ORAL PRESENTATION

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OPCBQ1 - POST-TRANSCRIPTIONAL REGULATION OF CRUZIPAIN BY THE ENDOGENOUS INHIBITOR CHAGASIN.

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Trypanosoma cruzi relies on cysteine proteases (CPs) for efficient growth and survival inside the host. The activity of the main parasite CP, cruzipain, has been directly or indirectly associated with intracellular amastigote survival, host cell invasion and inflammation. Cruzipain is encoded by a large gene family, whose members present variable polymorphism and are developmentally regulated. Studies on cruzipain expression showed that it is post-transcriptionally regulated, leading to increased levels of CP activity in the replicative stages. Although cruzipain has been extensively characterized at the biochemical and structural level, little is known about the mechanisms controlling its activity in living parasites. Recently, we have identified an endogenous CP inhibitor in *T. cruzi*, chagasin, which potently inactivates cruzipain, suggesting that it may regulate CP activity in the parasite. Notably, the expression of chagasin in the life stages of *T. cruzi* is inversely correlated with that of cruzipain. Here we investigate whether cruzipain and chagasin interact in living parasites and also evaluated the levels of functional enzyme and inhibitor in epimastigotes of six *T. cruzi* isolates. The cruzipain:chagasin complex was visualized by Western blot under partially denaturing conditions, using anti-chagasin antibodies. The detection of the complex immediately after parasite lysis suggests that they may interact in vivo. These complexes were more abundantly detected in the isolates Dm28c, Sylvio X10/6, Y and G, but are poorly detected in the isolates CL and Brazil. Analysis of steady-state levels of cruzipain, using monoclonal antibodies, showed a high expression of this CP in the isolates Dm28c, Sylvio X10/6, but a low expression in Y, CL, G and Brazil parasites. Likewise, quantification of functional enzyme in parasite lysates upon titration with Mu-Phe-hPhe-VSO (an inhibitor of cruzipain) revealed the occurrence of lower levels of active cruzipain in CL, Brazil and G parasites. Interestingly, although these three isolates presented similar steady-state levels of cruzipain by Western blot, the G isolate bears 3-5 fold less functional cruzipain than the others. Accordingly, the titration of functional chagasin revealed that a reduced concentration of the inhibitor is found in isolates presenting lower cruzipain expression (CL and Brazil), with the exception of the G isolate which showed a high content of chagasin. These observations indicate that in *T. cruzi*, low cruzipain expression is accompanied by a reduction in the chagasin levels, resulting in a reduced formation of the cruzipain:chagasin complex in living cells. As a consequence, these parasites present only a 2-fold difference in the amount of functional cruzipain when compared to isolates with abundant cruzipain expression (i.e. Dm28c, X10/6, Y). Parasites that do not compensate decreased cruzipain expression by lowering the amount of chagasin (i.e. G) present higher accumulation of cruzipain:chagasin complexes, resulting in 10- fold less functional cruzipain. These results suggest that chagasin acts a post-transcriptional regulator of cruzipain levels through its interaction with active enzyme.

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OPCBQ2 - FUNCTIONAL CLASSIFICATION OF *TRYPANOSOMA CRUZI* PROTEINS: THE APPLICATION OF THE BIONOTES SYSTEM

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Trypanosoma cruzi, the ethiological agent of Chagas disease, is an important human pathogen. Its genome is currently being sequenced in several laboratories around the world. High-throughput technologies are generating large numbers of sequences such as EST's and GSS's. One of the main goals of this project is to obtain a detailed classification of *T. cruzi* proteins based on their functional properties, which would provide useful insights on the biochemistry and genetics of this parasite. A viable approach to obtain this classification is to perform an automatic classification based on database searches by keywords and sequence similarity. Although a high degree of automation is essential when dealing with large amounts of data, there are inherent errors in databases that would be perpetuated without a careful analysis by human analysts. Our project aims to classify the *T. cruzi* sequences using the following procedure: sequence compilation and retrieval (SRS); sequence clusterization (cap3); sequence similarity search and analysis (BLAST, MSPcrunch, in-house scripts); protein analysis (GCG); curation of the results by searches in other databases (Swiss-Prot, PIR, PROSITE, PFAM) and literature review (PUBMED). Besides, we propose to design and develop a distributed annotation system allowing interested laboratories to develop and maintain annotations which are readily accessible to the community at large, the BioNotes system. The system needs to be easy to use, readily accessible by the community, and capable of representing annotations graphically. The objective is the maintenance of the data sources quality; therefore it is needed to be heavily curated to reflect new developments, additional knowledge, and continuing research efforts. First, we have analyzed 10,133 EST; 21,319 GSS and 998 genomic sequences (present at the GenEMBL subset IN). EST's and GSS's clusters were obtained using the cap3 program, resulting in 1,635 contigs and 3,257 singlets from the EST subset, and 1,921 contigs and 4,396 singlets from the GSS subset. These clusters and singlets were "blasted" against the GenBank nr (non-redundant) subset. Results indicated that the majority of the *T. cruzi* coding sequences are as yet of unknown function (approximately 80% of the EST clusters). Where function assignment was possible, sequences were classified in major categories such as metabolism, energy, cell growth and division, DNA synthesis, transcription, etc. Contamination with *E. coli* sequences or plasmids/cloning vectors was also found in some cases. Recently, 6,851 GSS sequences and 880,048 whole genome shotgun individual reads were deposited <http://tcruzidb.org/static/sources.shtml>, now under processing. The results of these analyses will also be available at <http://www.dbbm.fiocruz.br/TcruziDB/index.html>.

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OPCBQ3 - CLONING AND KINETIC CHARACTERIZATION OF *PLASMODIUM FALCIPARUM* POLYPRENYL DIPHOSPHATE SYNTHASE - A POSSIBLE NEW DRUG TARGET

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The isoprenoid biosynthesis of intraerythrocytic stages of *P. falciparum* has been studied and pointed out as an important target for the development of new antimalarial drugs. Endproducts of the isoprenoid metabolic pathway are prenylated proteins, dolichols and ubiquinones which derive from condensation reactions of 5-carbon linear prenyl diphosphates. The condensation is catalysed by a family of enzymes known as polyprenyl diphosphate synthases. In this study, we cloned and characterized a gene that encodes a putative polyprenyl diphosphate synthase. The gene is transcribed in ring and trophozoite stages of *Plasmodium falciparum*, but not in schizont stages. After expression in *Escherichia coli*, the recombinant GST-tagged protein was purified and its function characterized in prenylation assays. The expressed polypetide, but not GST alone, showed specific polyprenyl diphosphate synthase enzymatic activity,

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as did a fraction from partially purified parasites. Polyclonal antibodies raised against the recombinant GST-tagged protein also confirmed the presence of the native protein in trophozoite and schizont stages. On the basis of vast sequence differences to human polyprenyl synthases, we suggest that enzyme may be considered as an interesting target for antimalarial drugs.

OPCBQ4 - A NOVEL PHOSPHOLIPASE C CLEAVES ACYLATED GPI IN PROCYCLIC FORMS OF *TRYPANOSOMA BRUCEI*

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African trypanosomes make widespread use of glycosylphosphatidilinositol (GPI) to stabilize its glycoproteins on the parasite surface. The bloodstream form in the mammalian host is protected by a dense glycocalix of variant surface glycoprotein (VSG), and the so called procyclic forms, which multiply in the midgut of the tsetse fly vector, are covered by a glycoprotein called procyclin or PARP. Both glycoproteins are GPI anchored, but with a critical and possibly biologically important difference: the procyclin GPI anchor is directly acylated on the inositol and the GPI of the bloodstream VSG is not. When bloodstream trypomastigotes differentiate into prociclic forms a GPI specific phospholipase C (GPI-PLC) is upregulated, as well as a metalloprotease, to release the VSG coat (Gruszynski et al., J. Biol. Chem.*278*: 24665, 2003). Aiming to find out whether procyclics expressed activities capable of removing the lipid moieties of acylated GPIs, we initially used ^{3}H-Myristate labelled Glycolipid C (Gly C) as substrate and detected a membrane bound PLC which produced dimyristoylglycerol (DMG). This was indeed a very surprising finding since acylated GPIs are knowingly detected by their resistance to PI and GPI-PLCs. There are also two other prominent activities processing Gly C in procyclics: a PLA and a PLD. The unusual PLC, being only detected in procyclics and not in bloodstream forms, including GPI-PLC minus mutants, has thus been named Procyclic-PLC (P-PLC). This novel enzyme, which possibly defines an yet undiscoverd class of PLCs, was also very active towards PP1, the acylated GPI precursor in procyclics, but the inositol acylation is not a requirement for activity since P-PLC can also use glycolipid A as substrate. Judged by the appearance of the neoepitope CRD when membrane form VSG is treated by P-PLC *in vitro*, the enzyme can act not only on free GPIs but also on GPI-anchored proteins. At 30^{0}C P-PLC was active over a pH range of 7-10 and also in the presence of 5 mM of PCMPSA, the classical inhibitor of GPI-PLC. So far we have been unable to detect any Ca^{2+} or other ion dependency. Studies are underway to (i) determine whether P-PLC is also capable of cleaving PI and procyclin; and (ii) to develop an assay in conditions where the PLA and PLD are inhibited to allow purification of this novel enzyme. P-PLC might turn out to be biologically important in the metabolism of procyclin playing an important role in the biology of African trypanosomes and can also prove to be an excellent tool to detect acylated GPIs.

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OPCBQ5 - *TRYPANOSOMA CRUZI* PUTATIVE AMINO ACID TRANSPORTER GENES: IDENTIFICATION AND SEQUENCE ANALYSIS.

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The metabolism of *Trypanosoma cruzi* is largely based on the consumption of amino acids, mainly proline, and aspartic and glutamic acids, which are the main carbon and energy sources for several stages of the parasite life cycle. An arginine kinase that converts arginine in phosphoarginine, a phosphagen with a role as energy reservoir, was recently described. Also, the literature has established that Pro, Asp and Glu somehow participate in the *T. cruzi* differentiation process. The transport of amino acids into the parasite may be regarded as the first step of their metabolic pathways, making the identification of genes coding for transporters a major goal aiming at metabolic research and drug design. Notwithstanding, the mechanisms by which amino acids are transported into the cytoplasm in *T. cruzi* are poorly studied and, surprisingly, no protein with amino acid transport activity has been characterized, as yet, at the molecular level. In the present work, advantage has been taken from the information generated by the *T. cruzi* genome project in order to identify genes coding for putative amino acid transporters. Ten thousand partial sequences corresponding to ESTs and GSSs have been used to assemble CONTIGs containing 50-100 complete ORFs. The existence of such virtual ORFs was confirmed by PCR and sequencing, and the fact that they are effectively expressed were indicated by Northern blot and RT-PCR. Interestingly, some of the obtained CONTIGs contained more than one ORF coding for putative amino acid transporters. This observation raises the question whether amino acid transporter genes would be organized in clusters in the parasite.

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OPCBQ6 - PROTEOMIC ANALYSIS OF TRYPOMASTIGOTE AND AMASTIGOTE FORMS OF *TRYPANOSOMA CRUZI* USING ISOTOPE-CODED AFFINITY TAGGING

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Comparative proteome analysis of *Trypanosoma cruzi* developmental stages was carried out by isotope-coded affinity tag technology (ICAT). For that, protein extracts of trypomastigote and amastigotes were labeled with heavy (d8) and light (d0) ICAT reagents and subjected to cation exchange and avidin affinity chromatographies, followed by LC-MS/MS analysis. Sequence information and expression levels for 42 *T. cruzi* polypeptides, including metabolic enzymes, paraflagellar rod components, tubulins and heat shock proteins are reported. Twenty eight proteins displayed similar levels of expression in both forms of the parasite, eight proteins presented higher levels in trypomastigotes, while five were more expressed in amastigotes. The biological relevance of these findings in the life cycle of the parasite is discussed.

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OPCBQ7 - HISTONE H1 PHOSPHORYLATION DURING THE CELL CYCLE OF *TRYPANOSOMA CRUZI*

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In most eukaryotic cells, variable phosphorylation of histone H1 has been implicated in chromatin relaxation as the cells progress into the cell cycle. We have previously shown that *Trypanosoma cruzi* histone H1 becomes mostly phosphorylated in non-dividing trypomastigotes forms, or in cells arrested at the beginning of the S-phase with hydroxyurea (HU), and that the phosphorylated protein binds more weakly to the chromatin (Marques Porto, et. Al. 2002, MBP *119*,265). To further understand how this protein modification is regulated, here we identified the phosphorylation site and studied when dephosphorylation occurs. By using Q-TOF MS we found that histone H1 in epimastigotes forms of the parasite (Y-strain) has a predominant non-phosphorylated form of 7992 Da and a single monophosphorylated form of 8072 Da. We also verified that histone H1 may exist in mono, di and trimethylated forms. MS-MS analyses after trypsin hydrolysis indicated that the phosphate group is added to the serine 13 (SPKK), a typical cyclin dependent kinase (CDK) site. In addition, the N-terminus was found N-acetylated at serine 2 as shown for other histone H1 sequences. By removing HU from parasites arrested at the onset of the S phase, which predominantly have the phosphorylated form of histone H1, we observed that dephosphorylation only happened after cells divide. Finally we found that at minimal concentrations affecting growth, okadaic acid (1 mM), but not calyculin A (10 nM), prevented histone H1 dephosphorylation. We propose that histone H1 phosphorylation is promoted by a cyclin dependent kinase and that dephosphorylation promoted by a PP2A phosphatase occurs at the beginning of the cell cycle. This dephosphorylation occurs when the cells re-enter in the cell cycle, but not when parasites differentiate into infective forms.

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OPCBQ8 - APICAL REGULATED EXOCYTOSIS OF *PLASMODIUM* SPOROZOITES IS MEDIATED BY CAMP AND CA^{++}

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Migration of *Plasmodium* sporozoites through host cells is required for infection of hepatocytes because it induces apical regulated exocytosis in the sporozoites, a pre-requisite for hepatocyte infection. During migration through host cells, sporozoites enter in contact with the cytosol of host cells, which induces exocytosis in the parasite (Mota et al, Nat. Med. 2002, 11, 1318-22). Using host cell lysates as stimulus to induce exocytosis in *P. yoelii* sporozoites, we have characterized the signaling pathways involved in this process. Exocytosis is mediated by the activation of a G-protein coupled receptor that induces increases in cytosolic Ca^{++} and cAMP concentrations in sporozoites. Stimulation of exocytosis by increasing Ca^{++} or cAMP concentrations results in higher infectivity and decreased migration through host cells, confirming the role of exocytosis in infection. We are trying to identify the G-protein coupled receptor mediating exocytosis in sporozoites. A search in the *P. yoelii* sequence database provided several candidates that are being evaluated.

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OPCT1 - STUDIES ON *LEISHMANIA* VIRULENCE AND ANTIMONY RESISTANCE.

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Pentavalent antimonials (Sb^V) have been the first choice drugs against leishmaniasis for more than 50 years. As a consequence of systematic monotherapy over decades, Sb^V resistant *Leishmania* strains were selected and spread in endemic areas. In order to evaluate *in vitro* antimony resistance we have compared several *Leishmania* isolates for their capacity to survive to antimonial exposure in infected macrophage cultures. Parasites were obtained from different clinical forms of leishmaniasis and from responsive or refractory cases. Among seven isolates obtained from refractory cases, two were markedly resistant to antimonial treatment in *in vitro* macrophage cultures (45 and 50% reduction of parasitism); three others were partially resistant (60, 75 and 85% reduction) and two were susceptible (90 and 100% reduction). In attempt to isolate *Leishmania* antimony resistance genes, a cosmid library was constructed with the genomic DNA of the BA276 strain of *in vivo* (in the pacient and in Balb/c mice), and *in vitro* (in the amastigote-macrophage system), Sb^V resistant *L. (L.) amazonensis*. The BA125 Sb^V susceptible strain of *L. (L.) amazonensis* was electroporated with pools of about 400 individually prepared cosmids. After isolation of individual transfected *Leishmania* clones, pools of clones were used to infect footpads of Balb/c mice that were subsequently treated with Sb^V. Lesion growth was then monitored, *Leishmania* cells were recovered, cosmids extracted and analysed by *Sal I* restriction. No Sb^V resistant lesions were generated although more than 2000 cosmids were tested. For each cosmid pool, a maximum of 2 or 3 cosmids was selected after passage into mice and the same cosmids were found in the different individual mice infected with the same pool. After prolonged in vitro culture of the cells recovered from lesions, other cosmides were selected. When clones transfected with individual cosmids were used to infect mice, several patterns of lesion growth/antimony susceptibility were observed, from highly virulent/low responders to almost avirulent clones. However, these patterns appeared unrelated to the cosmids since they were also observed for different clones transfected with the same cosmid and for cellular clones of the untransfected susceptible strain BA125. The same patterns were furthermore observed with subclones of the cellular clones. In conclusion, it looks like every *Leishmania* lesion generates a population of different cells with different properties, even when one uses previously cloned populations. This fits well with the particularities of the biology of parasites such as *Leishmania*, which must adapt quickly to rather different conditions: a few cells are always better adapted and can propagate the infection.

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OPCT2 - ANTIPROLIFERATIVE EFFECT *IN VITRO* OF 22-HYDRAZONE-IMIDAZOL-2-YL-5Á-COLEN-3Â-OL ON *LEISHMANIA (L.) MEXICANA* PROMASTIGOTES

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In this study we have examined the effect on the growth rate and ultrastructure of a Hidrazone sterol derivative on *Leishmania (L.) mexicana* promastigotes, whose structure is analogous to an ergosterol intermediary biosynthesis in eucariotes. The promastigotes were cultivated at 26ºC in *Drosophilla´*s-Schneider medium supplemented with 5% inactivated fetal bovine serum. The experiments were carried out at the exponential phase of growth; at 10^7 parasites/ml the promastigotes were treated with the sterol derivative 22-hydrazone-imidazol-2-yl-5á-colen-3â-ol at 0.5, 1, 3, 6 and 10 mM; continuous cellular growth rate were evaluated on a Neubauer camera and indirectly in a spectrofotometer; in addition cellular shape and motility was analized. A preliminary transmision electron microscopic study were conducted with the effective lethal concentration. The compound induced an antiproliferative dose dependent effect. At sublethal doses the parasites showed

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a growth rate reduction, with an inhibitory concentration IC_{42} with 3 mM and an IC_{80} with 6mM in 60 hours. The estimated Minimum Inhibitory Concentration (MIC) was calculated by the minimum square method between the growth factor at the end of the experiment and the Hidrazone concentrations, yielding a MIC with 8 mM ($r^2 = 0,92$). At the light microscopic level, 10 mM (LD_{33}) of Hidrazone induced inmediately motility loss, cellular swelling at one hour treatment, and more severe effect with cell rupture and lysis at 18 hours. Preliminary study at the transmision electron microscope on parasites treated with LD_{33}, showed cytoplasm irregularly stained, cellular matrix coagulated with granular like, organelles vacuolization, cellular membrane separation, mitochondria and kinetoplast less dense and the cellular profiles irregularly arranged. These findings suggested an antiproliferative dose dependent effect associated with biomembranes alterations, which could be related to ergosterols biosynthesis metabolism blockage. Similar results were previously described on *Trypanosoma cruzy* epimastigotes $D^{24,(25)}$ sterol methyl transferase activity, treated with 22,26-azasterol and 24(R,S),25 epiminolanosterol combined with ketoconazole (Urbina et al., 1995. Mol. Biochem. Parasitol. 73:199-210). Furthermore the potent activity observed with 22-hydrazone-imidazol-2-yl-5á-colen-3â-ol on *L. mexicana* promastigotes are promising to begin *in vivo* studies on experimental model in the search of new and neccesary alternative to erradicate human leishmaniasis.

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OPCT3 - THE DUAL ACTION OF SB ON THIOL REDOX METABOLISM

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Despite the clinical use of pentavalent antimonials in the treatment of leishmaniasis for over fifty years, their mode of action and the basis for their selective toxicity remains uncertain. Paradoxically, more is known about the mechanisms of resistance to antimonials than the molecular basis for their mode of action. Recent studies on resistance to arsenite and antimonials have revealed a potential role for the major thiols of *Leishmania*, trypanothione (N^1, N^8-*bis*(glutathionyl)spermidine or $T[SH]_2$) and glutathione (γ-L-glutamyl-L-cysteinylglycine or GSH), in the detoxification of these heavy metals[1]. $T[SH]_2$, the principal low-molecular–mass thiol in *Leishmania* spp., is an essential intermediate in the regulation of thiol-redox homeostasis and also plays a crucial role in defence against oxidative stress. Trypanothione is maintained as a dithiol through the action of the enzyme trypanothione reductase (TR) which is essential for the survival of leishmania parasites[2]. TR has been shown to be sensitive to inhibition *in vitro* by Sb^{III} [3]. These reports prompted us to examine the effects of Sb^{III}on trypanothione metabolism in drug-sensitive *Leishmania*.

Following exposure to Sb^{III} levels of GSH and $T[SH]_2$ decreased rapidly to near undetectable levels over 4 h. Subsequent radiolabelling experiments demonstrated that this Sb^{III}-induced loss was mainly due to an efflux of glutathione-containing thiols from the parasites. In addition to the loss of reduced thiol in Sb^{III}-treated amastigotes and promastigotes, levels of trypanothione and glutathione disulphide were observed to increase, suggesting that the activity of TR was inhibited. Further evidence that TR was inhibited by Sb^{III} in the cell was provided by monitoring the rates of thiol regeneration following transient exposure to the membrane-permeable oxidising agent diamide. In promastigotes cultured in the absence of Sb^{III}, levels of $T[SH]_2$ and GSH rapidly returned to normal following exposure to diamide. However, in Sb-treated promastigotes, reduced thiol levels did not recover. The most likely explanation for this observation is that Sb^{III} inhibits TR within intact cells. Based on these results we propose that Sb-cidal action is due to synergistic effects on *Leishmania* thiol redox metabolism.

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OPCT4 - TRIPANOCIDAL ACTIVITY OF THE ESSENTIAL OIL OF *CROTON CAJUCARA*

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Chagas' disease (American trypanosomiasis), endemic in tropical America, affects more than 16 million people, 5 million in Brazil, and is responsible for 45000 casualities every year (1). The ethiological agent is the protozoan *Trypanosoma cruzi*. Transmission occurs either by insect vector or blood transfusion. *Croton cajucara* Benth. (Euphorbiaceae) is a shrub native to Amazon, localy known as "sacaca". It has been used for long time in folk medicine, generally as a tea made with the leaves, to treat liver and kidney disorders, stomachache, diabetes and other diseases (2). The essential oil from the leaves of *C. cajucara* is rich in linalool (circa 40%), nerolidol and other sesquiterpenic compounds (3). We have previously observed the essential oil to be active against the protozoan *Leishmania (L.) amazonensis*, the causative agent of cutaneous (4). Prompted by these findings, we decided to investigate the effect of the essential oil on *T. cruzi*. The essential oil was used to analyze the modulation of peritoneal mouse macrophage infection by *T. cruzi*. The nitric oxide production by the infected macrophages was also determined. *T. cruzi* cells were incubated in culture medium (RMPI), in the presence of the essential oil (30ng/mL), at 37ºC. Parasite survive and cell morphology were evaluated under transmission electronic microscopy. Nuclear and kinetoplast chromatin destruction, followed by cell lysis were observed within one hour. Pretreatment of peritoneal mouse macrophages with 30 ng/mL essential oil dilution reduced in 60% the association index between these macrophages and *T. cruzi*, with a concomitant increase of 175% on nitric oxide production by the infected macrophages, as compared to the control ones.

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OPCT5 - THE EFFECT OF TOPOISOMERASE II INHIBITORS ON THE KINETOPLAST ULTRASTRUCTURE

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A typical feature of the Trypanosomatidae family is the kinetoplast, an enlarged portion of the mitochondrion which contains DNA. Type II topoisomerases (topo II) play essential roles in the kinetoplast DNA (kDNA) replication, such as decatenation of interlocked minicircles and chromosome segregation. Several commercially available drugs are known to be inhibitors of topoisomerases. Quinolone based drugs, (such as nalidixic acid) and coumarins (like novobiocin), are specific inhibitors of DNA gyrase, a bacterial type II topoisomerase. Eukaryotic type II topoisomerases are inhibited by etoposide, an antitumor drug causing a decrease of cell proliferation. This drug increases the concentration of topo II-cleaved DNA complex, hence converting topoisomerase II into physiological toxins that fragments the genome. Since topoisomerases II inhibitors have wide clinical use, it is interesting to examine if some of these drugs can target trypanosomatid topoisomerase. In the present work we evaluated the effect of topoisomerase II inhibitors on the ultrastructure of some trypanosomatid species that present differences in their kDNA arrangement, such as *Blastocrithidia culicis*, *Crithidia fasciculata*, epimastigote forms of *Trypanosoma cruzi* and promastigote forms of *Leishmania (L.) amazonensis*.

Our results showed that topo II inhibitors promoted distinct effects on the trypanosomatid growth and ultrastructure. The quinolone nalidixic acid promoted a dose-dependent inhibition on cell proliferation of all species treated. This drug induced drastic ultrastructural modifications in the kinetoplast of B. *culicis* and *C. fasciculata*, which display a loosely arrangement of kDNA when compared to *T. cruzi* and *L. (L.) amazonensis*. *B. culicis* and *C. fasciculata* showed a striking condensation of their kDNA fibers, while in *T. cruzi* this drug slightly increased the level of kDNA compaction and promoted detachment of the kDNA from the kinetoplast membrane. In *L. (L.) amazonensis*, ultrastructural modifications were not seen on the kinetoplast when nalidixic acid was used. In the case of the inhibitor novobiocin, kDNA condensation was also observed in *B. culicis*; however the other species analyzed were not affected, with the exception of *T. cruzi* epimastigotes that suffered cell lysis after treatment for 2 or 3 days with higher concentrations of this drug. On the other hand, etoposide did not cause growth impairment or ultrastructural modifications in none of the species analyzed. Epithelial cells ($LLCMK_2$) were used as a positive control for etoposide treatment and in this case, DNA condensation was observed on the nucleus.

Our results demonstrated that drug concentrations required for growth inhibition and for ultrastructural alterations varied according to the inhibitor. These differences may be related to factors as cell permeability, kDNA arrangement and sensitivity of topoisomerases to drugs. Studies involving the action of topoisomerase inhibitors reinforce the use of these enzymes as a potential target to trypanosomal chemotherapy.

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OPCT6 - POLYMORPHISM AND THE SPREAD OF CHLOROQUINE RESISTANCE IN *PLASMODIUM FALCIPARUM* POPULATIONS ACROSS THE AMAZON BASIN

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The prevalence of drug-resistant malaria parasites in South America (SA) presents a formidable obstacle to controlling the disease in that region. In order to characterize the parasite populations and the chloroquine resistant profile of *P. falciparum* in the Amazon basin, we have analyzed a DNA segment of the *pfcrt* gene spanning codon 72-76 (a putative transporter associated with chloroquine resistance in *P. falciparum*) and genotyped 15 microsatellites (MS) on four chromosomes in 100 isolates from five different endemic areas of Brazil and Peru (and one from Columbia). The K76T mutation, which is critical for chloroquine resistance, was found in all isolates. Five different *pfcrt* haplotypes [S(tct)MNT, S(agt)MNT, CMNT, CMET, and CIET] were observed, four of which have been previously reported from SA parasites, and one of which was found in African/Asian isolates. MS genotyping shows relatively homogeneous genetic backgrounds among the isolates, with an average of 3.8 alleles per marker, compared with parasites from Asia and Africa. Isolates with identical 15-loci MS haplotypes were collected from different locations, suggesting that gene flow was not severely limited across the Amazon Basin. The results also show that allopatric isolates carrying SMNT and CMNT haplotypes have similar genetic backgrounds. The parasites carrying the CIET haplotype, however, have some unique MS alleles, suggesting that the parasites with CIET allele were likely introduced into Brazil from Asia or Africa. This study provides the first evidence of the Asian *pfcrt* allele in SA, and reprensents a detailed analysis of *P. falciparum* populations with respect to *pfcr*t haplotypes in the Amazon basin.

OPCT7 - CONFIRMATION OF DIFFERENTIAL GENE EXPRESSION LEVELS OF *TRYPANOSOMA CRUZI* POPULATIONS SUSCEPTIBLE AND RESISTANT TO BENZNIDAZOLE

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In this work, we investigated the differential gene expression in *T. cruzi* populations (BZS-BZR) and clones (clone4S-clone16R) susceptible and with resistance selected *in vivo* (Murta & Romanha, 1998) and induced *in vitro* (17WTS-17LER) (Nirdé *et al*., 1995) to benznidazole. Representation of differential display (RDE) (Krieger & Goldenberg 1998), mRNA differential display (DD) (Liang & Pardee, 1992) and microarray hybridization analysis (Nunes *et al*., 2001) were used to select *T. cruzi* drug-resistance or drug-susceptible specific genes. Using these approaches we identified 96 genes: 55 by RDE, 21 by DD and 20 by microarray, considering at least a 2.3-fold difference in expression. In order to confirm the differentially expressed genes obtained by these methodologies, northern blot analyses using 35 different gene fragments were performed. Our results showed that 2 genes presented higher levels of expression in the *T. cruzi* drug-susceptible and 11 in the *T. cruzi* drug-resistant populations/clones when

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compared to each other. 17 genes showed the same level of expression in resistant and susceptible populations and 5 showed very low level of expression that did not allow further comparative analysis. Additional studies are underway to better characterize the differentially expressed genes in order to establish their possible involvement with the drug resistance phenotype in *T. cruzi*.

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OPCT8 - NOVEL TRYPANOTHIONE ANALOGUES IN *TRYPANOSOMA CRUZI*

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Trypanosomatids synthesize a unique metabolite trypanothione [N^1,N^8-bis(glutathionyl)spermidine] which plays a central role in anti-oxidant defence, ribonucleotide metabolism and in resistance to some anti-trypanocidal drugs. The insect trypanosomatid *Crithidia fasciculata* synthesizes trypanothione in two sequential steps, which are catalysed by two distinct enzymes, glutathionylspermidine synthetase (GspS) and trypanothione synthetase (TryS), respectively [(1)]. However in *Trypanosoma cruzi,* an unusual TryS synthesizes both the intermediate glutathionylspermidine and trypanothione [(2)]. Since *T.cruzi* is unable to synthesize putrescine and is dependent on the uptake of exogenous polyamines by high affinity transporters, synthesis of trypanothione may be circumstantially limited by lack of spermidine. We show that *T.cruzi* is able to circumvent the potential shortage of spermidine by conjugating glutathione with other physiological polyamine substrates from exogenous sources (spermine, N^8-acetylspermidine, *N*-acetylspermine) [(3)]. Structures of these novel thiols purified from epimastigotes were determined by matrix-assisted laser desorption ionisation time-of-flight analysis to be N^1,N^{12}-bis(glutathionyl)spermine, N^1-glutathionyl-N^8-acetylspermidine and N^1-glutathionyl-N^{12}-acetylspermine, respectively. Recombinant TryS catalyses formation of all these novel thiols with kinetic parameters equivalent to or better than those of spermidine. N^1,N^{12}-bis(glutathionyl)spermine and its disulphide are physiological substrates of the amidase function of TryS and trypanothione reductase, respectively. However, all life-cycle stages of *T. cruzi* preferentially synthesize trypanothione, even when intracellular spermine and spermidine content are equivalent. Some (as yet) unexplained mechanism might account for the preference of spermidine over spermine and other polyamines by TryS in vivo. The broad substrate specificity of TryS could be exploited in the design of polyamine-based inhibitors of trypanothione metabolism.

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OPIM1 - DETERMINATION OF SPLEEN T LYMPHOCYTE SUBPOPULATIONS AND THEIR CYTOKINE PRODUCTION IN C57BL/6J MICE IMMUNIZED WITH IRRADIATED *TOXOPLASMA GONDII* RH STRAIN TACHYZOITES

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About 60% of the São Paulo population is infected with *Toxoplasma gondii*, an obligatory intracellular protozoon that infect birds and mammalian, with felines as definitive hosts, affecting the eye and immune-compromised patients. Currently, the only commercial vaccines are for use in ovine, with partial protection. *T. gondii* irradiated tachyzoites induced protection, with immune response similar to the chronically infected mice, with resistance to challenge. In this work, we study the production of cytokine and the proportion of spleen T lymphocytes subsets, after stimulation with *T. gondii* antigen in immunized mice with irradiated tachyzoites. C57Bl/6j mice were immunized with three sequential i.p. injection of 10^7 255 Gy RH strain irradiated tachyzoites. After 15 days of last dose, mice were sacrificed and the spleen was removed and mechanically dissociated. The spleen cells were added at a density of 2 X 10^6 cells per well of 96-well flat-bottom tissue culture plates in RPMI 1640 with 10% fetal calf serum and antibiotics, and stimulated with *T. gondii* antigen (10 µg/ml). After 72 hours of incubation at 37 ºC and 5% CO_2 cells were collected and centrifuged at 300*g* in PBS with 0,05% sodium azide and 1% fetal calf serum, and were incubated for 30 minutes at 4º C in dark chamber with anti-CD4 Cy-chrome conjugate and conjugated anti CD8 PE conjugate, for Flow Cytometry. Replicate cells had their culture media discarded and each well received TRIzol® with pooled triplicates stored in 1.5mL tubes at –80 ºC until mRNA extraction. Extracted mRNA was submitted to RT-PCR with oligo primers (Oligo $dT_{12\text{-}18}$) in the first step and specific PCR reactions to IL-10, γIFN and TNF-α mRNA, using murine β-actin (349 bp) segment as amplification control. Results were analyzed after PAGE (6%, silver stained), and dried gels digitalized for optical density quantitation of each band, using β-actin bands as pattern of mRNA production. The acute infection induces cellular response with high CD4 numbers but low CD8 numbers as compared to control mice, but chronically infected mice presented the same higher levels CD4 and restores and amplifies CD8 response. The antigen driven CD4 enhance is clearly seen in immunized mice, despite a lower extent than infection, and some animals presented also the CD8 response, which was more heterogeneous. The cytokine production, analyzed by mRNA RT-PCR showed higher levels of the three cytokines both in acute and chronic infection, and immunized mice produced the same pattern of cytokine production, despite expected lower levels. Our data shows irradiated tachyzoites induced similar immune cell response than usual disease, but with similar efficiency in the protection, due to absence of acute infection suppression.

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OPIM2 - A FACTOR PRODUCED BY *PLASMODIUM YOELII* INFECTED ERYTHROCYTES MODULATES DENDRITIC CELL FUNCION

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Plasmodium spp. parasites have adopted different strategies for survival, which render it one of the most successful human pathogens. One such mechanism, *Plasmodium*-induced immune suppression, is an immune evasion strategy utilized by the parasite. We have found that *Plasmodium yoelii*-induced immune suppression is mediated by dendritic cells that lose the ability to mature in response to activating stimuli and ultimately suppress T-cell responses. Using a murine malaria model, we show that one mechanism of DC suppression is induced by a soluble factor(s) produced by *P. yoelii*-infected erythrocytes. This soluble factor(s) inhibits DC maturation as measured by a decrease in the surface

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expression of co-stimulatory molecules and an inhibition of the secretion of immune activators, like IL-12. Interestingly, this active soluble factor(s) is smaller than 30 kDa and is heat resistant and stable. We have also determined that the soluble factor(s) of *P. yoelii*-infected erythrocytes interferes with the activation of the transcription factor NF-kB and is responsible for an increase in intracellular calcium levels in DCs. Detailed understanding of the signaling pathways and soluble mediators involved in malaria-induced immune suppression will provide novel drug targets for the treatment of disease.

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OPIM3 - HUMORAL AND CELLULAR IMMUNE RESPONSE AGAINST CDNA CONSTRUCTIONS FROM *LUTZOMYIA LONGIPALPIS* SALIVA IN THE GOLDEN HAMSTER

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The saliva of blood-sucking arthropods contains pharmacologically active substances that affect the vertebrate host's hemostatic, inflammatory and immune response. The activity of the saliva possibly benefits the parasite delivered by the vector. Since saliva contains an array of proteins, vertebrate host might mount an immune response (cellular and/or humoral) to these molecules. Mice exposed to salivary gland homogenate developed antibodies against saliva and a delayed-type hipersensitivity (DTH) reaction at the site of the bite. The identification and isolation of salivary molecules from sand flies has led to the discovery of novel proteins to test biologic activities that possibly promote a protective immune response against *Leishmania* infection. In this study we tested sixteen DNA vaccines containing full-length cDNA constructions isolated from the salivary gland of the sand fly *Lutzomyia longipalpis*. Each cDNA (20mg) were inoculated intradermally into the right ear dermis of the hamster using a 27-gauge needle in a volume of 20ml. Each group were boosted twice. Two weeks after the last booster, one pair of SGH was inoculated intradermally into the left ear dermis and 48 hours later, the evolution of DTH was monitored by measuring the diameter of the induration. Antibodies were evaluated by ELISA and Western blot using SGH as antigen. Our study shows that at least three cDNA (LLsp45, LLsp44 and LLsp61) induced high production of antibodies and DTH response. Sera from hamsters inoculated with cDNA LLsp45, 44 and 61 recognized bands from SGH that corresponded to 45, 44 and 61 kD. Characterization of the salivary components responsible for these activities is necessary to understand their mechanism of action. The study of these salivary molecules' role is an important target to better understand the control of *Leishmania* infection.

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OPIM4 - ROLE OF INTERLEUKIN-4 AND PROSTAGLANDIN E_2 IN *LEISHMANIA (L.) AMAZONENSIS* INFECTION OF BALB/C MICE

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The role of cytokines in *Leishmania (L.) amazonensis* experimental infection has not been well studied as in *L. (L.) major* infection model. Here we investigated the role of interleukin (IL)-4 and PGE_2 in *L. (L.) amazonensis* infection of susceptible BALB/c mice. Methods and Results: Interleukin (IL)-4 deficient (IL-4-/-) or wild-type (IL-4+/+) BALB/c mice were infected with *L. (L.) amazonensis* promastigotes and submitted to treatment with indomethacin, an inhibitor of PGE_2 synthesis. Cytokine (in lymph node cell culture supernatants) and serum anti-leishmania antibody levels were determined by ELISA. Lesions of IL-4 -/- BALB/c mice progressed similarly to those of IL-4 +/+ mice BALB/c upon infection with 5 x 10^6 parasites/footpad. Two weeks after infection, the production of interferon (IFN)-γ upon *L. (L.) amazonensis* antigen stimulation was significantly higher in IL-4 -/- mice than in IL-4 +/+ mice. The levels of anti-*leishmania* IgG2a antibodies were also significantly higher in serum from IL-4 -/- mice. In contrast, the levels of IgG1 antibodies were higher in IL-4 +/+ mice and almost undetectable in IL-4 -/- mice. However, IL-4 -/- mice developed smaller lesions upon infection with 10^5, 10^4 or 10^3 parasites, compared to IL-4 +/+ mice. Lymph nodes of IL-4 -/- mice had significantly less parasites than those of IL-4 +/+ mice by limiting dilution assay (2 and 4 x 10^6 geometric means, respectively). In both groups, the lesions contained macrophages with large vacuoles and parasites. The intensity of inflammation was lower in the IL-4 -/- animals. IL-4 +/+ e IL-4 -/- mice treated with indomethacin during the first three weeks of infection developed smaller lesions and lower parasitic load when compared to the control group. The lesions of indomethacin- treated groups contained most macrophages without vacuoles and small or absent necrotic areas. Conclusion: Our results indicate that IL-4 and PGE_2 are susceptibility factors to *L. amazonensis* infection.

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OPIM5 - ROLE OF NK CELLS IN RESISTANCE TO *TRYPANOSOMA CRUZI* INFECTION IN MICE

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Vertebrate host defense against *Trypanosoma cruzi* is mainly the T_H1 response. During the course of this intracellular infection activated macrophages produce the interleukin IL-12, which in turn induces IFN-gamma (IFN-γ) production in T_H1 cells. IFN-γ is an important cytokine produced in response to intracellular pathogens and plays a key role in activating macrophages to produce reactive nitrogen intermediates capable of eliminating the parasite intracellularly. In the early phase of the infection, however, NK cells are known to produce IFN-γ and are therefore thought to initiate activation of the macrophage population which leads to a positive feedback loop. In order to test this hypothesis, we used a reconstitution model of genetically engineered alymphoid RAG2 x IL-2R-gamma chain (RAG/gc)-KO mice on BALB/c background. These knock-out mice are devoid of NK, NKT, T and B cells and were reconstituted with cell suspensions from peripheral lymph nodes and spleen after removing NK cells. The absence of $DX5^+$ NK cells in recipient mice was verified 6 weeks after reconstitution with lymph node and spleen cells respectively. In later experiments, we used only lymph node cell suspensions to generate NK cell-deficient mice. After subcutaneous infection with 15 *T. cruzi* (Tulahuen) blood trypomastigotes, reconstituted RAG/gc-KO mice were able to control the infection and clear the parasites from the blood in contrast to unreconstituted controls. In addition, analysis of IFN-γ and IL-12p40 at days 14, 21 and 28 post-infection in reconstituted mice showed cytokine levels similar to those of wild type, leading to sufficient production of reactive nitrogen intermediates.

Moreover, reconstituted mice that survived primary infection were also able to mount a memory response which enabled them to survive a second challenge with a lethal dose of 500 *T. cruzi*.

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These results suggest that NK cells are not important for resistance and immunity to *T. cruzi* infection. Further reconstitution experiments with sorted T cell populations, such as ab-T cells, will provide more information as to the relevance of particular cell types involved in clearing these flagellates from the blood and controlling infection.

OPIM6 - GENETIC CONTROL OF THE HUMORAL IMMUNE RESPONSE AGAINST *PLASMODIUM FALCIPARUM* GLUTAMATE-RICH PROTEIN

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The *Plasmodium falciparum* Glutamate-Rich Protein (GLURP) is an exoantigen synthesized in all stages of the parasite in humans. Immuno-epidemiological studies performed in high transmission areas have demonstrated in adults a high prevalence of antibodies against GLURP and that high levels of GLURP-specific antibodies are significantly associated with low parasite densities and protection against clinical malaria. In this study we evaluated the IgG response against GLURP and used HLA antigens as genetic markers in an attempt to determine the presence of genetic control of the humoral responsiveness to GLURP in individuals living in Brazilian endemic area with low levels of transmission. HLA-DR and DQ was determined in 107 individuals living in rural area of Porto Velho (RO). Sera from these individuals were tested by ELISA for IgG against recombinant proteins (R0 and R2 regions) and synthetic peptides (R0: P3, P4, P5, P8, P9, P10, P11, S3; R2: S4) corresponding to different regions of the protein. We observed a high frequency of antibodies against R0 (67%) and R2 (79%) regions. We observed a higher frequency of responders to S4 (52%) and P11 (49%) peptides when compared with others peptides. The absence of anti-R0 response was associated with HLA-DR11 and HLA-DQ7 and the absence of anti-R2 response was associated with HLA-DR12. We also observed positive and negative associations between HLA and the immune response against GLURP-derived peptides (positive: P3 with DR4 and DQ8; P4, P8 and P9 with DR13; P10 with DR8; P11 with DR8 and DQ4; negative: S4 with DR7). Our results suggests that there are significant associations between HLA-DR and HLA-DQ and the humoral immune response against GLURP. Thus, naturally exposed individuals with different HLA class II antigens seem to respond differently to GLURP protein, indicating that the choice of relevant peptide sequences may have important consequences for subunit vaccine development.

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OPIM7 - IMPAIRED RESISTANCE TO ACUTE INFECTION WITH *TRYPANOSOMA CRUZI* IN MICE LACKING FUNCTIONAL MYD88

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Studies performed in vitro suggest that activation of Toll-like receptor (TLR) by parasite-derived molecules may initiate inflammatory responses and host innate defense mechanisms against *Trypanosoma cruzi*. Here, we evaluated the impact of TLR2 and MyD88 deficiencies in host resistance to infection with *T. cruzi.* Our results show that macrophages derived from TLR2-/- mice are unresponsive to tGPI-mucin, a parasite molecule with a potent pro-inflammatory activity. However, the same cells from TLR2-/- still produce TNF-α, IL-12 and reactive nitrogen intermediates (RNI) upon exposure to live *T. cruzi* trypomastigotes. Consistently, we show that TLR2-/- mice mount a robust pro-inflammatory cytokine response as well as RNI production during acute phase of infection with *T. cruzi* parasites. Further, deletion of functional TLR2 gene had no major impact on parasitemia or on mortality. In contrast, the MyD88-/- mice had a diminished cytokine response and RNI production upon acute infection with *T. cruzi.* More importantly, we show that MyD88-/- mice are more susceptible to infection with *T. cruzi* as indicated by the higher parasitemia and accelerated mortality, as compared to the wild type mice. Together, our results indicate that *T. cruzi* parasites elicit an alternative inflammatory pathway independent of TLR2. This pathway is dependent on MyD88 and is necessary for mounting optimal inflammatory and RNI responses that control *T. cruzi* replication during the early stages of infection.

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OPIM8 - IDENTIFICATION OF B13 AND CARDIAC MYOSIN EPITOPES RECOGNIZED IN MOLECULAR MIMICRY BY A CROSSREACTIVE T CELL CLONE: STRUCTURAL ANALYSIS

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Elucidating the structural features of T cell receptor interaction with its antigenic peptide imbedded in the HLA molecule is crucial to determine the T cell molecular mimicry in Chagas Disease Cardiomyopathy (CCC). T cells from CCC patients crossreactively recognize human cardiac myosin and the immunodominant B13 protein from *T. cruzi*. In order to identify crossreactive epitopes between B13 protein and human cardiac myosin, we established a T cell clone KJS15.4 from an HLA-DQ7+ individual specific to peptide S15.4 that is preferentially recognized by CCC patients. We tested the clone in proliferation assays against peptides at 25 mM bearing the HLA-DQ7 binding motif: ten 15mer peptides from the *T cruzi* B13 protein and 47 15mer peptides from human b-cardiac myosin heavy chain. Additionally we tested 15 Lys/His-substituted S15.4-derived peptides for TCR/HLA contact analysis. Interestingly, KJS15.4 recognized 4 B13 derived peptides and 13 partially homologous peptides from human cardiac myosin. The combination of Lys/His scanning analysis with HLA-peptide complex molecular modeling and previous HLA-binding data showed that Phe5, Gly6 and Ala8 are important HLA contact residues and Gln7, Ala10, Asp12, Lys13 and Pro15 are important TCR contact residues of S15.4 peptide. These results are the first description of the molecular recognition pattern of T cell molecular mimicry in CCC.

Supported by FAPESP

OPMB1 - IDENTIFICATION OF PDZ5, A CANDIDATE UNIVERSAL MINICIRCLE SEQUENCE BINDING PROTEIN OF *TRYPANOSOMA CRUZI*.

Coelho, E.R.; Santos, G.V.; Urmenyi, T.P., Rondinelli, E. and Silva, R.

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The order kinetoplastidae encloses a variety of protozoan parasites, such as the Trypasosomatidae family, and is characterized by the presence of the kinetoplast, a network-like structure that contains the DNA of the single mitochondrion of these organisms, called kDNA. kDNA is composed of two groups of interlocked DNA molecules, the maxicircles and the minicircles. Replication mechanism of the minicircles requires the detachment of the individual molecules from the disc-shaped DNA network and its migration to one of the two antipodal proteic sites, where they are replicated. The replication starts at the Universal Minicircle Sequence (UMS), a conserved nucleotide sequence represented by the dodecamer 5'-ggggttggtgta-3'. After the complete replication of the minicircles, they are reattached to the periphery of the DNA disc. There is a protein, characterized in *C. fasciculata*, which binds to the UMS and is thought to recruit other proteins responsible for the replication itself. This protein is called Universal Minicircle Sequence Binding Protein - UMSBP, and localizes to the kinetoflagelar zone.

We are studying a zinc finger protein from *Trypanosoma cruzi*, that we have named PDZ5, which presents a great percentage aminoacid sequence homology with *C. fasciculata* UMSBP. PDZ5 has 134 aminoacids, and five zinc finger domains of the consensus type CCHC. Western blotting analysis using an anti-*C. fasciculata* UMSBP antibody (gently given by Dr Joseph Shlomai - Hebrew University) revealed that it recognizes both the recombinant and the *T. cruzi* endogenous PDZ5, which is about 14 kDa. Eletrophoretic mobility shift assay (EMSA) using the recombinant PDZ5 indicated that this protein binds specifically to the Universal Minicircle Sequence (Coelho, et al, Int J Parasitol. 2003, Jul; 33(8): 853-8). PDZ5 encoding gene is present as a single copy gene in the *T. cruzi* genome, adjacent to the beta five proteasome subunit. We are investigating the cellular localization of PDZ5 through imunolocalization assays. Data from RNAi experiments may reinforce the characterization of PDZ5 as a *T. cruzi* UMSBP.

OPMB2 - THE COHESIN COMPLEX OF TRYPANOSOMATIDS

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New information on the architecture of protein complexes involved in the dynamic organization of chromosomes challenges existing models of their structures and provides fresh insight into their mechanisms of action. Cohesion between sister chromatids is established during S phase by a multisubunit complex called cohesin. The cohesin complex consists of Smc1, Smc3, Scc1 and Scc3 proteins and mediates the connection between chromatids. Smc1 and Smc3 belong to the Structural Maintenance of Chromosomes (SMC) family proteins, which are rod-shaped molecules with globular ABC-like ATPases at one end and dimerization domains at the other connected by long coiled coils. This unique protein structure confers them critical roles in mitotic chromosome organization. The ATPase domains are bridged by Scc1, creating a ring that can trap sister DNA molecules. Cohesion between chromatids is lost when Scc1 is cleaved by a cysteine protease called separase. Even though it is not part of the cohesin complex, the evolutionary conserved protein Eco1 is required for establishment of sister chromatid cohesion during S phase. Eco1 has a two-domain architecture with an N-terminal C_2H_2 Zn finger-like domain and a C-terminal domain with an acetyl coenzyme A binding motif. *In vitro* Eco1 acetylates components of the cohesin complex but not histones. The establishment of cohesion between sister chromatids appears to be regulated by this specific acetyltransferase. In trypanosomatids, the structural basis of mitosis, spindle organization and chromosome segregation is poorly understood. The chromatin of these organisms is known to be organized in classical nucleosomal filaments, but surprisingly, these filaments are less compact than in higher eukaryotes and do not fold into visible chromosomes. Besides, the nuclear envelope is preserved throughout the cell cycle and remains intact during cell division. We address the identification of the cohesin complex genes in the trypanosomatids *T. cruzi*, *T. brucei* e *L. (L.) major*. The SMC subunits of these organisms showed conserved N-terminal and C-terminal globular domains, as well as conserved dimerization domains. Scc1 is also conserved among the trypanosomatids, specially at the N-terminal and C-terminal ends. Scc3 has a high N-terminal conservation, for it is in this region that the SCD (Stromalin Conservative Domain), composed of about 90 aminoacids, is located. Eco1 has a conserved C-terminal catalytic domain, but in trypanosomatids it lacks the N-terminal zinc finger domain, which has been observed in all other eukaryotic organisms studied so far. Studies of the cohesin complex in these organisms may lead to the elucidation of chromosome organization events even beyond mitosis.

OPMB3 - *TC*RBP1 AND *TC*RBP2: TWO PUTATIVE RNA BINDING PROTEINS FROM *TRYPANOSOMA CRUZI*

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Trypanosomes are a group of eukaryotic organisms with many unusual characteristics in their molecular biology. These characteristics, together with the different cell forms trypanosomes present along their life cycle, makes the identification and characterization of RNA binding proteins even more important as they play key roles in the regulation mechanism of gene expression.

We have identified clones from a Cl Brenner *T. cruzi* normalized cDNA homologs to two *Trypanosoma brucei* RNA binding proteins, *Tb*p34 and *Tb*p37, previously reported by Zhang and Williams, 1997. The predicted proteins were named *Tc*RBP1 and *Tc*RBP2 as they present, each, two well-known RNA binding domains (RBD or RRM) and are very similar in their aminoacid sequence. The 5´ and 3´ UTRs were characterized and the trans-splicing and polyadenilation sites determined. The RBP genes are organized in a tandem which consists of at least 7 copies of the RBP genes alternated by a predicted ORF that does not present homology with any characterized protein. The RBP transcripts are present in all parasite cell forms, mainly in the spheromastigote stage suggesting a stage-specific regulation. Western blot analysis using heterologous antibodies raised against *Tb*p34 and *Tb*p37, gently given by Dr. Noreen Williams - State University of New York in Buffalo, revealed that the RBP proteins are present not only in *T. cruzi* but also in *Leishmania (V.) braziliensis* and *Crithidia fasciculata.* Preliminary imunofluorescence assays localize the RBP genes in the cytoplasm of the parasite cells suggesting. Both TcRBP genes were cloned in pGEX vectors and expressed in *E. coli* strains as fusion proteins. We are now, producing these proteins in large-scale for functional assays.

OPMB4 - TESTS OF RNA INTERFERENCE (RNAI) AND CONSTRUCTION OF A TETRACYCLINE-INDUCIBLE T7 PROMOTER SYSTEM IN *TRYPANOSOMA CRUZI*

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The technique of RNA interference (RNAi) is exceedingly useful for knocking down the expression of a specific mRNA in African trypanosomes and other organisms for the purpose of examining the function of its gene. However, when we attempted to apply RNAi in the Latin American trypanosome, *Trypanosoma cruzi*, to diminish expression of mRNA encoding the surface protein amastin, we found that the amastin double-stranded RNA (dsRNA) was

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not efficiently degraded in either epimastigotes or amastigotes to the short interfering RNAs required for the RNAi pathway, and the level of amastin mRNA remained unchanged. We generated a strain of *T. cruzi* CL-Brener in which the T7 promoter and tetracycline operator could be used to maximize tetracycline-regulated dsRNA synthesis and constructed plasmids that directed dsRNA against four different *T. cruzi* endogenous genes [encoding â-tubulin, GP72 (flagellar adhesion protein), ribosomal protein P0 and amastin] and an exogenously added gene [GFP; green fluorescent protein]. After either stable or transient transfection of these plasmids into *T. cruzi*, the expected RNAi phenotype was not observed for any of the five genes, although the *T. cruzi* â-tubulin RNAi plasmid did give the expected FAT cell phenotype in the African trypanosome, *Trypanosoma brucei*. These data indicate that, similar to *Leishmania*, *T. cruzi* lacks one or more components necessary for the RNAi pathway and that these components will need to be engineered into *T. cruzi*, or compensated for, before RNAi can be used to study gene function in this organism.

Supported by: NIH and CNPq

OPMB5 - REAL TIME PCR STRATEGY FOR *TRYPANOSOMA CRUZI* MAJOR LINEAGE IDENTIFICATION DIRECTLY ON HUMAN INFECTED TISSUES.

Jorge M. Freitas; Eliane Lages Silva, Sérgio D. J. Pena and Andréa M. Macedo

The protozoan *Trypanosoma cruzi* is the parasite agent of Chagas disease, which represents one of the major medical significance throughout Latin America. This disease has a variable clinical presentation during chronical phase and geographical variations in the prevalence of clinical forms and morbidity have been reported. These clinical and geographical differences might be related in part to host genetic aspects and immune competence, but most probably they are caused by the genetic diversity of the parasite. *T. cruzi* exhibits a great intra specific variability and a set of biochemical and molecular markers support the division of parasites into at least two major lineages – *T. cruzi* I and *T. cruzi* II, which present different epidemiological features. *T. cruzi* I strains are better adapted to marsupials and preferentially encountered in the sylvatic cycle of transmission of Chagas disease while *T. cruzi* II strains are strongly associated with the domestic cycle and are found in primates and humans. Until now all parasites isolated from soropositive individuals from endemic regions belong to *T. cruzi* II major lineage. However, despite of a lot of effort, a clear correlation between the *T. cruzi* diversity and the variable clinical outcomes remain to be demonstrated. One possible explanation for this is that most of genetic profiling techniques used for the genetic characterization of *T. cruzi* require parasite isolation from patient blood and growth in laboratory animals or cultures, and thus there is ample opportunity for clonal selection and consequently the trypanosome populations available for analysis can differ from those actually causing the tissue lesion. In the present study we developed a PCR strategy based on the amplification of the D 7 region of the 24sa rRNA gene in a Real Time PCR apparatus, that allows parasite quantification and lineage identification directly in host infected tissues. The methodology was initially validated by analyzing artificially double infected mice tissues with JG and Col1.7 G2. We found a perfect correlation between JG profile (rDNA group 1, *T. cruzi* II) in the hearts and the Col1.7G2 profile (rDNA group 2, *T. cruzi* I) in the recta of the same animals, as previously demonstrated by LSSP-PCR technique. When compared to LSSP-PCR, the present methodology is much faster and simpler. The analysis of the parasites present in 8 hearts and 8 esophagi obtained from patients resident in a Chagas endemic area, in Minas Gerais, demonstrated that all parasites exhibit the rDNA group 1 profile, indicating that *T. cruzi* II is the lineage responsible for Chagas disease.

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OPMB6 - QUANTIFICATION AND IDENTIFICATION OF *LEISHMANIA (VIANNIA)* PARASITES BY GLUCOSE-6-PHOSPHATE DEHYDROGENASE GENE BASED REAL-TIME PCR ASSAYS.

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Organisms belonging to the genus *Leishmania* are responsible for important emerging or reemerging zoonoses, leishmaniass. Given the species diversity of these parasites, their identification and quantification has become important for both epidemiological and chemotherapeutical studies. Standard techniques of parasite burden determination are time-consuming and not easily performed. New techniques based on real-time PCR are being implemented for different pathogens; this approach has the advantage that it can be performed quickly and can allow parasite identification. Unfortunately none of the real-time PCR approaches described to date identify and distinguish subgenus *L. (Viannia)* species. Recently, we described a new test to identify these parasites based on *Leishmania* G6PD locus (Castilho *et al.*, 2003). We present here two new assays to quantify and distinguish *L. (V.) braziliensis* from other *L .(Viannia)* species endemic on Brazil using SYBR-green and TaqMan assays that, in addition to parasite identification, allows us to determine the number of the parasites.

By comparing the *G6PD* sequence of *L. (L.) amazonensis, L. (L.) mexicana L. (L.) infantum*, *L. (V.) braziliensis*, *L. (V.) guyanensis*, *L. (V.) naiffi*, *L. (V.) lainsoni* and *L. (V.) shawi G6PD*-IVSPF1 and *G6PD*-IVSPR1 oligonucleotides were designed to only generate an amplicon from *L.(Viannia)* parasite DNA. These oligonucleotides successfully generate amplification products from *L.(V.) braziliensis, L. (V.) guyanensis, L. (V.) shawi, L. (V.) lainsoni* and *L. (V.) panamensis* reference strains' DNA. Also included were two *L. (V.) braziliensis* isolates, recently grouped in different clusters, that are from geographically distinct regions of Brazil that were positive in the assay. In contrast, amplification products were not generated from *L. (L.) amazonensis, L. (L.) infantum, L. (S.) adleri, T. cruzi* Y and human DNA. Plasmids carrying the amplicon of *L. (V.) braziliensis* and *L. (V.) guyanensis* were used to normalize a standard curve for the quantification assays, giving a good linear correlation within a range of 10^5 to 10 parasites in the presence or absence of human DNA.

Differences in the amino terminal sequence of G6PD between *L. (V.) braziliensis* and the other *L. (Viannia)* species were used to design two specific TaqMan probes, respectively, FAM_bra and VIC_nbra. Those probes together with the *L.(Viannia) species* specific oligonucleotides (described above) were used in a TaqMan assay. *L. (V.) braziliensis* DNA was specifically recognized by FAM_bra probe and *L. (V.) guyanensis* DNA by VIC_nbra probe. This assay also gives us a good linear correlation in a range of 10^5 to 10 parasites. Both probes were negative with *L. (L.) amazonensis, L. (L.) infantum, L. (S.) adleri, T.cruzi* Y and human DNA.

New assays to quantify *L. (Leishmania)* species and mammalian cells are being developed to be used together with the *L. (Viannia)* assays described here. These assays combined will allow us to determine parasite burden in host tissues or cell culture that will be a useful tool to investigate disease pathology and epidemiology.

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OPMB7 - FUNCTIONAL GENOMICS ANALYSIS OF DIFFERENTIATING EPIMASTIGOTES DURING TRYPANOSOMA CRUZI IN VITRO METACICLOGENESIS

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The differentiation of *T. cruzi* epimastigotes into metacyclic trypomastigotes involves the transformation of a replicative, non-infectious form into a non-replicative, infectious stage. During *in vitro* metacyclogenesis, differentiating epimastigotes adhere to the culture flasks and are released to the medium upon transformation into metacyclic trypomastigotes. To improve our comprehension of the mechanisms of gene regulation in *T. cruzi* and the biological role of genes specifically expressed in the course of the metacyclogenesis process, we have been doing a systematic analysis of differentially expressed genes by competitive hybridization using *T. cruzi* DNA microarrays. For the microarray construction a search of different *T cruzi* nucleotide sequences was made in international databases. These data was used for creating a database containing different *ESTs,* probably representing distinct genes. Clusterization of 9919 *T.cruzi* sequences using the CAP 3 program (Huang and Madan 1999) resulted in the grouping of 6995 sequences in 1655 putative unique *ORFs*, while 2924 had remained as singletons, without detectable similarity with other gene sequences of the parasite. These sequences have been used for primer design and the primers were then used for the amplification of the genes to be spotted onto the micro-array slides. Competitive hybridization assays of the microarrays with RNA isolated from parasites after 24 hours of differentiation allowed the selection of ESTs which are differentially expressed by this cell population. Genomic and cDNA libraries were screened with these specific probes; complete sequence of the ESTs was obtained and BLAST search was performed to identify the products encoded by these genes..

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OPMB8 - DNA MICROARRAYS FOR COMPARATIVE GENOMICS AND ANALYSIS OF GENE EXPRESSION IN *TRYPANOSOMA CRUZI*

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Trypanosoma cruzi isolates show remarkable differences in biological parameters. In spite of the broad genetic diversity, parasite isolates have been clustered into two major phylogenetic lineages, named as *T. cruzi* I and *T. cruzi* II. CL Brener has been chosen as the reference organism for genome sequencing. In this study we aimed at evaluating whether DNA microarrays containing predominantly CL Brener cDNAs can be used for comparative genomics and to investigate differential gene expression in *T. cruzi* isolates. We constructed the first prototype microarray with 710 ESTs of CL Brener and 20 characterized genes of various strains. These targets represent 665 unique sequences. For the initial studies we chose two pairs of isolates: CL Brener (*T. cruzi* II) and Silvio X10 cl1 (*T. cruzi* I); and two *T. cruzi* II strains isolated from a chagasic asymptomatic patient (Famema) and a patient with cardiac and digestive manifestations (Hem 179). To visualize the microarray data, we developed a new conceptual framework called S-P plot. Hybridization of the microarray with DNA of Silvio and CL Brener indicated that 44 targets (6%) have different representation in the parasite genomes. Several of these sequences were confirmed by Southern blot with genomic DNA of some strains. Sixty-six targets (9%) exhibited significant differences in the level of mRNA transcripts in epimastigotes of Silvio and CL Brener. The analysis of the transcript levels in Famema and Hem 179 epimastigotes showed that 68 genes (9.3%) were differentially regulated, whereas 48 genes (6.5%) were differentially expressed in metacyclic trypomastigotes. Some of these targets were confirmed by Northern blot with total RNA of parasite strains and developmental stages. Microarrays allowed the identification of several sequences for which differences in the abundance of gene copy number and/or developmentally regulated levels of RNA transcripts had been demonstrated by different approaches. In addition, this study provides further evidence for a high level of post-transcriptional regulation of RNA abundance in *T. cruzi*. It is concluded that microarrays are a powerful tool for gene discovery and for comparative analyses of genomics and gene expression in *T. cruzi* isolates.

Support: FAPESP, CNPq and CAPES.

OPVE1 - SALIVA FROM *LUTZOMYIA LONGIPALPIS* INDUCES MCP-1 EXPRESSION AND MONOCYTES RECRUITMENT *IN VITRO* AND *IN VIVO*

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Members of the genus *Leishmania* are sand fly vector-transmitted protozoan parasites that cause leishmaniasis in the mammalian host. The saliva of bloodfeeding arthropods contains a wide variety of molecules that modulate their host hemostatic, inflammatory and immune responses. In order to further explain the effect of salivary gland homogenate (SGH) from *Lutzomyia longipalpis* on the process of leukocyte migration, we used the air pouch model to study their potential chemotactic effect. SGH (0.5 pair/animal) was inoculated in the air pouch formed in the back of BALB/c mice. LPS and saline were used as positive and negative controls, respectively. After twelve hours, pouches were washed thoroughly with saline for total and differential leukocyte counts. Using this model, we could observe that there was a significant increase of monocytes and macrophages migration when SGH was inoculated in the air pouch when compared to the LPS treated mice. SGH's recruitment reached a peak twelve hours after inoculation. The same effect was not seen when C57BL/6 mice were used. This differential cell recruitment in BALB/c mice was directly correlated to an increase in MCP-1 expression in the air pouch lining tissue. The MCP-1 expression was also seen *in vitro* when J774 murine macrophages were exposed to the same SGH. The SGH effect was neutralized by pre-incubation with serum positive for anti-SGH IgG. This observations indicate that SGH from *Lutzomyia longipalpis* in BALB/c mice recruits monocytes/macrophages that may enhance the establishment of *Leishmania* in the host.

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OPVE2 - COULD THE ANAESTHETIC ACTIVITY OF THE TRIATOMINES SALIVA BE ORIGINATED FROM THE PARALYSING ACTIVITY OF THE HEMIPTERA PREDATORS?

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The insects of the genus *Belostoma* are aquatic predators of molluscs, other

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invertebrates as well as small vertebrates. The saliva of the species in this genus is whitish, viscous and a little soluble in water, indicating the presence of lipids. The presence of proteases conjugated with lipids would difficult the dilution of these enzymes in water. This characteristic would improve the external pre-digestion of the prey in the aquatic environment. Beyond the proteolytic activity, the *Belostoma* saliva presents the property of immobilizing the prey probably by acting on the nervous system. Previous work with saliva from other Hemiptera such as the haematophagous *Triatoma infestans* saliva showed an inhibitory effect on rat sciatic nerve compound action potentials (CAP) and Na^+ channels. The objective of the present work was to study the action of the semi-purified saliva of *Belostoma anurum* on the CAP. The saliva of *Belostoma* adults was collected by electric stimulation. After boiling during two minutes the saliva was centrifuged and ultrafiltered (cut off of 5 kDa). Measurements of the CAP were performed on rat sciatic nerve. The CAPs were obtained using the single sucrose-gap method. The addition of the ultrafiltered material, corresponding to the saliva of one insect, produced a progressive reduction of CAP. In five experiments using 40ml of ultrafiltered material, the reduction of the CAP amplitude was of 24.2 ± 1.4 % (mean ± EP) after 50 min of incubation. Partial recovery of the CAP amplitude was observed immediately after washing the preparation with Locke's solution. The CAP amplitude reached 87.1 ± 2.3 % of the control value thirty minutes after washing. It is believed that haematophagy evolved from predatory habits among the Hemiptera. Therefore, the paralysing activity in the predators could be precursor of the anaesthetic activity present in the haematophagous Hemiptera.

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OPVE3 - CHARACTERIZATION OF UBIQUITIN-ASSOCIATED-VITELLIN POPULATIONS FROM *RHODNIUS PROLIXUS*

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The insect eggs contain structures called yolk bodies which are rich in vitellin (VT), a glicolipophosphoproteins utilized by developing embryo during the process of embryogenesis.

In *Rhodnius prolixus* we have described three heterogeneous populations of VT. They were identified and named VT1, VT2 and VT3, according to their order of elution from the DEAE columm (Salerno, 1996).

Salerno et al (2001) demostrated that these populations of VTs are localized at different regions of the oocyte: VT1, close to the membrane, VT2, in the central region and VT3 between the others. Ubiquitin is a small protein (76 amino acids) that signals the protein degradation by proteasomes (poliubiquitin) or the fate of the protein in intracelular vesicles (monoubiquitin) (Schenell and Hicke, 2003). We used antibody against ubiquitin to verify its presence or not among different populations of VT. Western blot analysis showed clearly that only VT1 population contain ubiquitin associated to the protein. VT1 is sinthesized by follicle cells and in vitro the cells secrete the molecule to the culture medium already associated with ubiquitin. Purified molecules are taken by the oocytes. So, the presence of ubiquitin can explain in part why VT1 is located in the outer layer of the oocyte, since it is known that ubiquitin can be used as a signal to direct proteins to specific intracellular vesicles. The role of ubiquitin associated with a specific population of VT is dicussed.

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OPVE4 - FEEDING-INDUCED CHANGES IN PROTEIN PHOSPHORYLATION STATUS IN *AEDES AEGYPTI*

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Mosquitoes are the vectors of tropical diseases such as malaria, dengue, yellow fever and encephalitis. Many events are observed in adult females in reponse to a blood meal. These events include blood ingestion, formation of peritrophic matrix induction of protease synthesis and their secretion in the mosquito midgut. This complex set of events requires several signalling cascades, most of which are still unknown. The final target of every cell signalling pathway involves the reversible phosphorylation of proteins. Therefore, the present study was designed to understand the mechanism by which blood acquisition and digestion are triggered by these cascades. Our strategy includes the isolation, amino terminal sequencing and identification of the complete set of proteins either phosphorylated or dephosphorylated upon blood feeding. In this study we fed *Aedes aegypti* females with an artificial meal (AM) containing PBS pH 7.0, 50 mg/ml bovine albumin and 1 mM ATP at 37ºC. After 24 hours, all the females were homogenized in PBS pH 7.4 1 mM EDTA, 1 mM EGTA, 0.15 mM OKA, 0.18 mM PAO, 0.1% Triton X-100 and a cocktail of protease inhibitors. The homogenates were submitted to SDS-PAGE and transferred to nitrocellulose membranes. The membranes were blocked and incubated with rabbit primary polyclonal antibodies against phosphoserine and developed by ECL. A proeminent 40 kDa protein was detected by western blotting in sugar fed mosquitoes but it disappears after feeding with AM. Sugar fed and AM mosquitoes homogenates were applied to an ion exchange chromatography coupled to a HPLC system. Fractions were collected and submitted to a dot-blotting using phosphoserine antibodies. Three fractions (29, 30 and 31) were positive to phosphoserine in sugar fed mosquitoes but not in AM fed. This is the first report of a protein whose phosphorylation state is altered in response to a meal.

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OPVE5 - THE *BOOPHILUS MICROPLUS* PROTEIN TYROSINE PHOSPHATASE (BMPTP) CONTROLS VITELLIN DEGRADATION

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In this work we have identified and kinetically characterized a tyrosine phosphatase (BmPTP) in laid eggs of the cattle tick *Boophilus microplus*. The main kinetic properties of this enzyme include an optimum pH of 5.0, strong inhibition by ammonium molibdate, sodium vanadate and cupric sulfate, absence of inhibition by okadaic acid and a Km for pNPP of 0.34 mM. This inhibition by cupric sulfate matches the classification of this enzyme as a tyrosine phosphatase, once this class of enzymes bears a conserved cysteine residue in its catalytic site, which is central to the mechanism of catalysis. When developing eggs were fractionated by gel filtration chromatography the molecular mass determined for BmPTP was 45 kDa. The activity of this enzyme is 4-fold stimulated during embryogenesis. Besides, the profile of phosphotyrosine phosphorylated proteins was also determined and the BmPTP targets were identified. These targets are mainly two vitellin (VT) subunits, VT1 and VT2, the main yolk protein of the egg. These subunits are dephosphorylated *in vivo* during egg development. Next we studied the involvement of BmPTP in the regulation of proteolysis in this system. We verified that ammonium molibdate

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was the most powerful inhibitor of *in vitro* VT proteolysis. Furthermore, it protected from proteolysis the same VT polypeptides that were the targets of BmPTP found either *in vivo* and *in vitro*. Therefore, we propose that BmPTP action on VT engenders it susceptible for further degradation by egg proteases. Since tyrosine phosphatases are classicaly involved in signal transduction, the present study is the first demonstration of such a class of enzymes involved in yolk processing.

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OPVE6 - SEQUENCING OF A MIDGUT-SPECIFIC GENOMIC CHITINASE CLONE FROM *LUTZOMYIA LONGIPALPIS*

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Leishmaniasis are caused by *Leishmania* parasites, transmitted by the bite of infected sand-fly vectors. Current methods for fighting this disease are inefficient, the development of new techniques for vector control being necessary. Little is known about the physiology of digestion and about the sand-fly/ *Leishmania* interaction mechanisms in *Lutzomyia longipalpis*, the main visceral leishmaniose vector in Brazil. In our laboratory we are studying differentially expressed molecules after blood-feeding and infection by *Leishmania*. For that we are using molecular biology techniques as EST sequencing of gut cDNA under various conditions, and identifying differentially expressed gut genes by DDRT-PCR after blood-feeding or infection. One cDNA of interest identified by DDRT-PCR codifies a chitinase with high expression after 72 hours of blood feeding that may have a role in peritrophic matrix degradation. This sequence was used to isolate a genomic clone in EMBL3, that is being sequenced. This will allow the identification of introns and also the possible identification of promoters and regulatory sequences. The gene segment between the two codifying extremes was amplified by PCR. Although the cDNA sequence is approximately 1300bp in length, the fragment obtained had more than 3kb, indicating the presence of introns. This was confirmed by sequencing of the amplified fragment. Flanking regions of the gene are being amplified with specific primers, which anneal to the cDNA's 5' and 3' extremes, paired with random primers which putatively will anneal to regions flanking the gene. Promising PCR products were obtained and are being sequenced, and the sequences are being analyzed by computer programs.

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OPVE7 - IMMUNE RESPONSE AGAINST *LUTZOMYIA INTERMEDIA* SALIVA IN BALB/C MICE.

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Leishmania spp. parasites are transmitted to the vertebrate hosts through the bite of an infected phlebotomine sandfly. When an infected sandfly probes the skin for a blood meal, both its saliva and parasites are injected. Sandfly saliva contains a repertoire of substances with potent immunomodulatory effects and *Leishmania* parasites appear to exploit these effects in order to establish the infection in the vertebrate host. Recent reports have shown that the immunization of BALB/c mice with *P. papatasi* salivary gland components results in protection against leishmaniasis, probably involving a DTH reaction against salivary gland components. Accordingly, we have investigated the immune response generated against salivary gland components of *Lu. intermedia*, the vector responsible for *L. (V.) braziliensis* transmission in Brazil. We immunized mice, in the ear dermis with 1 pair of salivary glands, every 15 days, three times. Fifteen days after the last immunization, mice ears were probed for a DTH reaction which was not observed. However, draining lymph node cell cultures produced high levels of both IFN-γ and IL-4 upon stimulation with salivary glands. In terms of the humoral immune response, antibodies against components of the salivary glands were detected by ELISA and Western blot. Presently, we are evaluating the effects of *Lu. intermedia* salivary glands on human PBMCs and monocytes by in vitro priming assays. These studies could lead to important findings in terms of leishmaniasis pathogenesis and, consequently, to delineate new strategies for vaccine development.

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OPVE8 - SOME APPROACHES TO STUDY THE BLOOD DIGESTIVE PROCESS AND ITS CONSEQUENCES ON *CULEX QUINQUEFASCIATUS* MIDGUT.

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Blood is a nutritional requirement for *Cx. quinquefasciatus* in order to ensue its gonadotrophic cycle. However, blood ingestion is not an harmless process for the mosquito (which risks its life in each meal) neither for its midgut epithelial cells.

A huge distension of the posterior midgut is the first feature after blood feeding (abf), causing a striking flattening of epithelial cells made possible by the stretching of basal lamina and muscular bundles that involve the epithelium. Afterwards, various proteolytic enzymes are secreted (aminopeptidase, trypsin, chymotrypsin-like and an elastase-like, as demonstrated by using specific synthetic substrates and protease inhibitors), some of them probably triggered by an early trypsin-like enzyme. RT-PCR was used to amplify the mRNA for this enzyme from midguts of non blood-fed mosquitoes. Genomic PCR allowed the identification of one chymotrypsin gene, containing a 63 bp intron, with high similarity with the corresponding *Cx. pipiens pallens* sequence.

Using an anti-trypsin antiserum, we can follow the trypsin journey in the midgut during blood digestion. Labelled vesicles as well as enzyme activity are initially detected at 6 h abf; vesicles are abundant at 24 h abf, when activity is increasing and, at 48 h abf, labelling is restricted to the midgut lumen, correlating with the rapid decrease of trypsin activity at this time.

The digestive process, which biochemically ends around 72 h abf, provokes the death of some epithelial cells, mainly by apoptosis. This has never been reported for mosquitoes not infected with some pathogen. Surprisingly, between 76 and 120 h abf several regenerative cells seem to suffer differentiation: they develop an extracellular apical chamber filled with microvilli and with electrondense material. They seem to grow towards the midgut lumen, opening their microvilli chambers to the lumen and becoming typical columnar cells. As far as we know, this is the first time regeneration is described in midgut epithelial cells of adult mosquitoes.

It is known that, while *Aedes* and *Anopheles* are mainly associated with mammals, the members of the *Culex* genus have ornitophagic behavior. It was suggested that the association of *Cx. quinquefasciatus* with mammals is relatively recent (Chevillon et al., Evolution, 49: 997, 1995). This could explain the occurrence of cellular death and regeneration in the midgut after feeding with mammalian blood. Possible causes of both phenomena, such as mechanic stress produced by the stretching and/or toxic effects caused by the ingested mammalian blood, are being investigated in our lab.

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OPVPM1 - PRELIMINARY OBSERVATIONS ON THE MORPHOLOGY OF MURINE (BALB/C) PURIFIED EPIDERMAL LANGERHANS CELLS FOLLOWING INTERACTION WITH *L.*(V.) *BRAZILIENSIS* OR *L. (L.) AMAZONENSIS*

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Langerhans cells (LC) are MHC class II positive antigen-presenting cells that constitute 2-3% of all epidermal cells, which have been demonstrated to stimulate a vigorous T-cell response to *Leishmania (L.) major*. New world cutaneous leishmaniasis is caused by different species, presenting diverse clinical pictures, varying from cutaneous localized to mucous or anergic disease. Using a panning technique, we purified murine (Balb/c) epidermal LC to around 95% purity (pLC). Freshly prepared LC (fLC) presented small, delicate dendrites and the classic Birbeck granules. Parasites of the subgenera *Viannia* and *Leishmania*, which are genetically very distinct, have been suggested to have a possible species-specific response in cell mediated immunity. In this study, we cultured pLC and *L.*(V.) *braziliensis* or *L. (L.) amazonensis* and analyzed their morfology after 12h culture. Using Giemsa stain we detected different morphologic changes on LC after 12h culture on both cultures, LC and *L. braziliensis* or LC and *L. (L.) amazonensis*. After interaction with *L. (V.) braziliensis*, LC became very dendritic when compared to LC cultivated alone. In contrast, after interaction with *L. (L.) amazonensis*, LC became round shaped with a few cells showing some dendrites. These very preliminary results suggest that LC primary response against different species of *Leishmania* could be distinct upon the species involved in the interaction process.

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OPVPM2 - AFRICAN TRYPANOSOME INTERACTIONS WITH THE HUMAN BLOOD-BRAIN BARRIER

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The neurological manifestations of sleeping sickness in man are attributed to the penetration of the blood-brain barrier (BBB) and invasion of the central nervous system by *Trypanosoma brucei gambiense* and *T. b. rhodesiense*. However, how African trypanosomes cross the BBB remains an unresolved issue. Using an *in vitro* blood-brain barrier (BBB) model system constructed of human brain microvascular endothelial cells (BMEC) grown on Costar Transwell™ inserts, we examined the mechanisms used by African trypanosomes to cross the BBB. The human infective *T. b. gambiense* strain IL1852 was found to cross human BMEC far more efficiently than the animal infective *T. b. brucei* strains 427 and TREU 927. For example, *T. b. gambiense* traversal of the BBB by 3 hours was significantly greater than *T. b. brucei* traversal after 16 hours even when 10 times more *T. b. brucei* was used. Equally motile tsetse fly infective procyclic trypomastigotes did not cross even when co-incubated with bloodstream form *T. b. gambiense*. After overnight incubation, the integrity of human BMEC monolayer measured by transendothelial electrical resistance (TEER) was maintained on the inserts relative to the controls when the endothelium were incubated with *T. b. brucei*. However, decreases in TEER were generally observed when the BMEC coated inserts were incubated with *T. b. gambiense*. We also detected early and reversible changes in monolayer integrity with *T. b. gambiense* by measuring real-time resistance with Electric Cell-Substrate Impedance Sensing (ECIS). Light and electron microscopy studies revealed that the trypanosomes initially bind at or near intercellular junctions prior to crossing the BBB paracellularly. We envision that during contact with African trypanosomes that signaling pathways responsible for inducing rapid changes in $[Ca^{2+}]_i$ in human BMEC results in endothelial cell contraction causing tension at human BMEC tight junctions. With the possible help of parasite-associated hydrolases, the trypanosomes then cross through the barrier between the BMEC cells. Further studies are needed to determine the mechanism of BBB traversal by these parasites on the cellular and molecular level.

OPVPM3 - EFFECTS OF BONE MARROW CELL TRANSPLANTATION IN EXPERIMENTAL CHRONIC CHAGASIC MYOCARDITIS

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Chagas' disease is one of the main causes of heart failure in Latin American countries. A progressive destruction of the myocardium occurs in about 30% of *Trypanosoma cruzi*-infected individuals, causing chronic chagasic cardiomyopathy, a disease so far without effective treatment. Syngeneic bone marrow cell transplantation has been shown to cause repair and improvement of heart function in a number of studies in patients and animal models of ischemic cardiopathy. In this work, we studied the effects of bone marrow transplant in a mouse model of chronic chagasic cardiomyopathy. Bone marrow cells injected intravenously into chronic chagasic BALB/c and C57BL/6 mice migrated to the heart and caused a significant reduction in the inflammatory infiltrates and in the interstitial fibrosis characteristics of chronic chagasic cardiomyopathy. The beneficial effects were observed up to six months after bone marrow cell transplantation. Bone marrow cell transplantation did not affect parasitemia or tissue parasitism. A massive apoptosis of myocardial inflammatory cells was observed after the therapy with bone marrow cells. Transplanted bone marrow cells obtained from chagasic mice and from normal mice had similar effects in terms of mediating chagasic heart repair. These results show that bone marrow cell transplantation is effective for treatment of chronic chagasic myocarditis and indicate that autologous bone marrow transplant may be used as an efficient therapy for patients with chronic chagasic cardiomyopathy.

OPVPM4 - BIOLOGICAL BEHAVIOR OF *LEISHMANIA (LEISHMANIA) AMAZONENSIS* ISOLATED FROM DIFFERENT CLINICAL FORMS OF AMERICAN TEGUMENTAR LEISHMANIASIS IN BALB/C MICE

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Leishmania (L.) amazonenis has been considered as the causative agent of wide spectrum of cutaneous leishmaniasis in Brazil: localized cutaneous leishmaniasis (LCL), borderline disseminated cutaneous leishmaniasis (BDCL) and anergid diffuse cutaneous leishmaniasis (ADCL). These different clinical manifestations have been attributed to genetic and immunological differences of the host. Nevertheless, *in vitro* studies have also shown the important role of the parasite in determining of these distinct clinical forms (*Gomes et al. 2000, Medicina 60:71; Gomes et al. 2002, Rev.Soc.Bras.Med.Trop. 35:323*).

This study aims to evaluate the biological behavior of *Leishmania (Leishmania) amazonensis* isolated from different clinical forms of american tegumentar leishmaniasis in BALB/c mice using clinical, parasitological and histophatological criteria.

BALB/c mice (20 animals/group) were inoculated with 10^5 *L. (L.) amazonensis* promastigotes in the hind footpad. The development of lesions was monitored by measuring the footpad swelling using a metric caliper. The lesion progression was evaluated weekly during 98 days post infection (PI). Material from inoculation site was collected at 7 and 12 weeks PI to histopathological analysis and determination of parasite load. For quantification of parasite number, a limiting dilution *in vitro* culture was performed.

The hind footpad swelling was significantly lower in mice inoculated with LCL and BDCL compared to ADCL since 8 weeks PI. Mice inoculated with ADCL strain developed progressive and severe lesion, with a more rapid hind footpads swelling. The histophatological lesion of animals injected with LCL strain showed moderate parasitism and inflamatory infiltrate in the dermis formed mainly by mononuclear cells with few polymorphonuclear cells and focal necrosis. Mice infected with BDCL strain showed parasitism, inflammation and focal necrosis varied between moderate to intense and high number of vacuolized macrophages parasitized. By other side, ADCL strain induced a inflammatory infiltrate constitued only by vacuolized macrophages heavily parasitized. The number of parasites in the lesion from animals injected with ADCL strain was already significantly higher since 7 weeks of infection. The diference among different groups increased substantially at 12 weeks PI.

Our findings demonstrated that the differences observed among the various *L. (L.) amazonensis* isolates concerning to the course of infection and parasite load in the *in vivo* experiments are correlated with the distinct clinical manifestations in human.

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OPVPM5 - ROLE OF ENDOGENOUS INTERFERON-GAMMA IN CPG ODN- OR OTHER ADJUVANTS-ASSISTED IMMUNIZATIONS USING A RECOMBINANT MALARIA VACCINE CANDIDATE.

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Adjuvant selection will be critical for the development of new vaccine formulations as they exert unique influences on the magnitude and quality of immune responses. In spite of their importance, relatively little is known on the mechanisms of action of most adjuvants. In our study, we found that the antibody immune response induced by a recombinant malarial antigen administered in several distinct adjuvant formulations were similar to the one generated by CFA/IFA in terms of magnitude, affinity, IgG subclasses and longevity. In spite of the similar antibody immune responses, the *in vitro* interferon-gamma secretion by immune CD4 T cells from mice that received the adjuvants CpG ODN 1826 or MPL/TDM/CWS was significantly higher. The use of interferon-gamma KO mice identified their essential role in CpG-ODN- or MPL/TDM/CWS-assisted antibody responses. As oppose, the magnitude of the antibody response during QuilA–assisted immunization was not changed in these KO mice. CpG ODN-assisted immunization of IL-4 or IL-12 KO mice provided evidence that, in contrast to interferon-gamma, these cytokines do not interfere with the magnitude of the antibody immune response. Nevertheless, the absence of endogenous IL-4 or IL-12 caused significant shifts in the ratio of specific serum IgG1/IgG2a.

Our results support the notion that endogenous interferon-gamma plays a critical and unexpected role during CpG ODN- or MPL/TDM/CWS-assisted immunizations with a recombinant malaria vaccine candidate.

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OPVPM6 - THE LONG-LASTING PROTECTION CONFERRED BY MUCOSAL IMMUNIZATION AGAINST MURINE LEISHMANIASIS

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The induction of oral tolerance against disease-inducing antigens has emerged as a feasible strategy to prevent immunopathologies. Previously, we found that parenteral administration of adjuvant-free whole *Leishmania (L.) amazonensis* antigens (LaAg) promoted disease aggravation in mice and that not only oral immunization but also nasal immunization with LaAg protected BALB/c mice against homologous infection. In this work, we evaluated the long term duration of the protection induced by mucosal (oral and nasal) vaccination with LaAg against murine cutaneous leishmaniasis. Thus, BALB/c mice received intranasal or intragastric doses of 10 mg or 100 mg LaAg, respectively, and a booster vaccination 7 days later. Controls received PBS. Nineteen weeks after boosting, the animals were infected with *L. (L.) amazonensis* transfected with green fluorescent protein (GFP). The lesion growth was monitored up to 160 days after infection, the parasite loads were measured by fluorimetry and the production of cytokines was measured by ELISA. The results showed that both oral and nasal vaccination maintained significant protection in BALB/c mice even after infected 19 weeks after vaccination, as seen by lower lesion sizes and significantly lower parasite burden as monitored by decreased fluorescence in the infected feet. Both IFN-γ and IL-10 were greatly elevated in mucosa-draining and cervical lymph nodes whereas IFN-γ was prevalent in the peripheral lymph nodes. Nasal LaAg significantly induced in mice a cutaneous hypersensitivity response 19 wks after vaccination. In contrast, oral LaAg impaired the capacity of mice to mount this response. Moreover, since murine intestinal γδ TCR^+ T cells have been associated with mucosal tolerance and that their depletion during oral immunization with i.p. anti- γδ TCR reverted protection, it is tempting to speculate that the protection achieved by oral LaAg is associated with peripheral tolerance. These results demonstrate for the first time the feasibility of using the mucosal route immunization, which is the most convenient and acceptable means of vaccine delivery, to induce long lasting protection against cutaneous leishmaniasis using a crude parasite antigen in the absence of adjuvants.

OPVPM7 - PARTICIPATION OF T-$CD4^+$ AND T-$CD8^+$ LYMPHOCYTES IN THE IMMUNE RESPONSE OF IL-12P40 DEFICIENT MICE INFECTED WITH *LEISHMANIA (V.) BRAZILIENSIS*.

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Even though *L. (V.) braziliensis* is the major causative agent of cutaneous leishmaniasis in Brazil, few studies have used the murine model of this disease probably due to the fact that the majority of the laboratory mouse strains are resistant to this parasite. We used IL12p40 deficient mice in order to better understand the immune mechanisms involved in the control of the infection caused by *L. (V.) braziliensis*. Although IL12p40-/- mice infected by *L. (V.) braziliensis* presented dissemination to liver and spleen, the lesions at the site of inoculation of the parasite did not ulcerate and was partially controlled. This observation suggests the existence of a control mechanism that is independent of IL-12. Based on these observations, we decided to evaluate the role of T-CD4$^+$ and T-CD8$^+$ lymphocytes in the infection of IL-12p40-/- mice infected in the footpad with 1x10^7 promastigotes of *L. (V.) braziliensis*. Our results show that these mice did not present DTH reaction to the parasite antigen and that treatment with a-CD4 antibodies induced smaller footpad lesions, although the parasite load in both the footpad and the spleen was not altered. IFN-γ production by lymph node cells from IL-12p40 -/- mice treated with α-CD4 was similar to untreated mice. On the other hand, IL-4 production by spleen cells was ablated in treated animals. Furthermore, even though NO production was small in all groups, α-CD4- treatment induced increased production of this molecule. Treatment with a-CD8 antibodies induced a decrease in lesion size at the beginning of treatment that equaled the untreated group after 3 weeks. No differences in parasite load at the site of inoculation were observed. On the other hand, spleen parasitism was increased by depletion of CD8$^+$ cells. No differences were observed in IFN-γ and IL-4 production by spleen and lymph node cells between α-CD8-treated and untreated IL-12p40 -/- mice. Surprisingly, an increased NO production was detected in the spleen of CD8 depleted mice. Evaluation of iNOS expression by immunohistochemistry detected a large number of iNOS$^+$ cells in the footpad of infected animals. These results suggest that T-CD4$^+$ cells apparently do not have a role in the lesion control in the footpad. T-CD8$^+$ cells, on the other hand, appeared to be important in the control of parasite growth in the spleen of IL-12p40 -/- mice, but not in the footpad.

Apoio Financeiro:CAPES

OPVPM8 - EVIDENCE FOR IN SITU CELLULAR HYPERACTIVATION IN HUMAN MUCOSAL LEISHMANIASIS

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Human infection with *Leishmania braziliensis* can lead to at least two different clinical forms: cutaneous or mucosal. Despite the clinical outcome, lesions are one of the most important pathologic consequences of the disease. The different types of lesion are related to many factors, including, activation and recruitment of leukocytes to infection site, cytokine microenvironment and effector functions. The evaluation of the immunological profile of circulating cells from individuals with cutaneous and mucosal leishmaniasis have led to the hypothesis that, whereas in the mild cutaneous form a controlled immune response is mounted, an exacerbated immune response is responsible for the intense tissue destruction observed in mucosal patients. In this study, we performed an *in situ* comparative analysis of lesions from individuals with cutaneous and mucosal leishmaniasis, determining the expression of key inflammatory and anti-inflammatory cytokines and their cellular sources, using multiparameter confocal microscopy. We also evaluated the expression of granzyme A, to access cytotoxic activity in the lesions. We observed that lesions from patients with the mucosal form of the disease display a greater inflammatory infiltrate as compared to cutaneous lesions. The cellular infiltrate is predominantly characterized by an increased frequency of CD4$^+$ and CD8$^+$ T lymphocytes. The frequency of CD68+ cells did not change amongst the groups. Analyzing IFN-γ expression, we verified that patients with the mucosal clinical form present a higher number of cells expressing this cytokine when compared to cutaneous patients. Moreover, we observed a positive correlation between the frequency of CD4$^+$IFN$^-$γ+ cells and total IFN$^-$γ expression in both clinical forms, suggesting that CD4$^+$ T cells are important contributors to IFN$^-$ γ expression in cutaneous and mucosal lesions. The number of CD68$^+$TNF-α+ or CD68$^+$IL10$^+$ cells was similar in cutaneous and mucosal lesions. Additionally, lesions from individuals with mucosal leishmaniasis display higher frequencies of granzyme A$^+$ cells than lesions from patients with cutaneous leishmaniasis. Moreover, the commitment of the CD8$^+$ cell population to cytotoxic activity is higher in mucosal lesions, as shown by the higher portion of CD8$^+$granzymeA$^+$ cells within the total CD8$^+$ population. Further analysis of our data also showed that while in cutaneous lesions CD8$^+$ cells are responsible for 70% of the total granzyme A expression, in mucosal lesions, this population contributes to approximately 56% of the total granzyme A expression. This demonstrates that in mucosal lesions a larger portion of non-CD8 cells are responsible for the overall granzyme A expression. Taken together, our data shows evidence of an exacerbated *in situ* cellular immune response in human mucosal leishmaniasis, indicating that a lack of proper immunoregulation is related to pathology.

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