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

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

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

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

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

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

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

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

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

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

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

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