasite per macrophages (respectively) as follows: non activated macrophages, 24 h 46%, 2,5; and 48 h 76%, 4,4; and activated macrophages, 24 h 11%, 1,4, and 48 h 18%, 4,3. Activated macrophages produced 75 μM of NO_2^-. We can conclude that the MQ-NCSU can control the *T. gondii* proliferation by nitric oxide production.

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IM-103 – RESPONSE OF C57BL/6J MICE IMMUNIZED WITH IRRADIATED TACHYZOITES OF *TOXOPLASMA GONDII* RH STRAIN TO ORAL CHALLENGE WITH ME-49 STRAIN

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Toxoplasma gondii is an obligatory intracellular protozoan, with felines as definitive hosts and mammals and birds as intermediate hosts. Toxoplasmosis is acquired by the ingestion of food or water contaminated with oocysts from feline stools or raw meat containing cysts. The disease is usually asymptomatic in immune-competent individuals, occasionally with eye lesions. In immune-compromised (AIDS/organs recipients) patients or fetus, the disease can be extremely severe, leading frequently to death or disability. The ionizing radiation was demonstrated to be efficient to eliminate cysts of *T.gondii* in meat or to abolish the reproductive capacity of the parasite, allowing its use for immunization, with promising results. Here, we irradiated tachyzoites of the RH strain of *T.gondii* with 255Gy and immunized groups of C57Bl/6j mice with 3 sequential 10^7 i.p. shots, with posterior oral challenging with 20 cysts from the ME-49 strain. The mortality, immune response and histological lesions in brain, lung, liver, spleen were analyzed. No animal deaths were observed, in contrast with 30% that occurred in non-immunized groups. Specific antibody response was detected through ELISA, showing an increase in the IgG levels after the immunization. For cell cooperation, *in vitro* assays were performed with culture of purified spleen lymphocytes on *T.gondii* antigen coated microplates, with subsequent antibody detection by ELISA. Higher production of IgG was observed with cooperation between lymphocytes from immunized mice as compared with controls. Through histology, it was difficult to identify tissue cysts or lesions in immunized mice, as compared to controls, which presented several cysts and necrotic lesions in the brain, as well as other organs as lung and liver. Greater follicular response occurred in spleen of immunized and challenged mice. RT-PCR for ribossomal RNA on nucleic acids from brains of immunized challenged mice demonstrate small amounts of *T.gondii* rRNA, showing that the induced immunity protect from lesions, but allows brain colonization by cysts. Those promising results demonstrate that ionizing radiation can be important tool in the sterilization of *T.gondii* tachyzoites, allowing both immunity and protection from disease, in the prospects of a vaccine for toxoplasmosis, specially to at risk groups.

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IM-104 – STANDARDIZATION OF ANTI-*TOXOPLASMA GONDII* DOG IGG ELISA FOR THE DETECTION OF SEROPREVALENCE OF TOXOPLASMOSIS IN STREET DOGS FROM SÃO PAULO METROPOLITAN REGION

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Toxoplasmosis is a high prevalent protozoan disease, which affects at least 20% of the world population. Generally benign or asymptomatic, occasionally it can affect the eye or fetuses, with great social and economic burden, specially in countries with higher prevalence, as Brazil. This disease is transmitted by ingestion of oocysts in water or food contaminated with cat stools, the definitive host, or by ingestion of raw meat containing resting tissue cysts, from warm-blooded intermediate hosts. The dog, mainly the free living, could be used as an index of environmental contamination, as they usually fed in small preys like cats. The infection in dogs is generally asymptomatic, but occasionally causes systemic disease, often with respiratory and neurological involvement. We developed a specific anti T.gondii dog IgG ELISA, testing 128 samples from street dogs from São Paulo Metropolitan Region, that were captured by Zoonosis Health Services. ELISA was standardized using positive sera tested with indirect immunofluorescence assay; using microplates coated with saline extract of RH strain tachyzoites from experimentally infected mice. Microplates variation was corrected by a standard cut-off serum, and estimated as artificial units. There were a 77/128(60,15% - 51.5 – 68.4 95% C.I.) positivity in this assay. When dental age and sex were analyzed, the proportion of infection was similar in both sexes (24/44 female and 53/84 males), most age groups presented similar prevalence, showing that the infection occurs early in the dogs life. Take together, these data shows that the prevalence of toxoplasmosis in street dogs was similar to the human seroprevalence in same region, and reflects similar food source and environmental conditions, that could be used in epidemiological interventions for disease control.

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IM-105 – AMNIOTIC FLUID IN SUSPECTED CONGENITAL TOXOPLASMOSIS. IGA AND ANTIGEN DETECTION

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Toxoplasmosis is a worldwide infection, mainly asymptomatic, transmitted by oral ingestion of contaminated food. *Toxoplasma gondii*, an obligatory intracellular Apicomplexa protozoan, present a complex life cycle, involving cat and warm blood hosts. The main problem in toxoplasmosis is the vertical transmission of the agent to the fetus, during acute infection in pregnancy. This host is incapable to control the infection, which causes a severe infection, with destruction of brain and eye, with deaths or disability in the newborn. The diagnosis of this infection is complex, and usually performed by serological methods in maternal blood, often inconclusive. If diagnosed, the infection is treated with toxic drugs that also could induce fetal death. Here, we present data from analysis of amniotic fluid using specific IgA detection in amniotic fluid by ELISA and a *T.gondii* antigen capture detection ELISA. Those samples were collected, after informed consent, from mothers from the Fetal Infection Group of the Gynecology and Obstetrics Dept., HCFMUSP, on therapy with spiramycin. From 58 amniotic fluid samples, all presented positive for specific IgG and IgM, by indirect immunofluorescence assay(IFA), but few samples were IgA positive by IFA. Mice isolation was attempted in all samples without success. A quantitative IgA ELISA showed also a few samples highly positive. The antigen detection capture assay, using anti-*T.gondii* from mice as coating protein and anti *T-gondii* from rabbit as a secondary reagent, showed only 03 positive samples, concordant with higher IgA titers($p=0.010$). The efficiency of this assay was 1 µg/ml, estimated with saline extract from RH tachyzoites. There are also some higher IgA titers without elevated antigen levels. The main clinical features of concordant pregnancies were immunodeficient mother, with affected fetus and placenta, but one case delivered an healthy newborn, but the sorting system, IgM detection during pregnancy and not seroconversion, result in a low number of actual fetal infection, as detected by fetal death or ultrasound features of brain lesions. The main bias that could induce the low resolution of both assays was the therapy before any performed test. Those tests could have an auxiliary role for diagnosis of congenital toxoplasmosis.

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IM-106 – INVERSE CORRELATION OF PRO-INFLAMMATORY ACTIVITY AND VIRULENCE OF RH STRAIN OF *TOXOPLASMA GONDII* DERIVED FROM MICE AND TISSUE FIBROBLAST CULTURE

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Different studies suggest that the ability of *Toxoplasma gondii* to elicit IL-12/IFN-γ mediated cell-mediated immunity (CMI) is an important component of parasite:host equilibrium by protecting the intermediate host against uncontrolled parasite replication (Denkers & Gazzinelli, 1998, *Clin. Microbiol.Rev*. 11: 569). Different studies demonstrate that *T. gondii* strains can be grouped according to their virulence and capacity to establish chronic infection in mice (Sibley & Howe, 1996, *Curr. Topics Microb. 219:3*). Thus, we speculate that parasite molecules responsible for induction of CMI may be directly associated with the degree of virulence displayed by an specific parasite strain. In the present study we compared the ability of RH tachyzoites derived from either tissue culture fibroblasts (tachTCF) and from peritoneal cavity of mice (tachPCM) to elicit the production of IL-12, TNF-α Nitric Oxide (NO) and microbicidal activity by unprimed or IFN-γ primed murine inflammatory macrophages. Surprisingly, our results demonstrate that tachTCF but not tachPCM are potent stimulators of pro-inflammatory and microbicidal activity by macrophages. Similarly, tachTCF but not tachPCM stimulate the synthesis of IFN-γ by splenocytes from uninfected mice. Interestingly, tachPCM, but not tachTCF, were highly virulent *in vitro* and their replication was mostly uncontrolled in macrophages pre-activated with IFN-γ. Therefore, we decided to search for molecules present in the tachTCF and absent in tachPCM surface, that are responsible for pro-inflammatory activity and may account for parasite virulence. Based on solvent organic extraction and fractionation based on degree of hydrofobicity, our preliminary results indicate that membrane associated GPI/GIPL related structures may be responsible for the pro-inflammatory activity of tachTCF. We are currently evaluating differences *in vivo* virulence of tachTCF and tachPCM.

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