ORAL PRESENTATION
**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 BI NOTES 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 clusteringization (cap3); sequence similarity search and analysis (BLAST, MSPrunch, in-house scripts); protein analysis (GCC); 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://www.dbbm.fiocruz.br/Tcruzidb/index.html>, 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 polypeptide, but not GST alone, showed specific polyprenyl diphosphate synthase enzymatic activity,
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 glycoalex 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 trypanosomes differentiate into procyclic forms a GPI specific phospholipase (GPI-PLC) is upregulated, as well as a metalloprotease, to release the VSG coat (Gruszynski et al., J. Biol. Chem. 252; 24665, 2003). Aiming to find out whether procyclins expressed activities capable of removing the lipid moieties of acylated GPs, we initially used 3H-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 GPs are knowingly detected by their resistance to PI and GPL-PLCs. There are also two other prominent activities processing Gly C in procyclins: a PLA and a PLD. The unusual PLC, being only detected in procyclins and not in bloodstream forms, including GPI-PLC minus mutants, has thus been named Procyclin-PLC (P-PLC).

This novel enzyme, which possibly defines an as yet undiscovered class of PLCs, was also very active towards PI1, the acylated GPI precursor in procyclins, 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 GPs but also on GPI-anchored proteins. At 30°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++ 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 a 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 GPs.

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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 trypanastigote and amastigotes were labeled with heavy (d8) and light (d0) ICAT reagents and subjected to cation exchange and avidin affinity chromatography, followed by LC-MS/MS analysis. Sequence information and expression levels for 42 T. cruzi polypeptides, including metabolic enzymes, parafflagellar 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 trypanastigotes, 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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**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 genome.

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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 199,265). 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 (SbV) have been the first choice drugs against leishmaniasis for more than 50 years. As a consequence of systematic monotherapy over decades, SbV 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 patient and in Balb/c mice), and in vitro (in the amastigote-macrophage system), SbV resistant L. (L.) amazonensis. The BA125 SbV 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 SbV. Lesion growth was then monitored. Leishmania cells were recovered, cosmids extracted and analysed by Sot I restriction. No SbV resistant lesions were generated although more than 2000 cosmids were tested. For each cosmipool, 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-3Á-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 Hydrazole sterol derivative on Leishmania (L.) mexicana promastigotes, whose structure is analogous to an ergosterol intermediary biosynthesis in eucariontis. The promastigotes were cultivated at 26°C in Drossophilida’s-Schneider medium supplemented with 5% inactivated fetal bovine serum. The experiments were carried out at the exponential phase of growth; at 10³ parasites/ml the promastigotes were treated with the sterol derivative 22-hydrazide-imidazol-2-yl-5á-colen-3á-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 analyzed. A preliminary transmission electron microscopic study were conducted with the effective lethal concentration. The compound induced an antiproliferative dose dependent effect. At sublethal doses the parasites showed...
a growth rate reduction, with an inhibitor concentration IC_{50} with 3 mM and an IC_{90} 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 Hidazole concentrations, yielding a MIC with 8 mM (r^2 = 0.92). At the light microscopic level, 10 mM (LD_{10}) of Hidrazine induced immediately motility loss, cellular swelling at one hour treatment, and more severe effect with cell rupture and lysis at 18 hours. Preliminary study at the transmission electron microscope on parasites treated with LD_{10}, 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 ergosterol biosynthesis metabolism blockade. Similar results were previously described on *Trypanosoma cruzi* epimastigotes D^{(3)}G_5 (25) sterol methyl transferase activity, treated with 22.26-azasterol and 24(R,S),25 epiminoalanosterol combined with ketoconazole (Urbina et al., 1995. Mol. Biochem. Parasitol. 73:199-210). Furthermore the potent activity observed with 22-hydrazone-imidazol-2-yl-5a-colen-3a-ol on *L. mexicana* promastigotes are promising to begin in vivo studies on experimental model in the search of new and neccesary alternative to eradicate human leishmaniasis.

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**OPC3 - THE DUAL ACTION OF SB ON THIOI 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 arsenate and antimonials have revealed a potential role for the major thios of Leishmania, trypanothione (N^{8}, N^{6}-bis(glutathionyl)sermidine or T[SH]_{2} and glutathione (γ-L-glutamyl-L-cysteinylglycine or GSH), in the detoxification of these heavy metals. 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. TR has been shown to be sensitive to inhibition in vitro by SB^{43}. These results prompted us to examine the effects of SB on trypanothione metabolism in drug-sensitive Leishmania.

Following exposure to SB^{43} levels of GSH and T[SH]_{2} decreased rapidly to near undetectable levels over 4 h. Subsequent radiolabelling experiments demonstrated that this SB^{43}-induced loss was mainly due to an efflux of glutathione-containing thios from the parasites. In addition to the loss of reduced thiol in SB^{43}-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^{43} in the cell was provided by monitoring the rates of thiol regeneration following transient exposure to the membrane-permeable oxidising agent diamine. In promastigotes cultured in the absence of SB^{43}, levels of T[SH]_{2} and GSH rapidly returned to normal following exposure to diamine. However, in SB-treated promastigotes, reduced thiol levels did not recover. The most likely explanation for this observation is that SB^{43} 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.

References:


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**OPC4 - 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, locally 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 (RPMI), in the presence of the essential oil (30ng/mL), at 37°C. 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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References:


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 cotrimoxazole (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 an antitumor drug causing a decrease of cell proliferation. This drug increases the concentration of topo II-cleaved DNA complex, hence converting an antitumor drug causing a decrease of cell proliferation. This drug increases the concentration of topo II-cleaved DNA complex, hence converting an antitumor drug causing a decrease of cell proliferation. This drug increases the concentration of topo II-cleaved DNA complex, hence converting an antitumor drug causing a decrease of cell proliferation. This drug increases the concentration of topo II-cleaved DNA complex, hence converting an antitumor drug causing a decrease of cell proliferation.

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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 pfcr gene spanning codon 72-76 and further in vivo studies with parasites carriers of the pfcr gene spanning codon 72-76 (a putative transporter associated with resistance to resistance to chloroquine resistance in P. falciparum) and genotyped 15 microsatellites (MS) in four chromosomes in 100 isolates from five different endemic areas of Brazil and Peru (and one from Colombia). The K76T mutation, which is critical for chloroquine resistance, was found in all isolates. Five different pfcr haplotypes, (Sctc)MN7, (Sga7)MN7, CN77, CM77, and CI77), observed four of which have been previously reported from SA isolates, 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 population, 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 a mostfrequent allele carriering the S (Ala7) allele in SA, and represents a detailed analysis of P. falciparum populations with respect to pfcr 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 & Romana, 1998) and induced in vitro (17WTS-17LER) (Nird 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 expression levels of T. cruzi drug-resistant populations/clones when compared with parasites from Asia and Africa. This study provides the first evidence of the Asian pfcrt allele in SA, and represents a detailed analysis of P. falciparum populations with respect to pfcrt haplotypes in the Amazon basin.
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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OPC87 - NOVEL TRYPANOTHIONE ANALOGUES IN TRYPANOSOMA CRUZI

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Trypanosomatids synthesise a unique metabolite trypanothione [N²,N⁶-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, N⁶-acetyl spermidine, N⁶-acetyl spermine) (3). Structures of these novel thiols purified from epimastigotes were determined by bis(glutathionyl)spermine, synthetase (GspS) and trypanothione synthetase (TryS), respectively (1). However in sequential steps, which are catalysed by two distinct enzymes, glutathionylspermidine

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About 60% of the Sao 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 10⁵ 255 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 10⁶ 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 °C and 5% CO₂, cells were collected and centrifuged at 300g in PBS with 0.05% sodium azide and 1% fetal calf serum, and were incubated for 30 minutes at 4°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 °C until mRNA extraction. Extracted mRNA was submitted to RT-PCR with oligo primers (Oligo dT 12-18) 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.

This work was supported by FAPESP (99/04926-4), LIMHCFMUSP-49, CNpq and CAPES.

OPIM2 - A FACTOR PRODUCED BY PLASMODIUM YOELII INFECTEDERYTHROCYTES MODULATES DENDRITIC CELL FUNCTION

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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 expression of CD83 and CD86 and a decrease in IL-12 production. We also show that this soluble factor(s) induces DC dysfunction as measured by a decrease in the production of IFN-γ and TNF-α. Finally, we show that this soluble factor(s) inhibits DC maturation and function in vivo using a murine malaria model. These findings suggest that Plasmodium yoelii-infected erythrocytes produce a soluble factor(s) that inhibits DC maturation and function.

References:

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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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ORAL PRESENTATION
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-κB 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 CDN 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 hypersensitivity (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. DTH 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 104, 105 or 106 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 106 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 PGE2, 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 Th1 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 Th1 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 aliphithoid 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 DXS5+ 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 trypanomastigotes, 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.
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. Immunological 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, P3; 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 suggest 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 αGPI-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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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 αGPI-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.

Supported by: CNPq, FAPEMIG, Fundação Oswaldo Cruz (PAPES III), WHO.

**OPIM9 - IDENTIFICATION OF PDZ5, A CANDIDATE UNIVERSAL MINICIRCLE SEQUENCE BINDING PROTEIN OF TRYPANOSOMA CRUZI**

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This protein is called Universal Minicircle Sequence Binding Protein - UMSBP, and is thought to recruit other proteins responsible for the replication itself. Homology with have named PDZ5, which presents a great percentage aminoacid sequence eukaryotes and do not fold into visible chromosomes. Besides, the nuclear envelope filaments, but surprisingly, these filaments are less compact than in higher organisms. 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 immunolocalization 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 Chromosome (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 cyclindependent protein 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 C2H2 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 nucleosome 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 (Stromatin Conservative Domain), composed of about 90 aminoacids, is located. Ecol1 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 - TCRBP1 AND TCRBP2: 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, Thp34 and Thp37, previously reported by Zhang and Williams, 1997. The predicted proteins were named TbRBP1 and TbRBP2 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 Thp34 and Thp37, 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 immunofluorescence assays localize the RBP genes in the cytoplasm of the parasite cells suggesting. Both TbRBP 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
not efficiently degraded in either epimastigotes or amastigotes to the short interfering RNAs required for the RNAi pathway, and the level of amastin miRNA remained unchanged. We generated a strain of *Trypanosoma cruzi* CL-Brenner 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 *Trypanosoma cruzi* endogenous genes (encoding α-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* α-tubulin RNAi plasmid did give the expected FACS 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 chronic 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 murine 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 D7 region of the 24sgRNA 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 *T. cruzi* I and Col7.7G2. We found a perfect correlation between JG profile (rDNA group I, *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.

Financial support: FAPESP, CNPq and WHO

**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, leishmaniasis. 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. (V.) braziliensis*, *L. (V.) guyanensis*, *L. (V.) naffi*, *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. braziliensis*, *L. guyanensis*, *L. shawi*, *L. lainsoni* and *L. panamensis* reference strains’ DNA. Also included were two *L. 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 10^3 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. These 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 to 10 parasites. Both probes were negative with *L. (L.) amazonensis*, *L. (L.) infantum*, *L. (S.) adleri*, *Tcruzi 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.**

Cardoso, J.; Pavoni, D.P.; Probst, C.M.; Correa, A.; Ávila, A.R.; Monteiro-Góes, V.; Arauco, P.R.C.; Goldenberg, S.; Krieger, M.A.

Cardoso, J.1; Pavoni, D.P.1; Probst, C.M.1; Correa, A.1; Ávila, A.R.1; Monteiro-Góes, V.1; Arauco, P.R.C.1; Goldenberg, S.1; Krieger, M.A.1
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 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 determined. DNA of Silvio and CL Brener indicated that 44 targets (6%) have different gene expression. DNA microarrays were hybridized with total RNA of parasite strains and developmental stages. Microarrays allowed 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: CNPq, 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 understand 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 pL/pair/mice) 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.

This work was supported by CAPES, Fiocruz.

OPVE2 - COULD THE ANALGESIC 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
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 sciatric 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 sciatric 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 experiments using 40ml of ultrafiltered material, the reduction of the CAP amplitude was of 87.1 ± 2.3 % of the control value thirty minutes after washing. It is believed that haematomaphagy 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.

Supported by: CNPq and FAPEMIG.

**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 response 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 37°C. 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 predominant 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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**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 column (Salerno, 1996).

Salerno et al (2001) demonstrated 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 synthesized 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 intracelular vesicles. The role of ubiquitin associated with a specific population of VT is discussed.

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

This research is sponsored by: GORGAS/ASTMH, IFS, FAPERJ and CNPq.

**OPVE6 - SEQUENCING OF A MIDGUT-SPECIFIC GENOMIC CHITINASE CLONE FROM \textit{LUTZOMYIA LONGIPALPIS}**

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Leishmaniasis are caused by \textit{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/\textit{Leishmania} interaction mechanisms in \textit{Lutzomyia longipalpis}, the main visceral leishmniase vector in Brazil. In our laboratory we are studying differentially expressed molecules after blood-feeding and infection by \textit{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 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 \textit{LUTZOMYIA INTERMEDIA} SALIVA IN BALB/C MICE.**

de Oliveira, CI; Oliveira, F; Miranda JC; Miranda D; Passos E; Barral, A; and Brodskyn, C

\textit{Leishmania} spp. parasites are transmitted to the vertebrate hosts through the bite of an infected phlebotome sandfly. When an infected sandfly probe 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 \textit{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 \textit{F. 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 \textit{Lu. intermedia}, the vector responsible for \textit{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 \textit{Lu. intermedia} salivary glands on human PBMCs and monocytes by \textit{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.

Supported by: FAPESP, CNPq and TMRC-NIH.

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


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Blood is a nutritional requirement for \textit{Cx. quinquefasciatus} in order to ensue its gonadotropic 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 \textit{C. 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 \textit{Aedes} and \textit{Anopheles} are mainly associated with mammals, the members of the \textit{Culex} genus have ornithopaglic behavior. It was suggested that the association of \textit{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).

Using Giemsa stain we detected different morphologic changes on LC after 12h culture on both cultures, LC and L. (V.) braziliensis or L. (L.) amazonensis and analyzed their morphology 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 cultured 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 trypanosomes were shown to be able to cross the BBB by crossing the intercellular junctions. With the possible help of parasite-associated hydrolases, the trypanosomes then pass 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 TEGUMENTARY LEISHMANIASIS IN BALB/C MICE

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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...
Leishmania (L.) amazonensis 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 anergic 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 histopathological 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 for 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 histopathological lesion of animals injected with LCL strain showed moderate parasitism and inflammatory 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 in the dermis formed mainly of parasite number, a limiting dilution in vitro culture was performed.

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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OPVM5 - 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 opposed, 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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OPVM6 - 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 transplanted 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.
CD4+ and T-CD8+ lymphocytes in the infection of IL-12p40-/- mice infected in
of IL-12. Based on these observations, we decided to evaluate the role of T-


braziliensis


lesions a larger portion of non-CD8 cells are responsible for the overall granzyme


Expression, in mucosal lesions, this population contributes to approximately


Effector functions. The evaluation of the immunological profile of circulating


lesions are one of the most important pathologic consequences of the disease.


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.


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 demonstration 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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