POSTER

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BQ1 - CLONING AND CHARACTERIZATION OF DNA POLYMERASE BETA FROM *TRYPANOSOMA CRUZI*

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The DNA polymerase beta (DNA pol b) plays a central role in base excision repair and could also interfere in other aspects of DNA repair and metabolism when overexpressed in mammalian cells. Nothing is known about the role of this enzyme and its molecular significance for the *Trypanosoma cruzi* biology. We have cloned and sequenced the *DNA pol b* from this organism. The *TcDNA pol b* sequence has 66% homology with the *Chritidia fasciculata* and *Leishmania (L.) infantum DNA pol b* genes. The *TcDNA pol b* is able to clomplement the *Escherichia coli* clone SC18-12 deficient in the *polA*$^{-}$ gene. The expression of *TcDNA pol b* in those bacterial cells restores their ability to grow at 42ºC and diminishes their sensibility to U.V. radiation. In addition, the overexpression of *TcDNA pol b* in CHO cells provokes an increase in the cells mutation rate in the 6-TG resitence assay. The TcDNA pol b protein has been expressed in *E. coli* in fusion with MBP and purified in a maltose column. The *TcDNA pol b* fusion protein was capable to add the dNTPs to primer in a DNA extension assay. Futhermore, this enzyme was used an assay of ^{3}H-dTTP incorporation measured by CPM counting to characterize its behavior in relation to dNTPs concentration. The enzyme works in optimal concentration of 5mM of dNTPs. This same assay has been used to demostrate that this enzyme has the ability to incorporate the modified nucleotide AZTTP to DNA molecules. So, we have cloned and characterized the *DNA pol b* gene from *Trypanosoma cruzi* and have started biochemical characterization of this gene product.

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BQ2 - THE TELOMERASE REVERSE TRANSCRIPTASE COMPONENT (TERT) OF *LEISHMANIA* SPP.: BIOCHEMICAL CHARACTERIZATION AND GENE CLONING

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Telomeres are the protein-DNA complexes that protect linear chromosomes. In *Leishmania* spp. the telomeric DNA is composed by the conserved TTAGGG repeated sequence and is replicated by the action of telomerase. Telomerase is a multisubunit enzimatic complex, composed by a reverse transcriptase component (TERT), an intrinsic RNA molecule (TER) and associated proteins. It ensures the complete DNA replication by adding new telomeric sequences at the G-rich strand. The enzyme also works as part of the high order complex that regulates the capping/uncapping telomere states.

This work has two goals: i) to characterize the *in vitro* enzymatic activity of *Leishmania* telomerase and ii) to study the roles played by the TERT component in *Leishmania* life span. The main objective is to discover if telomerase can be a good target for future anti-parasite therapy.

To achieve the first goal, protein extracts of *L. (L.) amazonensis* with telomerase activity were purified using complementary chromatographic methods. Enzyme activity was tested in each purification step by using the "Two-tube TRAP" assay. The preliminary results showed that enzyme activity is found in fractions purified by anion exchange and heparin affinity chromatography. However, the activity is greatly enriched after affinity purification using a 2´-*O*-methylphosphoramidite oligoribonucleotide (Lingner & Cech, 1996). The 2´-*O*-methyl oligoprobe is complementary to the TER template sequence used by telomerase to copy the telomeric repeats, acting as a specific ligand to purify telomerase. In this case, the enzyme is eluted in native form using a displacement oligonucleotide complementary to the oligoprobe. The conventional telomerase assay is being standardized to study the enzyme catalyses features.

For the second goal, a search for sequences sharing similarities with conserved TERT domains, at the public *Leishmania* Genome Project database, resulted in a sequence from a non-assembled contig of chromosome 36 of *L. (L.) major*. A cloning strategy based on the design of primers from this putative *L. (L.) major* TERT (LmTERT) was used to amplify by PCR *L. (L.) amazonensis, L. (L.) major* and *L. (V.) braziliensis* DNA and *L. (L.) amazonensis* cDNA. PCR products of ~2.33 Kb, which correspond to two thirds of *L. (L.) major* sequence (~3.3 Kb), were amplified from all samples. The putative LaTERT, LmTERT and LbTERT share high sequence similarity, suggesting that in *Leishmania,* telomerase is a housekeeping gene. The chromosomal map of LaTERT by pulsed field gel electrophoresis, showed that it hybridized only with chromosome 36 of *L. (L.) amazonensis*, indicating that LaTERT is present in a low copy number and shows chromosome sinteny with *L. (L.) major*. RACE-PCR reactions are being standardized in order to complete the gene sequences. The genomic organization of *Leishmania* TERT and the expression of the gene during the parasite life cycle are underway.

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BQ3 - CHARACTERIZATION OF A CONSTITUTIVE NITRIC OXIDE SYNTHASE FROM *LEISHMANIA (V.) BRAZILIENSIS* AND *L. (L.) CHAGASI* PROMASTIGOTES: EFFECT OF SPECIFIC INHIBITORS

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Nitric oxide ($NO^{\cdot}$) is a free radical derived from molecular oxygen and the guanidine nitrogen of L-arginine. NO is involved in a variety of biological functions in different cells and is an important anti-microbial effector molecule in macrophages against intra- and extra cellular pathogens. The $NO^{\cdot}$ production is catalyzed by the enzyme NO synthase (NOS), which is presented at least in two types. One (with two subtypes) is a Ca^{2+}/calmodulin-dependent that is constitutively found in the endothelium (eNOS or NOS III) and in neuronal tissue (nNOS or NOS I). The other, is inducible (iNOS or NOS II) in vascular tissues, smooth muscle cells, neutrophils, hepatocytes and macrophages, is Ca^{2+} -independent. There are few data in literature in relation to NO produced by *Leishmania* parasites, and thus, the study of NO pathway in these parasites can shown important data in reference to metabolic steps as possible target of anti-*Leishmania* drugs. A NOS was already purified from *L. (L.) donovani* and *L. (L.) amazonensis* promastigotes, but the biological significance, as well as the relationship with the macrophage pathway continues being unknown (Basu e cols., 1997: Genestra e cols., 2003a). Results of our laboratory demonstrated the NO pathway in *L. amazonensis*, that is essential for the infection establishment in murine macrophages (Genestra e cols., 2003b) and thus, in this work we decided to assay some L-arginine analogs already described as inhibitors of NOS. Parasites (*L. (V.) braziliensis*/MHOM R616 strain and *L. (L.) chagasi*/ MHOD P142) were grown in Schneider´s medium supplemented with 10% of fetal calf serum at 26°C/pH 7.2 in 24-well plates (5 x 10^5 cells/well). The group test (triplicate) was grown in the presence of the following L-arginine analogs N-nitro-L-arginine (L-NNA); N-nitro-L-arginine methyl esther (L-NAME) and D-arginine. Other alternative includes one mixture of L-NAME with L-arginine

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and/or EGTA 40 mmol/L. After incubation of 12 to 148 hours, the supernatants were used to assay the NO production by Griess reaction. The absorption was determined at a wavelength of 540 nm and the NO_2^- (a byproduct of NO) concentration in samples was determined using a standard curve of $NaNO_3$ (0,009 to 100 mmol/L) in medium. The results pointed to a significative decrease on NO production by L-NAME. Furthermore, the presence of exogenous L-arginine did not increased the NO/NO_2^- concentration, and these substrate also did not reverts the effect of L-NAME, confirming the irreversibility activity of L-NAME on NO production. Additionally, immunofluorescence assays using antibody anti cNOS demonstrated a strong immunolabeling, in comparison with a antibody anti-iNOS.

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BQ4 - CHARACTERIZATION OF THE PHOSPHATIDYLSERINE SYNTHASE II CODING GENE OF *LEISHMANIA (L.) AMAZONENSIS.*

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The life cycle of the protozoan *Leishmania* is well known. It comprehends alternative cycles in the insect and the mammalian host. However some aspects of the infection of the mammalian macrophage remain to be elucidated. It has been sugested that parasite lipid organisation can play a role in the phagocytic process and on the ability to survive in the host organism. Phosphatidylserine (PS) on the exoplasmic leaflet of the plasma membrane could be one of the signals delivered by amastigotes to inhibit the antileishmanial activity of macrophages (Balanco et al., 2001). The phosphatidylserine synthase II (PSS II) is an enzyme that exchanges the headgroup of phosphatidylethanolamine from etanolamine to serine.

The main objective of the present comunication is to isolate the complete open reading frame of PSS II from *L. (L.) amazonensis*. For that, conserved regions of the PSS II were determined by the alingment of PSS II aminoacid sequence from some phylogenetically distant organisms (*Mus musculus, Anopheles gambiae, Arabidopsis thaliana, Caenorhabditis elegans, Drosophila melanogaster, Homo sapiens* and *L. (L.) major*), obtained from GenBank. Primers were designed based on these conserved regions using the *L. (L.) major* genomic data. PCR was performed using genomic DNA from *L. (L.) amazonensis* as a substrate and the expected fragment of about 700bp was obtained.

Reverse-transcription-PCR was then performed using total RNA purified from promastigotes. The 5' and 3' regions of the PSS II coding gene were obtained, the fragments were cloned in pGEM-T easy and sequenced. The identity of the sequences in GenBank was then assured with the BLASTx program. The degrees of similarity were 37% identity and 55% positives, in relation to the human PSS II. The whole nucleotide sequence of PSS II ORF of *L. (L.) amazonensis* was then analysed to define the strategies to knock-out the gene to enable us to study its role in macrophage infection.

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BQ5 - COMPARATIVE INHIBITORY STUDIES BETWEEN RECOMBINANT FORMS OF THE HUMAN AND *LEISHMANIA (L.) MEXICANA* GLUCOSE-6-PHOSPHATE ISOMERASE

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Glucose-6-phosphate isomerase (formerly called phosphoglucose isomerase; PGI; E.C. 5.3.1.9) is an intracellular enzyme that catalyses the reversible reaction of D-glucose 6-phosphate (G6P) to D-fructose 6-phosphate (F6P). The native *Leishmania* PGI is a homodimer molecule of 60 kDa per monomer with 47% sequence identity when compared to the human PGI. It has been shown to be present both in the cytosol and in the glycosome of *Leishmania* promastigotes. The present work describes the purification of two *Escherichia coli* expressed *L. (L.) mexicana* PGI constructs, one corresponding to the natural protein and the other to an N-terminally deleted form. The function and structure of this N-terminal segment is still unclear, but it may be related to its glycosomal localization. Four known high-energy intermediate analogue inhibitors of PGIs from *T. brucei*, *Bacillus stearothermophilus*, yeast, and/or rabbit muscle were evaluated on both recombinant human and *L. (L.) mexicana* PGIs for comparison purposes. Although the IC_{50} values obtained are 1 to 2 orders of magnitude higher than the corresponding K_i values obtained for other PGIs, probably because of the different conditions used, these compounds represent, to our knowledge, the first inhibitors ever evaluated on *L. (L.) mexicana* PGI.

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BQ6 - *TRYPANOSOMA CRUZI* EXPOSURE TO HYDROGEN PEROXIDE: EFFECTS ON GLUCOSE-6-PHOSPHATE DEHYDROGENASE AND TRYPAREDOXIN PEROXIDASE

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Trypanosoma cruzi has an effective and complex system to deal with oxidative stress. Different pathways are involved in detoxifying hydroperoxides showing different sub-cellular sites and substrate specificities. In this intricate net, that converges to trypanothione, the proteins involved, cytosolic tryparedoxin peroxidase (TcCPX) included, act in concert to mediate transfer of reducing equivalents to the hydroperoxide having NADPH as the initial donor. By its turn, NADPH is produced by glucose-6-phosphate dehydrogenase (G6PD) and 6-phosphogluconate dehydrogenase (6PGD) in the pentose phosphate pathway and is involved in antioxidants pathways and reductive biosynthesis.

In the present communication the G6PD activity / expression and TcCPX expression / steady state protein level were evaluated in two *T. cruzi* strains, submitted to hydrogen peroxide (H_2O_2) treatment for different periods of time. Among other distinct biological properties, these strains have different resistance to oxidative stress generated by H_2O_2. The Y strain is less resistant to oxidative stress in contrast to the resistance showed by Tulahuen 2. Epimastigotes (5.2×10^6 cells/ml) in early stationary phase, were incubated in PBS in the presence of 50mM H_2O_2 for 30, 90 or 150 min. Cells were then collected by centrifugation and aliquots were separated for G6PD activity determination, Northern and Western blotting analysis. In Y strain, no significant changes in G6PD activity were observed except when glucose was added to the incubation medium. On the other hand, Tulahuen

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2 G6PD activity and mRNA expression could be modulated by oxidative stress. Upon 30 and 90 min incubation in the presence of the oxidant, G6PD activity increased in 40 and 60%, respectively, in relation to control. 6PGD activity remained unchanged for all times tested. mRNA levels were altered and upon 150 min incubation, an increase of 72% was observed. In the beginning of treatment no correlation could be established between G6PD and TcCPX. Western blotting analysis of TcCPX showed, after 30 min, a 38% decrease in TcCPX protein level. After 90 min mRNA levels decreased and protein expression increased. These results indicate that under oxidative stress conditions, enzymes that work in correlated pathways can be coordinately modulated. The higher G6PD activity observed in Tulahuen 2 epimastigotes and the ability of these cells to respond to H_2O_2 simultaneously with changes in TcCPx expression / protein can contribute to the higher resistance to oxidative stress and also to the higher proliferation index observed in these cells when compared to the other strain.

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BQ7 - CHARACTERIZATION OF PRENYLATED AND DOLICHYLATED PROTEINS IN *PLASMODIUM FALCIPARUM*

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Malaria is one of the major public health problems in the world, killing over a million people each year (most of the victims are children) and infecting around 500 million (World Health Report, 2001). Therefore new drugs are urgently required for its control, since many *Plasmodium falciparum* strains have developed multi-drug resistance. Recently, it was demonstrated that the inhibition of the isoprenoid pathway (Jooma *et al*, Science 285, 1573-1576, 1999), responsible for the production of isoprenoid products, such as dolichol and isoprenic chains attached to certain proteins and coenzyme Q's isoprenic chain, could be an excellent target for the development of antimalarial drugs.

Recently Jooma *et al*. (Science 285, 1573-1576, 1999) demonstrated the presence of an isoprenoid biosynthesis pathway, the MEP pathway, in *P. falciparum* and two drugs (Fosmidomycin and FR-900098) that inhibit this pathway. Studies in our laboratory using drugs that inhibit downstream of Isopentenyl-PP (IPP) in isoprenoid pathway have been demonstrated good results, confirming this pathway as potencial target for develop new antimalarial drugs.

Other important points related about isoprenoid pathway are post-translational farnesylation, geranylgeranylation and dolichylation of protein. Prenyl modification appears necessary for the biologic activity of several proteins involved in cell cycle control, including the expanding family of *ras*-related small GTP-binding proteins (Cohen *et al*, Biochem. Pharmac., 60, 1061-1068, 2000). Our group demonstrated the existence of protein prenylation in *P. falciparum* (Moura *et al*, Antimicrob. Agents Chemoter. 45, 2553-2558, 2001) and the presence of coenzyme Q´s isoprenic chain (de Macedo *et al,* FEMS Microbiol. Lett., 207, 13-20, 2002). Couto *et al* (Biochem. J., 341, 629-637, 1999) also demonstrated the presence of dolichol of 11 and 12 isoprenic chain in the intraerythocytic forms of *P. falciparum.*

This work aimed to characterize the prenylated and dolichylated protein in the diferents intraerythocytic forms of *Plasmodium falciparum*, using the ESI-MS and QTOF mass spectrometry techniques, onto to characterized the isoprenic chain attached to this proteins.

We identified some proteins in *P. falciparum* cultures metabolic labeled with [1-(n)-^{3}H] farnesyl pyrophosphate ([^{3}H]FPP) or [1-(n)-^{3}H] geranylgeranyl pyrophosphate ([^{3}H]GGPP). When cultures were labeled with [^{3}H]FPP, we identified labeled proteins with molecular masses of approximately 14 kDa, 21-24 kDa and 50 kDa. When cultures were labelled with [^{3}H]GGPP, we identified labelled proteins with molecular masses of approximately 14 kDa and 21-24 kDa. Analysis of the isoprenic chain attached to this proteins, utilizing mass spectrometry technique (ESI-MS and QTOF), demonstrated the presence of Farnesyl group attached to proteins of 21-24 kDa and 50 kDa, Geranylgeranyl group attached to proteins of 21-24 kDa and dolichyl group attached to proteins of 21-24 kDa.

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BQ8 - THE METHYLERYTHRITOL PHOSPHATE PATHWAY IS FUNCTIONALLY ACTIVE IN ALL INTRAERYTHROCYTIC STAGES OF *PLASMODIUM FALCIPARUM.*

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Plasmodium falciparum synthesizes isoprenoids via a methylerythritol phosphate (MEP) pathway. Recently the first two enzymes, the PfDOXP*synthase* and PfDOXP*reductoisomerase,* were cloned and it was shown that the pesticide Fosmidomycin, a specific inhibitor of DOXP*reductoisomerase,* inhibited growth of parasite both *in vitro* and *in vivo*. In order do characterize other target enzymes of the same pathway we first demonstrated the metabolic intermediates downstream of DOXP by HPLC and Q-Tof analysis. When testing intermediates in the presence of Fosmidomycin, the levels of MEP decreased while DOXP accumulated, as expected. To our knowledge, this is the first report showing by direct biochemical detection that the MEP pathway is functionally active in all intraerythrocytic forms of *P. falciparum*. Besides, the inhibitory effect of Fosmidomycin on the isoprenoid biosynthesis (dolichols and ubiquinones) was dependent on the developmental stage: While inhibition of MEP production was observed in ring, trophozoite and schizont stages, the dolichol and ubiquinone synthesis was only affected in trophozoite and schizont stages, but not in ring stage. These results indicate that the parasite may have mechanisms to substitute isoprenoid precursors from the MEP pathway.

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BQ9 - *TRYPANOSOMA CRUZI* PROLINE RACEMASE MUTANTS : STRUCTURAL AND FUNCTIONAL STUDIES

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A recently described enzyme, the first eukaryotic proline racemase, is secreted by the infective forms of *Trypanosoma cruzi*. This enzyme, *Tc*PRAC, displays a mitogenic activity towards host B cells that become polyclonally activated contributing to parasite escape and persistence inside the host. The integrity of the active site would seem critical for the mitogenic activity of the parasite released enzyme. The peptide sequence of *Tc*PRAC presents a significant homology with sequences encoding putative proline racemases in microorganisms of agricultural and medical interest. The catalytic mechanism of the proline racemase, previously only described for the protobacterium

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Clostridium sticklandii, predicted that two cysteine residues, one from each homodimer subunits, are the catalytic residues responsible for the racemization of proline enantiomers. However, the comparative analysis of peptide sequences of the *Tc*PRAC and the one of diaminopimelate epimerase monomeric enzyme (DapE), suggested that another cysteine residue upstream of the active site could as well be involved in the reaction mechanism of racemization. Using molecular, biochemical and immunological studies, we have compared in the present work wild-type *Tc*PRAC and recombinant proteins mutated for key cysteine residues located both within and outside the active site of the enzyme. We show that *Tc*PRAC possesses two functional active sites per homodimer, thus challenging the previous proposed reaction mechanism. We propose here a new enzymatic mechanism for proline racemases and present evidences that the enzymatic and mitogenic properties of *Tc*PRAC are dissociated. In this context, we show that *Tc*PRAC in presence of specific inhibitors presents a different folding that certainly prevents the correct triggering of host B cells. The identification and characterization of essential putative proline racemases in other microorganisms may allow the broad use of potential therapeutic inhibitors of *Tc*PRAC.

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BQ10 - ARGININE KINASE OVEREXPRESSION IMPROVES *TRYPANOSOMA CRUZI* SURVIVAL CAPABILITY

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Arginine kinase catalyzes the reversible transphosphorylation between phosphoarginine and ADP. Phosphoarginine is a metabolite that is involved in temporal and spatial ATP buffering as well as inorganic phosphate regulation. Recently, we demonstrated that arginine kinase is present in the parasites *Trypanosoma cruzi* and *Trypanosoma brucei*. In turn, this enzyme is absent in their mammalian hosts. In this work we establish a relationship among the homologous overexpression of the *Trypanosoma cruzi* arginine kinase and the ability of the transfectant parasite cells to grow and resist stress conditions. Using the novel expression vector pTREX it was obtained more than one hundred-fold overexpression of the *T. cruzi* arginine kinase activity. The stable-transfected parasites showed an increased cell density since day 10 of culture, which resulted about 2.5-fold higher than the control group on day 28. These results suggest an improved capability of the transfected parasites to survive in a nutrient-depleted medium. Additional stress conditions were tested by incubating the parasites in different media. Arginine kinase transfected parasites revealed about 54% of increase in the cell number growing in Triatomine Artificial Urine medium, 91% in alkaline PBS and 79% in conditioned medium. We propose that arginine kinase and phosphoarginine are involved in the regulation of the *Trypanosoma cruzi* growth and its adaptation to environmental changes.

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BQ11 - MOLECULAR CLONING AND EXPRESSION OF THE *TRYPANOSOMA CRUZI* METHYLTHIOADENOSINE PHOSPHORYLASE GENE.

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Trypanosomatids have an absolute requirement for purines because they lack the machinery to synthesize their own purine ring, *de novo* synthesis. Purines serve as precursor molecules for DNA and RNA, as carriers of high-energy phosphate bonds, as constituents of coenzymes and as modulators of certain enzymes. Purines are found and available to these parasites as nucleobases, nucleosides, charged nucleotides, or polymers in nucleic acids. The methylthioadenosine (MTA), a byproduct from the polyamines production, is cleaved by a specific MTA phosphorylase (MTAP) into adenine and methyl-thioribose-1-phosphate (MTR1P). Adenine is converted to adenine nucleotides (purine salvage pathway) and MTR1P intermediate can be recycled into methionine. This pathway is potentially exploitable for chemotherapy target in protozoan parasites because of the needs of pre-formed purines. The MTAP from *Trypanosoma cruzi* has been poorly studied and needs better characterization to evaluate the possibility of its use as a drug target. Our objective is to characterize molecularly and functionally this enzyme. For this, we identified its gene that shows high identity with MTAPs from different organisms. It is present as a single copy per haploid genome of the parasite and expressed in all of its forms. To obtain active rMTAP successfully in *E. coli*, the full-length proteinase gene ORF was inserted into the pET-19b expression vector to generate an N-terminal His-tagged recombinant protein. The recombinant protein was then purified from soluble and insoluble fractions utilizing a column charged with nickel. The recombinant MTAP showed approximately a 33 kDa protein in SDS-PAGE both reducing or non-reducing conditions. The antibodies raised against the recombinant protein specifically recognized the native MTAP in immunoblots with total protein extract from trypomastigote, epimastigote or amastigote forms of *T. cruzi*. The rMTAP from the soluble fraction was shown to be fully active on MTAP substrate. The assay of rMTAP activity was done coupling the enzymatic reaction with xanthine oxidase, which converts free adenine to 2,8-dihydroxyadenine. The expression of an active recombinant allows a better inhibitor screening and the production of MTAP crystals to determine the three-dimensional structure. The complete *T. cruzi* MTAP characterization can elucidate its relevance to the metabolism of the parasite as well as the evaluation of its potential as a drug target.

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BQ12 - CHARACTERIZATION OF ECTO-ENZYMES IN SHORT AND LONG EPIMASTIGOTES OF *TRYPANOSOMA RANGELI.*

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Trypanosoma rangeli is transmitted through triatominae vectors, during a blood meal on man and other mammals. It develops predominantly as short epimastigotes forms in the gut of the insect, invades the haemocoel and a few days after infection they disappear to be replaced by a massive colonization by long epimastigotes forms. The long epimastigotes survive in the haemolymph and/or to get into the haemocytes, migrate to and complete their development in the salivary glands where the metacyclogenesis (trypomastigote formation) takes place. Data already published, demonstrated a higher defense reaction when short, but not long epimastigotes of *T. rangeli* were inoculated into the haemolymph of the insect vector, *Rhodnius prolixus*.

Surface membrane interactions between parasites and their host cells are of critical importance for the survival of the parasite. The plasma membranes of cells contain enzymes whose active sites face the external medium rather than

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cytoplasm. The activities of these enzymes, referred as ecto-enzymes, can be measured using living cells. The regulation of the complex interactions required for trypanosomatidae differentiation and proliferation is mediated in part by protein phosphorylation. In several protozoa parasites ecto-phosphatase have been described, although the physiological role has not been well established, it seems to be involved with nutrition and cell differentiation. Many enzymes working together are responsible to the phosphate transport. Phosphatases and ATPases are examples of these enzymes. The ATP hydrolysis offers energy for to get phosphate into the cells.

In the present study we described experiments about the development of short and long epimastigotes at different phosphate concentrations in LIT medium and also analized ecto-phosphatase and ecto-ATPase activities on the surface of *T. rangeli*. The observed results were: (i) short epimastigotes requires higher phosphate concentrations than long epimastigotes; (ii) both ecto-phosphatase activities Mg^{2+}dependent and independent from short epimastigotes dephosphorylated more efficiently *p*-nitrophenylphosphate (*p*-NPP) as well as b-glycerophosphate than long epimastigotes; (iii) in addition, ecto-ATPase activity of short epimastigotes was greather than long epimastigotes; (iv) the inorganic phosphate determination in the haemolymph of *Rhodnius prolixus* showed a low concentration of this compound. These findings will be discussed on the light of the knowledge of *T. rangeli* development in the triatominae vector.

This work was supported by CNPq and FAPERJ.

BQ13 - CHARACTERIZATION AND IMMUNOLOCALIZATION OF AN ATP-DIPHOSPHOHYDROLASE OF *TRYPANOSOMA CRUZI* AND ITS POSSIBLE ROLE IN VIRULENCE PROCESS

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In this work an ecto-NTPDase insensitive to inhibitors of other ATPases and phosphatases has been characterized on the surface of *Trypanosoma cruzi* intact parasites (strains Y and Be-78). The enzyme exhibits a broad substrate specificity for nucleotide hydrolysis, typical for the family of ATP-diphosphohydrolases, which are known to be present on the surface of other endoparasites such as *Schistosoma mansoni*, *Toxoplasma gondii* and *Leismania amazonensis*. Antibodies against a fusion protein from *T. gondii* ATP-diphosphohydrolase immunoprecipitated a single 58 kDa protein from ^{35}S-methionine labeled *T. cruzi* parasites, confirming that *T. cruzi* enzyme possesses epitopes which are common to the family of ATP-diphosphohydrolases. Epimastigotes (non-infective form) and tripomastigotes (infective form) from strain Y showed different ATP/ADP hydrolysis, infective form showed a preference for ATP and non-infective form hydrolyzed both nucleotides at the same rate. Confocal fluorescence microscopy analyses localized the NTPDase on the external surface of all forms of parasite, with trypomastigotes and amastigotes having higher contents of ATP-diphosphohydrolase than epimastigotes and metacyclic forms. The NTPDase on the surface of *T. cruzi* could be part of the *T. cruzi* purine salvage pathway and also might play a role in the escape mechanisms of the parasite by degrading ATP, ADP or other nucleotides eventually involved in different process such as inflammation and modulation of immune responses.

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BQ14 - PURIFICATION OF AN ECTO-ATPASE INVOLVED IN ADENOSINE ACQUISITION IN *LEISHMANIA (L.) AMAZONENSIS* PROMASTIGOTE.

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Leishmania spp., as others parasites of Trypanosomatidae family, is unable to synthesize purines *de novo*, requiring exogens purines for its growth. The metabolism and purines transport across parasites plasma membrane represent a promising target for diseases treatment caused by trypanosomatides. The plasma membrane of these cells contains enzymes whose active sites face the external medium rather than the cytoplasm. These enzymes are referred to as ecto-enzymes and their activities can be measured using living cells.

The capacity of *Leishmania (L.) amazonensis* intact promastigotes hydrolyzes nucleotides was analyzed by isocratic ion-pair reversed-phase HPLC. The parasites were incubated with ATP 100 mM, aliquots were took in variable times and analyzed in HPLC in order to determine concentration of ADP, AMP and adenosine, as well as, the reminiscent ATP present in the medium. It was verified that ATP concentration decreased rapidly with subsequent generation of ADP, AMP and adenosine. Initially the ADP concentration increased, but some minutes later it began to decrease due to surface ADPase activity of *L. (L.) amazonensis*.

A plasma membrane fraction was obtained and the enrichment was verified measuring 3'nucleotidase activity. The solubilized plasma membrane proteins was applied in poliacrilamida gel under nondenaturing conditions and stained for enzyme activity. The band that exhibited ATPase activity was excised, analysed by SDS-PAGE and stained with silver.

More studies are being performed for a better understanding of enzyme structure and functions, such as the relation with other ecto-ATPases and apyrases already described on literature.

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BQ15 - AN ECTO-ATPASE IN *ENTAMOEBA HISTOLYTICA* AND ITS POSSIBLE INVOLVIMENT WITH VIRULENCE

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Entamoeba histolytica is an enteric protozoa with high pathogen potential for human being due to its ability to disrupt and invade the colonic mucosa. The pathophysiology of amoebiasis is a multifactorial process which includes adhesion and colonization of the intestinal mucosa, damage in epithelial layer, lysis of inflammatory cells of host immune defense followed by penetration in other tissues.

During the last two decades, considerable progress has been achieved in the study of ecto-nucleotidases in general and ecto-ATPases in particular. Ecto-ATPases are glycoproteins present in the plasma membrane and have the active site facing the external medium rather than the cytoplasm. In different protozoa including some members of *Trypanosomatidae* family, it has been described the presence of ecto-ATPases able to hydrolyse extracellular ATP. Our group characterized a Mg^{2+}-dependent ecto-ATP diphosphohydrolase activity present in the surface of *E. histolytica* which is inhibited by DIDS and Suramin and stimulated by galactose, one of the recognized sugar by the surface lectin Gal/GalNAc of amoeba. In this work, the possible involvement of this activity in parasite-host cell interaction was evaluated by the influence of different modulators of this enzymatic activity in the cytotoxic effect of this parasite under HeLa cells. In these interaction experiments, epithelial cells were previously prepared and incubated with [^{3}H]Timidine, so that the lysis of monolayer can be measured by counting the radioactivity in coculture supernatant by liquid cintillation. After 30 minutes of

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interaction, the time course was linear with time at least four hours of incubation and with cell density until the ratio of 3:1 (trophozoite:HeLa cells) achieving 40% of lysis of the monolayer. To test the efficiency of the interaction system used in this work to measure citotoxicity, the effect of galactose was tested. This result shows that the addition of galactose (50 mM) in the interaction medium reduced the lysis of HeLa cells in 40%. Similar results were observed when the monolayer was pretreated with PCLEC (10 µg/mL) and TEL (10 µg/mL), two different lectins specific to galactose and GalNAC, respectively. The effect of ATP, preferential substrate to *E. histolytica* ecto-ATPases, was analysed. This nucleotide added to the interaction medium reduced in 40% the citotoxic effect of the parasite. The same protector effect was observed to DIDS (1 mM), the inhibitor agent of the ecto-ATPase activities observed in E. histolytica. When DIDS and galactose were added concomitantly in the reaction mixture the protective effect was enhanced. These results suggest a possible role of ecto-ATPases in the interaction process between *E. histolytica* and host cell.

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BQ16 - CHARACTERIZATION OF AN ECTO-PHOSPHATASE ACTIVITY PRESENT IN EPIMASTIGOTES OF *TRYPANOSOMA RANGELI* AND ITS POSSIBLE ROLE IN NUTRIENTS ACQUISITION

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Trypanosoma rangeli is a digenetic hemoflagelated parasite widely distributed on the Central and South Americas. It's able to infect several animal groups, as well as humans. The life cycle begins with the ingestion of trypomastigote forms present in vertebrate bloodstream by the triatominae. Inside of the vector gut, parasites differentiate to epimastigote forms and pass through the gut epithelium, achieving the hemocoel. Parasites proliferate in the haemolymph or into the haemocytes and migrate to the salivary glands where trypomastigote is formed. This stage is able to infect the vertebrate host during new blood feed.

In protozoa parasites, surface protein phosphorylation and dephosphorylation events are involved with host-cell interaction, cell differentiation, secretion of toxic factors and nutrients acquisition. Thus, the detection of kinases and phosphatases activities on the parasaites surface is of great relevance to the comprehension of its biochemistry.

This work proposes the characterization of an ecto-phosphatase activity present in *T. rangeli* and its possible involvement in nutrients acquisition. Living cells of *T. rangeli* are able to hydrolyze the artificial substrate *p*-nitrophenylphosphate (*p*-NPP), linearly with time and with cell density. *p*-NPP hydrolysis presented a Michaelian kinetic with apparent K_m and V_{max} values of 2.6 ± 0.26 mM and 6.62 ± 0.17 nmol *p*-NP / h x 10^7 cells, respectively. $MgCl_2$ and $CuCl_2$ stimulated the phosphatase activity and Mg^{+2} stimulation was dose-dependent. Levamizole and tartrate, alkaline and secreted phosphatase inhibitors, respectively, were not able to modulate this activity. However, NaF, molybdate and vanadate, three classic acid phosphatase inhibitors, decreased considerably the control activity. This ecto-phosphatase activity was also able to dephosphorylate phosphoaminoacids. Phosphoserine hydrolysis was stimulated by Mg^{2+} as well as *p*-NPP hydrolysis, suggesting the presence of more than one enzyme on the parasite surface with different specificities. Our findings demonstrated that, at low phosphate concentrations, the epimastigote cells of *T. rangeli* did not grow efficiently. Nevertheless, when β-glycerophosphate, a usual substrate for phosphatase activities, was added in the culture medium the growth rate became higher. These observations suggest that this phosphatase activity present on the surface of *T. rangeli* may participate to the mechanisms of nutrients acquisition in this trypanosomatid.

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BQ17 - PRESENCE OF ACIDOCALCISOME IN *LEPTOMONAS WALLACEI*.

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Acidocalcisomes are acidic calcium-storage organelles found in several microorganisms. They are characterized by their acidic nature, high electron density, high content of polyphosphates, and a number of pumps and exchangers responsible for the ion uptake into these organelles. In this work, we show that *Leptomonas wallacei*, a trypanosomatid isolated from the phytophagous insect *Oncopeltus fasciatus*, possess a electron dense organelles with structural, chemical and physiological properties similar to the acidocalcisomes. Pyrophosphate-driven H^+ uptake was measured in cells permeabilized by digitonin using acridine orange, a dye that changes its absorbance when accumulated in acidic compartments, as a probe. The H^+-pyrophosphatase activity was inhibited by sodium fluoride (NaF), imidodiphosphate (IDP), H^+-pyrophosphatases inhibitors. H^+ was released with the addition of 250µM Ca^{2+}, suggesting the presence of a Ca^{2+}/ H^+ antiport in internal compartments. However, Na^+ was unable to release protons from these organelles. The H^+-pyrophosphatase activity was optimal in the pH range of 7.0 to 7.5. This activity was completely dependent of ion K^+ and independent of Na^+ and sucrose. The maximal activity occurred with 130 mM of KCl. The pyrophosphate-driven proton uptake was dependent on the PPi concentration. However, due to the fast substrate consumption, we were not able to obtain a value for half-maximal activation. Maximal values for H^+ transport were obtained at concentrations of PPi above 50 µM. In addition, X-ray elemental mapping associated with energy-filtering transmission electron microscopy showed that most of the cations, namely Na, Mg, P, K, Fe and Zn are located in the acidocalcisome matrix.

These results suggest that *Leptomonas wallacei* possess an organelle that is able to accumulate H^+ using the energy coupled from pyrophosphate hydrolysis. This organelle possess a Ca^{2+}/ H^+ antiport and a H^+-pyrophosphatase which is inhibited by IDP and NaF, as described in acidocalcisomes. In addition, electron-dense organelles with structural properties and elemental composition similar to the acidocalcisomes were identified. However, in contrast to the other trypanosomatids so far studied, we did not identify the presence of a H^+-ATPase sensitive to bafilomycin A (V-H^+-ATPase) neither a Na^+/ H^+ antiport in *Leptomonas wallacei*.

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BQ18 - INTERACTION BETWEEN PLATELET ACTIVATING FACTOR (PAF) AND *TRYPANOSOMA CRUZI*: BIOLOGICAL ACTION AND MOLECULAR ASPECTS OF A POSSIBLE RECEPTOR.

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Chagas disease is caused by the protozoan *Trypanosoma cruzi* (*T. cruzi*). It represents one of the main public health concerns in Latin America. Data from the literature have suggested the presence of a platelet activating factor receptor

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(PAF) in *T. cruzi.* This lipid mediator induces, by direct action on the parasite, enzyme secretion, cell differentiation, and signal transduction. The goals of this work were to study some biological effects of PAF on *T. cruzi* and to obtain a preliminary molecular analysis of a possible receptor gene for this lipid mediator in *T. cruzi*. Trypomastigote suspensions were treated with PAF and the resulting biological effects were monitored by *in vitro* morphological transformation assays, evaluation of parasite migration to artificial cavities in mice pre-infected with this parasite, as well as spectrofluorimetric assays to quantify calcium influx. Results from these experiments show that PAF (10^{-6}M) induces transformation of 30% of the trypomastigote forms into amastigote-like forms, after incubation for 120 minutes. Interestingly, this effect was blocked by pertussis toxin or Veragensin in approximately 50% of the parasites. Moreover, PAF induced parasite migration to the dorsal air pouch in mice, in a dose-dependent manner, with the most effective dose being 10^{-6}M. This mediator was also able to induce calcium influx in trypomastigotes. This influx was affected by different doses and incubation times. In order to investigate the presence of a PAF receptor gene in *T. cruzi*, polymerase chain reaction (PCR) assays were performed using cDNA and/or genomic DNA from different forms of *T. cruzi*. Oligonucelotides, homologous to the PAF receptor gene from mammals, were utilized in these reactions. It was possible to amplify DNA fragments (350 and 1450 base pairs) with these oligonucleotides from trypomastigote cDNA. Based on the results obtained, we suggest that PAF may modulate some biological events in *T.cruzi* infection by interacting with a possible G protein-coupled receptor. These findings may add some insights to studies on the interaction of *T.cruzi* with the vertebrate host immune defense system and on the physiopathology of Chagas disease.

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BQ19 - INVOLVEMENT OF CYCLIC AMP AND PROTEIN KINASE A IN CELL DIFFERENTIATION TRIGGERED BY PLATELET-ACTIVATING FACTOR IN *HERPETOMONAS MUSCARUM MUSCARUM*

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Herpetomonas muscarum muscarum is a house fly flagellate parasite of the family Trypanosomatidae that presents three evolutive forms: promastigote, paramastigote and opisthomastigote. These parasites have been widely used as a model for cell biology and biochemistry of lower as well as higher eukaryonts. These trypanosomatids resemble higher eukaryonts in several aspects, including the fact that their cellular functions are mediated by signaling pathways involving protein kinases and phosphatases, G proteins and second messengers. Cyclic AMP (cAMP) is an important second messenger that regulates functions such as cell proliferation, differentiation and host cell invasion by parasites. Most of the effects induced by cAMP are mediated through protein kinase A (PKA), a protein kinase dependent on cAMP, whose targets have not been identified in parasites yet. Platelet-activating factor (PAF) is a potent phospholipid mediator of several cellular functions in diverse biological and pathophysiological processes, such as cell differentiation, inflammation and allergy. In earlier studies, we have demonstrated that PAF triggers the process of cell differentiation in *H. m. muscarum* and in *Trypanosoma cruzi.* Recently we showed that PAF triggers a cascade of signal transduction events, leading to the activation of casein kinase II (CKII), through protein kinase C (PKC) in *H. m. muscarum.* In this work, the concentration of intracellular cAMP was assayed in the cytoplasmic content of *H. m. muscarum* grown in the absence or in the presence of PAF (10^{-7}M), showing that this lipid mediator promoted a two-fold increase in the concentration of cAMP. Taking this result into account, we decided to study the effect of cAMP on cell differentiation of *H. m. muscarum*. The parasites were grown for periods ranging from 1 to 3 days, in the absence or in the presence of the following drugs: PAF (10^{-7}M) and/or cAMP (10mM) and/or the PKA inhibitor H89 (10mM). The percentage of non-differentiated forms (promastigotes) and differentiated forms (paramastigotes plus opisthomastigotes) was daily determined by using Giemsa stained preparations. On the third day of incubation, parasites grown in the presence of these modulators presented the following percentage of differentiated forms: PAF (70%), cAMP (75%), H89 (42%), PAF + cAMP (75%), PAF + cAMP + H89 (43%), cAMP + H89 (38%), PAF + H89 (32%), as compared to the control parasites, which presented 40% differentiated forms. Together, this set of results suggests that PAF and cAMP stimulate cell differentiation in *H. m. muscarum* in a PKA activity-dependent fashion.

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BQ20 - PLATELET-ACTIVATING FACTOR-TRIGGERED CELL DIFFERENTIATION IN *TRYPANOSOMA CRUZI* IS DEPENDENT ON CYCLIC AMP AND PROTEIN KINASE A

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Trypanosoma cruzi, a protozoan parasite that exhibits developmental regulation of virulence, is transmitted by reduviid insects. These insects become infected by ingesting trypomastigotes from the blood of the mammalian hosts; the parasites then multiply as epimastigotes and differentiate into metacyclic trypomastigotes in the lumen of the crop and midgut. Mechanisms underlying the differentiation of the parasite are poorly understood, although we do know that platelet-activating factor (PAF) triggers the differentiation of *T. cruzi* from epimastigotes into trypomastigotes. PAF is a potent phospholipid mediator of several cellular functions in diverse biological and pathophysiological processes, such as cell differentiation, inflammation and allergy. *T. cruzi*, like other eukaryonts, present cellular functions mediated by signaling pathways involving protein kinases and phosphatases, G proteins and second messengers. Cyclic AMP (cAMP) is an important second messenger that regulates functions such as cell proliferation, differentiation and host cell invasion by parasites. Most of the effects induced by cAMP are mediated through protein kinase A (PKA), a protein kinase dependent on cAMP, whose targets have not been identified in parasites yet. Recently we showed that PAF triggers a cascade of signal transduction events, leading to the activation of casein kinase II (CKII), through protein kinase C (PKC) in the trypanosomatid *Herpetomonas muscarum muscarum.* In this work we studied the effect of cAMP on cell differentiation of *T. cruzi*, clone Dm28c *in vitro*. The parasites were maintained in TAUP medium for periods ranging from 1 to 6 days, in the absence or in the presence of the following drugs: PAF(10^{-6}M) and/or cAMP (10mM) and/or the PKA inhibitor H89 (10mM). The percentage of epimastigotes and trypomastigotes was daily determined by using Giemsa stained preparations. On the third day of incubation, parasites maintained in TAUP medium in the presence of these modulators presented the following percentage of trypomastigotes: PAF (81%), cAMP (76%), H89 (35%), PAF + cAMP (82%), PAF + cAMP + H89 (43%), cAMP + H89 (45%), PAF + H89 (52%), as compared to the control parasites, which presented 48% trypomastigotes. Together, this set of results suggests that PAF and cAMP stimulate cell differentiation in *T. cruzi*, which is inhibited by the PKA inhibitor H89. Taking this result into account, we decided to study the effects of these modulators on the protein profile of *T. cruzi* extracts by SDS PAGE. Accordingly, it was observed that the protein extract from *T. cruzi* parasites maintained in TAUP medium for one hour in the presence of 10^{-6} M PAF or cAMP presented a

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major difference in the amount of a protein of 60 kDa and of both this protein and another one of 105 kDa in PAF or cAMP-treated parasites, respectively, as compared to the control flagellates. This phenomenon was suppressed by H89, which correlates well with the cell differentiation results.

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BQ21 - CASEIN KINASE 2 (CK2) AND PROTEIN KINASE C (PKC) ACTIVITIES IN *LEISHMANIA (V.) BRAZILIENSIS*: A COMPARATIVE STUDY BETWEEN INFECTIVE AND NON-INFECTIVE STRAINS

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Parasites of the genus *Leishmania* are transmitted by the bite of sand flies and infect cells of the mononuclear phagocyte lineage of their vertebrate hosts. Depending both on the virulence factors of the parasite itself and on the immune response established by the host, a spectrum of diseases known as leishmaniasis can appear, and these can be cutaneous and/or visceral. Approximately 350 million people live in areas of active transmission of *Leishmani*a, with 12 million people throughout Africa, Asia, Europe, and the Americas directly affected by leishmaniasis. The metacyclogenesis process (acquiring of infectivity), is very important to the *Leishmania*-macrophage interaction. This process may modify the molecules of the parasite surface and, by thus, promoting the activation of signal transduction pathways. The study of the enzymes related to phosphorylation and dephosphorylation of proteins present on the external surface of these parasites is of fundamental importance. Several ecto-enzymes have been described in the trypanosomatids, including ecto-phosphatases and ecto-kinases. Casein kinase 2 activities have been described both on the cell surface and as secreted enzymes of *Leshmania (L.) major* and *L. (L.) amazonensis*. These enzymes seem to be involved with cell growth, differentiation and infectivity. In the present work, we have identified two kinase activities (CK 2 and PKC) in two strains of *Leishmania braziliensis*: an infective and non-infective one. These enzymes are present on external cell membrane of these parasites, in the cytoplasm and as a secreted form. The *Leishmania (V.) braziliensis* infective strain (MHOM/BR/2002/EMM-IOC-L2535) was recently isolated from a patient. This strain was inoculated in hamster footpad and recovered 9 weeks after infection. The *Leishmania (V.) braziliensis* non-infective strain (CT-IOC-238-L566) has been kept axenically in culture for several years. The ecto-CK2, the intracellular CK2 and the secreted CK2 activities were much higher in the infective strain than in the non-infective one. However, the enhancement induced by the addition of the substrate casein (1mg/mL) on the secreted CK2 activity was more pronounced in the non-infective strain (4-fold). The addition of casein promoted 37% inhibition of the CK2 activity present on the external surface of *L. (V.) braziliensis* infective parasites. The PKC activity presented an opposite pattern, as it was 30-fold higher in the non-infective strain than in the infective one.

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BQ22 - ENDOSYMBIOSIS IN TRYPANOSOMATIDS: THE SYMBIOTIC BACTERIUM INFLUENCES THE POLYAMINE METABOLISM IN THE HOST PROTOZOAN

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Some protozoa of the Trypanosomatidae family presents an endosymbiotic bacterium which is enclosed by two unit membranes and is usually found close to the host cell nucleus. A cured strain of such species can be obtained after antibiotic treatment, allowing a better understanding of this symbiotic association. The endosymbiont furnishes essential nutrients to the host cell, resulting in higher proliferation capacity of the endosymbiont-bearing trypanosomatid when compared to cured strains. The ornithine decarboxylase (ODC) is involved in polyamine biosynthesis and its activity is related to the cell division capacity. Western blotting analysis showed that both strains have similar amounts of ODC, while endosymbionts obtained after cell fractioning did not present such enzyme. However, the ODC activity is higher in endosymbiont-bearing trypanosomatids when compared to the results obtained for the cured strain. In order to better understand the differential ODC activity, the cured strain of *Crithidia deanei* was grown in conditioned culture medium, which was obtained after 24 h of cultured endosymbiont-bearing strain. The results showed an increase in cell proliferation and in the ODC activity, when the cured strain was cultivated in conditioned medium or after addition of endosymbiont extract to the culture medium. Taken together, these data suggest that the endosymbiont can enhance the protozoan ODC activity by providing factors that increase the polyamine metabolism. Regarding the ODC localization, the endosymbiont-bearing strain displayed immunolabeling in the cytoplasm and in the flagellar pocket after incubation with a polyclonal antibody anti-ODC. The cured strain showed a different ODC localization, since immunofluorescence was only observed over the cytoplasm. The cultivation of the aposymbiotic strain in the conditioned medium resulted in a new distribution of the enzyme, since the flagellar pocket was also labeled, as in the normal strain. These results suggest that the ODC activity is important for *C.deanei* growth, which is dependent on factors eventually produced by the endosymbiont.

Supported by: CNPq, FAPERJ and FUJB.

BQ23 - THE USE OF AN ALKYL-LISOPHOSPHOLIPID TO STUDY THE SYMBIOTIC RELATIONSHIP IN TRYPANOSOMATIDS

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Alkyl-lysophospholipids (ALP), originally developed as anticancer drugs, have shown a significant antiproliferative activity against trypanosomatids. The effect of these compounds have been related to perturbation of the alkyl-lipid metabolism and to the biosynthesis of phospholipids, as well as damage to celullar membranes. In this study we verified the effects of ET-18-OCH3, an ALP, in *Crithidia deanei*, an endosymbiont-harbouring trypanosomatid. There is a controversy involving the origin of the endosymbiont envelope; it can be derived from the host trypanosomatid or it can presents prokaryotic features. Thus, the lipid composition of this symbiotic bacterium has been investigated in order to elucidate this question. Phosphatidycholine (PC), is the major membrane phospholipid in eukaryotes, however it is found in only a few species of bacteria, including the symbiotic ones. Enzymatic methylation of phosphatidylethanolamine (PE) is the main biosynthetic pathway to yield PC in bacteria. However, in symbiotic associations this phospholipid can be directly

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synthesized from choline produced by the host cell. Taken together, these data suggest that PC may be required for a successful interaction of the symbiont with the host. Previous studies revealed that the major phospholipid in the endosymbiont of *C. deanei* is phosphatydilcoline, followed by cardiolipin, phosphatidylinositol and phosphatidylethanolamine. Recent data showed that the ET-18-OCH3 had a dose-dependent effect on cell proliferation and also promoted ultrastructural modifications in *C. deanei*. Ultrastructural analysis by transmission electron microscopy showed plasma membrane shedding, mitochondrion swelling and damage of the endosymbiont envelope. Recent biochemical analysis showed that such morphological alterations are related to the drug effect on phospholipid biosynthesis. Now, we are verifying the effect of ET-18-OCH3 on the phospholipid composition of the endosymbiont and the mitochondrion, an organelle with symbiotic origin, which is used as a comparative model in our study.

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BQ24 - PROTEIN DEPHOSPHORYLATION MAY BE INVOLVED IN *TRYPANOSOMA CRUZI* DIFFERENTIATION

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Differentiation of the infective trypomastigote form of *Trypanosoma cruzi* to the replicative amastigotes normally occurs in the cytoplasm of infected cells. A preliminary study on the involvement of protein phosphatases type 1 and 2A in the extracellular differentiation of trypomastigotes to amastigotes was undertaken at 37°C and 33°C using the CL-14 clone of *T. cruzi*. Calyculin A, an inhibitor of protein phosphatases 1 and 2A, triggers the transformation of trypomastigotes to amastigotes at neutral pH through epimastigote-like intermediate forms. Treatment of trypomastigotes for 6 hours with 1 nM or 5 nM Calyculin A resulted in the differentiation of more than 50% of trypomastigotes to epimastigote-like forms. After 8 hours, all trypomastigotes were differentiated to amastigotes or epimastigotes-like forms. Taken into account previous results of our laboratory (Almeida-de-Faria et al., Exp. Parasitol. 92, 263-274, 1999) these results suggest that the epimastigote-like form is an intermediate stage in both directions of the amastigote – trypomastigote inter-transformation. Interestingly, metacyclic trypomastigotes from the CL-14 clone submitted to the same treatment with Calyculin A showed no morphological changes.

Financial support: FAPESP

BQ25 - *TRYPANOSOMA CRUZI* TRANSFORMATION INDUCED BY PI-PLC LEADS TO CALCIUM MOBILIZATION AND AMASTIN EXPRESSION

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Trypanosoma cruzi trypomastigotes treated with exogenous phosphatidyl inositol-specific phospholipase C (PI-PLC) of *B. thuringiensis in vitro* are rapidly induced to differentiate into round forms very like amastigotes. Upon contact with PI-PLC, morphological changes occur very readily in the parasite. In the present work we have studied the mechanisms underlying these morphogenetic changes, and the signals that are generated and that induce this phenomenon. We have shown that treatment of trypomastigotes of *T. cruzi* with genistein, an inhibitor of protein tyrosine kinase, blocked the differentiation of the parasites. The inhibitor of protein kinase C, calphostin C and the inhibitor of adenylate cyclase, 2,5-dideoxyadenosine, do not block the transformation. The Ca^{2+} response induced in trypomastigotes upon contact with PI-PLC is discrete, with a slight variation in the intracellular calcium concentration of the parasite. Pre-treatment of trypomastigotes with calcium chelator BAPTA/AM also have a slight effect in the transformation of trypomastigotes into amastigotes. We also show, by Northern-blotting, that the treatment of trypomastigotes of *T.cruzi* with PI-PLC induces the expression of amastigote-specific genes. After six hours of the treatment with PI-PLC, the amastigote-like forms express the mRNA for amastin, a *T. cruzi* amastigote-specific gene. Taken together, these data indicate that *T. cruzi* transformation induced by PI-PLC activates protein tyrosine kinases pathway with calcium mobilization and expression of amastigote-specific genes.

Financial Support: CAPES

BQ26 - HEME REQUIREMENT AND ITS POSSIBLE INTRACELLULAR TRAFFIC IN *TRYPANOSOMA CRUZI* EPIMASTIGOTES.

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Trypanosoma cruzi, the ethiologic agent of Chagas disease, is transmitted through triatomine insects vectors during the blood-meal on vertebrate host. These hematophagous insects usually ingest in a single meal about 10 mM heme bound to hemoglobin. Heme is a powerful generator of reactive oxygen species, including free radicals, and can damage a variety of biomolecules. *T. cruzi*, in the course of their evolution history, had to develop adaptations to avoid the deleterious effects of high concentrations of free heme found in this environment and there is a large lack in literature about its mechanisms of uptake, its effects and degradation inside the parasites. The first transformation into epimastigotes occurs in the stomach and initiates few hours after parasite ingestion. We have been investigating the effects of hemin on *T. cruzi* growth. Hemin concentration in the medium varied from 0 to 1 mM. Addition of hemin drastically increased the parasite proliferation in a dose-dependent manner. Ultrastructural analysis of parasites grown in high heme concentration are in course. Pd – Mesoporphyrin IX (an analogous of heme: Fe - Protoporfirin IX) intrinsic fluorescence was used as a label to trace the fate of heme taken up by the parasite. We followed the time course of Pd-mesoporphyrin IX internalization in parasites from three to five-day-old cultures incubated with globin-Pd-Mesoporfirin IX. The fluorescence signal was initially associated with anterior vesicle compartments, reaching reservosomes after less than a minute of incubation. On the other hand, when eight-day-old parasites were starved by a 24 hour incubation in medium without serum, the images showed intense fluorescence in the kinetoplast. Taken all together, our data suggest the need of heme in the development of *T. cruzi* and points to the existence of a specific pathway for heme absorption from medium, which would be consistent with the utilization of host heme for assembly of mitochondrial proteins of the parasite. Besides this we have also described the use of Pd-Mesoporfirin IX, a new tool to study cell metabolism of heme.

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BQ27 - L-GLUTAMINE INDUCES *TRYPANOSOMA RANGELI* DIFFERENTIATION *IN VITRO*

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The difficulty in obtaining infective trypanosomes has been a break point for the study of several aspects of *Trypanosoma rangeli* infection in mammals. For that, our group has developed a simple method to induce *T. rangeli* differentiation *in vitro,* obtaining differentiation rates over 75%. However, the mechanism or mechanisms and factors involved in the differentiation phenomenon are barely known. In this study, we have identified the amino acids responsible for induction of *T. rangeli* differentiation *in vitro*. For that, epimastigotes of *T. rangeli* Choachi strain harvested in LIT medium were washed twice in PBS and cultivated in the MEM amino kitÒ (Gibco, USA), which is basically a DMEM medium, depleted of all amino acids. The assays were performed three times in triplicate in 1.5 ml micro tubes using 10^6 parasites/mL at 26°C by adding one single amino acid at a time. Differentiation was assessed in each testing medium at 0, 2, 4 and 6 days of incubation by random counting of 300 parasites in Giemsa stained smears to determine the number of trypomastigotes. On the 6^{th} day of incubation, parasites cultivated in the presence of L-glutamine, presented a differentiation rate of 83.8±8.9%, while in the control medium (DMEM plus all amino-acids) the differentiation rate was 75.7±4.5%. Among the other 12 amino acids tested, significant lower differentiation or higher mortality rates of the parasites were observed. These herein described results suggest that L-glutamine plays an important role on *T. rangeli* differentiation *in vitro*, probably involved in the polyamines pathway. The study and comprehension of the *T. rangeli* differentiation process *in vitro* is of great importance for the study of the parasite biology as well as for comparative studies with *T. cruzi.*

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BQ28 - THE METABOLIC CONTRIBUTION TO *HERPETOMONAS ROITMANI* WITH A BACTERIUM-LIKE ENDOSYMBIONT IN THE CYTOPLASM.

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The Tripanosomatidae family has singular importance due to its capacity to infect many organisms even men's, which results in efforts for its eradication, that is difficult to achieve due to lack of knowledge. For biological studies of the family, the group Hepertomonas has been regarded as a good comparative model. The incoming work evaluates the ergogenic potential in *Herpetomonas roitmani* through conduction microcalorimetry. The protozoons were cultivated in Roitmani, to keep them for microcalorimetry and for its control. To find the cure, several changes of blood cultures, containing clorafenical, to Roitman's chemically defined minimum were done. We also did experiments in microcalorimetry using 0,5 ml of energetic substratum, 0,5 ml of physiological serum and 0,8 ml of cellular suspension. The statistic analysis was conducted based on linear minimum squares. The heat conduction resulted in a value of 29,3 -/+ 12,9 pw/cell of heat liberation by the protozoon Trypanosomatid with a bacterium-like endosymbiont in the cytoplasm (R=0,794). The experiments show that the medium value of the produced heat by *Herpetomonas roitmani* matches with other reported values from other cells such as the Hybridoma 30-50 pW/cell;3T3, 53 pW/cell, and is superior than the human ordinary cells.

BQ29 - CLONING, EXPRESSION AND CHARACTERIZATION OF THE CYSTEINE PROTEASES FROM *TRYPANOSOMA BRUCEI BRUCEI* (BRUCIPAIN) AND *LEISHMANIA (L.) DONOVANI*

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Pathogenic trypanosomatids express high levels of papain-like cysteine proteases (CPs), which are important for their pathogenicity and survival in the host. The use of potent synthetic irreversible CP inhibitors in experimental Chagas' Disease and leishmaniasis has helped the validation of these enzymes as promissing targets. The development of more effective and selective inhibitors depends on the detailed structural and functional characterization of these molecules. The only trypanosomatid CP characterized at the structural level is the one of *Trypanosoma cruzi*, cruzipain, obtained in its recombinant form truncated at the C-terminus. In trypanosomatids, these proteases are usually encoded by polymorphic gene families, whose members may present variable kinetic properties. Therefore, the fine characterization of a particular member requires its functional expression in a heterologous system, which has been recently achieved for the CPs of *Leishmania (L.) mexicana* and of *Trypanosoma brucei rodhesiense* (rodhesain). Here, we report the expression of functional CPs from *Trypanosoma brucei brucei* (brucipain) and from *Leihmania (L.) donovani*, followed by a preliminary characterization of their biochemical properties. Degenerated oligonucleotides based on the sequence of rodhesain were used as primers in PCR reactions with genomic DNA of *T. brucei* as a template, leading to the amplification of a single fragment, which presents 95% similarity with rodhesain. A gene fragment spanning the pro and central domains of brucipain were cloned in the vector pQE30 for the *E. coli* expression of a fusion protein bearing a 6 histidine residue-tag at the N-terminus of the enzyme. Recombinant pro-brucipain, obtained as inclusion bodies, was denatured in urea buffer, purified in Ni-agarose resin and refolded "in vitro". Active enzyme was produced upon incubation of the precursor forms at low pH and low temperature and subsequently affinity purified in the tio-propyl sepharose resin. Using a similar strategy, complementary oligonucleotides based on of the CP from *L. (L.) donovani* (GenBank), were used in PCR reactions and the recombinant mature enzyme was obtained as described above. The preliminary characterization of brucipain and of r- CP revealed that they efficiently hydrolize Z-Phe-Arg-MCA. Interestingly, brucipain displayed strong substrate inhibition when assayed with this substrate at room temperature, a feature described with cruzipain. Similarly to cruzipain, brucipain did not display substrate inhibition when assayed at 37ºC, suggesting that it also bears a second substrate binding site at the central domain, which is modulated by temperature. If present with natural substrates, the parasite could use temperature-dependent substrate inhibiton to modulate enzyme activity when it changes from the invertebrate to the vertebrate host. Detailed characterization of the enzymes' substrate specificity using long synthetic substrates is now underway.

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BQ30 - POTENT INHIBITION OF TRYPANOSOMATID CYSTEINE PROTEASES BY THE PRO DOMAIN OF CRUZIPAIN

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Pathogenic protozoa bear high levels of papain-like cysteine proteases (CPs),

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which have been associated with parasite virulence and survival. Similarly to their mammalian counterparts, these enzymes are expressed as inactive precursors, which undergo maturation by proteoliytic removal of the pro segment. Although there is a large body of information on the biochemical properties of trypanosomatid CPs, little is known about the mechanisms controlling their activity "in vivo". The rate of zymogen processing could be one way of modulating the levels of mature enzyme. In mammalian cells, it was demonstrated that free intact pro segment of lysosomal CPs, cathepsins L and B, selectively inhibit the respective mature enzyme with high affinity in a pH-dependent manner. It has been suggested that, if intact pro segment is liberated upon pro-enzyme processing in vivo, it could act as an inhibitor of mature enzyme until the complex is delivered to acidic compartments. A similar mechanism could regulate the levels of active CPs in trypanosomatids. Along these lines, it has been recently demonstrated that the pro segment of congopain, the CP from *Trypanosoma congolense*, inhibits congopain and cruzipain (the CP from *Trypanosoma cruzi*) with moderate affinity. The mapping of the inhibitory regions within the pro segment of congopain using synthetic overlapping peptides associated the YHNGA sequence (partially conserved among trypanosomatid CPs) with the inhibitory activity. In contrast with mammalian CPs, where pro domain-dependent inhibition is highly selective, it is possible that there is pro segment cross-inhibition among CPs of different trypanosomatids Here, we set out to investigate the inhibitory properties of the pro segment of cruzipain. A DNA fragment corresponding to the pro segment of cruzipain (Cys X-Gly X) was generated by PCR using complementary oligonucleotides and subsequently cloned into the pQE30 expression vector. The recombinant pro segment containing a 6 histidine resisue–tag at its N-terminus was produced in *E. coli*, purified in affinity resins and tested for inhibitory activity towards cruzipain, using Z-Phe-Arg-MCA as a substrate. We demonstrate that the recombinant pro domain of cruzipain strongly inactivates mature enzyme (K_i = 0.03 nM) at pH 6.5. This result indicates that if the pro segment is present intact in reservosomes, or liberated to the extracellular environment, it could act as a key regulator of cruzipain function. Interestingly, we observed that the recombinant protein also potently inactivates recombinant brucipain (the CP of *T. brucei*), the CP of *Leishmania donovani*, as well as lysates of other trypanosomatids such as *T. rangeli* and *Chritidia fasciculata*, while it did not inhibit papain, and cathepsins L or B. The properties of this molecule could be exploited as an alternative to generate a potent selective inhibitor of trypanosomatid CPs.

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BQ31 - SPECIFICITY OF SERINE PROTEASES OF *L. (L.) AMAZONENSIS*

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AIMS: Serine protease of parasites play crucial roles in their physiology and in the interaction with their hosts. The inhibition of these enzymes by specific protease inhibitors can interfere in the process of invasion and survival of the parasites within the hosts. Serine proteases of *Leishmania amazonensis* were characterized in our laboratory and their specificity was analysed with the purpose of developing specific inhibitors which could interfere in the parasite physiology.

MATERIALS AND METHODS: Serine proteases were purified from cellular extracts and cell-free extracts of *L. amazonensis* promastigotes using affinity chromatography on aprotinin-agarose and high performance liquid gel filtration chromatography. The enzymatic specificity was analysed using synthetic peptides. The enzyme hydrolyzed substrates in basic pH alkaline range at 28°C.

Results and Conclusions: The enzymes have shown greater specificity for peptides containing aromatic and aliphatic groups at P1 position, such as leucine, valine, alanine and tyrosine. The results showed that serine proteases from *Leishmania (L.) amazonensis* have a similar enzymatic specificity chymotrypsin.

Financial support: FAPERJ, FIOCRUZ, CNPq

BQ32 - DIFFERENTIAL PROTEINASE PROFILES OF *TRICHOMONAS VAGINALIS* ON A WELL-ESTABLISHED STRAIN AND A FRESH ISOLATE

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The parasitic protozoan *Trichomonas vaginalis* is the causative agent of human trichomoniasis, a common infection of the urogenital tract, and exerts its pathogenic effect when interacting with the surface of epithelial cells. This infection is globally considered one of the most frequent sexually transmitted diseases, with approximately 180 to 200 million cases annually (Rendón-Maldonado *et al*., 1998, *Exp.Parasitol*., 89: 241-250). Several molecules have been identified as virulence factors involved in the mechanism of tissue damage by *T. vaginalis* i. e. adhesins, a cell-detaching factor, a laminin-biding receptor, pore-forming proteins and proteinases. *T. vaginalis* proteinases have been related to nutrient acquisition, immune evasion and cytotoxicity. In this work we aim to characterize the protease expression profile among microorganisms of a fresh isolate of *T. vaginalis* (FMV1 strain) and of a long-term culture (FF28JT-Rio strain). Proteinase activity was detected on immobilized gelatin substrate copolymerized with polyacrilamide gel electrophoresis and in solution with a chromogenic substrate. Both FF28JT-Rio and FMV1 strains were cultivated in TYM medium supplemented with 10% fetal bovine serum and maintained at 37 °C for 24 hours. Parasites were washed in PBS pH 7.2 and lysed in 10 mM Tris-HCl pH 6.8 containing 1% of Triton X-100 (30 minutes, 4 C). The insoluble fraction was removed by centrifugation (12 000 g, 30 minutes, 4 C) and the enzymatic activity was evaluated in the soluble fraction. Qualitative electrophoretical analyses have showed that a constant pattern of proteinase bands with molecular mass between 93 – 8.7 kDa might be detected in both parasite samples in acid pH. However, a differential profiles was detected in both parasite extract: 93.7, 81.8, 62.4, 52.0, 41.6, 31.1, 22.3, 11.8 and 8.2. kDa from FF28JT-Rio and 89.1, 71.3, 65.3, 40.0, 26.6, 19.2, 13.1 and 8.7 kDa from FMVI parasite. In the inhibition assay we observed that only the cysteine-proteinase inhibitor (E-64) had an effect on total proteinase band profile of both parasites. An additional qualitative assay was performed with synthetic peptide substrate. The specific chromogenic substrate pGlu-Phe-Leu p-nitroanilide; (0,1mM) was digested in 50mM Sodium Acetate pH5.0 containing 1mM dithiotreitol, 10mg of soluble protein fraction in final volume of the 500ml (25 C, 10 minutes). In this last experiments it was also possible to quantify the activity of protienase on the FF28JT-Rio strain (36,0 nmol minute^{-1} mg of protein^{-1}) and on FMV1strains (35,0 nmol minute^{-1} mg of protein^{-1}). The implications of preliminary results are discussed.

Financial Support: CNPq, FAPERJ, FINEP and PRONEX.

BQ33 - IDENTIFYING SERINE AND CYSTEINE PROTEINASES IN *ACANTHAMOEBA* ISOLATES

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Free-living amebae of the genus *Acanthamoeba* multiply as phagotrophic trophozoites and encyst under unfavorable conditions. *Acanthamoeba* spp. have been identified as the causative agents of granulomatous amebic encephalitis and amebic keratitis in man (Marciano-Cabral & Cabral, Clin. Microbiol. Revs.16: 273, 2003). Tissue damage and invasion by the amebae still remain poorly understood, but contact-dependent cytopathic effects and proteinase secretion seem to be involved. We have shown that *A. polyphaga* trophozoites (ATCC 30461) constitutively secrete multiple cysteine proteinases (CPs) and serine proteinases (SPs) (Alfieri *et al.*, J. Parasitol. 86: 220, 2000). We have extended the observations to several *Acanthamoeba* isolates (*A. castellanii* ATCC 30234, 30011, 30868; *A. polyphaga* ATCC 30871, 30872, 30873, 30461), including three from brazilian cases of keratitis (U/E 2, U/E 8x, U/E 10). Proteinase activities were monitored by azocasein assays and gelatin-containing SDS-polyacrylamide gels, using as enzyme source cell lysates and medium conditioned for 24 hours by trophozoites (*Acanthamoeba* conditioned medium, ACM).

Azocasein hydrolysis was detected over a broad pH range (4.0-10.0) in ACM, and with maximal rates at pH 6.0 and above; substrate hydrolysis at near neutral/alkaline pH was potently inhibited by PMSF, thus indicating the involvement of SPs. Serine proteinases were detected in low levels in cell lysates; assays performed at pH 5.0 with 2 mM DTT indicated high CP activity in the latter.

SPs were identified in ACM following electrophoresis in gelatin gels and incubation in 0.1M Tris-HCl, pH 8.0. In *A. castellanii*, there were bands common to all isolates (50, 60 and 100 kDa) and others (55 and 170 kDa) visualized only in isolates 30011 and 30234. In *A. polyphaga*, in addition to a band near 100 kDa common to isolates 30871, 30873, and 30461, SPs distributed as follows: 50, 57, 60 kDa (30871); 44, 80 kDa (30872); 47, 51, 56 kDa (30873); 52, 60 kDa (30461). In the three clinical isolates, SPs resolved identically to the enzymes of *A. polyphaga* 30461. SPs apparently equivalent to those identified in ACM were detected in cell lysates; visualization of tracks of activity in gels fixed and stained immediately after running indicated enzyme activation during electrophoresis.

Blockage of SPs with PMSF prior to electrophoresis and gel incubation at pH 3.8 with 2 mM DTT was the condition used to examine CPs in activity gels (Alfieri et al., 2000). Bands inhibited by E-64 and thus associated with CPs, although more intensely visualized in cell lysates, were also identified in ACM: four were detected in *A. castellanii* 30011 and 30234 (42, 45, 75, 116 kDa), and five (36, 45, 66, 75, 116 kDa) in isolate 30868. There were differences between *A. polyphaga* isolates, but all displayed activities around 70 and 100 kDa. Again, in the clinical isolates, CPs resolved identically to the enzymes of *A. polyphaga* 30461.

Support: CNPq

BQ34 - CLONING AND EXPRESSION OF A RECOMBINANT CYSTEINE PROTEASE (TFCP1) FROM *TRITRICHOMONAS FOETUS*

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Tritrichomonas foetus, the causative agent of bovine trichomonosis, inhabits the mucosal surfaces of urogenital cavities where it is found adhering to the subjacent epithelium. This parasitic cytoadhesion may result in cytotoxicity to the host tissue. Previously data obtained in our laboratory indicate that cysteine proteases (CP) might play an important role in the disruption of epithelial monolayers by *T. foetus*. In order to further study the biochemical and biological attributes of CPs in *T. foetus* we have designed experiments having as tool a recombinant cysteine protease in *Escherichia coli*.

Using primers based upon the sequence of the gene *tfcp1* (accession number U13153) we obtained a fragment of 928 kb by PCR amplification of genomic DNA of parasites from the K strain of *T. foetus*. The sequence corresponds to an open reading frame which encodes a protein of 35 kDa showing 56% similarity with human cathepsin L. Comparison with the original sequence led to the identification of three aminoacid divergences that may correspond to strain variation. This fragment was cloned into pQE30 vector and transfected into *E. coli* M15. A recombinant 6xHis tagged protein of approximately 40 kDa was obtained after induction of expression by adding IPTG in bacteria culture medium and purification from *E. coli* extracts using Ni-affinity chromatography. Experiments are now being carried out to determine ideal refolding conditions for the recombinant enzyme and to obtain policlonal antisera against TFCP1.

Supported by: CAPES-MEC, CNPq, FAPERJ, FUJB-UFRJ & MCT-PRONEX

BQ35 - *LEISHMANIA (L.) MAJOR* INHIBITOR SCREENING GUIDED BY ADENINE PHOSPHORIBOSYLTRANSFERASE (APRT) INHIBITIONS

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Leishmaniasis is a serious disease caused by parasites of the order Kinetoplastida. According to the World Heath Organization (WHO, 1998), 88 countries are affected, with 12 million people infected, and approximately 350 million at risk of infection. The need for new drugs for the treatment of leishmaniasis comes from a lack of safe drugs and the serious secondary effects observed in the available chemotherapy. In order to look for new anti-*Leishmania* drugs we used recombinant APRT from *L. tarentolae*, as model system, to screening the inhibitory capacity of both plant and marine animal extracts through exploration the Brazilian biodiversity. This investigation has been done using an easy and fast spectrophotometric enzymatic assay. The best inhibitors of APRT were selected to be used in an *in-vivo L. (L.) major* inhibition study. The obtained results from enzyme screening lead us to identification of eighteen promising compounds. The best three IC_{50} values from selected molecules are 151 μM, 142 μM and 50 μM and the respective *L. (L.) major* killing capacity LD_{50} are 109 μM, 96,5 μM and 30 μM. The enzyme APRT and the three best inhibitors had their structures determined by X-ray crystallography. In a next step we intend to use these selected inhibitors in experiments of rational drug design based on structural information. The approach includes: co-crystallization of protein-inhibitor, computational molecular docking and QSAR (Quantitative Structure Activity Relationships). The information will be used to orient the molecular synthesis of new compounds toward the improvement of the inhibitory activity.

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BQ36 - INHIBITION OF *TRYPANOSOMA CRUZI* GROWTH BY *PTERODON PUBESCENS* OILY EXTRACT

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Trypanosoma cruzi is the etiologic agent of Chagas´ disease, an endemic disease in Central and South America. Chemotherapy of this disease is still very unsatisfactory, being based in nitrofurans and nitroimidazoles. These compounds are inadequate due to frequent toxic side effects and limited efficacy having little or no activity in the chronic phase of the disease. These facts show us the urgency for the development of new drugs more effective and less toxic for Chagas´ disease. *Pterodon pubescens* Benth (leguminosae), known as Sucupira branca is a native tree specie of Brazil and its seeds are used as hydroalcoholic infusion presenting anti-rheumatic, analgesic, anti-inflammatory and cercaricide properties. Toxicological studies demonstrated that the *P. pubescens* seeds extracts did not present acute or sub-acute toxicity. Geranylgeraniol and related substances (14,15-epoxygeranylgeraniol and 14,15-dihydro-14,15-dihydroxygeranylgeraniol) have been associated to the cercaricide activity of Pterodon pubescens oil. Isoprenoids are involved in cell proliferation and differentiation, and much work is being done nowadays to study these compounds, especially farnesol and geranylgeraniol, that are also involved in post-translational prenylation of proteins, facilitating protein-protein interactions and membrane-associated protein trafficking. In this work we study the effects of oleaginous extract of *P. pubescens* seeds (Ppoe), the hexanic fraction (Hex) and geranilgeraniol in the growth of *T. cruzi* epimastigotes from Y strain. Ppoe was obtained by maceration of *Pterodon pubescens* seeds in ethanol at room temperature for 15 days. A hexanic extract (Hex) from Ppoe was obtained by liquid-liquid extraction. Geranilgeraniol was further obtained from Hex by HPLC in a C8 column and characterized by GC-MS and NMR. The epimastigotes were grown in BHI, with 10% SFB, 10mg l^{-1} hemin and 20mg l^{-1} folic acid for 25ºC for 7 days in the presence and in the absence of Poep or H2. The EOPp, Hex and H2 presented a dose-dependent inhibition of epimastigotes growth with an IC50 of 12.31; 13.64 and 31.84 mg/ml respectively. Studies to the mechanism of action of the geranilgeraniol in the cellular division of the *T. cruzi* are in course in our laboratory.

Acknowledgments: CNPQ, FAPERJ.

BQ37 - LEISHMANICIDAL ACTIVITY OF POLYPHENOLICS-RICH EXTRACT FROM HUSK FIBER OF *COCOS NUCIFERA* LINN. (PALMAE)

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The available therapy for leishmaniasis, which affects 2 million people per annum, still causes serious side effects. The polyphenolics-rich extract from the husk fiber of *Cocos nucifera* Linn. (Palmae) presents antibacterial and antiviral activities, also inhibiting the lymphocyte proliferation, as shown by our group in previous works. In the present study, the *in vitro* leishmanicidal effects of *C. nucifera* on *Leishmania (L.) amazonensis* were evaluated. The minimal inhibitory concentration of the polyphenolics-rich extract from *C. nucifera* to completely abrogate the parasite growth was 10 mg/ml. Pretreatment of peritoneal mouse macrophages with 10 mg/ml of C. *nucifera* polyphenolics-rich extract reduced in approximately 44% the association index between these macrophages and *L. (L.) amazonensis* promastigotes, with a concomitant increase of 182% on nitric oxide production by the infected macrophage in comparison to non-treated macrophages. These results provide new perspectives on drug development against leishmaniasis, since the extract of *C. nucifera* at 10 mg/ml is a strikingly potent leishmanicidal substance, which inhibited the growth of both promastigote and amastigote developmental stages of *L. (L.) amazonensis* after 60 min, presenting no in vivo allergenic reactions or in vitro cytotoxic effect in mammalian systems.

Financial Support: CNPq, FAPERJ, FUJB, CAPES.

BQ38 - α-TOMATINE TOXICITY ANALISIS ON TRYPANOSOMATIDS PARASITES OF PLANTS AND INSECTS.

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The plants accumulate a great variety of secondary metabolites that have important functions on plant protection against pathogens. The glycoalkaloids are a class of substances that are involved in this protection and a better understanding about its role against diverse plant parasites has great practical interest. The glycoalkaloid α-tomatine exists in high concentrations in green tomato fruits (*Lycopersicum esculentum*), whereas in the ripe fruits it is changed to its aglycone form, tomatidine. In the case of *Phytomonas serpens*, a trypanosomatid parasite of tomato fruits, preliminary results showed that its development occur only in ripe fruits, what could be an evidence of involvement of α-tomatine in the defense of the tomato plant. Its protective function against fungal phytopathogens is well known and here it was evaluated against trypanosomatid parasites of plants and insects. We used logaritmic fase culture forms in GIPMY medium of seven strains of lower Trypanosomatids (genera *Phytomonas*, *Herpetomonas*, *Crithidia* and *Leptomonas).* To determine the 50% lethal dosis (LD50), we mixed equal volumes of 10^7 of the various culture forms with concentrations between 10^{-3} and 10^{-8} M of α-tomatine in PBS (pH 7,2, 150 mM with 10% ethanol), during thirty minutes at room temperature. The test was evaluated by the% of motility of the protozoan culture forms observed with optical microscopy in a Newbauer chamber. The results after the LD50 determination showed differences between the strains, with the LD50 oscilating between 10^{-3} and 10^{-5}M of á-tomatine. The same tests realized with the tomatine aglycone form, tomatidine, showed little toxicity on the same evaluated trypanosomatid culture forms. The mode of action on membranes and the antifungic action of α-tomatine is well known, but with protozoans, is poorly understood and this is the first step to clarify its role in plant defense against trypanosomatid phytopathogens.

Financial support: CNPq and CAPES

BQ39 - A COMPARATIVE STUDY OF MESOIONIC DERIVATIVES AGAINST *LEISHMANIA (L.) CHAGASI* AND *LEISHMANIA (V.) BRAZILIENSIS*

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In a previous study searching for new and highly effective antileishmanial drugs with low toxicity, we reported the *in vitro* activity of a series of mesoionic derivatives against *L. (L.) amazonensis* (Silva et al., 2002). As a part of our research program on chemotherapy against diseases caused by *Leishmania* spp., we decided to assay some 1,3,4-thiadiazolium-2-aminide class of mesoionic derivatives against other two different species of *Leishmania*, which has been associated to the others clinical forms of disease. The species used in this work were *L. (V.) braziliensis*

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that usually caused mucocutaneous disease and is endemic in the state of Rio de Janeiro and *L. (L.) chagasi* that is the causal agent of visceral leishmaniasis. These compounds, 4-phenyl-5-(4' and 3'-methoxy-cinnamoyl)-1,3,4-thiadiazolium-2-phenylamine chlorides were assayed against *L. braziliensis* (MCAN/BR/98/R619 strain) and *L. (L.) chagasi* (MCAN/BR/97/P142 strain) promastigotes and using Pentamidine Isethionate as reference drug. Parasites in the late log phase culture were incubated with several concentrations of the drugs solubilized in dimethyl sulphoxide (DMSO) and then counted in a Neubauer's chamber. Controls without the drugs and with DMSO were done in parallel. The preliminary results showed that mesoionic compounds such as 4'-methoxy and 3'-methoxy were effective against both *L. (V.) braziliensis* and *L. (L.) chagasi*. However, these compounds were about seven fold more effective against *L. (L.) chagasi* than *L. (V.) braziliensis* in promastigotes forms. Concerning the *L. (L.) amazonensis* promastigotes (MHOM/BR/77/LTB0016 strain), data from our previous work showed significantly difference in the sensitivity between both 3'- and 4'-methoxy mesoionic compounds; the 3'-methoxy derivative (IC_{50}/24=0.02±0,01µg/mL) was more efficient than 4'-methoxy compounds (IC_{50} /24=0.07±0.01 µg/mL). The present data showed that comparing the effect of those derivative against the three species and it was observed that the most sensitive was *L. (L.) amazonensis,* while *L.* chagasi (4'-OCH3 IC_{50}/24= 1,25±0,35 µg/mL; 3'-OCH3 IC_{50} /24= 1,73±0,27µg/mL) had a sensitivity between *L. (L.) amazonensis* and *L. (V.) braziliensis*, the last having the highest (4'-OCH3 IC_{50} / 24 = 6,5 ±0.70 µg/mL; 3'-OCH3 IC_{50} /24 = 8,3±1.55 µg/mL) Furthermore, in a comparative analysis using four mesoionic salts against *L. (L.) amazonensis* and *L. (L.) chagasi* promastigotes, it was observed that again *L. (L.) amazonensis* had lower IC_{50}/24 (4'-H IC_{50}/24 =0,18±0,01 µg/mL; 4'-OEt IC_{50}/24 =0,65±0,09 µg/mL; 4'-F IC_{50}/24 =0,38±0,03 µg/mL; 3'-Cl IC_{50}/24 =0,21±0,01 µg/mL) than *L. (L.) chagasi* (4'-H IC_{50}/24 =3,19±0,65 µg/mL; 4'-OEt IC_{50}/24 =4,75±0,51 µg/mL; 4'-F IC_{50}/24 =2,26±0,09 µg/mL; 3'-Cl IC_{50}/24 =2,261±0,09 µg/mL). The difference of effectiveness would be explained by the differences in the culture conditions such as higher concentration of fetal calf serum, since some amount of drug could be linked to the serum albumin, resulting in a decrease in the drug availability. Besides, it would be expected that the metabolism of the three species should be different.

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BQ40 - ANTILEISHMANIAL ACTIVITY OF NEROLIDOL

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Isoprenoids are essential compounds required for cell proliferation and differentiation. Isoprenoid biosynthesis in most eukaryotes derives from the mevalonate pathway. From acetyl-coenzyme A as the main substrate, most organisms synthesize hydroxymethylglutaryl-CoA (HMG-CoA) which is reduced to mevalonate by HMG-CoA reductase. Mevalonate is converted into isopentenyl pyrophosphate (IPP) which generates geranyl pyrophosphate and farnesyl pyrophosphate, the main precursors of polyisoprenoids such as dolichol, ubiquinone, cholesterol and prenyl groups transferred to prenylated proteins. The inhibition of isoprenoid biosynthesis leads to suppression of cell growth and death.

Terpenes are isoprenoids found in oils derived from fruits and herbs and their antibactericidal, antitumoral and antiprotozoal activity have been under study. The effect of nerolidol against *Plasmodium falciparum* has been shown to be due to the interference in the isoprenoid biosynthetic pathway (Macedo et al., FEMS Microbiol. Let., 207:13-20, 2002).

To investigate a possible leishmanicidal activity of nerolidol, in vitro cultures of *L. (L.) amazonensis* promastigotes and amastigotes were grown in the presence of increasing concentrations of the drug. The growth of both life cycle stages was arrested by nerolidol with IC50s of 90 mM for promastigotes and 53 mM for amastigotes. The effect of nerolidol was also demonstrated against *L. (L.) major* (IC50 67 mM), *L. (V.) braziliensis* (IC50 70 mM) and *L. (L.) chagasi* (IC50 73 mM) promastigotes and *T. cruzi* epimastigotes (IC50 105 mM).Metabolic labeling of promastigotes and amastigotes of *L. (L.) amazonensis* with [2-^{14}C] mevalonate followed by analysis of the hexane fraction by HPLC, showed that the synthesis of dolichol and of the isoprenic chain of ubiquinone are 91% inhibited after treatment with nerolidol 30 mM. These results suggest that terpenes may have great potential in the development of new antileishmanial chemotherapeutic agents.

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BQ41 - PARTIAL CHARACTERIZATION OF A UDP-GLCNAC: POLYPEPTIDE A-*N*-ACETYLGLUCOSAMINYLTRANSFERASE IN *CRITHIDIA FASCICULATA*.

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In *Trypanosoma cruzi*, the first step of mucin-like molecules glycosylation occurs in the Golgi complex and consists in the transfer of *N*-acetylglucosamine (GlcNAc) unit from UDP-GlcNAc to threonine (Thr) residues, in a reaction catalyzed by a polypeptide *O*-a-*N*-acetylglucosaminyltransferase (*Previato et al.*, 1998). In contrast, the O-glycans of mammal's mucins are linked to hydroxylated aminoacids Thr and serine (Ser) in the peptide backbone through a a-*N*-acetylgalactosamine (a-GalNAc) unit. Since polypeptide GlcNAc-transferases have been only characterized in *T. cruzi* and *Dictyostelium discoideum* (*Jung et al.*,1998), we decided to investigate the presence of this transferase activity in *Crithidia fasciculata,* a member of the Trypanosomatidae family. *C. fasciculata* is a monogenetic parasite that normally colonize the gut of various fly hosts, and that can be easily grown axenically. In this study, we detected the polypeptide *O*-GlcNAc transferase activity and optimized it's assay. The enzymatic assay was done using microsomal membranes of *C. fasciculata*, a synthetic peptide $KP_2T_8KP_2$ and UDP-[^{3}H]GlcNAc as acceptor and donor substrates respectively. After purification of the O-glycosylated peptide, the incorporation of [^{3}H]GlcNAc was measured by liquid scintillation counting. The enzyme activity showed to be dependent of incubation time and concentration of enzyme and acceptor substrate. The transference has an optimal pH of 7,5-8,5, an optimal temperature of 20°C and requires Mn^{2+}. The glycosylated $KP_2T_8KP_2$ product is susceptible to base-catalyzed b-elimination, and the efficiency and level of glycosylation were determined with chromatographic methods. These results demonstrated a great efficiency in the transfer reaction of the GlcNAc residue to the synthetic acceptor, with a polypeptide *O*-GlcNAc transferase activity comparable of the one previously described in epimastigotas and trypomastigotes forms of *T. cruzi*.

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BQ42 - GLYCOSPHINGOLIPIDS (GSLS) FROM AMASTIGOTE FORMS OF *LEISHMANIA (LEISHMANIA) AMAZONENSIS*: CHARACTERIZATION OF A GSLS-BINDING PROTEIN IN MURINE MACROPHAGES

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The biologycal cycle of *Leishmania* parasites alternates between a flagellated promastigote in the sandfly midgut and a nonflagellated amastigote stage in the

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mammalian macrophage. Several receptors have been described to *Leishmania* promastigote-macrophage interaction, however the uptake of *Leishmania* amastigote by macrophages remains poorly understood. It has been described two classes of cell-surface receptors that mediate phagocytosis: opsonic and nonopsonic receptors. The *L. (L.) amazonensis* amastigote-macrophage interaction mediated by stage-specific GSLs is a typical example of phagocytosis that do not require parasite opsonization in order to occur macrophage adhesion and internalization, as described by Straus *et al* (J Biol Chem, 1993:268;13723-30). Recently, it was demonstrated in our lab, that a 30 kDa mouse peritoneal macrophage protein interacts either with micelles containing GSLs of amastigote forms of *L. (L.) amazonensis* or with whole amastigote parasites. By Western blotting it was verified that this 30 kDa receptor is not solubilized when macrophages were extracted with PBS containing 0.5% Triton X-100 plus protease inhibitors, remaining in the insoluble fraction. In order to better characterize this receptor, macrophage surface proteins were labeled with biotinamidocaproate N-hydroxysuccinimide ester at 4^0C, and a strong labeling was observed for these proteins confirming their expression at cell surface. Since a variety of lectin-like macrophage receptors have been identified, the purification and the analysis of NH_2-terminal sequence of this 30 kDa macrophage protein are currently been carried out.

Supported by FAPESP and CNPq.

BQ43 - INHIBITION OF TRYPOMASTIGOTES INFECTIVITY BY MONOCLONAL ANTIBODY BST-1, DIRECTED TO EPIMASTIGOTE GIPCS. CROSS-REACTIVITY WITH A HIGH MOLECULAR WEIGHT ANTIGEN PRESENT IN TRYPOMASTIGOTES

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Recently it was a produced in our lab a monoclonal antibody (mAb), termed BST-1, against glycosylinositolphosphoceramides (GIPCs) present in *T. cruzi* epimastigotes. By indirect immunofluorescence a strong parasite labeling was detected with epimastigotes, amastigotes and trypomastigotes. By Western blot it was verified that GIPCs are the only antigens recognized by BST-1 in epimastigotes, on the other hand, when amastigotes and trypomastigotes isolated from culture of VERO cells were analyzed it was observed that besides GIPCs a high molecular weight component (160-200 kDa) was also recognized by the BST-1 antibody. This component was also detected in culture medium of trypomastigotes infected VERO cells.

In order to characterize the synthesis and secretion of the high molecular weight antigen, recognized by BST-1, VERO cells were infected with trypomastigotes (strain Y) and after 24, 48, 72, 96 and 144 hours, the parasites present in VERO cells and in the conditioned medium were collected. The parasite-free supernatant of conditioned medium was also analyzed. The number of trypomastigotes and amastigotes were determined and the BST-1 reactivity analyzed by Western Blot. It was observed that the level of 160-200kDa component recognized by BST-1 increases during infection indicating that amastigotes are able to synthesize the component of 160-200 kDa. On the other hand, the presence of the 160-200 kDa antigen in the parasite-free supernatant was detected after 96 hours of infection, when trypomastigotes start to be liberated from VERO cells to culture medium.

In infectivity assays it was verified that mAb BST-1 at a concentration of 2.5 µg/ml was able to inhibit about 70% of trypomastigotes infectivity of VERO cells. These results suggest that the epitope/antigen recognized by mAb BST-1 is involved in adhesion/internalization of trypomastigotes.

A better characterization of this trypomastigote high molecular weight component recognized by BST-1 and the mechanism of secretion are under study.

Supported by FAPESP and CNPq

BQ44 - PURIFICATION AND PARTIAL CHARACTERIZATION OF A FLAGELLAR GLYCOPROTEIN FROM *LEISHMANIA (VIANNIA) BRAZILIENSIS*

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Glycoconjugates are usually involved in *Leishmania*-macrophage interactions. Studies carried out in our lab demonstrated the involvement of flagellar 180 kDa glycoprotein specific of *Leishmania (Viannia) braziliensis* in the interaction of promastigotes with macrophages during the infection (Silveira et al, Int J Parasitol., 31:1451-8, 2001). Promastigote forms of *L. (V.) braziliensis* (WHOM/BR/1987/M11272) were cultivated in LIT medium supplemented with 10% of heat inactivated fetal calf serum. The flagella were separated from promastigote bodies by gasose nitrogen cavitation (350 psi) and purified by sucrose gradients. The flagellar proteins were solubilized with n-Octyl glucoside 50mM, PMSF 0.2mM and EDTA 1mM in PBS. The solubilized proteins were diluted in sample buffer and polyacrylamide gel electrophoresis (SDS-PAGE) was performed. A high molecular weight flagellar glycoprotein was purified by SDS-PAGE. After gel staining with Coomassie blue the proteins were electroeluted. The purified 180 kDa flagellar glycoprotein was analyzed by SDS-PAGE and tested by Western blotting with 7 biotinylated lectins and with monoclonal antibody (mAb) SST-3, which is specific for this flagellar glycoprotein. *Helix pomatia* lectin and mAb SST-3 recognized only the 180 kDa flagellar glycoprotein while Concanavalin A bound to the 180 kDa glycoprotein and various other parasite glycoproteins. By indirect immunofluorescence carried out using SST-3 and biotinylated *Helix pomatia* lectin it was detected a positive fluorescence only in flagella. When promastigotes were pre-incubated with mAb SST-3 and than with biotinylated *H. pomatia* lectin and streptoavidin conjugated to fluorescein, no fluorescence was detected, indicating that mAb SST-3 blocked *H. pomatia* lectin binding to gp180, and that the this glycoprotein presents a-GalNAc residues in the glycan structure. Further purification of gp180 is required to improve its characterization by determination of the isoelectric point and NH_2-terminal region sequence.

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BQ45 - THE ROLE OF SULFATED GLYCOSAMINOGLYCANS IN THE INVASION OF CARDIOMYOCYTES BY *TRYPANOSOMA CRUZI* AND BIOCHEMICAL CHARACTERIZATION OF ITS RECEPTOR

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The ability of *Trypanosoma cruzi* to recognize molecules on the surface of phagocytic and non-phagocytic cells is determinant to its survival within vertebrate host. Evidences of the participation of sulfated proteoglycans in the recognition process have been reported in many human pathogens, including *T. cruzi* via a heparin-binding protein (penetrin). Recently, we have demonstrated that heparan sulfate proteoglycan (HSPG) mediate the invasion of trypomastigotes in cardiomyocytes (Calvet et al., 2003). However, the structure of HSPG molecule implicated in the receptor-ligand binding and the role of others sulfated glycosaminoglycans (GAGs) in this interaction is not completely

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understood. Additionally, we were interested to isolate the *T. cruzi* surface receptor in order to verify its interaction with the mammalian cells and the peritrophic membrane of its vector, *Rhodnius prolixus*.

To test the effect of GAGs on *T. cruzi* invasion, culture-derived trypomastigotes, Dm28c clone, were pretreated with 20µg/ml of heparin, keratan sulfate (KS) or heparan sulfate (HS) fragments, which were obtained by enzymatic treatment. In the competition assays, the parasites were incubated for 2h at 37ºC with cardiomyocytes and the percentage of infection evaluated. Treatment of trypomastigotes with heparin decreased considerably the parasite invasion achieving an inhibition of 80% in the infection ratio. The HS fragment obtained by heparitinase II cleavage reduced the infection level, while KS displayed no inhibitory effect.

These parasites receptor was isolated by the association of the Triton X-114 method with heparin-sepharose and DEAE-cellulose chromatography. SDS-PAGE analysis of the parasites hydrophobic protein extracts revealed two distinct proteins bands, 65.8 kDa and 59.0 kDa, eluted from the heparin affinity chromatography, which were in the majority eluted at 0.5M and 1.0M of NaCl for trypomastigotes and epimastigotes, respectively. The radioactive analysis suggests that the surface receptor is 2.7-fold higher in trypomastigotes compared to levels found in epimastigote forms. Furthermore, similar molecular mass duplet was also detected in both parasite forms after the heparin or heparan sulfate recognition on the parasites proteins extract by Western blot. The anionic chromatography data show evidence that these two proteins exhibit similar charge net in both *T. cruzi* forms. These results also suggest that a homogeneous sample occur in the low salt concentration, 0.075M of NaCl. Further studies are in progress to verify the ability of these isolated proteins to recognize mammalian and the *R. prolixus* peritrophic proteins.

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BQ46 - PRELIMINARY ANALYSIS OF VESICLE PROTEINS FROM *LEISHMANIA* SPECIES.

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With the aim of identifying secreted proteins in both Old World and New World *Leishmania* species, intrecellular vesicles were extracted by centrifugation from cell lysates of promastigotes forms of *L. (L.) major* and *L. (L.) amazonensis*. After disruption of the vesicles by ultrasonication, soluble proteins were separated by ultracentrifugation, and were analysed by SDS-PAGE and 2D-PAGE. Protein spots were analysed by ESI-MS/MS mass spectometry of peptides generated by tryptic digestion, and the amino acid sequences of these peptides were compared with protein and DNA sequence databases using BLAST and FASTS. Preliminary results show that the protein extracts from both species includes the cell surface secreted proteins Gp46 and Gp63, which demonstrates the viability of whole vesicle extract proteomic analysis for the identifation of secreted proteins in trypanosomatid parasites. Furthermore, differences in the vesicle protein profiles were observed comparing extracts from *L. (L.) major* and *L. (L.) amazonensis*, which suggest that this methodology may be applied to a subcellular proteomic approach to study differential expression patterns of the parasite.

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BQ47 - ANALYSIS OF THE PROTEOME OF *TRYPANOSOMA CRUZI* USING TWO-DIMENSIONAL GEL ELECTROPHORESIS AND MALDI TOF

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Trypanosoma cruzi, the protozoan which causes Chagas disease, possesses a complex life cycle involving different developmental stages. Experimental conditions for two-dimensional electrophoresis (2-DE) analysis of *T. cruzi* trypomastigote, amastigote and epimastigote proteomes were optimized. Comparative proteome analysis of the life stages were done, revealing that few proteins included in the 2-DE maps displayed significant differential expression among the three developmental forms of the parasite. In order to identify landmark proteins, spots of the trypomastigote 2–DE map were subjected to MALDI-TOF MS peptide mass fingerprinting, resulting in 26 identifications that corresponded to 19 different proteins. Among the identified polypeptides there were heat shock proteins (chaperones, HSP 60, HSP 70 and HSP 90), elongation factors, glycolytic pathway enzymes (enolase, pyruvate kinase and 2,3 bisphosphoglycerate mutase) and structural proteins (KMP 11, tubulin and paraflagellar rod components). The relative expression of the identified proteins in the 2-DE maps of the three *T. cruzi* developmental stages is also presented.

Supported by CNPq, CAPES, UnB

BQ48 - DIFFERENTIAL PROTEIN EXPRESSION PROFILE IN HEARTS OF CHRONIC CHAGAS'DISEASE OR IDIOPATHIC DILATED CARDIOMYOPATHY

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Chronic Chagas'disease cardiomyopathy (CCC) is an often fatal outcome of *Trypanosoma cruzi* infection, having shorter survival than idiopathic dilated cardiomyopathy (IDC), a clinically similar cardiomyopathy showing less inflammatory phenomena. In order to analyze the molecular changes in the affected myocardium in response to chronic inflammation in CCC, we used proteomic analysis to compare the protein expression profile in heart samples from CCC and IDC patients and identify differentially expressed proteins. Myocardial homogenates of heart samples from end-stage CCC and IDC hearts explanted during heart transplantation were subjected to bidimensional electrophoresis (3-10 pI gradient and 12% polyacrylamide gel electrophoresis). Differentially expressed Coomassie blue-stained protein spots were selected with imaging software; selected spots in gels were excised, subjected to tryptic digestion and processing in a robotic workstation. Protein identification was performed by peptide mass fingerprinting with the aid of MALDI-ToF mass spectrometry and virtual tryptic digestion of proteins in sequence databanks. Results have shown more than 200 Coomassie blue-stained spots in each gel. Preliminary analysis has identified mitochondrial creatine kinase as the protein with most significantly reduced expression in the myocardium of a CCC patient, as compared to a paired IDC patient. Given the fundamental role of mitochondrial creatine kinase in the translocation of high-energy phosphate from the mitochondrion to generate cytoplasmic ATP, this finding may be related to the shorter survival observed in CCC as compared with IDC.

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BC1 - THE MEDIAN BODY OF *GIARDIA LAMBLIA*: ULTRASTRUCTURE BY HIGH RESOLUTION SCANNING ELECTRON MICROSCOPY

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Giardia lamblia is an intestinal parasite of several mammals. It is a parasitic protozoan that infects thousands of people all over the world, causing a disease known as giardiasis. *Giardia* possesses several cytoskeletal structures composed of microtubules, such as the disc, median body, axonemes of eight flagella and the funis. The median body is formed by an irregular set of microtubules situated dorsally to the caudal axonemes, resembling a comma in a face.

In the present study we have used a new technique that allows the removal of the plasma membrane using detergents and observation of cytoskeletal structures by either conventional scanning electron microscopy or field emission scanning electron microscopy (FESEM). Cells were adhered to poly-L-lysine-coated glass coverslips and then treated with the permeabilization buffer (0.5% Nonidet-40 P-40, 0.1M Pipes, 1mM $MgSO_4$, 2 mM glycerol, 2 mM EGTA, 1 mM PMSF (phenylmethylsulfonyl fluoride), 0.5% Triton X-100) for different times (10 min-2 h). The cells were washed, fixed in 2.5% glutaraldehyde, post-fixed 1% OsO_4, dehydrated, critical point dried and coated with gold. The samples were examined in a Jeol JSM-6340F Field Emission or JSM-5800 Scanning Electron Microscope. Upon these procedures the median body was better visualized. When the plasma membrane was completely extracted the median body was not visualized suggesting the connection between these two cells structures. FESEM revealed that the median body was constituted by several small fascicles formed by microtubules forming larger bundles. The bundle number was variable as well the microtubules number found in each fascicle. There was a variation in number, disposition and location of the median body fascicles in different organisms found in the same culture preparation. Each fascicle was formed by parallel microtubules with different lengths, but the different fascicles were not parallel. Some fascicles were connected, leading a misunderstanding by light microscopy of the presence of one or two median bodies. In the caudal axonemes region a thicker median body bundle is seen. In conclusion, there are not two median bodies, and they were present in about 70% of the cells population, with connections with the ventral disc.

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BC2 - MYCOPLASMA OBSERVATIONS IN *TRICHOMONAS VAGINALIS*: THE ENEMY WITHIN.

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The protist *Trichomonas vaginalis* is a well described early eukaryote and its infection is the most prevalent nonviral sexually transmitted disease worldwide. In recent studies it has been proposed that Trichomonads presents different infectious organisms within the cell such as, virus like particles and bacteria. Among them, mycoplasm-like organisms are the most easily detectable in some *Trichomonas* isolates.

The genus *Mycoplasma* presents the smallest organisms lacking cell walls that are capable of self-replication and cause various diseases in humans, animals, and plants. Although mycoplasmas are recognized primarily as extracellular parasites or pathogens of mucosal surfaces, recent evidences suggested that certain species may invade the host cells. They have also been recognized as a major contaminant of cell cultures where they thrive in close association with host cell plasma membrane and may cause drastic alterations (Stanbridge, 1982).

The aim of the present study is to further characterize this relationship between these two human pathogens that even being long time known, but still not well defined. Since it is known that *Trichomonas* is used to feed from bacteria, it is not clear whether *Mycoplasma* or *Trichomonas* benefits from one another or if they work together in a co-parasitism.

By means of electron microscopy, thin sections observations of these organisms revealed that they present a pleomorphism characterized by a size ranging from 90 to 200mm, probably due to its lack of a rigid cell wall, which may favour their adhesion and fusion with the host membrane (Scholtyseck *et al*., 1985). This variation variation in shape was distributed in ovoid, spherical and peak-like protrusion forms. Once in a while some budding sites could be seen and also, many places of adherence invaginations visible on the *Trichomonas* surface. On some of the budding vesicles we could also visualize an electrondense area, which could be nucleic acid material, strongly suggesting that the mycoplasma-like organism may have some kind of cycle within the *Trichomonas* cell. Mycoplasms also showed to cause some damage to the host, like intense cytoplasmic vacuolization sometimes harboring several mycoplasm-like structures.

Its has been shown for many years that the co-existence of different sexually transmitted diseases in the same individual it is quite common, vaginal infections by *T. vaginalis* were associated *with Mycoplasma fermentans* since 1985 by Scholtyseck *et al*.

It is still unknown if these new observations have significance with respect to virulence or pathogenicity of *T. vaginalis*, however we cannot discard the possibility of trichomonads playing the role of a new vector for infectious agents. We are directing our efforts towards the discovery of new labeling techniques and markers that could help us to elucidate these questions. Further studies with immunoelectron microscopy will be needed to confirm this possibility and to extent our knowledge on this relationship.

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BC3 - ENDOCYTIC PATHWAY IN PHAGOCYTIC AND NON-PHAGOCYTIC CELLS DURING THEIR INTERACTION WITH *TRYPANOSOMA CRUZI*

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The internalization of small molecules, macromolecules, large particles and even whole cells is a process collectively called endocytosis. Following internalization, endocytic vesicles fuse with sorting endosomes localized throughout the cytoplasm, where several members of the Rab family of Ras related small GTP-binding proteins participate in the transpont events such as budding, docking and vesicle fusion. The intracellular protozoan pathogen *Trypanosoma cruzi* causes Chagas' disease in humans, a chronic inflammatory conditions caracterized by cardiomyopathy and digestive disorders. As (i) several alterations have been reported during the infection of host cell by parasites and (ii) endocytosis has important roles in cellular events, the aim of the present study is characterize the endocytic pathway in professional phagocytic (peritoneal macrophages-PM) and non-phagocytic cells (cardiomyocytes-CM) during their in vitro interaction with *T. cruzi*. We started our studies by analyzing the fluid-phase endocytosis in uninfected host cells through fluorescent assays using dextran conjugates (FITC-Dx) by ultrastructural approaches using albumin adsorbed to gold particles (BSA-Au). After 5-15 min of incubation at 37ºC, the fluid-phase ligands were noted within early endosomes mostly localized in the

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periphery of both CM and PM. Longer periods of incubation resulted in the perinuclear accumulation of the ligands in late compartments in both cell types. We next investigated the distribution of early and late endosomes by fluorescent assays using monoclonal antibodies raised against Rab 7 (marker for late endosomes-LE), Rab 11 (recycling endosomes-RE) and EEA 1(sorting endosomes-SE) molecules. In uninfected CM, the EEA 1 labeling showed a puntual distribution throughout the cytoplasm and around the nuclei. The Rab 11 analysis in CM showed positive labeling in large vesicles and in tubular elements throughout the cytoplasm besides a bright perinuclear spot. When anti-Rab 7 was probed, CM displayed a bulk labeling surrounding the cell's nuclei and a spotted labeling in cytoplasm. However, during the interation (24-48h) of non-phagocytic cells with trypomastigotes of *T. cruzi* (Y and Dm28c stocks) we observed a striking decrease and almost loss of the LE and RE expression in the host cells. As expected, the infection with both parasite stocks also altered the usual distribution and number of FITC-Dx-labeled late compartments in infected-CM. Flow cytometry and ultrastructural approaches are underway in order to deep analyze and compare the altered expression of endocytic compartments in both phagocytic and non-phagocytic cells during their invasion by *T.cruzi*. The observed endocytosis impairment during the infection of host cells by the parasite can contribute to the overall physiologic failure by modifying normal incoming of nutrients as well as interfering with other important events related to the endocytic pathway.

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BC4 - REDISTRIBUTION OF CYTOPLASMIC ORGANELLES FROM PHAGOCYTIC AND NON-PHAGOCYTIC CELLS AFTER INVASION BY *TOXOPLASMA GONDII*: A STUDY BY LASER SCANNING MICROSCOPY.

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Toxoplasma gondii, the causative agent of toxoplasmosis, is an obligatory intracellular parasite that establishes a most unique relationship with its host and is capable of active penetration and multiplication in any nucleated cell of warm blooded animals. Its survival strategies include the partial exclusion of host cell membrane proteins, what allows the escape from fusion of the parasitophorous vacuole with host cell lysosomes, and the rearrangement of host cell organelles in relation to the parasitophorous vacuole. In this work we report the rearrangement of host cell organelles (mitochondria and endoplasmatic reticulum) around the parasitophorous vacuole of two distinct cell types: a phagocytic and a non-phagocytic cell. We used the mitochondrion-selective dye CMXRos (Mitotracker) and the carbocyanine dye DiOC6 in high concentrations (2mg/ml) to label mitochondrion and endoplasmic reticulum of LLCMK2 cells (epithelial cells from *Macaca mulata* kidney), and CF1 mice peritoneal resident macrophages, both challenged with *Toxoplasma* and allowed to interact for 1 and 24 hours. Under Laser Scanning Microscopy observation Mitotracker labeling showed filamentous mitochondria all over the cytoplasm. In some cells, specially 24 hours after challenge, enhanced fluorescence around the PV was observed, but could be the result of PV growth pushing mitochondria around it. This was observed both in LLCMK2 cells and in macrophages. Ultrathin sections of infected cells observed at the transmission electron microscope also did not show strict association of mitochondria and PV membrane. So, our results on organelle association to the PVM agreed less with other authors when mitochondria were considered both for macrophages and LLCMK2 cells. On the other hand, in cells labeled with DiOC6 an area of higher fluorescence around the PVs was always observed, showing that association of ER profiles with the growing PV, as observed by electron microscopy, indeed occurs in all cell types. These results indicate that the the organelle association with the PVM, described by Sinai et al. (*J Cell Sci.* 17, 2117[1997]) may not be mandatory for all cell types, at least in what concerns mitochondria.

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BC5 - THE CYTOSKELETON PARTICIPATES ON *TRYPANOSOMA CRUZI* EPIMASTIGOTES ENDOCYTIC PATHWAY

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Although actin filaments are known to be essential for endocytosis in yeast and mammalian cells, there is few information concerning their presence in trypanosomatids: actin gene was found and it also seems to be expressed (De Souza et al., 1983. J. Parasitol. 69:138; Mortara, 1989. J. Protozool. 36:8) but no role for actin filaments has yet been described in *Trypanosoma cruzi*. *T. cruzi* is a very useful cell model to investigate the influence of the cytoskeleton on endocytosis, as it possesses a very special cytoskeleton. Among the most conspicuous cytoskeleton components are the subpellicular microtubules (SPMT), underneath the plasma membrane. This parasite is a polarized cell where endocytic organelles have a very well defined cytoplasmic localization, distributed from the cell anterior region, where the cytostome, the main site of endocytic cargo entry, is placed to the posterior end where reservosomes, the lysosome-like organelles, are found. As the endocytic pathway must span for such a distance along the parasite body, it is highly feasible that microtubules and microfilaments are involved in guiding endocytic transport vesicles.

We processed for transmission electron microscopy *T. cruzi* epimastigotes that have previously uptaken colloidal gold-conjugated transferrin (Tf-Au), using a special protocol to emphasize cytoskeleton structures. We could observe connections between endocytic organelles and SPMTs. We have also used drugs that act on cytoskeleton to evaluate the different participation of microtubules and microfilaments on endocytosis. Oryzalin and cytochalasin D were used as disrupting agents of stable microtubules and actin filaments, respectively. As stabilizing agents, we used taxol for microtubules and jasplakinolide for actin filaments. After drug treatment, cells were incubated with transferrin–fluorescein (Tf-FITC) or Tf-Au, processed and observed. We verified some ultrastructural alterations, mainly at the cytostome. Unexpectedly, the connections between endocytic organelles and SPMTs were maintained in oryzalin and taxol treated cells. We have also quantified endocytosis using radioiodinated transferrin (^{125}I-Tf) in the presence of the drugs. Oryzalin and taxol caused a decrease of about 50% in ligand endocytosis. The effects of cytochalasin and jasplakinolide were more drastic, suggesting that, like in other eukaryotic cells, actin plays a fundamental role in *T. cruzi* epimastigote endocytosis. While jasplakinolide-treated cells presented some internalized TF-Au, cytochalasin treatment completely blocked ligand entry, entrapping Tf-FITC and Tf-Au at the cytostome and rendering ^{125}I-Tf accessible to tripsin digestion. We demonstrated, for the first time, that epimastigotes do recycle internalized Tf. The drugs, mainly the stabilizing ones, affected recycling of ^{125}I-Tf to the plasma membrane. Degradation of transferrin inside reservosomes also occurred and was almost completely blocked by cytochalasin.

In conclusion, endocytic organelles are connected to SPMTs that may guide their positioning. Actin filaments seem to be implicated in the initial events of endocytosis in epimastigotes. Transferrin recycling may depend on both cytoskeleton components – microfilaments and microtubules.

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BC6 - IMMUNOCYTOCHEMICAL LOCALIZATION OF THE C-TERMINAL EXTENSION FROM THE CYSTEINE PROTEINASE LPCYS2 OF *LEISHMANIA (L.) PIFANOI.*

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Little is known about protein sorting in trypanosomatids. We are studying the mechanisms of lysosome sorting of cysteine proteinases in leishmania. Cysteine or thiol proteinases have been identified in a variety of pathogenic microorganisms and have been implicated in processes that can be important for infection and virulence. Cysteine proteinases are highly conserved among trypanosomatids,. These proteinases have three characteristic domains: the prepro sequence, the pre part being a putative signal peptide, the pro domain containing a short peptide responsible for targeting to the megasoma; the second one is the catalytic moiety, and the last one is a C-terminal extension, the function of which, until the moment, is not known. In previous studies, immunolocalization using a monoclonal antibody anti C-terminal extension, had shown the presence of this domain in the lysosome. We have produced a polyclonal antibody against this domain and used it to determine the final localization of the cysteine proteinase C-terminal extension in *Leishmania*. *Leishmania* axenic amastigotes, when exposed to this antibody, showed lysosomal labeling and also strong labeling of the flagellar pocket. Interestingly, when *Leishmania (L.) pifanoi* in early stages of macrophage infection were labeled, the C-terminal extension localized mostly to the surface of the parasite. On the other hand, a control using an antibody agains the catalytic domain of Lpcys2, marked the lysosome and, to a lesser extent the flagellar pocket, as expected. This indicates that the cysteine proteinase suffers a gradual processing in the lysosome, preserving its active mature region inside this organelle, and secreting the C-Terminal domain through the flagellar pocket route. Nevertheless, there seems to be an accumulation of this domain in the flagellar pocket in the culture forms of the parasites, and a release of the C-terminal domain after contact with macrophages. This route of secretion may be used by the parasite to discard proteins that possibly already fulfilled their metabolic function and are no more necessary, or, on the other hand, it may also be that some of these proteins or proteolytic fragments have an role at the immunoregulation level, helping parasites to survive in the hostile environment of the mammalian host.

BC7 - ENDOCYTIC PATHWAY IN EVOLUTIVE FORMS OF *T. CRUZI:* CHARACTERIZATION OF THE COMPARTMENTS

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Endocytosis represents the uptake of extracelular material through multiple roads, including the phase fluid, receptor-mediated endocytosis and the phagocytosis. Endocytic pathway of evolutive forms of the *T. cruzi* is not well known, controversies exist about the existence of early endosomes in these parasites. In the present study, we investigate evolutive forms of *T. cruzi* (Y strain), the expression and the distribution of early (sorting and recycling) and late endosomes through the expression of specific GTPases by electron microscopy and flow cytometry approaches. Bloodstream trypomastigotes were obtained from *T. cruzi* infected Swiss mice [1], epimastigotes from axenic medium and amastigotes from the supernatant of J774G-8 macrophage cell lines [2]. For ultrastructural characterization, the parasites were washed and fixed with 0.01% paraformaldehyde (PFA), 0.2% glutaraldehyde, 0.1% picric acid, dehydrated in methanol crescent series and then embedded in Lowicryl resin. Unstained ultrathin sections were incubated for 1h/37°C in a blocking buffer and further incubated for another 1h/37°C with monoclonal antibodies against Rab7 (for detecting late endosomes), Rab 11 (for recycling endosomes) and EEA1 (for sorting endosomes). After washing the samples were incubated for 30 min with the secondary antibody (goat anti-rabbit IgG) coupled to 5nm colloidal gold particles and then analyzed by transmission electron microscopy using EM10C Zeiss microscope. For flow cytometry assay, the parasites were fixed in PFA 0.2% and incubated with specific antibodies, followed by incubation with the secondary antibody (anti-rabbit IgG-TRITC) and analyzed in a FACSCalibur. All the negative controls were performed by the omission of the primary antibody. Ultrastructural analysis of the anti-EEA1 showed a dispersed labeling in the whole cytoplasm mostly near the cell periphery and within the flagellar pocket in both amastigotes and trypomastigotes. The staining with anti-Rab11 in amastigotes and trypomastigotes forms were localized close to the nucleus, kinetoplast. The labeling with anti-Rab 7 were preferentially visualized near the nucleus and the kinetoplast of the parasites. Our data showed that, the percentages of positive parasite population for each labeled endosome were similar among epimastigotes and amastigotes whereas the trypomastigotes forms displayed lower labeling, suggesting and confirming literature data concerning the higher endocytic activity in the former parasites [3]. Our data also suggest the expression of early endosomes corroborating the data of Porto-Carreiro et al (2000). Another approaches are under way to deep investigate and compare the endocytic pathway in the three evolutive stages of *T. cruzi.*

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BC8 - DIVIDING *LEISHMANIA (L.) AMAZONENSIS* AND *HERPETOMONAS MEGASELIAE* PRESENT A FLAGELLA CONNECTOR

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The cytoskeleton of trypanosomatids presents some particularities, such as the subpellicular microtubules (SPMT), a cage-like parallel array of singlet microtubules, which are connected to each other and to the plasma membrane by short proteic filaments. The SPMT is a stable network that confers cell shape and mechanical resistance. The trypanosomatids owns a single flagellum that emerges from the flagellar pocket. In addition to the classical "9+2" microtubule axoneme, the flagellum contains a lattice-like structure named paraflagellar rod (PFR) which runs alongside the axoneme. The PFR is evolutionary conserved among the members of the Kinetoplastida (except for the endosymbiont-bearing ones) and immunologically relevant. Recently it was described, exclusively in the procyclic form of *Trypanosoma brucei*, a new structure during cell division that attaches the new flagellum to the old one, the flagella connector (FC). The FC was described with three subcomponents: a "fuzzy" component at the distal tip of the new flagellum, a short link structure and a plate-like lamellar domain. The FC connects the new flagellum to the lateral side of the old flagellum

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axoneme, and not to the PFR. It remains present even in *T. brucei* lacking flagellum-cell body attachment, and it is resistant to detergent extraction and high Ca^{2+} concentration treatment.

In this work we report the presence of a flagella connector in promastigote forms of *Herpetomonas megaseliae* and *Leishmania (L.) amazonensis*. We verified the presence of FC in 97% and 75% of *H. megaseliae* and *L. (L.) amazonensis* dividing cells, respectively. Using high-resolution field emission scanning electron microscopy (FESEM) we observed that the FC anchors the tip of the new flagellum since it shows up from the flagellar pocket and runs forward along the old flagellum until close to its distal end. In negative staining, it was possible to readily observe that the connector has a single detergent-resistant amorphous component apposed to the old flagellum axoneme. The new flagellum appears to be thinner than the old one and seems to have the PFR not completely assembled. However, immunocytochemistry using antibodies anti-PFR A (L8C4) and anti-PFR A and PFR C (L13D6) revealed both PFR major proteins distributed in the PFR of the new flagellum.

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BC9 - ISOLATION OF LIPID DOMAINS FROM THE MEMBRANES OF *TRYPANOSOMA CRUZI* RESERVOSOMES

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Reservosomes are acidic compartments that store nutrients, found in epimastigote forms of *Trypanosoma cruzi*. They were first described as multivesicular bodies (De Souza et al, 1978 Exp. Parasitol, 45:101). Further studies (Soares and De Souza, 1988 J Submicrosc Cytol Pathol, 20:349) using ultrastructural cytochemistry and stereology characterized reservosomes as single-membrane bound organelles ranging from 0.4 to 0.6 mm in diameter that lack true internal vesicles and contain lipid inclusions dispersed in an electron-dense protein matrix. The lipid inclusions may present unusual aspect, forming sharp angles that sometimes seem to rupture the organelle. The frontier between lipids and the dense protein matrix may assume the trilaminar aspect of a lipid bilayer. It is relevant to investigate if reservosomes present or not internal membranes when we discuss the participation of Golgi elements and early endosomal vesicles in the formation or renewal of the organelle. To start answering this question, intact epimastigotes were slam frozen and freeze-fractured. Reservosomes fractured inside intact cells present flattened lipid inclusions, similar to those usually found in reservosome ultrathin sections, as well as round inclusions, resembling internal vesicles. We have recently isolated reservosomes (Cunha-e-Silva *et al.* FEMS Microbiol. Lett 7-14, 2002) and, in an initial lipid analysis, found high levels of colesteryl ester and ergosterol, as well as phosphatidylcoline and phosphatidylethanolamine as the major phospholipids. The reservosome purified fraction was submitted to 0.25% Triton X-100 for 30 minutes at 4ºC or 37°C and layered on the top of a continuous sucrose gradient. Transmission electron microscopy of the fractions obtained showed that, while detergent treatment at 37°C completely solubilized reservosome membranes, at low temperature detergent treatment rendered a membrane preparation formed by planar membranes, akin to the trilaminar profiles found in the interior of intact organelles. Alternatively, reservosome membranes were prepared by ultrasonic disruption of purified reservosomes previously incubated in 150 mM sodium carbonate pH 11 at 4°C for 1h. Using this protocol we obtained a preparation where planar membrane profiles and round closed membrane vesicles coexist. Lipid analyses of reservosome membrane fractions are in course. Based on ultrastructural data and differential detergent solubility, we suggest that reservosomes possess internal membranes with a distinct composition that may reflect function and origin different from the organelle external membrane.

Supported by PRONEX/MCT, CNPq and FAPERJ.

BC10 - HEME REQUIREMENT AND ITS POSSIBLE INTRACELLULAR TRAFFIC IN *TRYPANOSOMA CRUZI* EPIMASTIGOTES

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Trypanosoma cruzi, the ethiologic agent of Chagas disease, is transmitted through triatomine insects vectors during the blood-meal on vertebrate host. These hematophagous insects usually ingest in a single meal about 10 mM heme bound to hemoglobin. Heme is a powerful generator of reactive oxygen species, including free radicals, and can damage a variety of biomolecules. *T. cruzi*, in the course of their evolution history, had to develop adaptations to avoid the deleterious effects of high concentrations of free heme found in this environment and there is a large lack in literature about its mechanisms of uptake, its effects and degradation inside the parasites. The first transformation into epimastigotes occurs in the stomach and initiates few hours after parasite ingestion. We have been investigating the effects of hemin on *T. cruzi* growth. Hemin concentration in the medium varied from 0 to 1 mM. Addition of hemin drastically increased the parasite proliferation in a dose-dependent manner. Ultrastructural analysis of parasites grown in high heme concentration are in course. Pd – Mesoporphyrin IX (an analogous of heme: Fe - Protoporfirin IX) intrinsic fluorescence was used as a label to trace the fate of heme taken up by the parasite. We followed the time course of Pd-mesoporphyrin IX internalization in parasites from three to five-day-old cultures incubated with globin-Pd-Mesoporfirin IX. The fluorescence signal was initially associated with anterior vesicle compartments, reaching reservosomes after less than a minute of incubation. On the other hand, when eight-day-old parasites were starved by a 24 hour incubation in medium without serum, the images showed intense fluorescence in the kinetoplast. Taken all together, our data suggest the need of heme in the development of *T. cruzi* and points to the existence of a specific pathway for heme absorption from media, which would be consistent with the utilization of host heme for assembly of mitochondrial proteins of the parasite. Besides this we have also described the use of Pd-Mesoporfirin IX, a new tool to study cell metabolism of heme.

Supported by FAPERJ, CNPq, PADCT, and HHMI (to PLO) and PRONEX.

BC11 - MEMBRANE FORM VSG IS SECRETED IN VESICLES BY *TRYPANOSOMA BRUCEI*

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African trypanosomes display 10 million copies of a glycoprotein (VSG) which is anchored to the plasma membrane by glycosylphosphatidylinositol (GPI) anchor. A GPI specific phospholipase C (GPI-PLC), by clipping off the

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dimyristoylglycerol from the membrane bound VSG (mfVSG), promotes VSG shedding in a soluble form (sVSG). This process can be monitored by immunodetection of an epitope called CRD on the sVSG and also by loss of mfVSG anfifilicity assessed by Triton X-114 phase separation. It has been proposed that access of GPI-PLC to its substrate requires tetramerization and depends on thioacylation occurring within a cluster of three cysteine residues (Paturiaux-Hanocq et al., JBC 275:12147, 2000; Armah et al. JBC 275:19334,2000). Indeed, a trypanosome mutant whose GPI-PLC gene has been modified on the acylated cysteines (Triple Mutant, TM) lost the hallmark behaviour of releasing VSG upon hipotonic lysis. Incubation of live wild type (WT) trypanosomes for up to 60 min at 4^0C and 37^0C in saline phosphate buffer pH7.5 suplemented with glucose (PSG) indicated that both s and mfVSG were released in the supernant in a time and temperature dependent manner. On the other hand, GPI-PLC minus mutant (PLC^-) released only mfVSG and TM released mostly intact VSG, with just a small fraction of CRD positive VSG signalling a mildly functional enzyme. WT parasites released about 0.6% of total ^{125}I-labelled VSG after 60 min at 37^0C whereas TM and PLC^- mutants released 10 times less VSG. Aiming to examine whether mfVSG was released in vesicles, the material shed by both WT and PLC^- trypanosomes was submitted to 100.000g centrifugation for 60 min. Analyses of the pellet revealed VSG which was CRD negative unless treated with GPI-PLC and transmission electron microscopy of material fixed either prior or following ultracentrifugation revealed a quite homogeneous population of vesicles with about 60nm of diameter, obviously containing some type of material in its lumen and covered with a slightly electrondense material resembling VSG. Surprisingly an occasional "filopodium-like" structure with a "beaded" aspect was observed, suggesting that filopodia could be fragmented into vesicles. Immunolabelling experiments are being carried out to detect VSG on the vesicles released *in vitro* and also on those recovered from infected mice sera. We hypothesize that the release of mfVSG-bearing vesicles is physiologically relevant firstly, to allow the GPI anchor with its lipid moiety to modulate the host immunosystem (Magez et al. J. Immunol. 160:1949, 1998), and secondly, fostering the transference of VSG to erythrocytes (Rifkin et al., PNAS 87:801, 1990) following vesicle fusion, a possibly significant event in the context of African trypanosomiais pathogenesis.

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BC12 - GRISEOFULVIN INDUCES CELL DEATH BUT NOT MICROTUBULES DEPOLYMERIZATION IN *TRITRICHOMONAS FOETUS*

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Tritrichomonas foetus is an extracellular parasitic protist that inhabits the urogenital tract of cattle causing the bovine trichomonosis. Like other trichomonads species *T. foetus* possesses very peculiar cytoskeletal structures such as the costa and the pelta-axostyle complex, responsible for maintaining the cell body shape. The axostyle, a ribbon of longitudinally-oriented microtubules which runs from one pole of the cell to the other, has been subject of controversy concerning its stability. We have previously demonstrated that the axostyle is maintained through the entire cell cycle of trichomonads, and does not depolymerize [1]. Recently, Noel et al. showed the effects of the anti-fungal compound griseofulvin on the cytoskeleton of *Trichomonas vaginalis* [2]. These authors stated that the drug irreversibly affects tubulin assembly in trichomonads, suggesting thus that the axostyle is composed of labile microtubules. In view of these contradictory observations, we decided to observe the effects of this anti-fungal on *T. foetus* by other methods.

We took advantage of complementary techniques in order to observe the effects of griseofulvin on *T. foetus* ultrastructure. The reversibility of the compound was also tested. After incubation with 50µg/ml griseofulvin at different times the cells were fixed and processed for fluorescence microscopy, scanning (SEM) and transmission (TEM) electron microscopy. By SEM and TEM we found that the drug induced a cell death with characteristics commonly observed during apoptosis, such as membrane blebbing. Autophagic features were also observed (eg. intense cytoplasmic vacuolization). The axostyle-pelta complex was fragmented, but not depolymerized. By light and fluorescence microscopy membrane blebbing and axostyle fragmentation were found, as well as nucleus condensation and fragmentation. Phophatidylserine was translocated from the inner to the outer leaflet of the plasma membrane, as detected by annexinV. The effects of the drug were time-dependent, and reversible until six hours of incubation.

Altogether the data herein presented show that griseofulvin induces a type of cell death in *T. foetus* with characteristcs resembling both apoptotic and autophagic processes. Besides, the effects of the anti-fungal griseofulvin can be reversible and it never leads to depolymerization of microtubular structures, corroborating our previous observations that the axostyle-pelta complex is composed of stable microtubules [1].

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BC13 - EXPRESSION OF ANIONIC SITES IN THE WALL OF TISSUE CYSTS OF *TOXOPLASMA GONDII*

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Toxoplasma gondii is an obligate intracellular protozoan parasite, member of phylum Apicomplexa that have ability to infect a wide range of hosts, including mammals and birds. (Coppin et al, 2003). To survive within infected hosts, *T. gondii* undergoes profound metabolic and morphological changes by differentiation into a cyst characterized by its resistance to the immune system and chemotherapy (Bohne et al, 1999; Lyons et al, 2002). The cyst wall is thought to be important in providing stability to the tissue cysts and in facilitating cyst persistence in chronically infected hosts. However the composition and biogenesis of the cyst wall in *T. gondii* has not been fully elucidated (Coppin et al, 2003).

Our present aims were to study the expression of anionic sites in the wall of tissue cysts. C57BL/6 mice were infected with 50 cysts/animal of *T. gondii* ME-49 strain type II. After 4-8 weeks of infection, the brains were harvested, homogenized and purified in dextran gradients. The cysts were incubated with two markers cationic: cationized colloidal gold (C-Au) and cationized ferritin (CF) for 20 min 4°C and additionally for 1, 6 and 24 hours at 37°C followed by processing for transmission electron microscopy. Assays were also performed

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using fixed cysts. The incubation of tissue cysts at 4°C with CF showed a homogeneous distribution of anionic sites on the wall cyst, which was followed by their incorporation after raising the temperature (37°C). The CF internalized was localized within vesicles, tubules and in the cyst matrix and sometimes, in close proximity or in direct contact with bradyzoite membrane inside the tissue cysts. Fixed cysts presented lower labeling when compared with non-fixed cysts. The expression of anionic sites using cationized colloidal gold particles showed a discontinuous labeling at 4°C, but after 6 and 24h at 37°C the particles were localized attached on the cystic wall.

The endocytic activity of the cystic wall revealed by anionic sites incorporation using cationized ferritin argues for its role during uptake of the nutrients by tissue cysts and represents a potential target for chemotherapy tests.

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BC14 - ANALYSIS OF THE SHEDDING PROCESS IN TRIPOMASTIGOTE FORMS OF *TRYPANOSOMA CRUZI*

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Cleavage or shedding of functional proteins is an important process on cell surface modulation and particularly important in parasite infections, where different mechanisms for evading the immune response are request. Previous studies have shown that two kinds of host immunoglobulins can interact with the surface of *Trypanosoma cruzi*, a non-specific bind to the parasite surface trough either Fc or Fab fragments of IgG, and a specific human and animal IgG antibodies, capable to induce mobility of surface antigens of live blood forms to form a cap in the anterior and posterior poles of the parasite in a process dependent of parasite strain, time, temperature and inhibited by sodium azide. Aggregated membrane components shedded from parasite surface, explain the immunophatological alterations described in the kidney of mice infected. However this process was described as a spontaneous process involving a plasma membrane vesiculation, results obtained by previous researches do not exclude that some antigens may have a differential rate of shedding or be enhanced in the presence of some substances or molecules. The aim of present study is the analysis of the shedding process in two different strains of tripomastigote forms of *T. cruzi*, and under the influence of different molecules. Briefly, tripomastigote forms from CL Brener clone and Y strain, were incubated in the presence of non-related IgG-gold complexes during 30 minutes at 37ºC or 4ºC. Experiments were also realized in the presence of Cationized Ferritin (FeCat) or with Concanavalin A (ConA) with previous incubations for 30 minutes at 4ºC and subsequently during 10, 20 and 30 minutes at 37ºC. Some samples were pre-fixed or pre-treated with sodium azide. All cells were processed and observed in scanning and transmission electron microscopy. In the presence of non-related IgG, parasites of both strains showed a small pattern of shedding when incubated at 4ºC. Although, at 37ºC, an intense shedding process could be observed with numerous vesicles distributed on the cell body of both strains. Vesicles aggregated could also be observed in extracellular milieu in both experimental conditions. Experiments realized with FeCat showed a discrete shedding process in samples incubated at 4ºC. Despite that, when these samples were submitted to a second incubation of 30 minutes at 37ºC, we could observe a little increase of the process. Similar results were observed in both strains. In the presence of Com-A, CL Brener tripomastigote forms didn't show shedding vesicles as described for FeCat. Complementary experiments using Con-A or PMA still in process.

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BC15 - PRODUCTION OF AN ANTIBODY AGAINST *L. (L.) AMAZONENSIS* ARGINASE

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Leishmania is an intracellular protozoa that, to survive inside the host macrophage, escapes from its microbicidal mechanisms. Amongst these mechanisms are the production of nitric oxide (NO) and superoxide radicals (Bogdan et al., 1996). The production of NO by inducible nitric oxide synthase (iNOS) requires L-arginine as substrate. In addition to, arginase requires this aminoacid to generate ornithine and urea. Arginase may play an important role in parasite survival by decreasing the concentration of the iNOS´s substrate and reducing the NO generation, as well as in the production of ornithine, a precursor for polyamines synthesis and consequently involved in DNA synthesis and cell proliferation (See Alves et al, this meeting). Moreover, there is a study showing that the inhibition of arginase by N^{ω}-hydroxy-L-arginine enhances NO production and increases the killing of the parasite (Iniesta et al., 2001).

In order to better understand the physiological role of arginase and its importance in *Leishmania´*s infection, the subcellular localization of the enzyme should be known. In the present communication, we describe the first step to get this localization, the production and characterization of a specific antibody against arginase.

The arginase coding gene from *L. (L.) amazonensis* was described and the open reading frame was cloned into a modified expression vector, pRSET, and used to transform *E. coli* BL21. Arginase was purified for an IPTG-induced culture of transformed bacteria. BALB/c mice were then inoculated with 18,5ìg of purified arginase and complete adjuvant subcutaneously. After 30 days, the burst was performed by another inoculation using 18,5ìg of purified enzyme with incomplete adjuvant. The antiserum was prepared after collecting the animals blood. To test its specificity for arginase, the antiserum obtained were used in Western blot experiments, containing PAGE fractionated cellular extracts of *L. (L.) amazonensis* as well as the transformed *E. coli* producing arginase, with and without IPTG induction. A significant stained band, with the same apparent molecular weight of the arginase was obtained. This result indicates that the antiserum obtained recognizes arginase with enough especificity.

Cytological preparation will now be submitted to the antiserum to localize the enzyme in different conditions of infection.

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BC16 - *TOXOPLASMA GONDII:* ENDOCYTIC ACTIVITY

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Toxoplasma gondii, an obligate intracellular parasite, has elaborated a battery of specialized secretory organelles (micronemes, rhoptries and dense granules) that mediate host cell attachment and invasion, and the formation and maturation of parasitophorous vacuole [1]. Endocytosis mechanisms are poorly known in

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these Apicomplexan parasites [2]. Little information about the lysosomal system in *Toxoplasma* has been reported, which raises a number of questions regarding parasite's basic cellular processes. The only acidic compartment described in *Toxoplasma* tachyzoites is the forming and the mature rhoptries [3]. Some extra cellular tachyzoites have been observed to slowly internalize the fluid phase endocytic tracer, horseradish peroxidase (HRP), through vesicles formation at the base of the micropore, the single structure that has been identified in nutrient acquisition in *T. gondii* [4]. The present work was undertaken to study the endocytic activity of *T. gondii.*

RH strain *Toxoplasma* tachyzoites were obtained from peritoneal exudates of Swiss mice 3 days after parasites inoculation. Exudates were washed with PBS (Phosphate Buffered Saline) and incubated for 20 min at 37°C with 100 mg/ml HRP-Au at 37°C and after 5 min to 24 h, the samples were washed twice with PBS and parasites were fixed for 30 min, at 4°C in a solution of 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.2, supplemented with $CaCl_2$. The parasites were washed three times in the same buffer and then post-fixed in 1% OsO_4. The cells were dehydrated in crescent concentrations of acetone and embedded in Epoxy resin. Thin sections were obtained, stained with uranyl acetate and lead citrate and examined by transmission electron microscopy, in a Zeiss EM10.

The ultrastructural analysis showed with relative frequency, HRP-Au inside rhoptries. It was commonly observe in the same parasite more than one rhoptry containing Au-particles. We obtained up to now no evidences of HRP-Au particles uptake through the micropore or any other parasite organelle. Our preliminary data suggest that rhoptries are involved in nutrient acquisition by *Toxoplasma*, despite their well-known role as secretory organelle during host cell invasion. Further studies will be developed to elucidate the mechanisms involved in the nutrients uptake by *Toxoplasma*, a phenomena not yet understood and of great relevance for the development of drug delivery targeting into the tachyzoites.

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BC17 - INVASION AND EGRESSION FROM BLOOD MONOCYTE OF AN *HAEMOGREGARINE* SP. FROM THE LIZARD *AMEIVA AMEIVA* (REPTILIAN: TEIIDAE).

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Parasites from the apicomplexan group have an obligate intracellular existence. Thus, active invasion and egression of host cells are a necessity and have been described for a few species. However, for a better understanding of this group, new descriptions of these processes from different species are necessary. Gliding is a process used by these parasites to move through the environment and to invade cells. We have been working with an Amazonian haemogregarine found in blood monocytes of the *Ameiva ameiva* that induces monocytosis positively correlated with parasitemia. Here we describe the final moments of the evasion and the whole invasion process of this parasite. Blood was collected by cardiac puncture and leukocytes separated by centrifugation. Live cells were mounted under a slide, observed and photographed under a Zeiss Axiophote using an immersion 100 x objective. Parasite was seen attached by its posterior end with most of its body out of the cell rotating furiously. It was impossible to determine the rotation side because of the high speed. After a few seconds, the parasite started to pull a portion of the monocyte plasma membrane, which formed a "cup" around the posterior portion of the parasite body. The parasite also moved down stretching the plasma membrane cup that increased in size. After 2 minutes, the parasite was released from the plasma membrane of the monocyte and immediately stopped the rotation movement and started the gliding motion through the slide. The other observation was the infection process. Noninfected monocytes were spread at this time. The parasite approached the monocyte using the gliding motion. Its anterior portion moved from one side to the other in a touching process. There was no evidence of attachment to a special region of the monocyte membrane. Suddenly the conoid extruded and the parasite invaded the monocyte within two minutes. Later the spreaded monocyte started to retract. After twenty-five minutes, all the monocyte fillipodium retracted and the cell rounded up. Fifty-seven minutes later, the infected monocyte assumed a round shaped. These results show that the egression process used by this parasite is unique and is based on rotating movement. It also supports the notion that the gliding motility capacity is conserved among apicomplexan parasites and illustrates how this invasion process alters the monocyte spreaded morphology.

Supported by: CAPES, CNPq, FAPERJ, PRONEX.

BC18 - MODULATION OF NEUTROPHIL ADHESION TO VASCULAR ENDOTHELIAL CELLS BY INACTIVE MEMBERS OF *TRYPANOSOMA CRUZI TRANS*-SIALIDASE FAMILY

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Trypanosoma cruzi, the etiologic agent of Chagas' disease, is a major cause of acute and chronic myocarditis and cardiomyopathy in endemic areas in Latin America. Host resistance to *T. cruzi* infection depends on both innate and acquired immunity. Polymorphonuclear neutrophils (PMN) constitute the first line of defense against invading microorganisms, however, they have been considered one of the major contributors to host damage in subacute and chronic inflammatory states. Migration of PMN from blood is a multi-step event dependent on lectin/sialylglycoproteins interactions mediating neutrophil-endothelial cell adhesion. *T. cruzi* express on its cell surface an inactive member of *trans*-sialidase family that physically interacts with sialic acid containing molecules on host cells. Inactive *trans*-sialidase binds to molecules containing sialic acid on endothelial cell surface inducing NF-kB activation and adhesion molecules expression. In this work we aim to elucidate the roles of iTS in PNM recruitment during Chagas' disease. Using immunofluorescence microscopy we demonstrate that iTS binds a2,3-sialic acid on CD43 from human PMN surface inducing actin cytokeleton mobilization and reorganization. iTS binding to CD43 inhibits PMN adhesion to activated endothelial cells, *in vitro* suggesting that members of the TS family are involved in the recruitment of circulating leukocytes and play a major role in the inflammatory response during *T. cruzi* infection, contributing to the pathogenesis of chagasic disease.

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BC19 - PARASITIC INFECTION DISRUPTS CX43, BUT NOT TIGHT JUNCTION PROTEINS IN AN EPITHELIAL CELL LINE

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We have previously shown Connexin 43 (Cx43) gap junction protein to be associated with the tight junction protein ZO1 in membranes of cardiac cells, and that infection with the protozoan parasite, *Trypanosoma cruzi*, the causative agent of Chagas disease, disrupts these proteins in the membranes of these cells. Interestingly, when infected cultures of MDCK type II cells (canine kidney derived cell line) were examined, although dye spread from the infected cell to its neighbors was completely abolished (indicating disruption of gap junctional communication), neither surface membrane ZO1 labeling nor transepithelial resistance of the monolayer were affected (indicating that the infection does not disrupt tight junctions). We therefore decided to examine the effect of *T. cruzi* infection on the expression of Cx43 and 4 members of the family of Tight Junction proteins: Zonula Ocludens 1(ZO1), Zonula Ocludens 2 (ZO2), Claudin and Ocludin in cultures of epithelial MDCK type II cell line. Cultured cells were infected with the Y strain of *T. cruzi* at a multiplicity of infection of 20 parasites/cell and the degree of coupling and surface expression of Cx43, ZO1, ZO2, Claudin and Ocludin were examined by immunofluorescence under conventional and confocal microscopy 48-72 hours after infection. As expected infected cells in the culture were uncoupled from their neighbors, based on spread of Lucifer yellow. Surface labeling for Cx43 was markedly decreased in infected cells, but the tight junction proteins ZO1, ZO2, Claudin and Ocludin were not affected. The non-infected cells in the infected cultures displayed normal expression of Cx43 with co-localization of the Cx labeling in membrane appositional areas with ZO1. We conclude that infection with *T.cruzi* disrupts Cx43 protein, but does not alter the expression of tight junction proteins in epithelial cells.

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BC20 - FUSION OF *TRYPANOSOMA CRUZI* VACUOLES TO *COXIELLA BURNETII* VACUOLES: LIFE-CELL IMAGING STUDIES IN DOUBLY INFECTED VERO CELLS

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Background: *Coxiella burnetii*, the agent of Q fever, is an obligate intracellular bacterium that multiplies within vacuoles of phagolysosomal origin. *Trypanosoma cruzi* trypomastigotes invade a wide variety of cells and in the process recruitment of lysosomes and parasitophorous vacuole formation is observed. We have studied the fate of different *T. cruzi* trypomastigote forms after invading Vero cells persistently colonized with *C, burnetii.* When the invasion step was examined, we found that persistent *C. burnetii* infection *per se* reduced only tissue-culture trypomastigote (TCT) invasion, whereas raising vacuolar pH with Bafilomycin A1 and related drugs, increased invasion of both metacyclic and TCT trypomastigotes when compared to control Vero cells. Kinetic studies of trypomastigote transfer indicated that metacyclic trypomastigotes parasitophorous vacuole (PV) are more rapidly and efficiently fused to *C. burnetii* vacuoles. Endosomal-lysosomal sequential labeling with EEA1, LAMP-1, and Rab7 of the PVs formed during the entry of each infective form revealed that the phagosome maturation processes are also distinct.

Metodology: We have transfected Vero cells (colonized or not with *C. burnetii*) with fluorescent EGFP-tagged-LAMP-1 plasmids and observed lysosomal dynamics under confocal microscopy. We also observed the invasion and fusion events of metacyclic trypomastigote vacuoles within transfected Vero cells colonized with *C. burnetii.* Video-microscopy was employed to observe the dynamics of parasitophorous vacuole fusion.

Results: We have observed that EGFP-LAMP-1 is recruited by metacyclic trypomastigotes in Vero cells colonized with *C. burnetii* and remained associated with metacyclic trypomastigotes parasitophorous vacuoles until their fusion with *C. burnetii* vacuoles. We also observed that metacyclic trypomastigotes parasitophorous vacuoles have an unexpected mobility, roving around *C. burnetii* vacuoles without losing GFP-LAMP protein.

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BC21 - GAP JUNCTIONS DISTRIBUTION IN MICE EMBRYOS CARDIOMYOCYTES DURING *IN VITRO* INFECTION WITH *TRYPANOSOMA CRUZI*.

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Gap junctions are molecu1es belonging to the connexin multigene family that in hearts provide a low resistance pathway for electrical coupling between adjacent cardiomyocytes [Yeh, H.I. et al., J. Histochem. and Cytochem., 51 259-266, 2003]. We developed an experimental system of primary culture of heart muscle cells (HMC) for studies of *T. cruzi*-cardiomyocyte interaction. Hearts of 18 days old mouse embryos were dissected, minced and incubated in a trypsin-collagenase solution. [Meirelles, M.N.L. et al., European Journal of Cell Biology, 41 198-206, 1986]. Previous work from our laboratory using ultrastructural and electrophysiological approaches showed that cardiac cells present the same basic characteristics found in mammalian cells *in vivo* such as specialized structures including sarcotubu1ar system, intercalated discs, myofibrilles, coupling interaction among the cells and spontaneous contractility. We noticed that in 3-day-old cu1tures that have been infected with *T. cruzi* the rate of spontaneous beating reached a peak value significantly greater than the 3-day-old control cu1tures, showing an increase in automaticity, higher spontaneous firing rate [Aprigliano, O. et al., J Mol Cell Cardiol., 25 1265-1274, 1993].

In the present work, we investigate the distribution of gap junctions in heart cells infected or non infected with *T. cruzi*, from two different strains (Y and Dm28c) during development of the parasite's cell cycle in HMC. For the immunofluorescence studies, *T. cruzi* infected and non infected cardiomyocytes grown on coverslip were fixed for 5 min. at room temperature in 1% paraformolaldehyde and were then incubated in a 1/400 dilution for 1h/37°C with a polyclonal antibody anti-connexin 43. After rinsing, the cells were incubated in a 1/800 dilution of TRITC-coupled anti-rabbit IgG. Cytoskeleton of the cells were stained with Falloidin FITC 1:200 for 30min. at 37°C. For u1trastructural cytochemistry, cells were fixed with 0.01% paraformolaldehyde, 0.2% glutaraldehyde, 0.1% picric acid, dehydrated in methanol crescent series and embedded in Lowicryl resin. Unstained ultrathin sections were incubated for 1h/37°C in a blocking buffer and further incubated with the polyclonal anti-connexin 43 antibody. After washing, the grids were incubated for 30min. with the secondary antibody coupled to 10nm colloidal gold particle. The fluorescence assays showed in groups of 4-8 coupled cells the distribution of connexin in the cell' sarcolemma; highly *T. cruzi* infected cardiomyocytes showed that punctate gap junctional staining was much reduced in infected cells besides reduced fluorescence among the cells. Ultrastructural approaches showed that coupled cardiomyocytes displayed gold particles organized as round structures at the sarcolemma of adjoining cells. These studies may contribute for the understanding of how the parasite affects the functional coupling of mouse cardiomyocytes *in vitro*.

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BC22 - MOLECULAR BASIS OF NON VIRULENCE OF *TRYPANOSOMA CRUZI* CLONE CL-14

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We investigated the properties of metacyclic trypomastigotes of non virulent *T. cruzi* clone CL-14, as compared to the parental isolate CL. In contrast to the highly infective CL isolate, which produces high parasitemias in mice by intraperitoneal or oral administration, metacyclic forms of clone CL-14 failed to produce patent infection. In vitro, the number of clone CL-14 parasites that entered epithelial HeLa cells, after 1 h incubation, was ~4-fold lower than that of the CL isolate and, at 72 h post-infection, intracellular replication was not apparent whereas HeLa cells infected with CL isolate contained large number of parasites replicating as amastigotes. The stage-specific surface glycoprotein gp82 plays a central role in mucosal invasion of CL isolate metacyclic forms, leading to systemic infection upon oral challenge (Neira et al., 2003, Inf. Immun. 71: 557-561) and is involved in host cell invasion *in vitro* (Ruiz et al., 1998, Biochem. J. 330: 505-511). Analysis by flow cytometry revealed that metacyclic forms of clone CL-14 express reduced levels of gp82 on the surface, although its overall expression, detectable by immunoblotting, is comparable to that of CL isolate, indicating that in clone CL-14 gp82 is mostly localized intracellularly. Otherwise, the surface profile of CL isolate and clone CL-14 was similar. Internally located clone CL-14 gp82, wich is not accessible to MAb 3F6, had its reactivity augmented by permeabilization of parasites with saponin. HeLa cell entry of clone CL-14 metacyclic forms increased upon treatment of parasites with neuraminidase, which removes sialic acid from the surface glycoprotein gp35/50, a mucin-like molecule previously shown to be engaged in cell invasion of poorly infective *T. cruzi* isolates (Ruiz et al., 1998). Activity of cystein proteinase cruzipain, reported to participate in host cell invasion and intracellular multiplication (Meirelles et al., 1992, Biochem. Parasitol. 52: 175-184), was similar in CL isolate and clone CL-14. Taken together, these data suggest that the lack of virulence of clone CL-14 is associated with the reduced expression of surface gp82 and the possible participation of gp35/50 in host cell invasion.

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BC23 - INVASION OF MDCK EPITHELIAL CELLS WITH ALTERED EXPRESSION OF RHO GTPASES BY *TRYPANOSOMA CRUZI* AMASTIGOTES AND METACYCLIC TRYPOMASTIGOTES OF STRAINS FROM THE TWO MAJOR PHYLOGENETIC LINEAGES

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Rho GTPases have been shown to regulate three separate signal transduction pathways linking plasma membrane receptors to the assembly of distinct actin filament structures. Rho GTPases which comprises Rho (formation of stress fibers), Rac (lamellipodium), Cdc42 (filopodia), TC10, RhoG, and RhoE have been shown to mediate extracellular signals to produce distinct microfilament-associated host cell responses. Here we examined the role of these small GTPases on the interaction between different *T. cruzi* infective forms of strains from the two major phylogenetic lineages with MDCK cells, transfected with different Rho GTPases constructs. We compared the infectivity of amastigotes isolated from infected cells (intracellular amastigotes) with forms generated from the axenic differentiation of trypomastigotes (extracellular amastigotes) and also with metacyclic trypomastigotes. Recent studies with different *T. cruzi* isolates have grouped the parasite in two major phylogenetic lineages: *T. cruzi* I associated with the domestic cycle and human disease designated and *T. cruzi* II linked to the sylvatic cycle of the parasite and other mammalian hosts. Besides the different genetic markers, *T. cruzi* I and II infective forms engage characteristic signaling pathways upon invasion of cultured cells that lead to distinct infectivities.

No detectable effect of GTPase expression was observed on metacyclic trypomastigote invasion and parasites of Y and CL (*T. cruzi* I) strains invaded to similar degrees all MDCK transfectants, and were more infective than either G or Tulahuen (*T. cruzi* II) forms. Intracellular amastigotes were complement sensitive and showed very low infectivity towards the different transfectants regardless of the parasite strain. Complement-resistant extracellular amastigotes, especially of the G strain, were highly infective for the constutively active GTPase transfectants, particularly Rac1V12. The fact that invasion was inhibited in Rac1N17 dominant negative cells suggested an important role for Rac1 in this process. Moreover, we also observed a discrete accumulation of both actin and Rac1 protein at the sites of extracellular amastigotes invasion, reinforcing the notion that Rac1 may be involved with the process of extracellular amastigotes invasion in these cells.

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BC24 - ROLE OF SMALL GTPASES IN *TRYPANOSOMA CRUZI* INVASION IN MDCK MUTANT CELL LINES.

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Trypanosoma cruzi is an intracellular parasite that modulates a great number of host intracellular responses like changes into the actin cytoskeleton and the recruitment of lyssome to its entry site. The small GTPases, RhoA, Rac1 and Cdc42 are examples of molecules that could be activated at this point of the invasion. The activation of these GTPases by signaling molecules modulates changes in the pattern of actin cytoskeleton that leads to the formation of structures like stress fibers, lamellipodium and fillopodium, respectively. Here we investigated the role of the small GTPases RhoA, Rac1 and Cdc42 in the cytoskeletal rearrangement in the host cell during *Trypanosoma cruzi* infection. For that we observed the behavior of MDCK cell lines using the tetracycline repressible transactivator to regulate RhoAV14, RhoAN19, Rac1V12, Rac1N17, Cdc42V12, and Cdc42N17 expression.

T. cruzi adhesion and internalization indexes after 2 hours and the infection rate after 48 hours in the MDCK cell lines TET, Rac1V12, Rac1N17, RhoAV14, RhoAN19, Cdc42V12 and Cdc42 N17 were analyzed. Comparative analysis showed that Rac1V12 and Rac1N17 mutants presented the highest adhesion and internalization indexes, but the lower infection rate after 48 hours, when compared with TET control group.

Confocal laser scanning microscopy showed changes in the pattern of actin distribution in these clones suggesting that there is actin reorganization at the site of trypomastigote entry. Our results suggest that changes in the actin cytoskeleton are caused not just due to the *T. cruzi* presence but for the c-DNA expression of each transfected lineage and, this reorganization, somehow affects the parasite rate of infection. Taken together, these observations suggest that the

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small GTPases RhoA, Cdc42 and mainly Rac1, act in the signaling mechanism modulating host cell responses involved in *Trypanosoma cruzi* invasion.

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BC25 - INFECTION BY *TRYPANOSOMA CRUZI* METACYCLIC FORMS DEFICIENT IN THE EXPRESSION OF SURFACE MOLECULE GP82

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Trypanosoma cruzi metacyclic trypomastigotes have been reported to invade and replicate in the gastric mucosal epithelium upon oral infection. Here we analysed the process of infection by *T. cruzi* isolates deficient in the expression of gp82, the metacyclic stage-specific surface glycoprotein implicated in target cell entry in vitro and in promoting mucosal infection in mice upon oral challenge. Gp82-deficient metacyclic forms of isolates 569 and 588 infected mice by oral route, producing patent parasitemia, but to greatly reduced levels when compared to the gp82-expressing isolate CL. Metacyclic forms of both isolates expressed gp30, a surface glycoprotein detectable by MAb 3F6 directed to gp82. Otherwise, the gp82-deficient isolates displayed a surface profile similar to that of the CL isolate and also entered epithelial HeLa cells in a manner inhibitable by monoclonal antibody (MAb) 3F6, and dependent on the parasite signal transduction that involved activation of protein tyrosine kinase and Ca^{2+} mobilization from thapsigargin-sensitive stores. Like gp82, gp30 triggered the host cell Ca^{2+} response required for parasite internalization. Purified gp30 and the recombinant gp82 inhibited HeLa cell invasion of metacyclic forms of isolates 569 and 588 by ~90% and ~70%, respectively. Cell invasion assay performed in the presence of gastric mucin, mimicking the in vivo infection, showed an inhibition of 70-75% in the internalization of gp82-deficient isolates, but not of the CL isolate. The recombinant gp82 exhibited an adhesive capacity towards gastric mucin much higher than that of gp30. Taken together, all these findings suggest that gp30 mediates target cell entry of gp82-deficient metacyclic trypomastigotes but it fails to promote an efficient mucosal infection due to its poor interaction with gastric mucin, which may be a preceding step for parasite invasion of underlying epithelial cells.

SUPPORTED BY FAPESP

BC26 - *TRYPANOSOMA CRUZI TRANS*-SIALIDASE ACTIVATES ENDOTHELIAL INDUCING VASCULAR ADHESION MOLECULE EXPRESSION AND RECRUITMENT OF NEUTROPHILS

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Chagas' disease, caused by the protozoa *Trypanosoma cruzi*, is a major cause of acute and chronic myocarditis and cardiomyopathy in endemic areas in Latin America. Host resistance of *T. cruzi* infection depend on both innate and acquired immunity. Polymorphonuclear neutrophils (PMN) constitute the first line of defense against invading microorganisms, however, they have been considered one of the major contributors to host damage in subacute and chronic inflammatory states. Migration of PMN from blood is a multi-step event dependent on lectin/sialyloligosaccharides interactions mediating neutrophil-endothelial cell adhesion. Activation of NF-kB is associated with inflammation and expression of adhesion molecules, which are involved in the recruitment of leukocytes, a critical factor in the initiation of inflammation. *T. cruzi* expresses the active and inactive forms of *trans*-sialidase (TS), the active form transfer sialic acid residues á2,3-linked to b-galactopiranose (b-Galp*)* on host donor molecules to mucin-like acceptor glycoproteins while TS inactive bind a2,3 sialic acid-glycoconjugate acting like a lectin. As the vascular endothelium is an early target to parasite invasion, here we tested the influence of *T. cruzi* TS in human bone marrow endothelial cells (HBMEC) and porcine aortic endothelial cells (PAEC). We demonstrate that inactive TS binds to HBMEC and the binding could be abrogated by the addition of a2,3-sialyllactose. Both active and inactive TSs trigger NF-kB activation and expression of the endothelial cell adhesion molecules E-selectin, ICAM-1 and V-CAM in both type cells. We also demonstrate that activation of endothelial cells by both aTS and iTS increases neutrophil-endothelial adesion in PAEC. These findings suggest that members of the TS family play a major role in the inflammatory response during *T. cruzi* infection, contributing to the pathogenesis of chagasic cardiomyopathy.

Supported by CNPq (PRONEX), CAPES, FAPERJ, HHMI, CAPES-COFECUB.

BC27 - THE KNOCKOUT OF A SINGLE COPY ARGINASE GENE OF *LEISHMANIA (L.) AMAZONENSIS* PREVENTS ITS SURVIVAL AND MULTIPLICATION IN MOUSE MACROPHAGES.

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Leishmania is an intracellular parasite that escapes microbicidal mechanisms such as nitric oxide (NO) and superoxide radical production in order to survive inside host macrophages (Bogdan et al., 1996). The inducible NO-synthase (iNOS) of macrophages acts on the substrate L-Arginine to convert it to NO and citruline. L-Arginine is also the substrate for another enzyme, arginase, to produce ornithine and urea (Krebs et al 1932). The inhibition of arginase by N-W-hydroxy-L-arginine down-regulated NO production, preventing killing of *Leishmania* inside cultured macrophages (Iniesta et al., 2001). In *Leishmania,* arginase was initially described as taking part of the urea cycle (Camargo et al., 1978). Our hypothesis is that *Leishmania* arginase could also have a major role in securing the survival of this protozoa inside macrophages by depleting the pool of L-arginine available to macrophage iNOS activity and, in consequence, decreasing NO generation.

A single copy arginase knockout *L. (L.) amazonensis* was constructed and characterized. *In vitro* and *in vivo* infection experiments in murine macrophages (BALB/c and C57BL6) showed that the *L. (L.) amazonensis* Darg::hyg/ARG mutant, in comparison with the wild type parasite, was much more readily destroyed inside macrophages and was not able to cause lesions when injected in the mouse footpad. However, experiments carried out in C57BL6 mice deficient in iNOS (iNOS-KO) showed that amastigote growth of mutant *Leishmania* in iNOS-deficient macrophages was impaired and footpad lesions did not develop when mutant *Leishmania* was injected in iNOS-KO mice (Alves et al., 2002).

In order to further investigate these findings, the *in vivo* infection experiments in iNOS-KO mice were repeated. *L. (L.) amazonensis* Darg::hyg/ARG or the

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corresponding wild type *Leishmania* strain (10^6 - promastigotes) were injected in iNOS-KO and WT C57BL6 mice. At selected times after infection (11 and 22 weeks) mice were killed and the footpads and draining lymph nodes (LN) removed. Parasites numbers in the LN were determined by limiting dilution technique. DNA was extracted from footpads and LN and submitted to PCR using *Leishmania* specific primers. The results show that no parasites were detected after 11 or 22 weeks in tissues from iNOS KO mice infected with the mutant *Leishmania*, in contrast to the very large number of parasites found in the mice injected with wild-type *Leishmania*. Furthermore, parasite DNA was not detected by PCR in the former group. These results indicate that the *L. (L.) amazonensis* Darg::hyg/ARG besides being more susceptible to intracellular destruction inside macrophages is not capable of establishing a lasting infection of these cells even in the absence of host NO production. This could be explained by a deficiency in the parasite's replication, probably due to insufficient polyamine synthesis. Indeed, the rate of DNA synthesis of the mutant *Leishmania*, as determined by ^{3}H-thymidine incorporation, was much slower than that of the wild type strain.

Altogether, the results indicate that arginase is important for *Leishmania* to escape from the macrophage microbicidal mechanisms by reducing the availability of L-arginine as iNOS substrate as well as providing ornithine to the polyamines' synthesis pathway essential for DNA replication.

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BC28 - REGULATION OF EXTRACELLULAR MATRIX EXPRESSION DURING *TRYPANOSOMA CRUZI*-CARDIOMYOCYTE INTERACTION

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Fibrosis, an outstanding manifestation of Chagas disease, occurs as a result of exacerbated accumulation of extracellular matrix (ECM) leading to myocardium hypertrophy and heart failure (Andrade et al, 1989). Fibrosis process occurs in association with inflammatory infiltrates and degenerating cardiomyocytes (Rossi, 2001). Several reports have demonstrated that cytokines synthesized during inflammation can mediate fibrosis process. TGF-b, a multifunctional cytokine which elicits a strong fibrogenic response is associated with Chagasic myocardiopathy (Araújo-Jorge et al, 2002). However, the regulatory mechanisms of fibrosis remain to be elucidated.

To evaluate the kinetics of extracellular matrix (ECM) expression and the role of cytokines on its regulation, primary culture of heart muscle cells were infected with *T. cruzi*, Y strain, and the interaction was interrupted after 24 to 96h. The involvement of TGF-b in the modulation of ECM was investigated by treating both uninfected and *T. cruzi*-infected cardiomyocytes with 5, 10 e 15 ng/ml of this cytokine. The fibronectin (FN) and laminin (LN) expression was detected by indirect immunofluorescence and the samples were analyzed by convencional and confocal laser scanning microscopy.

An intense deposit of FN and LN was observed in uninfected cardiomyocytes. No substantial alterations in the FN and LN expression patterns were detected after 24 and 48h of *T. cruzi* infection. However, fibronectin was absent or present only in reduced amounts in highly infected cardiomyocytes (72-96h), while adjacent uninfected cells displayed an intense network. Confocal microscopy analysis also revealed alterations in LN distribution in later times of infection. Our preliminary data demonstrated that treatment of the cultures with TGF-b lead to a general enhancement in FN matrix, which became more interconnected and exhibited thick deposits around adjacent cells. Nonetheless, the cells displaying high number of intracellular parasites reacted weakly to TGF-b stimulation, showing a reduced FN expression when compared to neighboring uninfected cells. Altogether, these results indicate that the enhancement of FN expression occurring *in vivo* (Andrade et al., 1989) may be an effect of TGF-b secretion by inflammatory cells. The differential FN expression displayed by infected cardiomyocytes *in vitro*, even after TGF-b treatment, may be related to cytoskeletal alterations already reported in infected cardiomyocytes (Pereira et al., 1993) or low expression of integrins or TGF-b receptors induced by *T. cruzi* infection.

References: Andrade SG et al 1989. *Am J Trop Med Hyg* 40:252; Araújo-Jorge TC et al 2002. *J Infect Dis 186(12)*: 1823; Pereira et al., 1993. J Submicrosc. Cytol. Pathol. 25(4): 559; Rossi MA, 2001 *Med Sci Monit 7(4)*: 820

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BC29 - *TRYPANOSOMA CRUZI* INFECTION ALTERS FOCAL ADHESION PROTEINS DISTRIBUTION IN CARDIOMYOCYTES

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Focal adhesion proteins play an important role in the contractile transmission force to the extracellular matrix during the contraction-relaxation process. Alterations in the contractibility and cytoskeleton organization have been demonstrated in Chagasic cardiomyopathy (De Carvalho et al., 1994; Fernandez at al., 1992, Pereira et al., 1993). However, the integrity of focal adhesion proteins in this cardiomyopathy is unknown. Our goal was to evaluate the focal adhesion expression during *Trypanosoma cruzi*-cardiomyocyte infection.

Indirect immunofluorescence was performed to investigate the distribution of focal adhesion proteins in uninfected and *T. cruzi*-infected mice heart cryosections and cardiomyocytes cultures. The samples were fixed with 4% paraformaldehyde in PBS. Thereafter, the cells were washed and incubated overnight at 4°C with anti-vinculin or anti-a-actinin antibody. The antigen-antibody complex was revealed with the appropriated secondary antibody. Controls were performed in the absence of the primary antibody.

In the health myocardium, vinculin was detected in striated pattern called costameres and also in the intercalate disk of cardiomyocytes. Preliminary data revealed a similar distribution of vinculin in the infected tissue during the acute experimental Chagas' disease. The immunolocalization of vinculin in cultured cardiomyocytes revealed its distribution anchoring the myofibrils to sarcolemma, co-localized at the Z-line (costameres) and also localized at the cell-substrate adhesion sites, while á-actinin was visualized in the Z-line and similarly associated to the cell-substrate adhesion sites. Both focal adhesion proteins, vinculin and á-actinin, displayed changes in the striation pattern in *T. cruzi*-infected cardiomyocytes. These data suggest that alterations reported in the contraction mechanism may also be related to the disturbance in the localization of focal adhesion proteins.

References:
De Carvalho et al., 1994. J Cardiovasc Electrophysiol. 5(8):686; Fernandez et al., 1992. Acta Physiol Pharmacol Ther Latinoam. 42(3):197; Pereira et al., 1993. Submicrosc. Cytol Pathol 25(4): 559

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BC31 - ANALYSIS ON THE FUNCTION OF MANNOSE-FUCOSE RECEPTOR IN THE UPTAKE OF *LEISHMANIA (L.) AMAZONENSIS* BY MICROGLIOCYTES IN GLIAL MIXED CULTURES

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Microglia are resident macrophages of the brain, which normally are quiescent or down-regulated when compared to other tissue macrophages. However, in chronic neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease and prion disease they are activated and express molecules not seen on the normal resting microglia. In recent years, there is much interest in studying the interaction of microglia cells with protozoa. *Leishmania* are pathogenic obligatory intracellular parasites, which must gain entrance into mononuclear phagocytes to successfully complete their cell cycle. Actually is well known that receptors interacting with terminal sugars as ligands are involved in the binding of *Leishmania* promastigotes to the macrophage surface and their subsequent internalization. In the present study, we have evaluated the involvement of the Mannose-Fucose Receptor (MFR) in the uptake of *Leishmania* by microgliocytes in mixed glial cultures.

Microglia primary cultures were obtained from newborn mice brains and the cells were dissociated by successive cycles of mild incubation with trypsin. The cell suspension was plated and maintained at 37°C in Dulbecco's modified Eagle medium supplemented with 10% fetal calf serum. For *in vitro* modulation of MFR, the cultures were pre-incubated for 24h with 0.1 mg/ml DM or 1 mg/ml LPS. Thereafter, the cultures were maintained for 2 h at 37°C in the presence of *L.(L.) amazonensis* promastigote forms in a 10:1 ratio. After this period, cultures were rinsed with PBS to remove extracellular parasites and fixed after 0, 2, 6, 12, 24, 48 and 72h. Infected cells were fixed with Bouin's solution, stained with Giemsa and observed by light microscopy.

To analyze the expression of MFR during the infection periods, the infected microglial cells were labeled with the neoglycoprotein, mannosyl bovine serum albumin (mannose-BSA) coupled to FITC and their morphology was compared with DM-treated cultures and non-infected cultures. The samples were then fixed for 5 min with 4% paraformaldehyde (PFA) at room temperature and incubated with mannose-BSA for 1h at 37°C.

Our preliminary results showed that all types of microglia (ramified and ameboid) expressed the MFR on their surface, with infected cells tending to display a different morphology in comparison to control and DM-treated cells. In the case of DM-treated cells, we detected punctual MFR fluorescence distributed close to the nucleus and around the parasitophorous vacuole of the host cells.

These results confirm the involvement of the MFR in the uptake of *Leishmania* by microgliocytes. An indirect evidence for this fact is that addition of D+-mannose to mixed DM-treated cultures impairs both microglial infection and internalization of the mannose-BSA.

BC32 - THE LAMININ-1 RESIDING ADHESION SEQUENCES FOR THE PARASITIC PROTOZOA *TRITRICHOMONAS FOETUS* AND *LEISHMANIA (LEISHMANIA) AMAZONENSIS*.

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The Laminins are large basement membrane matrix glycoproteins with multiple domains each one of them may determining different cell functions. The isoform 1 of Laminin (LMN-1) is known to be recognized by several pathogens including parasitic protozoa such as *Tritrichomonas foetus*. Our preliminary results showed that promastigote forms of *Leishmania (L.) amazonensis* like *T. foetus* recognized LMN-1. The Laminin-1 peptides A13, A208, AG73 (a chain), and C16 (b chain) have already been sequenced and characterized in terms of what cell functions they are preferentially involved. Here, we designed experiments looking for which are the LMN-1 peptides preferentially recognized by the parasites *T. foetus* and the promastigote forms of *L. (L.) amazonensis*. LMN-1 (20mg.mL^{-1}) was adsorbed onto plastic slides, and overlaid or not with each one of the four peptides. Parasites which have been coated or not with each one of the peptides were allowed to interact with the LMN-1-coated surfaces. Both *T. foetus* and *L. (L.) amazonensis* adsorbed onto LMN-1-coated surfaces and the peptides revealed to be inhibitory at different degrees when they were found recovering LMN-1-coated surfaces. These results lead us to conclude that both parasites could recognized LMN-1 through each one of the four assayed peptides. We are now searching for the cell functions determined by the peptides in the parasites, and identifying the LMN-1 surface receptors in *T. foetus* and *L. (L.) amazonensis*.

This research has been supported by PIBIC-UFRJ/CNPq, MCT-PRONEX, FAPERJ, and FUJB-UFRJ.

BC33 - *TRICHOMONAS VAGINALIS* VIRULENCE AGAINST EPITHELIAL CELLS AND MORPHOLOGIC VARIABILITY: COMPARISON BETWEEN A WELL-ESTABLISHED STRAIN AND A FRESH ISOLATE.

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The FMVI strain of *Trichomonas vaginalis* was freshly isolated from an asymptomatic patient and its morphological properties and virulence *in vitro* compared with the well-established JT strain. The morphology variability of the parasites was assessed by differential interference microscopy and both scanning and transmission electron microscopy. The FMV1 strain presented nearly 20% ameboid cells whereas the JT strain presented high percentages of ellipsoidal cells and no ameboid ones. The FMV1 morphotype population was not altered for at least one year sub-culturing. Electron microscopy studies revealed that this strain produced numerous pseudopod structures which mediated intimate contact among trophozoites. Dead FMV1 parasites were often phagocytosed by the conspecifc cells. We also compared the cytolytic capacity of these two populations against epithelial MDCK cells and its contact-dependence. The FMV1 strain rapidly adhered to plastic or glass surfaces and to MDCK monolayers. This strain destroyed about 93% of the epithelial cells in 90 min whereas the cytolytic activity of JT parasites was remarkably lower (about 41%). Parasite supernatants displayed no cytolytic activity indicating a contact-mediated lysis. The protozoan virulence *in vitro* did not correlate well with the clinical observations. The implications of these results are discussed.

Financial Support: CNPq, FAPERJ, FINEP and PRONEX.

BC34 - DIFFERENCES IN THE NITRIC OXIDE PRODUCTION IN SPLEEN AND LIVER OF *CALOMYS CALLOSUS* INFECTED WITH *TRYPANOSOMA CRUZI*.

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Nitric oxide (NO) has a major role in macrophage defense against intracellular microorganism, including parasites such as *Trypanosoma cruzi*. On the other hand, NO is also known as a double edged sword, generated at high levels it causes intense tissue necrosis. The aim of this work was to determine the NO-production in *Calomys callosus* infected with two different strains of *T. cruzi*. Male *C. callosus* were i.p. infected with $4x10^3$ blood derived trypomastigote forms of the Bolivia (BOL, group 1) and the Boliva-sobrenadante (BOL-SB, group 2) strain. At day 5, 7, 9, 12, 14 and 45 after infection, hepatocytes and splenocytes were prepared from whole organs and cultured for 42h in appropriate medium either with LPS (1mg/ml) or without. Nitrite levels of the supernatants were measured by the Griess-reaction. Parasitemia curves were obtained at the same days after infection. Splenocytes of *C. callosus* were unable to produce NO during the infection with Bol-SB, whereas Bol infected cells showed enhanced values at day 9 and 12 (highest parasitemia) compared to controls without infection. Stimulation with LPS leads to an earlier (day 5 /7) and higher production of NO, being most severe on day 14 in both infection models. In contrast, hepatocytes infected with Bol showed no significant differences during infection compared with non-infected controls. Remarkably, stimulation with LPS led to a significant decrease at day 12 (peak of parasitemia), followed by an increase to the highest values at day 14. Bol-SB strain caused a decrease of NO in hepatocytes already during an early stage after infection (day 5/7) reaching control levels at day 12 and 14. This decrease was even more pronounced after LPS stimulation. The *T. cruzi* strain Bol, with a predominance of broad structured forms, caused significant higher values of NO in splenocytes as the slender Bol-SB strain according to the different morphology and pathology described in literature, which is more severe in Bol-infected animals. In hepatocytes increased levels of NO were not observed as they were detected in splenocytes. During the course of infection with *T. cruzi* it seems that the production of NO is decreased in liver-cells. Since the NO-system has to be triggered very accurately, increased levels of NO already detected in non-infected cells compared to splenocytes, indicate that further stimulation, provoked by the parasites, may turn on other mechanism to prevent an overproduction of NO in these highly specialized metabolic cells. This could be one reason for the observed decrease in NO production during infection with two different strains of *T. cruzi*. Further experiments are carried out to analyze the triggering of NO production in this resistant animal model.

BC35 - INFECTION OF MOUSE DERM – DERIVED FIBROBLASTS BY MONOXENOUS TRYPANOSOMATIDS – *HERPETOMONAS ROITMANI* AND *CRITHIDIA DEANEI*.

Silva; J. S.*; Franco,L. S. *;Corte-Real, S.**; Soares,M.**; Bourguignon, S.C*.and Santos, D.O.*

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Introduction: Flagellate protozoa of the Trypanosomatidae family, which includes agents of important parasitic diseases such as leishmaniosis and Chagas' disease, infect a wide range of hosts including animals, plants and protists (Vickerman, 1994). Monoxenous trypanosomatids are usually found in insect hosts and are considered to be not capable to cause parasitic diseases in vertebrates (Wallace,1966). To investigate whether the trypanosomatids *C. deanei* and *H. roitmani* are able to infect vertebrate cells, we have used derm-derived mouse fibroblasts as experimental model. Skin fibroblasts were chosen as putative vertebrate hosts cells first due to some reports pointing to a role for trypanosomatids other than *Trypanosoma* and *Leishmania* in some opportunistic cutaneous infections observed in immunocompromised human individuals (Boisseau-Garsaud et al. 2000; Dedet et al. 1995), and second due to a previous report of fibroblast infection by *Leishmania (L.) amazonensis* (Corte-Real et al., 1995).

Materials and Methods: *C. deanei* and *H. roitmani* were incubated for determined times (2 hr to 48 hr) with mouse derm-derived fibroblasts at 28 ºC in DMEM medium. Thereafter, the cultures were rinsed with PBS and processed for light or electron microscopy. For light microscopy, the cells were fixed with Bouin's solution and stained with Giemsa. The percentage of infection was analysed under a Zeiss photomicroscopy.

For scanning electron microscopy (SEM), the glass coverslips containing infected fibroblasts cultures were fixed for one hour with 2.5% glutaraldehyde diluted in 0.1 M cacodylate buffer, pH 7.2, washed in buffer and then post-fixed with 1% osmium tetroxide (OsO_4) diluted in cacodilate buffer, pH 7.2. After the coverslips were processed to be analyze in a Zeiss DSM-640 scanning electron microscopy operating at 15kV. To be observed by Transmission electron (TEM), infected fibroblasts, as well as control parasites and uninfected fibroblasts were fixed for one hour with 1% paraformaldehyde /1% glutaraldehyde diluted in 0.1 M cacodylate buffer, pH 7.2. And, then, washed and post-fixed with 1% OsO_4 in cacodylate buffer. After, dehydrated in graded acetone, and embedded in PoyBed resin, ultra-thin sections were obtained in a Reichert OmU3 ultramicrotome and observed in a Zeiss EM-10C transmission electron microscope, operating at 80kV.

Results and conclusions: According to traditional protozoological concepts, monoxenous trypanosomatid protozoa do not infect vertebrate cells. We show that the monoxenous trypanosomatids *C. deanei* and *H. roitmani* are able to infect derm-derived mouse fibroblasts in vitro, as observed by light and electron microscopy. Our data are the first experimental evidence showing the phagocytosis and survival of two known monoxenous trypanosomatid parasites into vertebrate cells. We consider the possibility that *C. deanei* and *H. roitmani*, and perhaps other monoxenous trypanosomatid species, can cause opportunistic infections in immunocompromised individuals and possibly cause cutaneous lesions in vertebrate hosts.

Financial support: FAPERJ/ FIOCRUZ.

BC36 - CHEMOKINES AND INFLAMMATION DURING EARLY RESPONSE TO *L. (V.) BRAZILIENSIS* INFECTION.

Teixeira, M.J[1,2]; Teixeira, C.R[1,2]; Andrade, B.B[1,2]; Brodskyn, C.I[1]; Silva, J.S[3]; Barral-Netto, M[1,2]; Barral, A[1,2].

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In this study, we investigated the early immune response of *L. (V.) braziliensis* isolates that display different biological behavior in BALB/c mice, in order to understand the early inflammatory events and the pathogenesis of *L. (V.) braziliensis* infection. Of utmost interest, we want to establish whether the development of cutaneous leishmaniasis due to different *L. (V.) braziliensis* isolates can be associated with a specific chemokine profile and inflammatory response. Mice were infected with two *L. (V.) braziliensis* (CE-3227 and BA-788) and disease evolution was determined through weekly measurement of lesion sizes and assesment of parasite load. Cytokines, chemokines and chemokine receptors mRNA expression in footpad lesions was evaluated at various days post infection. The results shown that the infection with CE-3227 always resulted in larger lesions than that with BA-788 (0.129 0.013 vs 0.085 0.006). CE-3227 isolate seems to induce a more intense inflammatory reaction than BA-788, since parasite burden did not correlate with lesion development in the inocularion site. IFN-γ mRNA was detected earlier in BA788-infected mice than in CE3227-infected mice while IL-4 mRNA was observed at 3 days post infection with both isolates. The level of the chemokines and chemokines receptors expression in lesion tissue of infected mice was markedly different between the two parasite isolates. CE-3227 isolate upregulated the expression of more chemokines and chemokines receptors than BA-788 mice. Of interest, the expression of CCL2/JE/MCP-1, CCL3/MIP-1 and their receptors was more

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strongly induced in CE3227-infected mice than in BA788-infected one. These findings suggest that differences in virulence presented by *L. (V.) braziliensis* isolates can induce specific chemokine expression at the inoculation site, which would implicate in immunogical effects in the early inflammatory events and development of the differentiated pathogenesis in the *L. (V.) braziliensis* infection at a later time.

This work was supported by PROCAD/CAPES/FAPESB.

BC37 - PRELIMINARY SURVEY OF FREE-LIVING PROTOZOAN SPECIES FROM THE FURNAS LAKE (MG, BRAZIL): MORPHOLOGICAL AND MOLECULAR APPROACHES.

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The Furnas lake is an important artificial reservoir located in the south of Minas Gerais, Brazil. It plays a significant role in human activities as recreation, economical interest and human health. Free-living protozoa have a large distribution and a very significant role in aquatic microecosystems as they take part in a complex food web including all trofic levels, thus collaborating for nutrient recycling.

To study the protozoa species from the lake, water samples from 3 points of the Furnas lakes near the city of Alfenas were collected with plankton nets and bottles. The samples were examined one or two days later. The organisms were observed *in vivo* under a stereoscopic microscope where each organism could be isolated using glass micropipetes. In order to have a large number of organisms of each species for identification, different media were tested. The most successful ones were 997 (ATCC Bank) and a medium enriched with rice. For each species different fixative agents and some staining methods were tested in order to identify them by means of morphological characters useful for taxonomy. For a flagellate species we have also performed Normanski differential interference contrast microscopy.

Molecular techniques using DNA extraction and PCR reactions with primers specific for the mini exon donor RNA (medRNA) were used for the identification of an organism from the Bodonidae family.

In the present study we have found three great groups of protozoa in the Furnas lake region examined: ciliates (the most frequently and the group that shows the major diversity of morphospecies), flagellates and amoeba species. For the ciliates, species identification was done after silver impregnation technique by Dieckmann (1995) and Fernadez-Galeano (1976), revealing somatic and oral infraciliature and the nucleus. These characteristics lead us to identify 9 genus of ciliates by comparison with key identification and figures (Silva & Silva-Neto, 2001; Sleigh, 1989; Sorokin, 1999). For the flagellates, 3 genus could be identified, including a *Bodo* species, confirmed by molecular analysis that shows a PCR product of ~450bp using primers specific for the medRNA, whose size was already described for this genus by Santana *et al.* (2001).

Our preliminary results clearly shows that water samples from Furnas lake exhibit a large diversity of protozoa morphospecies, all of them already described and commonly found in freshwater samples and sewerage discharge (Silva & Silva-Neto, 2001; Sleigh, 1989). Our work also described for the first time the isolation of a *Bodo* species in an artificial reservoir, with its identity confirmed by molecular analysis.

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BC38 - DIMORPHISM IN *TRYPANOSOMA CRUZI*: A COMPARATIVE STUDY OF DISTINCT STRAINS

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One of the unsolved aspects of the cell biology of the *Trypanosoma cruzi* is the occurrence of slender and broad forms. Several studies were dedicated to explain the biological reason of the dimorphism in *T. cruzi*. They analyzed the following aspects concerning the presence of slender and broads forms: a) appearance and persistence in the bloodstream; b) infectivity to mammals and vectors; c) prevalence of each form in different strains; d) tissue tropism; e) motility and; f) behavior in tissue culture. In the present study a comparative analysis of the morphology, motility and cell surface antigenic composition of slender and broad forms were done. Trypomastigotes forms of the Y strain and CL-Brener and Dm28c clones were used. To analyze the influence of the cell size on the number of trypomastigotes produced at the end parasite cycle and the mechanical resistance of the cell to the movement of trypomastigote forms LLC-MK_2 cells were used. The first free trypomastigotes forms were harvested from the culture medium after 5-6, 6-7 and 3-4 days post-infection with the Y strain and CL-Brener and Dm28c clones, respectively. Preliminary studies using of the Dm28c clone showed that the length of trypomastigotes liberated from day 4-8 post-infection tends to increase whereas the width tends to reduce. The mean length of Dm28c trypomastigotes obtained after the first liberation (4^{th} day post-infection) is 10.7 ± 2.9mm and in the 8^{th} day post-infection is 15.2 ± 3.4mm. The width varies from 1.6 ± 0.4 mm to 1.2 ± 0.2mm in the 4^{th} and 8^{th} days post-infection, respectively. Analyses of the Y strain and CL-Brener clone are in progress. Preliminary data have shown that Y strain is mainly composed by thin trypomastigotes presenting a mean of 19.1 ± 3.1mm in length and 1.0 ± 0.2mm in width. Broad forms present 12.9 ± 2.3mm in length and 1.4 ± 0.2mm in width. Thin trypomastigotes of CL-Brener clone are 17.9 ± 2.3mm long and 1.0 ± 0.2mm in width, whereas broad form are 11.9 ± 2.4mm and 1.6 ± 0.4mm in length and width, respectively. Thin forms of Dm28c clone trypomastigotes present 15.9 ± 2.1mm in length and 2.1 ± 0.2mm in width whereas broad forms are 8.3 ± 1.0mm and 1.4 ± 0.2mm in length and width, respectively. Immunocytochemical analysis of the cell surface of slender and broad forms using antibodies against SAPA, 80kDa, LPPG and a-Gal showed that the broad forms of the different samples were always more intensely labeled than slender ones. Comparisons about the velocity of displacement and kind of movement on slender and broad forms of the three samples, using video-microscopy are in course.

Supported by: CNPq, CAPES, FUJB/UFRJ

BC39 - FURTHER ULTRASTRUCTURAL STUDY ON THE ENDOMASTIGOTE FORM OF A TRYPANOSOMATID ISOLATED FROM SOLANACEAE

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We have previously reported on the isolation and cloning of a trypanosomatid from a tomato fruit which presented a endomastigote form (Coelho et al., Rev. Inst. Med. Trop. S. Paulo, 44 Suppl.: 97, 2002). Here we report on a initial ultrastructural analysis of this isolated based on transmission electron microscopy (TEM). Cells were fixed for 2 h with 2.5% glutaraldeyde and 4% paraformaldehyde in 0.1 M cacodylate buffer, pH 7.2, containing 50 mM $CaCl_2$. The cells were then

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washed in the same buffer and postfixed for 1 h at room temperature in a 1% OsO_4 solution in 0.1 M cacodylate buffer, pH 7.2, containing 0.8% potassium ferricyanide. After postfixation, cells were dehydrated in acetone and embedded in Epon. Ultrathin sections were briefly stained with uranyl acetate and lead citrate and observed in a Zeiss EM-900 transmission electron microscope operating at 80 kV. Results showed cells with a long curved flagellar pocket, characteristic of the endomastigote stage of the *Wallaceina* genus (Podlipaev et al., Parazitologia, 30: 324-332, 1990, *in Russian*). However, some distinctions were also observed as a lack of a double set of four microtubules in the area of the flagellar pocket, and a presence of a large mitochondrion. Previous molecular analysis showed that *Leptomonas peterhoffi* and *Blastocrithidia gerricola* are close related with *Wallaceina brevicula* and *Wallaceina inconstans*, though they also show morphological differences. Thus, we conclude that the presence of the endomastigote stage strongly suggests that the isolated from Solanaceae may belong to the *Wallaceina* genus but biochemical and molecular analysis will be required to better characterize this trypanosomatid.

Supported by FINEP and CNPq (Pibic/Pronex)

BC41 - HUMAN STERILE URINE AS *LEISHMANIA* ENRICHMENT FACTOR FOR PRIMARY GROWING. INFLUENCE OF DIFFERENT SOURCES OF URINE AND THE COLLECT BIOLOGICAL SAMPLES FROM DOGS NATURALLY INFECTED IN KALAZAR ENDEMIC REGION.

Araújo, M.F.L.,Oliveira, O.R., Garcia, A.S., Castelão, K.G., Bisugo, M.C., Cunha, E.A., Garcia, E.L., Garcia, R.A., Taniguchi, H.H., Barbosa, J.A.R., Barbosa, J.E.R. and Tolezano, J.E.

Instituto Adolfo Lutz – São Paulo/S.P.

The aim of this study were investigate the importance of: a. the source of human urine to be used as enrichment factor of growing *Leishmania* in acellular culture media without fetal serum calf; b. the procedure for collecting biological samples from naturally infected dogs in canine kalazar endemic region.

Human urine collected from four different donors, a 47 years old man; a 40 years old woman; a 8 years old boy; a 6 years old girl, were sterilized by filtration in 0,22µ filter.

Four groups of 16x160 tubes of biphasic media (blood agar base as solid phase and brain heart infusion as liquid phase) were constituted. Each group was supplemented, respectively, with 5% of different human sterile urine described above, calculated to the final concentration of the liquid phase.

Spleen aspirate was obtained from 44 dogs with clinical signs of canine visceral leishmaniasis. All dogs examined were from Araçatuba region in São Paulo State most of them presented previous confirmed positive diagnosis. Two procedures were utilized in order to collect spleen aspirate: a. vacuum aspiratory system that turned possible direct inoculation in medium culture tube working in field condition; b. aspirate transferred to a recipient containing antibiotic saline solution and the inoculation into culture tube was did under laboratory condition in a sterile flow cabinet.

Vacuum aspiratory system permitted to us isolate *Leishmania* from 90% of infected dogs, while the second procedure maked possible isolation from 70% of the same animals. Qui square test showed no statistical significance for observed differences.

In previous studies we demonstrated that human sterile urine could optimize 2-3 times more *Leishmania* isolation than without any supplementation (Rev. Inst. Med. trop. S. Paulo, 44:2002; Rev. Soc. Bras. Med. Trop., 36 :2003). In this study we obtained again the high performance of *Leishmania* isolation when human sterile urine was utilized as growing factor, but no statistical differences were obtained, by qui square test, when considered the source of urine, for either different sex or age.

Again we concluded that the advantages of human urine utilization, are low costs and excellent technical performance, applied also for vacuum system, as alternative for *Leishmania* isolation in field conditions.

BC42 - GAMMA IRRADIATION AS A TOOL FOR METACYCLIC *LEISHMANIA (L.) AMAZONENSIS* SELECTION

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Leishmania spp. causes a spectrum of human diseases, ranging from self-healing skin lesions to severe and lethal visceral disease. The parasites are transmitted sand fly bites, and have two morphologically distinct forms on its life cycle: promastigotes, a flagellated that occurs in the insect gut, and amastigote form, an small obligatory intracellular parasite. In previous work we demonstrated that the protein and nucleic acid metabolism and oxidative respiration were severely affected by irradiation, in a dose response way, but a small but representative fractions are relatively radio resistant, surviving after 800 Gy of ^{60}Co irradiation. The best explanation could be a selection of metacyclic promastigotes. In these forms, the Go state allows the adequate correction of DNA repair after the irradiation insult. In this work, we are looking for the ideal radiation dose to select the higher proportion of metacyclic forms of *L.. (L.) amazonensis* in culture. Parasites were grown in RPMI 1640 medium, plus 20% fetal calf serum, at 24^{o} C, in monophasic medium until log stage were achieved. For the determination of metacyclic forms, we used the infection of mammalian cells, RAW (macrophage-like) cells, aside to the classical complement resistant behavior of the metacyclic form. Parasites were cultured for 7 days and their growth determined in Neubauer chambers. Initially, the parasites grow exponentially, procyclic forms, until the fourth day, while on the fiftieth and sixth we observed a declining growth that disappears at the 7th day, when the *Leishmania* numbers became stable or reduced. Those forms were recovered by centrifugation, suspended in fresh TC-100 medium without serum, adjusted to 10^7/ml promastigotes and irradiated with doses ranging between 25 and 400 Gy of ^{60}Co gamma-radiation. The number of surviving intact parasites was counted in hematocytometer chamber. As expected, there is a dose response effect on parasite numbers, with 400 Gy shown the lowest number of parasites. Irradiated promastigotes were challenged to $2x10^5$/ml RAW cells in round cover slips, in RPMI 1640 medium, plus 10% fetal calf serum, at 37ºC 5%CO_2 in 24-well plates by 3 hs. The cover slips were fixed, stained with Giemsa, with determination of the number of infected cells. Parasites irradiated at 400 Gy infected, proportionally, more cells than parasites irradiated at other doses. To confirm this metacyclogenesis, a complement lysis assay was performed with 5, 10 and 20% of male guinea pig blood serum at 20ºC for 3 hours, and parasites counted. Guinea pig serum a 10% promotes more lysis, with 200 Gy irradiated parasites being less affected, probably due to metacyclic selection. These preliminary results suggests that the ionizing radiation, specially between 200 and 400 Gy, could be a alternative tool for the selection of metacyclic forms of *Leishmania (L.) amazonensis* in culture.

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BC43 - SURAMIN TREATMENT LEADS TO FLAGELLAR DETACHMENT IN *TRYPANOSOMA CRUZI* TRYPOMASTIGOTES

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Suramin, a symmetrical polysulfonated naphthylamine derivative of urea, is a potent antagonist to some P2 receptors and is able to inhibit a large number of cellular enzymes, such as ecto-ATPases, retrovirus reverse transcriptase, DNA polymerase, among others. It had initially been used in sleeping sickness treatment and is under study for therapeutic treatment of some cancers. Morphological changes such as plasma membrane disruption of chick embryo neural retina, axonal degeneration or atrophy, which may lead to neuronal apoptotic cell death, alterations on distribution of actin filaments in epithelial cells and inhibition on formation of stress fibers and focal contacts in endothelial cells, which may interfere on cellular migration were observed after treatment with suramin. In this study we analyzed suramin effects on *T. cruzi* trypomastigotes flagellar adhesion to cell body. Y strain parasites were obtained from the supernatant of infected LLC-MK_2 cells cultivated in RPMI-1640 medium supplemented with 2% of fetal calf serum. 500 µM suramin were added to culture medium 24 h after infection and was maintained during *T. cruzi* intracellular cycle. Trypomastigotes cultivated in the presence of suramin are about 20% shorter and about 25% broader when compared to control cells and present parts of the flagellum or the whole flagellum detached from cell body. It was observed by videomicroscopy that suramin treated trypomastigotes present cellular movements about 3-fold slower when compared to control cells. In preliminary studies we observed, in drug treated trypomastigotes with the flagellum detached from cell body, an intense labeling both in cellular body and in the flagellum when we used the polyclonal antibody, for the repetitive flagellar antigen (FRA), which is an antigen located on the side of epimastigotes and trypomastigotes flagella that faces parasite body. This result was different from that observed with control cells, which present an intense labeling in the flagellum and just a weak labeling in cell body. Preliminary results show that suramin addition to culture medium during *T. cruzi* intracellular cycle may affect parasite division, since the presence of two flagella in some treated trypomastigotes was observed. This result probably is due to a disorganization on the arrangement of internal flagellar attachment zone structures in cells with the flagellum detached from cell body, since the correct organization on the arrangement of these structures is essential for the cell shape and division plane definition.

Supported by: CNPq, CAPES, FUJB/UFRJ

BC44 - ANALYSIS OF THE CYTOTOXICITY OF MNNG ON THE PROLIFERATION OF *HERPETOMONAS SAMUELPESSOAI* USING THE MTT ASSAY

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Herpetomonas samuelpessoai corresponds to a Trypanosomatidae parasite from the *Herpetomonas* genus characterized by three different differentiation forms, promastigota, paramastigota, and opistomastigota. These parasites can be grown in culture medium *in vitro*. Morphological, biochemical, growth, differentiation and motility alterations can be observed in these organisms after treatment with various drugs. MNNG (N-methyl N-nitro N-nitro guanidine) is an established carcinogenic substance with confirmed *in vivo* neoplasic action, and also with cytotoxic and mutagenic effects on *in vitro* mammalian cells. MNNG was shown to be less cytotoxic and highly mutagenic in mammalian MT1 cells. The objective of this study is to evaluate the cytotoxic effect of MNNG on *Herpetomonas samuelpessoai* growth. This effect was evaluated through the MTT assay using an automatic colorimetric method with a tetrazolium salt and spectrophotometric quantification. The inoculum was performed with 0,2 ml at the concentration of $2{,}4x10^6$ cells/ml. The addition of MNNG was performed at the concentration of 10, 50, 100, 150 mg/ml. A sample without treatment was used as control. The experiments were repeated three times. The samples were incubated for 48 h at 28ºC, and the parasites were thereafter submitted to the MTT assay and quantified spectrophotometrically with an ELISA reader, followed by the evaluation of absorbance at a wavelength of 560 mm. The results from the absorbance reading demonstrated in the control group and in the samples treated with 10, 50, 100, 150 mg/ml of MNNG, the following values: 301, 30, 12, 10, and 6, respectively. The results showed that, even at lower concentrations of MNNG (10 mg/ml), there was a 90% reduction of the absorbance of *H. samuelpessoai* reading. It can be concluded from this study, that following a 48 h incubation with MNNG, there was a reduction of the number of the parasites, demonstrating a marked cytotoxic effect of MNNG on *H. samuelpessoai* using the MTT assay.

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BC45 - DIBUCAINE INDUCES A DOWN-REGULATION OF THE CRUZIPAIN EXPRESSION IN *TRYPANOSOMA CRUZI* EPIMASTIGOTE FORMS

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Dibucaine, a local anesthetic, was found to inhibit a Ca^{+2} independent phospholipase A2 (iPLA2), an enzyme that releases aracdonic acid (AA) from phosphatidylethanolamine. Experiments in diferent cell types have shown that AA is involved in membrane fusion, degranulation, secretion and phagocytosis. Previous study of our group have shown that dibucaine arrest the growth of *Trypanosoma cruzi* epimastigote forms *in vitro* and also disturb the degradation of proteins in the reservosomes, indicating a possible role of dibucaine in the inhibition of the proteolytic process that occurs inside these organelles. In the present study, the role of dibucaine in the protein and proteolytic activity expression in *T. cruzi* epimastigotes were investigated. After growth of epimastigote forms of *T. cruzi* in LIT medium in the absence and in the presence of 50 and 100 mM of dibucaine, for 48 h at 28ºC, the protein and proteinase contents were analyzed on SDS-PAGE and Western-blotting. Epimastigotes grown in the presence of both dibucaine concentrations, showed qualitative changes in the protein profile with marked preferential expression of polypeptides of 95 and 56 kDa when compared with non treated cells. Gelatin-SDS-PAGE analysis revealed two major proteolytic activities in epimastigotes of *T. cruzi*, when incubated in acidic pH: a 40 kDa cysteine proteinase (cruzipain activity), inhibited by 10 mM E-64, and a 52 kDa metaloproteinase, restrained by 1 mM 1,10-phenantroline. The parasites cultivated in the presence of 50 and 100 mM of dibucaine demonstrated a significant reduction (approximately 4-fold) in the cruzipain activity, a known virulence factor of this human pathogen. In order to characterize if the dibucaine exerts its inhibitory effect directly in the enzyme structure by producing conformational changes, non-treated epimastigote cellular extract were incubated with distinct concentrations of the drug (50, 100, 250 and 500 mM) for 1 h and 4 h. In this case no reduction in cruzipain activity was observed. Moreover, no alteration in the proteolytic activity was observed when samples were separated by gelatin-SDS-PAGE and incubated for 20 h at 37ºC, in digestion buffer (pH 5,5) supplemented with 100 mM of dibucaine. Therefore, we hypothesized that dibucaine might be influencing the cruzipain synthesis, once this drug interferes with signal transduction pathways. Western-blotting analysis showed a dramatic reduction in the cruzipain polypeptide synthesis in dibucaine-treated parasites in comparison with non-treated ones. Here, we demonstrated that dibucaine inhibits the major cysteine proteinase activity of *T. cruzi*.

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BC46 - EVIDENCE FOR NITRIC OXIDE ROLE IN *T.CRUZI*-TRIGGERED NEURONAL LESIONS *IN VITRO*

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Objectives: Neuronal lesion and peripheric denervation in Chagas´ disease are related to local inflammation which is triggered by *T. cruzi* infected macrophages and activated lymphocytes. Although the human disease is associated with autonomic and enteric nervous system lesions, the pathogenic mechanisms of neuronal lesions in the heart and megavisceras are still unclear. We have previously developed an *in vitro* co-culture model of sympathetic cervical ganglion (SCG) neurons and macrophages (Arantes et al, 2000) and we have showed the role of macrophages in reducing *in vitro* neuronal survival average to 70%. At the present work, we investigate the interaction of nitric oxide production, neuronal lesion and survival in *T. cruzi* infected neurons and IFN-g activated macrophage co-cultures. Methods: Primary neuron cultures of SCG neurons were prepared as described previously (Blennerhasset & Bienenstock, 1998). Briefly, after the removal of C57BL/6 newborn mice ganglia, the neurons were isolated by enzymatic and mechanic dissociation. The cells were then cultured on Matrigel (Becton Dickinson, NJ) and 50ng/ml of NGF and were maintained for 48 hours before co-cultured with macrophages and infected with Y strain of *T. cruzi*, in the presence or absence of IFN-g.

Neuronal survival was assessed as the percentage of surviving neurons. Results: *T. cruzi* infected pure neuronal cultures didn´t produce nitric oxide (NO) and didn´t show reduction on neuronal survival when compared to control. Co-cultures of neurons and macrophages showed 77% of neuronal survival according to previous results, and the NO production of these co-cultures was not relevant. Infected co-cultures of neurons and IFN-γ macrophages showed 44% of neuronal survival associated with increase of NO production. Conclusion: We believe that *T. cruzi* infected pure neuron cultures do not produce NO. The addiction of IFN-γ to infected co-cultures is related to increased NO production by macrophages and significant neuronal death which suggests a role for macrophage derived NO in neuronal lesions.

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BC47 - MAST CELLS QUANTIFICATION AND HISTOPATHOLOGICAL ANALYSIS IN THE HEART, SPLEEN AND LIVER DURING THE EXPERIMENTAL ACUTE INFECTION WITH *TRYPANOSOMA CRUZI* IN RATS

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Mast cells are multifunctional cells capable of secreting a wide variety of mediators. Following activation, these cells express mediators such as histamine, serotonine, leukotrienes and prostanoids, as well as proteases and many cytokines and chemokines, all essential to the genesis of the inflammatory response. During *Trypanosoma cruzi* infection, it has been suggested that mast cells could contribute to the control of the parasite by recognizing and killing IgG-opsonized trypomastigotes and through secretion of mediators. Increased numbers of mast cells have been demonstrated in chagasic patients with chronic disease and in experimental models. In this work, we studied the occurrence of parasitism, inflammatory processes and distribution of the mast cells in different organs (heart, spleen and liver) during the acute experimental infection with *Trypanosoma cruzi* in rats. Female Holtzman rats (16 animals) infected with Y strain of *T. cruzi* (300.000 trypomastigotes, i.p.) and 4 controls were sacrificed at 12 and 20 days of infection. Fragments of heart (atrium and ventriculum), spleen and liver were fixed in Carnoy's fluid and embedded in glycolmethacrylate for histopathological study and quantification of mast cells. Semi serial 3-µm-thick sections were stained with toluidine blue/basic fucsin or toluidine blue, pH 3.0. Amastigote nests were counted in the heart (420 fields for both atrium and ventriculum) and liver and spleen (240 fields/organ), for each group. Mast cells numbers were quantified in 300 fields/group. Acute *T. cruzi* infection did not induce an increase of mast cell numbers neither in the heart nor in liver and spleen. However, a clear increase of degranulated mast cell numbers, in parallel to a diffuse mononuclear inflammatory process and high parasitism, was observed in the heart, at day 12 of infection. In the liver and spleen, mast cells were rarely observed and if so they were seen around vessels. In the heart, mast cells were scattered in the myocardium. Parasite nests were scarce in the heart, at day 20 of infection. Amastigote forms were not observed in the liver and spleen at different times of infection. Our data suggest that the acute phase of experimental infection with *T. cruzi* induce mast cell degranulation, process that seems related to the presence of high parasite load. These cells may be involved in the parasite control or even in pathogenetic mechanisms during acute Chagas disease.

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BC48 - COMPARISON OF TWO RAT MODELS USED IN EXPERIMENTAL STUDIES DURING ACUTE CHAGAS DISEASE

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Different experimental models have been used in Chagas' disease studies, but most investigations are carried out in murine model and/or *in vitro*. While different lineages of mice have been compared regarding the resistance to infection, there are few studies evaluating rat models. In the present work, Holtzman and Wistar rats were inoculated with Y strain of *T. cruzi* (300,000 trypomastigotes, i.p.) and studied at days 6, 12 and 20 post-infection (4-6 animals/group) to evaluate different patterns of acute infection (parasitemia, heart parasitism and myocarditis). For each rat, 100 plastic 5-mm sections, obtained from both atria and ventricles and stained with toluidine blue – basic fuchsin, were analyzed at an interval of 70mm. In parallel, the number of peripheral blood leucocytes was evaluated. Both rat models presented a prominent parasitemia with peak at day 10. However, when the pattern of involvement of heart, a target organ of the disease, was compared, we observed a significant and dramatic difference between the models. Holtzman rats showed a higher parasitism than Wistar rats at day 6 and especially at day 12 of infection (mean ± SE of respectively 1542.25 ± 460.40 and 56.50 ± 19.50 per animal). At this time, it was observed an Intense and diffuse mononuclear myocarditis in both rats. Parasitism dropped drastically in the heart of Holtzman and Wistar rats at day 20 of infection, but remained significantly higher in Wistar model. Both rats showed a significant increase of the total number of peripheral blood leukocytes compared to controls but in different days of infection. At day 6, the total number of peripheral blood leukocytes and the absolute number of blood monocytes, cells involved in the heart parasite clearance as macrophages, was higher in Wistar rats than in Holtzman ones, but the opposite was observed at day 12. At day 20, the total number of blood leukocytes showed no difference between the rats but the number of blood monocytes was higher in Holtzman rats. Our data

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indicate that Holtzman rats are more susceptible to *T. cruzi* infection, showing a prominent heart involvement compared to Wistar model. In both rats, the acute disease induced a great mobilization of cells from the monocytic lineage, but this response was more intense in Holtzman rats. In addition, Holtzman rats showed a more efficient resolution of the acute infection compared to Wistar. In spite of both rat models being able to respond to infection, our data highlight the Holtzman rat as a more reliable model for *in vivo* experimental Chagas disease studies, especially those ones focused on the monocyte/macrophage system involvement.

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BC49 - ADULT RAT INFECTIONS WITH JG STRAIN OR CL-BRENER CLONE: PARASITEMIA, HISTOPATHOLOGY AND CYTOKINE SERUM LEVELS

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The juvenile Hotzman rat has been used in our laboratory as a model for Chagas disease acute and chronic indeterminate phases, the heart being the main studied organ. It was demonstrated that the juvenile rat susceptibility depends on *T. cruzi* population. Six different populations have been tested and two of them present opposite virulence and pathogenicity, Cl-Brener clone and JG strain. JG strain provokes low parasitemia, null mortality and focal myocarditis that is maintained for several months with very low if any cardiac sympathetic denervation. CL-Brener clone causes higher parasitemia with high mortality and severe myocarditis accompanied by sympathetic denervation, both of which are resolved in acute phase-surviving animals. Adult rats are known to be resistant to *T. cruzi* infection. However, despite the null or very low parasitemia, Y strain-infected adult rats develop acute myocarditis with sympathetic denervation followed by recovery. Aiming at having adult rat models for the indeterminate chronic phase of Chagas disease, we now compared JG and CL-Brener clone infections. Two-month-old rats were inoculated with 10,000 trypomastigotes/ 50g body weight, and sacrificed at days 5, 10, 15, 20, 37 or 120 post-inoculation. Parasitemia was very low in both infections, the values remaining below 50 parasites/5 µl of blood. Mortality was null even in CL-Brener clone infection that kills 100% of juvenile rat after inoculation of 10,000 parasites. No histological alterations were found in the brain, esophagus and rectum. Skeletal muscle (diaphragm) presented amastigotes nests only in CL-Brener clone-infected animals during the acute phase. The heart was affected by both infections. However, the JG strain-induced acute myocarditis was predominantly focal (mild or moderate) and mild focal myocarditis persisted during the chronic phase. In contrast, CL-Brener clone-induced acute myocarditis was diffuse and severe at day 15, but normality occurred at day 120. Cardiac sympathetic denervation was observed only in CL-Brener clone-infected rats at day 20 of infection (glyoxylic acid-induced histofluorescence). TNF-α serum levels (ELISA) increased significantly during the acute phase in both infections. However, at day 120, only in JG strain-infected animals the levels remained higher than control values. In JG infection, higher serum levels of IFN-γ occurred only at day 15 of infection. In CL-Brener clone infection, IFN-γ levels remained elevated throughout the acute phase but the values were similar to control values at day 120. In conclusion, despite significant differences between juvenile (previous studies) and adult rats with regards to parasitemia and mortality, in adult rats the two tested populations provoked distinct myocarditis pattern as in juvenile animals. Recovery was faster in the CL-Brener clone infection in adult rats. This finding could explain the CL-Brener infection null mortality in adult rats. Cytokines levels during the infections in juvenile rats are in course.

CNPq and PRONEX

BC50 - IMMUNOCYTOCHEMICAL STUDY OF CANINE LIVER OF DOGS NATURALLY INFECTED. WITH *LEISHMANIA (LEISHMANIA) CHAGASI* FROM BELO HORIZONTE, MG

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Canine visceral leishmaniasis (CLV) is an endemic disease in Brazil and dogs are the principal reservoir domestic of this parasite and play a central role in the transmission cycle to humans by phlebotomine sand flies. In the New World is caused by specie *Leishmania (Leishmania) chagasi*. The aim of this study is to quantify the tissue parasitism in order to make correlations among with different clinical status of the animal. Eighty-five infected animals with positive serological exams to *Leishmania* (IFAT, Complement fixation and ELISA) were divided in four clinical groups: controls, asymptomatic, oligosymptomatic and symptomatic (weakness, cutaneous lesions, alopecia, and clinical anemia) animals. The dogs were sacrificed with lethal dose of Thionembutal 33% (1,0mL/Kg). During necropsy, small samples of liver were obtained to prepare tissue touch preparations (smears) for LDU analysis. Other liver fragments were collected and fixed in formalin buffered solution 10% for histopathological and immunohistochemical studies. The immunocytochemistry technique (streptoavidin-peroxidase method – Tafuri, et al., 2003) was carried out to determine the amastigote forms of *Leishmania* in paraffin tissue sections. For morphometrical analysis, the parasitism was analyzed with a 40x objective of an Axiolab light microscope (Zeiss). The images viewed on a computer video screen were obtained by means of the software and relayed to a computer-assisted image analysis system (Kontron Electronic/ Carl Zeiss, Germany). The results were expressed in mm^2. There was no statistical differences of the parasitism load among the defined clinical animal status (asymptomatic, oligosymptomatic and symptomatic) ($p>0,05$).

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BC51 - ISOLATION OF *TRYPANOSOMA CRUZI* STRAIN FROM SYLVATIC TRIATOMINAE

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Since 1981, the incidence of Chagas'disease decreased due to the effective control of its natural transmission in Brazil (Dias, 2000). However, considering the wide number of vectors and sylvatic reservoirs, it is necessary a constant epidemiological vigilance to keep under control the disease occurrence. In this aspect it is important the vigilance in regions where triatomine are present. In Brazil, the species *Triatoma baziliensis* and *T. pseudomaculata* can be found in the northeast, otherwise, *Panstrongylus megistus* can be found in many Brazilian areas. Another very susceptible species to *Trypanosoma cruzi* is *T. rubrovaria* (Perlowagora-Szumlewicz *et al*., 1988), which is widely dispersed in Rio Grande do Sul state, except in the north mountain region. As *T. rubrovaria* is in colonization process (Ruas-Neto *et al.* 1991), it keeps the sylvatic cycle of Chagas's disease

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and it is strongly susceptible to *Trypanosoma cruzi* it was decided to study this species in details. In 2002, *T. rubrovai* was collected in six Rio Grande do Sul towns. Two *T. cruzi* strains were isolated from 5th nymphal instar of *T. rubrovaria* collected in Passo do Guapurá, Quaraí-RS. In April/2003 another bugs capture was carried out in Quaraí in order to verify the contaminated sylvatic triatomine rate and to study the characteristics of *T. cruzi* strains isolated. From 23 to 25 April/2003 during 15 hours seven people collected by active search in six points of Quaraí-RS 684 *T rubrovaria* individuals, 53 *T. circummaculata* and 6 *Panstrongylus tupynambai*. Out of 26 *T. circummaculata* individuals examined no Trypanosomatidae forms were found. Out of 351 *T. rubrovaria* nymphs examined, 14 (3,98%) have had Trypanosomatidae forms in their intestinal contents (13 of 5th nymphal instar and one of 4th instar). Intestinal contents of 12 *T. rubrovaria* positive were inoculated in mice *Mus musculus* Swiss. Five strains were isolated in mice and culture medium LIT nominated QB I, QJ I, QJ III, QM I, QM II. These strains are in process of morphological and molecular characterization. In this field work six nymphs of *P. tupynambai* were also collected in the same ecotope of *T. rubrovaria* as well as a wizard *Tupinambis merinae*.

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BC52 - OBSERVATIONS ON A NATURAL INFECTION OF MONOXENOUS TRYPANOSOMATIDS IN CALIPHORIDAE

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Insect trypanosomatids comprise a large number of species mostly founded in the orders Hemiptera and Diptera, where about 300 species of trypanomatids have been described. Several aspects of the host-parasite association have been studied in insects naturally infected with trypanosomatids but only in a few cases investigations involved monoxenous species. The present study reports, on light and electron microscopy, observations of flies (Caliphoridae) naturally infected with trypanosomatids. Forty wild flies were collected in the vicinity of Alfenas city, Minas Gerais, Brazil. The dissection of the insects was carried out on sterile saline, and the presence of flagellates was detected by phase-contrast microscopy. Infected intestines segments were fixed in paraformaldeyde 0.4% (W/V), and embedded in Historesin (Leica). Semithin sections were stained with basic fucsin 0.1% for 30 min, and toluidine blue 0.1% (W/V) in borax 1% (W/V) for 15 min. For scanning electron microscopy the guts were ?xed in a solution containing 2.5% (V/V) glutaraldehyde and cacodylate buffer 0.1 M, pH 7.2, post-?xed in 1% (W/V) osmium tetroxide in 0.1 M cacodylate buffer, pH 7.2, dehydrated in ethanol series 50 to 100% (V/V), dried by the CO_2 critical point method, and sputtered with gold. Observations and micrographies were made with a Jeol JSM-5310. Up to 70% of the flies were founded parasited by trypanosomatids. Elongated flagellated cells were mostly observed as free parasites in the endoperitrophic space, always near the perithrophic membrane, but never attached to it. Few parasites were observed in the ectoperithrophic space as well as in the gut. However in the former region, some parasites were seen putting on the flagella between epitelial cells. The infection was also detected in the region of the rectum where flagellates could be seen lined with a thick cuticular layer. Ectoperithrophic infection was only observed when the region of the rectum was founded parasited. No parasites were seen attached to the epithelial cells of the rectal pads, although the infection was always observed near them. The morphology of the parasites attached to the cuticula layer was markedly distinct from those described in the endoperitrophic space since rounded forms predominated in the rectum region. A great number of cells observed by SEM showed a protusion in the end of the flagellum including non-attached cells.

Supported by FINEP and CNPq (Pronex)

BC53 - DYNAMICS OF *TRYPANOSOMA CRUZI* CIRCULATION IN NATURAL FORESTED ENVIRONMENT IN SÃO SEBASTIÃO ISLAND (ILHABELA), NORTH SHORE OF SÃO PAULO STATE, BRAZIL.

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Since 1997 we have studied the ecoepidemiology of *Leishmania* in natural forested areas in São Sebastião Island (Ilhabela), north shore of São Paulo State. Secondarily we have had opportunity to observe circulation of *Trypanosoma cruzi like* in this environment.

Between October 2000 and November 2002, monthly, we have caught wild animals in order to identify natural infection and isolate *T. cruzi.* After made identification and ear sign (a code built with a hole in the ear), for each animal, xenodiagnosis it was performed to attend this purpose. Then the animal was turned free in the same place of capture. Until now, from 551 captures, we had caught 298 different mammals (162 Marsupialia, being 139 *Philander opossum*, 22 *Didelphis aurita* and 1 *Gracilinamus agilis*; 136 Rodentia, being 122 *Proechimys iheringi*, 8 *Oxymycterus incanus*, 5 *Cavia aperea* and 1 *Nelomys thomasi*). The others 253 captures corresponding recaptures of several animals caught, marked and released before. In each recapture a new xenodiagnosis was done.

The results revealed as high is the circulation of this protozoan group, from 13.7% (19 out 139) *P. opossum*, 17.2% (21 out 122) *P. iheringi*, 4,5% (1 out 22) *D. aurita* and 25% (1 out 8) *O. incanus, T. cruzi like* was isolated. It is interesting to report that parasitemia was not constant. Some times several infected animals showed their positivity and some times not.

Even though the rates of parasite circulation in natural environment have been as high as we observed, a great number of animals rested still negative after 6, 12 and until 20 months and with 2 to 12 captures/recaptures.

It is clear to us that the geographic dispersion of parasite depends on the environment where the vertebrate and/or invertebrate host relationship occurs.

BC54 - COMPARATIVE EVALUATION OF THE INFECTIVITY AND DEVELOPMENT PATTERN OF GFP-EXPRESSING *TRYPANOSOMA RANGELI* IN *RHODNIUS DOMESTICUS* AND MICE

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Trypanosoma rangeli is a protozoan parasite transmitted in both Central and South America by triatomine vectors among a variety of mammal species, including humans, acting as a complicating factor for Chagas disease diagnosis. Aiming the study of the parasite life cycle, in this study we have characterized parasites of *T. rangeli* SC-58 strain transfected with the pTEX-GFPmut/SSUrRNA plasmid by comparison with non-transfected parasites concerning their infectivity and evolution in triatomine bugs. For that, two groups of Swiss mice were infected by i.p. inoculation of $2.5x10^6$ trypomastigotes/mice with transfected or non-transfected parasites of *T. rangeli* SC-58 strain. The infection course was evaluated daily by both fresh and Giemsa stained smears, observing the maintenance of GFP expression by transfected parasites. Positive mice were used for feeding 2 groups of 20 4th-5th instar nymphs of *Rhodnius domesticus* which were searched for the presence of parasites after 30 days of the infective blood meal by microscopic evaluation of the hemolymph, salivary glands, feces or digestive tract. Our results revealed no significant differences concerning the presence of flagellates in feces/digestive tract of *R. domesticus* infected with non-tranfected (52.63%) and transfected parasites (68.42%). The infection of hemolymph and

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salivary glands also not revealed significant differences between the experimental and control groups. Both transfected and non-transfeceted parasites were transmitted by triatomine bite, revealing patent parasitemia in Swiss mice. Quantitation of the GFP-expression in triatomines was performed during 60 days by microscopic evaluation of the hemolymph obtained from triatomines infected by intracelomic of culture forms, showed a decreasing expression level of the GFP, which corroborates former studies *in vivo*. Our results confirm the maintenance of biological characteristics of GFP-expressing *T. rangeli* as well as the reduction of the expression during the infection time course in absence of selective drug (G-418). Further studies using linearized plasmids are in progress in order to obtain stable tranfections and a constitutive GFP expression.

Supported by Pibic/CNPq and UFSC.

BC55 - PRELIMINARY STUDY OF THE INCIDENCE OF PARASITES IN GROUPS OF RISK (STUDENTS DINERS, SALESPERSONS OF FOODS AND CONSUMPTION PRODUCTS) INSIDE AND OUTSIDE OF SAN SIMÓN UNIVERSITY IN 100 METERS RADIOUS.

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The Transmitted Illnesses for Foods (ETA) they constitute, according to the World Organization of the Health, one of the most extended problems of health in the contemporary world, and an important cause of reduction of the economic productivity (Quevedo and cabbage., 1990), because it determines a discharge morbilidad rate affecting the health and quality of life (Báez and cabbage., 1993). The morbilidad for intestinal parasitosis is located in third place at world level (Kancha and cabbage., 2000), the same one that is caused by contamination of the foods, being this one of the main causes of illnesses diarréicas and of bad nutrition associated to them (Motarjemi and cabbage., 1994). Some of the intestinal protozoosis transmitted by more common foods that affect the man are those taken place for: *Giardia* spp., *Entamoeba histolytica*, *Entamoeba coli*, *Cryptosporidium parvum* and *Isospora* spp. that are transmitted primarily for via fecal-oral, and I eat consequence, the biggest source of contamination of foods and it dilutes it is through the pollution of these with fecal matter that contains cysts or ooquistes of these parasites (Motarjemi and cabbage, 1994; Lees-Gutiérrez, 1995); this infection modality is known as infection by faeces and it happens in parasites whose evolutionary cycles are only completed in a host (Atías, 1991). One of the causes of of morbilidad in bolivia are the illnesses diarreicas taken place by bacterias,virus or parasites transmitted mainly by foods and it dilutes (Almanza Gutiérrez, 2002) According to data provided by the Institute Gastroenterológy Bolivian Japanese of Cochabamba city in 1999 they were carried out 2072 analysis coproparasitologic, in this study 1380 (63%) t were negative and 764 (36%) were positive, of those which (11.53%) were *Giardia lamblia*. (Bustillos 2001) Therefore, the present study has as purpose to determine the presence of eggs, cysts and trofozoitos in samples of human grounds, samples of vegetables of short shaft (lettuces, carrots, radishes, turnips, spinaches), strawberries, expended in establishments of consumption public in the interior and areas inside a radius of 100 meters of San Simon University as well as students diners of the University. This study will allow us to know the existence of the problem in the student population and in the group of people that you/they elaborate and they sell foods inside and outside of the University. Through this study we will be able to know the incidence of parasites, to contribute in the diffusion of knowledge and implementation of education measures and later investigations. For the explain our objective is Istudy th the incidence of parasites in groups of risk (students diners, salespersons of foods and consumption products) inside and outside of the San Simón University and aroung the University in a 100 meters radious.. The examples to study come to our laboratory very early in special pockets and the methods that we are using for the study is the direct method and the We suited at this time 1906 and have the next results in the students 82 (4,3%) for the *E. hystolitica*, 4 for *Giardia lamblia* (0,21%), 1 (0,10%), 3 for *Ascaris lumbricoides* (0,21%) and *Ancylostoma duodenale* 2 (0,21%). Our study in of short shaft (lettuces, carrots, radishes, turnips, spinaches), strawberries show for us that in 20 examples, we get 1 *Balantidium coli* and lettuces we get *Ascaris lumbricoides*.

BC56 - PHOSPHATIDYLSERINE EXTERNALIZATION AND CASPASE-3-LIKE ACTIVITY IN AMASTIGOTES OF *LEISHMANIA* SPP.: AN APOPTOTIC MIMICRY

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Programmed cell death, although is clearly beneficial to multicellular organisms (1), its occurrence in unicellular organisms is still a debatable issue. It has been proposed that altruistic individual death can benefit clonal population of yeasts (2) and of infective trypanosomatids (3). Amastigotes of *Leishmania* spp. are the mammalian infective forms of the parasite and they multiply inside phagolysosssomes and propagate infection by sequentially infecting new macrophages when relesead from ruptured cells. We have shown that *Leishmania (L) amazonensis* amastigotes display phosphatidylserine (PS) at the outer leaflet of their membrane and they are able to induce inibition of macrophage proinflammatory activity by a strategy similar to that used by apoptotic cells (4). Some of the biochemical events underlying the apoptotic phenotype are effected by members of a family of cysteine proteases displaying a mandatory requirement for cleavage after aspartic acid, collectively known as caspases (5). In the present study, we examined PS exposure and caspase-3-like activity in amastigotes of *L. (L) amazonensis (*3 different strains*), L. (L.) major* and *L. (L.) chagasi*. PS exposure was positive in all species above, assessed by flow cytometry using FITC-labelled annexin V as ligand; as in mammalian cells, annexin V binding to the parasites was calcium-dependent. Caspase-3-like activity in the cytosol of amastigotes was detected by a fluorimetric assay, using the synthetic fluorogenic substrate Ac-DEVD-AMC in *L. (L.) amazonensis* and *L. (L.) major*. Hydrolysis of capase-3 substrate (Ac-DEVD-AMC) was inhibited by caspase-3 specific inhibitor (Z-DEVD-FMK) and not by E-64 or EST, a class-specific inhibitor of cysteine-proteinases. These results seems to indicate that the apoptotic mimicry is a characteristic not only of *L. (L.) amazonensis* but also others species of *Leishmania*. Apoptosis in this case, seems to be a mechanism for survival within the mammalian hosts and not for death (6,7).

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BC57 - RNA POLYMERASE II HAS A RESTRICTED LOCALIZATION IN *TRYPANOSOMA CRUZI* NUCLEUS WHICH IS DEPENDENT ON THE TRANSCRIPTIONAL STATE

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It has been shown that RNA polymerase II (RNA Pol II) of trypanosomes catalyzes transcription of protein encoding genes, as well as the splice leader gene, which donates the miniexon attached to the 5'end of all mRNA in these organisms. Splice leader transcription has a defined promoter, while specific initiation sites are not yet recognized for most of protein encoding genes. To understand how the same enzyme transcribes both types of genes, we started to investigate the nuclear localization of the largest subunit of RNA polymerase II of *Trypanosoma cruzi* by using specific antibodies raised against a recombinant carboxy terminal domain of this enzyme. By immunofluorescence analysis, we found that the RNA Pol II labeling was not distributed homogenously in the epimastigote nuclei. In some cells, the labeling surrounded the nucleolus region; in others, the enzyme was found in restricted areas of the nucleus. However, treatment with 20 mg/ml a-amanitin, which inhibits the enzyme, completely disperse the nuclear labeling, even though we detected the same levels of the enzyme by Western blot analysis. In contrast, treatment with actinomycin D, which blocks transcription by preventing polymerase movement, promotes the appearance of several foci of RNA Pol II labeling, in addition to the major foci near the nucleolus. We conclude that large amounts of RNA Pol II are concentrated in particular nuclear domains. When the enzyme is inhibited, it seems to disperse in the nucleoplasm. But when the polymerase movement is blocked, the enzyme could be detected in many other regions. We propose that the domain found in normal conditions could be due to the transcription of highly expressed gene, probably the splice leader promoter, as the splice leader genes showed a similar localization pattern, while the foci revealed by actinomycin D treatment represent accumulation of pol II in the remaining genes.

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BC58 - FINDING A ROLE FOR HISTONE ACETYLATION/ DEACETYLATION IN TRYPANOSOMATIDS

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Histone acetylation has been shown to play important roles in transcription control in most eukaryotes by allowing binding of transcription factors to the chromatin. In trypanosomes, the genes encoding for histone deacetylases (HD) are present. We found that several classical inhibitors for these enzymes are unable to affect *Trypanosoma cruzi* growing, suggesting that either histone acetylation does not occur, or the enzymes involved are insensitive. To answer this question, we probed *T. cruzi* for histone acetylation. We found that histone H4 and H2a are labeled when *T. cruzi* are maintained in the presence of tritiated acetic acid and cycloheximide to inhibit *de novo* protein synthesis. No effect of trichostatin was observed. Mass spectrometry analysis showed two *T. cruzi* histone H4 species distinguished by the mass of one acetylation. Recently, histone deacetylase genes HD1 and HD3 from *Trypanosoma brucei* have been shown to be essential, and HD4 is important for progression from G2 to mitosis. Therefore, we cloned and expressed HD1, HD2 and HD4 from *T. brucei* and HD2 from *L. major* in *Escherichia coli* to study their biochemical and structural properties.

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BC59 - IS RNAI FUNCTIONAL IN *TRYPANOSOMA CRUZI*?

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We have previously shown that *Trypanosoma cruzi* transfected with double strand RNA (dsRNA) decreased the expression of several targets. To further confirm whether this inactivation was due to the degradation of specific mRNAs, through the process of RNA interference (RNAi), we now generated and characterized several *T. cruzi* cell lines expressing double strand RNAs. We used vectors expressing the same portion of the *trans*-sialidase (TS) gene in the sense and antisense orientation separated by 60 nucleotides. In one set of vectors the expression was driven by the ribosomal promoter, while in the other by the T7 polymerase promoter in a cell line containing this polymerase. These constructs were transfected into the parasite and stable lines selected by antibiotic resistance. We found that these stable cell lines expressed large amounts of dsRNA, which remained stable, with no decrease in the amount of TS RNA, or activity, suggesting that RNAi was not occurring. As the expressed dsRNAs were quite stable, we hypothesized that the first step in the degradation of dsRNA is absent in *T. cruzi* and therefore we tested whether transfection of small RNAs (siRNAs) was able to induce RNAi in the parasite. Thus, *in vitro* transcribed and annealed dsRNA of green fluorescent protein (GFP) was treated with DICER, generating small interfering RNAs (siRNAs), which were delivered by electroporation to epimastigote cultures of *T. cruzi* expressing GFP. Again, no decrease in GFP expression was detected. These results support the notion that *T. cruzi* is not able to perform RNAi, at least under our experimental conditions, and the previous effects might be due an anti-sense effect.

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BC60 - HEPATIC FIBROSIS IN CANINE VISCERAL LEISHMANIASIS: A MORFOMETRICAL STUDY OF COLLAGEN FIBERS STAINED BY PICROSIRIUS RED

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Canine visceral leishmaniasis (CLV) is an endemic disease in Brazil and dogs are the principal reservoir domestic of this parasite and play a central role in the transmission cycle to humans by phlebotomine sand flies. In the New World is caused by specie *Leishmania (Leishmania) chagasi*. The aim of this study was to evaluate the parenchyma hepatic collagen deposition of dogs naturally infected with *L chagasi*. In human beings, the hepatic fibrosis is depicted in Indian Kala-azar (Rogers et al., 1908) and in Brazil (Bogliolo, 1956). Eighty-five infected animals with positive serological exams to *Leishmania* (IFAT, Complement fixation and ELISA) were divided in four clinical groups: controls, asymptomatic, oligosyntomatic and syntomatic (weakness, cutaneous lesions, alopecia, and clinical anemia) animals. The dogs were sacrificed with lethal dose of Thionembutal 33% (1,0mL/Kg). During necropsy, small samples of liver were obtained to prepare tissue touch preparations (smears). Other liver fragments were collected and fixed in formalin buffered solution 10% for histopathological and immunohistochemical studies. In all animals we observed a diffuse intralobular hepatic collagen deposition. It was characterized by Picrosirius red

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staining method of all liver tissue sections under microscopic optic with polarized light. A stronger yellow-red birefringence was observed indicating one collagen type fibers deposition. For morphometrical analysis, the deposition of the collagen (fibrosis) was analyzed with a 40x objective of an Axiolab light microscope (Zeiss). The images viewed on a computer video screen were obtained by means of the software and relayed to a computer-assisted image analysis system. (Kontron Electronic/Carl Zeiss, Germany). The results were expressed in m2. There was no statistical differences among the defined clinical animals status (asymptomatic, oligosyntomatic and syntomatic) (p>0,05). However, infected animals showed higher hepatic collagen deposition than the controls (p<0,05). Future studies using immunocytochemistry methods for collagen will be done to confirm a type 1 collagen fibers deposition

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BC61 - FURTHER ULTRASTRUCTURAL STUDY ON THE ENDOMASTIGOTE FORM OF A TRYPANOSOMATID ISOLATED FROM SOLANACEAE

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We have previously reported on the isolation and cloning of a trypanosomatid from a tomato fruit which presented a endomastigote form (Coelho et al., Rev. Inst. Med. Trop. S. Paulo, 44 Suppl.: 97, 2002). Here we report on a initial ultrastructural analysis of this isolated based on transmission electron microscopy (TEM). Cells were fixed for 2 h with 2.5% glutaraldeyde and 4% paraformaldehyde in 0.1 M cacodylate buffer, pH 7.2, containing 50 mM $CaCl_2$. The cells were then washed in the same buffer and postfixed for 1 h at room temperature in a 1% OsO_4 solution in 0.1 M cacodylate buffer, pH 7.2, containing 0.8% potassium ferricyanide. After postfixation, cells were dehydrated in acetone and embedded in Epon. Ultrathin sections were briefly stained with uranyl acetate and lead citrate and observed in a Zeiss EM-900 transmission electron microscope operating at 80 kV. Results showed cells with a long curved flagellar pocket, characteristic of the endomastigote stage of the *Wallaceina* genus (Podlipaev et al., Parazitologia, 30: 324-332, 1990, *in Russian*). However, some distinctions were also observed as a lack of a double set of four microtubules in the area of the flagellar pocket, and a presence of a large mitochondrion. Previous molecular analysis showed that *Leptomonas peterhoffi* and *Blastocrithidia gerricola* are close related with *Wallaceina brevicula* and *Wallaceina inconstans*, though they also show morphological differences. Thus, we conclude that the presence of the endomastigote stage strongly suggests that the isolated from Solanaceae may belong to the *Wallaceina* genus but biochemical and molecular analysis will be required to better characterize this trypanosomatid.

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BC62 - *LEISHMANIA (VIANNIA) LAINSONI*, AN ATYPICAL PARASITE OF THE *VIANNIA* SUBGENUS

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INTRODUCTION - *Leishmania (V.) lainsoni* was described in Pará, Brazil (Fernandes et al., 1987). It has been isolated from cutaneous lesions in humans and wild reservoirs, as well from the insect vector *Lutzomyia ubiquitalis*. This parasite presents hybrid morphological and biochemical characteristics from both the *Viannia* and *Leishmania* subgenera. Its biological behavior in axenic cultures and the way the intracellular amastigotes interact with the phagolysosomes of infected macrophages *in vivo* and *in vitro* do not coincide with the peripilary adhesion of the parasites in the insect vector, which a characteristic that has been used to include this protozoan in the *Viannia* subgenus. Aim of this work was to characterize the growth of *L. (V.) lainsoni* in axenic medium, as compared to *L. (L.) amazonensis* and *L. (V.) braziliensis*. Furthermore, we have analyzed by transmission electron microscopy the interaction between *L. lainsoni* and phagocytic cells *in vitro* and *in vivo*.

MATERIALS AND METHODS – Promastigote forms of *L. (V.) lainsoni, L. (L.) amazonensis* and *L. (V.) braziliensis* were grown at 25ºC in NNN-LIT medium supplemented with 10% fetal calf serum. Cells were harvested daily and counted in Neubauer chamber, in order to establish the growth curves. *L. lainsoni* promastigotes were used to infect *in vitro* mouse peritoneal macrophages, as well as J774-G8 macrophages. After different times of interaction, the infected cells were collected, fixed with 2.5% glutaraldehyde in cacodylate buffer 0.1 M, post-fixed with 1% osmium tetroxide, dehydrated in graded acetone, embedded in epoxy resin, and observed by transmission electron microscopy (TEM). Chronic lesions were experimentally produced by the inoculation of *L. lainsoni* promastigotes in hamsters (*Mesocricetus auratus*). Lesion fragments were obtained and processed for TEM as described above.

RESULTS – The growth curve profile of *L. (V.) lainsoni* presents a distinct pattern for digenetic trypanosomatids, with promastigotes being produced in higher numbers during all the observation time (14 days), as compared to *L. (V.) braziliensis*, a typical *Viannia* member, and *L. (L.) amazonensis*, a typical *Leishmania* member. Observation by transmission electron microscopy allowed to demonstrate the close adhesion between the amastigotes and the phagolysosomal membranes, in both *in vitro* and *in vivo* infected macrophages. This morphological feature is typically observed in infections with amastigotes from the *Leishmania* subgenus.

CONCLUSION – Our results suggest that *L. (V.) lainsoni* presents biological characteristics from both the *Viannia* and the *Leishmania* subgenera. Furthermore, we demonstrate that axenic cultures of *L. (V.) lainsoni* can be easily maintained, and appear as an alternative to obtain in laboratory large cell masses for biological, biochemical and immunological assays. Further biochemical and molecular investigations are underway in order to better characterize the taxonomic position of *Leishmania (Viannia) lainsoni.*

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BC63 - PHYSIOPATHOLOGY OF *BABESIA BOVIS*: ADHESION MOLECULES EXPRESSED ON ENDOTHELIAL CELLS (ICAM-1, VCAM, PECAM-1, E-SELECTIN AND THROMBOSPONDIN

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Endothelial cells from bovine umbilical vein (BUVECs) were isolated with the purpose of determining the expression of ICAM-1, VCAM, PECAM-1, E-selectin and thrombospondin in the physiopathology changes of babesiosis caused by *B. bovis*. Later, this expression was confirmed in a *in situ* study, in tissue samples (brain, lung and kidney) of animals that died after inoculation with a pathogenic strain of *B. bovis* (BbovUFV1 7th passage). Erythrocytes of infected animals were tested in order to observer capacity of binding to BUVECs and its adhesion kinetics. The same adhesion tests were made on BUVECs

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stimulated with plasma of animals infected with *B. bovis* and culture supernatant of bovine PBMC, stimulated with one synthetic peptide (SBb23290) derived from RAP-1 of *B. bovis* containing quantified cytokines (IFN-γ, TNF-α and IL-12). There was a significant increase in the adhesion of erythrocytes of animals inoculated in BUVECs stimulated with plasma and supernatant of PBMC. However, adhesion was observed only on non-parasitised erythrocytes, suggesting that free antigens of *B. bovis* in the serum can prime erythrocytes non-parasitised, or still a possible expression of an isoform of VESA-1 non adherent. Adherence was not observed in the tests with samples of the negative animals. Cells stimulated with infected animals plasma and with supernatant of PBMC showed stronger expression of ICAM-1, VCAM, PECAM-1, E-selectine and thrombospondin, the cells that didn't receive stimuli not showed expression of adhesion molecules. In the same way, it was observed strong expression of ICAM-1, VCAM, PECAM-1, E-selectine and thrombospondin in tissue samples of brain, lung and kidney in bovines infected with *B. bovis*, when comparing to the control group. These results suggest that Interleukins, liberated in the acute phase of babesiosis, stimulate the expression of adhesion molecules related to the physiopathology of babesiosis caused by *B. bovis*, as demonstrated by the expression of molecules in BUVECs and erythrocytes cytoadhesion. These date demonstrate physiopathologycal similarities between *B. bovis* and *Plasmodium falciparum*.

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QT1 - INHIBITION OF THE OUABAIN-INSENSITIVE NA^+-ATPASE ACTIVITY FROM EPIMASTIGOTES OF *TRYPANOSOMA CRUZI* BY MILTEFOSINE

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Miltefosine, initially developed as an anti-tumor agent, is the newest drug for the treatment of visceral leishmaniasis. Several laboratories have shown that this ether-lipid analogue is also toxic against different forms of *T. cruzi*, but its mechanism(s) of action is(are) still unclear. In leukemic cells, Miltefosine seems to interfere with cellular signal transduction pathways by inhibition of protein kinase C (PKC) and Na^+, K^+-ATPase. In order to verify if Miltefosine is able to act in a similar way on *T. cruzi*, we initially tested its effects against on the ouabain-insensitive and furosemide-sensitive Na^+-ATPase activities of the parasites (1).

The Na^+-ATPase activitiy was measured as described previously (1-2) using an enriched plasma membrane fraction from epimastigotes obtained after sub-cellular fractionation by differential centrifugation in sucrose-containing buffer. The addition of increasing amounts of Miltefosine (0,003-0,3 mg/mL) resulted in a dose-dependent inhibition of the Na^+-ATPase activity. In a second stage, the inhibition of the Na^+-ATPase by Miltefosine was confirmed by performing activity measurements in the presence of increasing amounts of NaCl, containing or not 0,015mg/mL of Miltefosine. As expected, there was a Na^+-dependent increase of the Na^+-ATPase activity, with a $K_{0.5}$ and V_{max} of 13.7±1.9 mM Na^+ and 11.4±0.5 nmol Pi x mg^{-1} x min^1, respectively. In the presence of Miltefosine there was a change in the kinetic parameters, with the $K_{0.5}$ for Na^+ raising to 28.5±5.3 mM and the V_{max} dropping to 7.6±0.5 nmol Pi x mg^{-1} x min^1. When the membrane fractions were pre-incubated with Miltefosine (0,015 mg/mL) and diluted to a condition containing four times less drug before performing the assay, no inhibition was observed when compared to controls. Taken together, the results suggest that Miltefosine act as a reversible inhibitor of the *T. cruzi* Na^+-ATPase.

Supported by: CNPq, FAPERJ, TWAS and IFS.

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QT2 - ATIVIDADE ANTIPARASITÁRIA DA HEMOLINFA DE DUAS ESPÉCIES NATIVAS DE MOLUSCOS BIVALVES

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Peptídios ou proteínas antimicrobianas (PAM) são componentes do sistema imune inato, presentes na maioria dos seres vivos, que podem apresentar uma atividade microbicida potente contra um amplo espectro de microrganismos. Entretanto, dos PAM conhecidos atualmente, muito poucos têm sido avaliados quanto a sua atividade antiparasitária contra protozoários. Neste trabalho foi investigada a atividade antiparasitária da hemolinfa de duas espécies nativas de moluscos, o mexilhão *Perna perna* e a ostra do mangue *Crassostrea rhizophorae*, contra formas promastigotas de *Leishmania (V.) braziliensis* e formas epimastigotas de *Trypanosoma cruzi.* Foram utilizadas as duas frações hemolinfáticas: plasma (PL) e sobrenadante de lisado de hemócitos (SLH). Culturas de ambos parasitas (10^7 células/mL, concentração final) foram incubadas em microplacas de 96 poços, com um volume igual das frações hemolinfáticas de ambos bivalves em diferentes concentrações, por um período de até 48 h. A atividade antiparasitária foi determinada pelo ensaio colorimétrico do sal de tetrazolium (MTT). A inibição do crescimento dos parasitas foi avaliada a partir da concentração protéica mínima das frações hemolinfáticas capaz de inibir em 50% o crescimento normal do parasita (EC_{50}). Foi detectada uma atividade inibitória contra ambos parasitas, no PL tanto de *C. rhizophorae* (EC_{50} = 300mg/mL para *L. (V.) braziliensis* e EC_{50} = 150mg/mL para *T. cruzi*) como de *P. perna* (EC_{50} = 240 mg/mL para *L. (V.) braziliensis* e EC_{50} = 600 mg/mL para *T. cruzi*), após um período de incubação de 24h. O SLH de *C. rhizophorae* não apresentou atividade antiparasitária até 48 h, enquanto o de *P. perna* mostrou-se inibitório após 48h (EC_{50} = 45 mg/mL para *L. (V.) braziliensis* e EC_{50} = 150 mg/mL para *T. cruzi*). Controles adequados mostraram que a concentração salina da hemolinfa não interfere no crescimento de ambos protozoários. Os resultados obtidos apontam para ocorrência de uma atividade inibitória da hemolinfa de ambos bivalves sobre *L. (V.) braziliensis* e *T. cruzi.* Análises ulteriores são necessárias para melhor avaliar esta atividade e o interesse de se partir para uma purificação dos eventuais fatores antiparasitários.

QT3 - ATIVIDADE ANTIPARASITÁRIA DE PEPTÍDIOS DA HEMOLINFA DO CAMARÃO PENEÍDEO *LITOPENAEUS VANNAMEI*

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Peptídios antimicrobianos (PAM) são moléculas integrantes do sistema imune inato de vertebrados e de invertebrados que funcionam como antibióticos naturais e que apresentam geralmente uma atividade microbicida rápida e potente contra um amplo espectro de microrganismos. Dos PAM conhecidos atualmente, muito poucos têm sido avaliados quanto a sua atividade antiparasitária contra protozoários, sendo principalmente caracterizados em relação a sua atividade antibacteriana e antifúngica. Neste trabalho foi investigada a atividade antiparasitária do peptídio recombinante peneidina (Pen 3a), expresso em leveduras, e do fragmento C-terminal da hemocianina (C-ter), ambos isolados e caracterizados da hemolinfa de *L. vannamei*, espécie altamente cultivada em países da América Latina. A Pen 3a caracteriza-se principalmente pela sua atividade anti-bactérias Gram positivas, enquanto o C-ter pela sua atividade antifúngica. Em nossos ensaios, utilizamos formas promastigotas e epimastigotas de *Leishmania (V.) braziliensis* e *Trypanosoma cruzi*, respectivamente. Culturas de ambos parasitas (10^7 células/mL, concentração final) foram incubadas em microplacas de 96 poços com diluições seriadas (concentraçao inicial de 100 mM) de ambos peptídios. Os ensaios foram realizados em meio de cultura Schneider (*L. (V.) braziliensis*) ou TC-100 (*T. cruzi*) com suplementação de glicose (1%) e as placas foram incubadas por 20 h para avaliação do efeito inibitório sobre o crescimento (proliferação) dos parasitas. No caso da Pen 3a, foi também testada uma incubação de 5 h para verificar um possível efeito lítico/citotóxico. A atividade antiparasitária foi determinada pelo ensaio colorimétrico do sal de tetrazolium (MTT), quantificando a viabilidade celular das culturas de ambos parasitas. Foi detectada uma pequena atividade inibitória da Pen 3a e do C-ter contra ambos protozoários. O efeito mais significativo foi o da Pen 3a contra *T. cruzi*, causando uma redução de 30% na viabilidade dos parasitas a uma concentração de 12,5 mM, após 20h de incubação. Após esse mesmo período, a incubação com o C-Ter causou uma pequena redução (10%) na viabilidade celular a uma concentração de 100 mM contra ambos parasitas. A incubação por um período de 5 h com Pen 3a (ate 80 mM) não causou nenhum efeito sobre *T. cruzi* e uma pequena inibição sobre *L. (V.) braziliensis* (redução de 20% na viabilidade celular). Os resultados obtidos até o momento apontam

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para a ocorrência de uma fraca atividade antiparasitária dos peptídeos, sendo um pouco mais relevante a ação da Pen 3a sobre o *T. cruzi.* Um maior número de análises é ainda necessário para possibilitar conclusões mais definitivas.

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QT4 - ACTIVITY OF OLEANOLIC ACID AGAINST *LEISHMANIA (L.) MAJOR IN VITRO*

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Leishmaniasis is a major world wide health problem, with around 3 million people infected and 600 thousand new cases appearing each year. In Brazil, 30 thousand new cases appear annually only in the Northeast region. Since 30's pentavalent antimonals are the first line treatment for leishmaniasis. Disadvantages such as costs, long-term treatment, side effects and low efficacy against many strains are reported. Although great efforts had done along the last century to develop new drugs for leishmaniasis treatment, a drug with high efficacy and low side effects is still need. Furthermore, a drug for oral administration is desirable. All this, prompted the search for new chemotherapeutic agents. Among all strategies used to develop new agents against leishmaniasis, the research of natural products produced good results.

This work investigated the leishmanicidal activity of oleanolic acid (OA), a triterpene isolated from fruits of *Licania tomentosa* Benth, a Brasilian plant. Oleanolic acid was dissolved in DMSO then diluted in media for use. The leishmanicidal activity of oleanolic acid was investigated against the two evolutive forms of *Leishmania (L.) major* (LV39 strain). Promastigotes of *L. (L.) major* (10^6/well) was incubated in 96 well plate with different drug concentrations (1 to 50 µg/ml) or with DMSO at the same concentratrions carried by the drug. After 24 and 48hr, parasite survival was determined by MTT, a technique based on mitochondrial activity. Treatment with 20 µg/ml OA for 24h or 48h inhibited parasite survival in 88% and 95%, respectively. The activity on amastigotes was tested on *L. (L.) major* infected mouse peritoneal macrophages (10:1 parasite:macrophage ratio) by counting the number of intracellular parasites. Infected macrophages were treated with different drug concentrations and 24h later infection was evaluated by optical microcopy. Treatment with 20 µg/ml of OA resulted in 88% inhibition of parasite infection. The viability of mouse peritoneal macrophages was not affected by a 24h treatment with 20 µg/ml or lower doses of OA.

The results presented herein, showing that oleanolic acid is able to kill the two evolutive forms of *L. (L.) major* without affecting macrophages viability, points to this triterpene as a strong candidate for the development of a new leishmanicial drug.

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QT5 - "IN VITRO" AND "IN VIVO" TRYPANOCIDAL POTENTIAL OF *VERNONIA* SPECIES (ASTERACEAE)

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The protozoan parasite *Trypanosoma cruzi* is responsible to determinate Chagas' disease, a serious debilitating disease that affects millions of people in Latin America. Tripomastigotes are capable of invading and replicating in different types of cells causing a chronic and multifocal disease. Benznidazole is only drug employed to treatment of this infection in Brazil but its acts are dubious still. The efficacy depends on strain of *T. cruzi.* In the present work we evaluated the trypanocidal potential of different plant extracts of the genus *Vernonia* "in vitro" and "in vivo" assays, against two strains of *T. cruzi* (Y and Bolivia). The "in vitro" assay was carried out using blood with 10^5 trypomastigotes forms/mL and it was added in extract solutions (*V. cognata, V. fruticulosa, V. lacunosa* and *V. rupestris*) prepared in dimethyl sulfoxide 5% (DMSO): saline (1:20) to provide different concentrations 2.000, 1.000 and 500 mg/mL. The bioassays were performed in triplicate on microtitle plate (96 wells); negative and positive controls containing either DMSO or gentian violet respectively were run in parallel. The plate was incubated at 4°C during 24 hours and the number of parasites determined according to Brener method. The better results found by the "in vitro" assay were evaluated "in vivo" assay. The better crude extract analyzed were ethyl acetate and hexanic of *Vernonia fruticulosa* and *V. rupestris*, extracted of leaves. Male, healthy and young mice (BALB/C) were inoculated intraperitoneally with 4 x 10^3 tripomastigotes forms and 48 hours after that the extracts were administrated. The extracts were prepared in and administered by oral way at 8 mg/kg/day for 20 days. Positive control (benznidazole) and negative control (DMSO 5%) groups were done. The count of parasites was performed according to Brener method. We observed that the strains showed different behaviors. "In vitro" assay there were a difference between Y and Bolivia strains and *V. fruticulosa* showed the lowest value of IC_{50} by the two strains. To "in vivo" assay with extract of *V. fruticulosa* in Y strain reduced the parasitemia but the mice had precocious death while compared with positive control. Bolivia strain showed a high parasitemia curve and survival period even the controls the same occurred with *V. rupestris* to Y strain. To Bolivia strain this crude extract showed lower parasitemia and reduced the survival of animals if compared with positive and negative controls. We concluded that the genus *Vernonia* is not capable to treat the parasite infection by the Y and Bolivia strains. To prevent the Chagas's disease *V. fruticulosa* need more studies to know that one fraction can be capable to it.

Supported: CNPq

QT6 - PIPERACEAE AND SOLANACEAE SPECIES AGAINST *LEISHMANIA (L.) AMAZONENSIS*

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The number of new cases of leishmaniasis has increased in the last years in Brazil. Vassouras is a small town situated in an endemic region in the interior of the Rio de Janeiro state, where there is a high number of infected people in rural areas. The present work is part of a project that aims to research the therapeutical potential of the Rio de Janeiro state flora. Plant extracts of several polarities from different organs of the families Piperaceae, Solanaceae, Cucurbitaceae and Rubiaceae have been evaluated. Promastigotes of *Leishmania (L.) amazonensis* were cultivated with extracts in several concentrations for 96h at 26°C. To evaluate the antiamastigote activity, murine peritoneal macrophages were infected with *L. (L.) amazonensis* and treated with extracts for 48h at 37°C. Leaf extracts of *Aureliana angustifolia* (Solanaceae), *Piper mallacophylum* and *Peperomia scandens* (Piperaceae) at 100 mg/ml inhibited in more than 90% the growth of both forms of the parasite. The solvents used in the extration have also influenced the result, being the polarity directly proportional to the leishmanicidal activity. The microscopical analysis of the macrophages treated with extracts indicated variable degree of cytotoxicity in concentrations higher than 100mg/ml. These

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results indicate that the phytochemical study guided by leishmanicidal assays of the active plants can result in the isolation and identification of new phytomedicines for leishmaniasis treatment. FUSVE.

QT7 - ANTI-LEISHMANIAL ACTIVITY FROM *TITHONIA DIVERSIFOLIA* LEAVES EXTRACT

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Leishmania is a protozoan parasite responsible for a group of diseases whose symptoms range from mild cutaneous lesions to fatal visceral involvement. The unavailability of appropriate drugs to treat the disease is a result of a combination of factors including the lack of ongoing "research and development" into neglected diseases, such as leishmaniasis for which there is virtually no market. With that in mind, we decided to search for anti-protozoan activity in crude extract from the leaves of a plant, *Tithonia diversifolia,* known in Brazil as "margaridão". Dicloromethane leaves extract (DLE) was prepared and tested for its leishmanicidal activity against *Leishmania (L.) major* axenic promastigotes. The DLE was shown to be cytotoxic, acting on promastigotes and the IC_{50} value lies between 1,5-2 mg/mL. Furthermore, scanning electron microscopy experiments demonstrate drastic morphological alterations in *L. (L.) major* promastigotes. We are currently testing the DLE effect on mammalian cells and on parasites' invasion kinetics.

Supported by FAPESP.

QT8 - PRELIMINARY ANALYSIS OF THE TRYPANOCIDAL AND LEISHMANICIDAL PROPERTIES OF XANTHONES DERIVATIVES.

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Xanthones have a broad spectrum of biological activities including cytotoxic and anticancer, anti-inflammatory and analgesic, antifungal, antiprotozoal and antidepressant activity. The present study was designed to determine the *in vitro* leishmanicidal and trypanocidal activity of the xanthone (C1) and six substituted xanthones, including 4-methyl-xanthone (C2), 1-carboxy-xanthone (C3), 2-carboxy-xanthone (C4), 3-carboxy-xanthone (C5); 4-carboxy-xanthone (C6) and 3-methoxy-xanthone (C7). Epimastigotes forms of *Trypanosoma cruzi* (Y strain) and promastigotes of *Leishmania (L.) amazonensis* (579 strain) were washed twice in cold PBS and their concentration was adjusted to $5x10^6$ cells/mL in LIT medium. Different concentrations of each compound (500, 300, 100, 30, 10, 3 and 1mg/mL) were incubated in the presence of 200?L of the parasites suspension in 96 microwell plates at 26°C for 72 hours. As controls, parasites were incubated in the absence of any drug and in the presence of benznidazole (50mg/mL) or amphotericin B (50ng/mL). Three to four experiments were carried out in triplicate and the number of surviving parasites was determined in Neubauer chambers. The substituted xanthones showed a concentration-dependent inhibitory effect on the growth of *L. amazonensis* promastigotes. Among the seven xanthones tested, C1, C5 and C10 revealed a major inhibitory effect on the growth of *L. (L.) amazonensis*, with IC50 values of 317.6mg/mL, 235.9mg/mL and 180.2mg/mL and inhibition rates of 87±2%, 90±1% and 85±4%, respectively. Except for C5, which revealed IC50=319.1mg/mL and 70±8% of growth inhibition, all other xanthones presented no effect against *T. cruzi.* Along with xanthone C5, which has a methyl substituent group in position 4 and revealed both leishmanicidal and trypanocidal activities, our results also showed promising leishmanicidal activity in some substituted xanthones.

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QT9 - ANTI-*LEISHMANIA (L.) AMAZONENSIS* ACTIVITY OF SUPERCRITICAL CO_2+ETHANOL EXTRACTS FROM *TABERNAEMONTANA CATHARINENSIS.*

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Leishmaniasis is a worldwide disease still treated with expensive compounds that present severe side effects, and are frequently ineffective, emphasizing the importance to search new compounds against this disease. *Tabernaemontana catharinensis* is a species belonging to the *Apocynaceae* family that is rich in indole alkaloids, used in folk medicine as antimicrobial, anti-inflammatory, antitumor and analgesic. Coronaridine, an indole alkaloid isolated from *T. catharinensis*, exhibited a potent leishmanicidal effect (Delorenzi et *al,* 1998 and 2002). In this work we used supercritical fluid extraction [SFE] to obtain *T. catharinensis* extracts. The easy removal of solvent from the final extract, the high selectivity and the use of moderate temperatures in the process, are the most significant advantages of this methodology.

Alkaloids, coronaridine and voacangine, were quantified in both extracts by gas chromatography [GC-FID]. The leishmanicidal effect of the two extracts, E2 and E4, was evaluated, as well as, their cytotoxicity to macrophages. Mouse murine macrophages infected with *L. (L.) amazonensis* stationary phase promastigotes for 24 hs were treated with E2 and E4 during 24 hs more. Our results showed that E2 (10 and 100 µg/ml) inhibited 20 and 80%, respectively, of amastigotes survival, while E4 (10 and 100 µg/ml) inhibited 26 and 100%, respectively, of parasite growth. In order to test the safety of this extracts, viability of mouse macrophages treated with both extracts was checked by Trypan dye exclusion and XTT assays. Both tests showed that E2 and E4 were not toxic for macrophages. These results point to the use of this methodology as a possibility to easily produce compounds to treat leishmaniasis.

Supported by: CNPq, FAPESP.

QT10 - INHIBITION OF *TRYPANOSOMA CRUZI* GROWTH BY *PTERODON PUBESCENS* OILY EXTRACT

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Trypanosoma cruzi is the etiologic agent of Chagas´ disease, an endemic disease in Central and South America. Chemotherapy of this disease is still very unsatisfactory, being based in nitrofurans and nitroimidazoles. These compounds are inadequate due to frequent toxic side effects and limited efficacy having little or no activity in the chronic phase of the disease. These facts show us the

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urgency for the development of new drugs more effective and less toxic for Chagas´ disease. *Pterodon pubescens* Benth (leguminosae), known as Sucupira branca is a native tree specie of Brazil and its seeds are used as hydroalcoholic infusion presenting anti-rheumatic, analgesic, anti-inflammatory and cercaricide properties. Toxicological studies demonstrated that the *P. pubescens* seeds extracts did not present acute or sub-acute toxicity. Geranylgeraniol and related substances (14,15-epoxygeranylgeraniol and 14,15-dihydro-14,15-dihydroxygeranylgeraniol) have been associated to the cercaricide activity of Pterodon pubescens oil. Isoprenoids are involved in cell proliferation and differentiation, and much work is being done nowadays to study these compounds, especially farnesol and geranylgeraniol, that are also involved in post-translational prenylation of proteins, facilitating protein-protein interactions and membrane-associated protein trafficking. In this work we study the effects of oleaginous extract of *P. pubescens* seeds (Ppoe), the hexanic fraction (Hex) and geranilgeraniol in the growth of *T. cruzi* epimastigotes from Y strain. Ppoe was obtained by maceration of *Pterodon pubescens* seeds in ethanol at room temperature for 15 days. A hexanic extract (Hex) from Ppoe was obtained by liquid-liquid extraction. Geranilgeraniol was further obtained from Hex by HPLC in a C8 column and characterized by GC-MS and NMR. The epimastigotes were grown in BHI, with 10% SFB, 10mg l^{-1} hemin and 20mg l^{-1} folic acid for 25ºC for 7 days in the presence and in the absence of Poep or H2. The EOPp, Hex and H2 presented a dose-dependent inhibition of epimastigotes growth with an IC50 of 12.31; 13.64 and 31.84 mg/ml respectively. Studies to the mechanism of action of the geranilgeraniol in the cellular division of the *T. cruzi* are in course in our laboratory.

Acknowledgments: CNPQ, FAPERJ.

QT11 - TRYPANOCIDAL ACTIVITY OF THE GALLIC ACID DERIVATIVES

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Gallic acid (3,4,5-trihydroxybenzoic acid) is a phenolic compound commonly found in plant species as an ester or hydrolyzable tannins, presenting some important biological activities such as inhibition of tumoral cells proliferation and anti-parasite activity against *Trypanosoma brucei* and *Leishmania* spp. In this work we have evaluated the "in vitro" trypanocidal activity of 25 derivatives of the gallic acid which were synthetized by the Department of Chemistry (UFSC). The assays were performed in triplicate in 96 well microplates by incubating 180mL of each parasite form suspension with 20mL of the testing compounds in distinct concentrations (100, 50, 10, 5 e 1mM). Assays were performed with *Trypanosoma cruzi* Y strain culture epimastigotes and blood trypomastigotes. Epimastigotes harvested in LIT medium were washed in PBS and had their concentration adjusted to $5x10^6$ parasites/mL. Trypanocidal assays with these forms were carried out for 72h at 27ºC using benznidazole (100mM) as positive control. Blood trypomastigotes obtained from experimentally infected mice had their concentration adjusted to $1x10^6$ parasites/mL and were incubated with each compound concentration for 48h at 4ºC using crystal violet as positive control. As negative controls, parasites were incubated in the absence of any drug and in the presence of DMSO 2%. The trypanocidal activity was carried out by determining the number of live parasites in Neubauer chambers for epimastigotes and according to Brener method (1962) for trypomastigotes. Four out of 25 assayed compounds showed trypanocidal activity against epimastigotes: eptyl gallate IC_{50}= 4.1 mM (2.8 - 6.1), octyl gallate IC_{50}=33.9 (28.1 - 40.9), dodecyl gallate IC_{50}=2.3 (1.7 - 2.9) and undecyl gallate IC_{50}=4.24 (3.0 - 5.8). None of these active compounds showed activity against *T. cruzi* blood trypomastigotes and no erithrocyte lysis was observed. The absence of activity against these forms may be attributed to hydrolysis of the alkyl chain by blood components. Further studies are in progress in order to modify the active compounds in order to avoid the action of blood components in the molecule structure.

Supported by CNPq and UFSC.

QT12 - TRYPANOCIDAL ACTIVITY OF *EUGENIA JAMBOLANA* LAM.

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Chagas' disease is an important endemic illness in Latin America, caused by the protozoan parasite *Trypanosoma cruzi*. Although transmission has been reduced, an effective treatment for the infected population is lacking. Chemotherapy of Chagas disease is limited to the drugs benznidazole and nifurtimox, which present low efficacy and several side effects. Moreover, the increased resistance of the parasite to these drugs is one of major problems for the successful of the treatment. Myrtaceae is a family belonging to the superorder Myrtiflorae, order Myrtales (*sensu* Dahlgren, 1982). This family consists of 130 genera and about 3000 species, many of which are used in popular medicine as diuretic, anti-inflammatory and anti-hipertensive. *Eugenia jambolana* was collected at FIOCRUZ campus, Rio de Janeiro State, Brazil. Essential oil of *E. jambolana* was extracted and tested *in vitro* against trypomastigotes forms from Y strain of *T. cruzi*, showing a dose depended trypanocidal effects in 24h of incubation (IC50 0,8mg/ml). When tested on release of trypomastigotes forms by infected macrophages, and development of amastigotes inside these cells, the essential oil also demonstrate a dose dependent effect. Expression of NO and TNF-a production by infected and noninfected macrophages treated with jambolana essential oil, indicates that the toxicity against the parasite is independent of cellular activation. These results suggest *E. jambolana* essential oils as potentially useful in the Chagas disease therapy.

QT13 - EVALUATION OF THE BIOLOGICAL ACTIVITY *IN VITRO* OF PROPOLIS EXTRACT OVER PROMASTIGOTES AND AMASTIGOTE FORMS OF LEISHMANIA (VIANNIA) BRAZILIENSIS.

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Leishmaniosis is a parasitic infection caused by a protozoan of *Leishmania* gender. It causes a social and economic impact, being considered the second infection in incidence after malaria. Antimonials are nowadays the first choice drugs, although display several side effects and parasistic resistance. For these reasons many other substances are being tested among them propolis, an apitherapic product that has been used recently with susccessfull results against protozoan. In this work, it was evaluated the biologic activity of propolis extract through *in vitro* assays over the promastigotes and amastigote forms of *Leishmania (Viannia)*

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braziliensis. The assays were done in triplicate, being used for promastigote forms the following propolis concentrations: 1, 10, 30, 50, 100, 250, 500, 750 mg/mL, in axenic media (LIT modificado) with aproximatelly 10^6 parasites/mL. For amastigotes forms, concentrations of propolis were 10, 100, 250 mg/mL using a macrophage culture infected with $1.5x10^6$ parasites/mL. Biological activity was seen for promastigotes forms, demonstrating a noteworthy leishmanicyd role for propolis. Although it was not observed any statistical difference concerning to positive control (Anfotericin B). Any biological activity was noticed for amastigote forms in none of the used concentrations.

SUPPORTED - CAPES

QT14 - TRYPANOCIDAL ACTIVITY OF DIFFERENT PROPOLIS SAMPLES FROM BRAZIL

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Propolis is a resinous substance that honeybees collect from different plant exudates and is used to block cracks, seal their honeycombs and also to prevent growth of microorganisms and protect the hive entrance against intruders [1]. Our laboratory is involved for some years on the investigation of propolis activity against *Trypanosoma cruzi* [2-4], inserted in a more wide multidisciplinary study, which aims to perform a systematic analysis about the chemical composition and biological activity of Brazilian propolis. In temperate zones the main sources of propolis are different poplar buds and the bioactivity of this bee product has been associated mainly to flavonoids and phenolic acids and esters. The samples from tropical zones, such as Brazil with its vast biodiversity, have become a subject of increasing scientific and economic attention. In Brazilian samples, several new compounds with microbicidal and cytotoxic activities have been already identified [5].

In this context we analyzed the chemical composition of 26 ethanolics extracts from different localization of Brazil by high temperature high resolution gas chromatography coupled to mass spectrometry [6] and high performance liquid chromatograpy [4] and assayed their activity against bloodstream trypomastigotes. To monitor such activity the parameter used was the value of ED_{50}/24 h that correspond to the concentration of the extract that lysis 50% of the parasites after 24 h of treatment. The values these the ED50/24 h varied between 186 e 2200 mg/mL. Multivariate analysis will be applied to correlate the trypanocidal activity with the chemical composition of each extract.

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[1] De Castro (2001) *Ann Rev Biol Sci 3*:49-83; [2] Higashi & De Castro, (1994) *J. Ethnopharmacol* 43*:*49; [3] De Castro & Higashi (1995) *J. Ethnopharmacol* 46:55; [4] Marcucci et al (2001) *J. Ethnopharmacol* 74:105; [5] Bankova, De Castro, Marcucci (2000) *Apidologie* 31:3; [6] Pereira & Aquino Neto (1999) Trends Anal Chem 18:126.

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QT15 - CHEMICAL COMPOSITION AND MICROBICIDAL ACTIVITY OF EXTRACTS FROM BRAZILIAN AND BULGARIAN PROPOLIS

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The chemical composition of ethanol extracts from a Brazilian (Et-Bra) and a Bulgarian (Et-Blg) was determined by high temperature high resolution gas chromatography coupled to mass spectrometry and assayed *in vitro* against against *T. cruzi*, several fungi and bacteria species were determined. *Candida albicans, Sporothrix schenckii* and *Paracoccidioides brasiliensis* were selected due to their importance as etiologic agents of mycosis in Brazil and *Neisseria meningitidis, Streptococcus pneumoniae* and *Staphylococcus aureus* are three common inhabitants of the human nasopharynx that has the potential of causing fatal diseases. The agar cup method was used for analysis of fungicidal activity and diameters of less than 15 mm were considered as lack of activity. The minimal bactericidal concentration was determined by means of the broth microdilution method and corresponds to the lowest concentration of the extract that yields negatives subcultures.

Et-Blg presents a predominance of flavonoids (42%), aromatic acids and esters (12%) and fatty acids (7%) (Prytzyk *et al.* (2003), J. Ethnopharmacol, in press), while in Et-Bra it was determined only one flavonoid, pinostrobin (1%), aromatic acids and amyrins. Both extracts showed a similar content of aromatic acids and derivatives, but different individual compounds were detected.

In assays with bloodstream trypomastigotes the ED_{50}/24 h for Et-Bra were 66.2±3.7, 380.6±54.5 and 452.6±53.9 mg/mL in assays performed in the presence of 0%, 5% and 100% blood, and the corresponding values for Et-Blg were 143.0±15.0, 187.4±10.4 and 534.9±52.4 mg/mL. Both extracts showed a similar activity against trypomastigotes and a decrease in activity due to presence of blood. Et-Blg and Et-Bra showed a strong and similar activity against *C. albicans*, *S. schenckii* and *P. brasiliensis* Although both extracts present striking differences in chemical composition they showed similar effect against *T. cruzi* and fungi, indicating that other compounds besides flavonoids are responsible for these microbicidal effects, such as the high content of aromatic acids, and possibly the presence of amyrins. In the assays with bacteria Et-Blg was more effective, particularly *N. meningitidis* and *S. pneumoniae*. It is possible that the bactericidal activity is directly associated with flavonoids, since the main difference between the two extracts is the high level of these bioactive compounds in the Bulgarian extract.

Due to the complexity and diversity of propolis, correlation between chemical composition and biological activity is an approach that can lead to the standardization and categorization of different samples, allowing considerations for a more ample therapeutic use of this bee product

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QT16 - THE IMMUNOLOGICAL PROFILE OF *T. CRUZI*-INFECTED MICE TREATED WITH PROPOLIS

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In vivo models have been employed in studies with propolis, usually by administration by oral route of ethanolic or aqueous extracts. In murine models propolis showed a potent activity as anti-inflammatory, hepatoprotective and tissue regenerative agents, effects associated with its anti-oxidative (1). Our laboratory is involved on the investigation of the activity of propolis against *Trypanosoma cruzi* (2-4). Treatment of infected mice with 50 mg/kg of propolis led to lower parasitemia in relation to control group, without interfering with the survival, and also leading to no toxicity effect up to 700 mg/kg.

In this study we determine the immunological profile of 4 groups of mice:

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non-infected non-treated (G1), non-infected treated (G2), infected non-treated (G3) and infected treated (G4). Propolis (50 mg/kg) was administered daily by gavage for 7 and 14 days (corresponding to 8 and 15 dpi for the infected groups). Differential counting of leukocytes was determined after blood staining with hematoxilin-eosin and spleens (3 animals/day) from each group were collected and weighted to determine the absolute mass and for flow cytometry analysis using a panel anti-mouse monoclonal antibodies (5). The distribution of leukocytes and the main lymphocyte subsets in the spleen of the four groups after 7 days of treatment (dt) showed that in G3 the number of lymphocytes increased and neutrophils decreased, in relation to G1 and G2, maintaining this profile at 14 dt. Comparison of G3 and G4 showed that the cellular immune response, detected at 8 dpi (7 dt) in non treated mice, occurred only later in infected and treated mice and that such treatment led to a delay in the switching from innate to adaptative immune response. At 14 dt, despite showing increased spleen mass, the splenocyte number of G3 was similar to G1, and the frequency of $CD8^+$ cells of G4 was higher than in G3 in spite of $CD4^+$ cells present no difference between both groups. Also at 14 dt, the percentage of expression of $CD69^+$, in $CD4^+$ and $CD8^+$ subsets, in G4 was lower than in G3, indicating that cell activation due to infection was partially inhibited by propolis treatment.

Based on these results, we suggest that the treatment of mice with propolis rich in flavonoids (6) seems to modulate the immune response during *T. cruzi* infection, leading to a decrease of parasitemia levels and encouraging us to continue this research of alternative natural products for Chagas disease.

(1) De Castro SL (2001) Ann Rev Biomedical Sci 3:49-83; (2) De Castro SL, Higashi KO (1995) J Ethnopharmacol 46: 55-58; (3) Bankova VS et al. (2000) Apidologie 31:3-15; (4) Marcucci MC et al. (2001) J Ethnopharmacol 74: 105-112; (5) Olivieri BP et al. (2002) Antimicrob Agents Chemother 46: 3790-3796; (6) Prytzyk E et al. (2003) J. Ethnopharmacol, in press.

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QT17 - EFFECT OF ATORVASTIN TREATMENT ON *LEISHMANIA (L.) MAJOR* INFECTION

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Statins, inhibitors of HMG-CoA Reductase, are used to control cholesterol in humans. It has been shown that they can modulate inflammatory diseases and interfere with immune response diminishing Th1 and augmenting Th2 responses. *Leishmania (L.) major* is a well-studied parasite that induces type 1 immune response in resistant models (C57BL/6 mice) and type 2 immune response in susceptible mouse strains (BALB/c mice). In the present study the effect of atorvastatin (AT) on *L. (L.) major* infection was investigated. C57BL/6 mice were treated with atorvastatin (10 mg/Kg/day, *per os*) beginning at –2 days (ATLm) or 14 days (LmAT) of infection with *L. (L.) major* ($1x10^6$ stationary forms in hind footpads). Control groups were infected and treated with vehicle (PBS). The evolution of the lesions was accessed weekly for 10 weeks and no differences between groups were found. At the end of experiment, the animals were sacrificed and the parasitism was quantified. The treated groups presented a significant higher parasitism (more than 2 log fold increase per mg of lesion). The IFN-γ production at draining lymph node was also accessed by ELISA. Interestingly, only the ATLm group presented statistically higher IFN-γ levels whereas LmAT presented a tendency of higher IFN-γ production when compared to the PBS-treated group. These results may be associated to a higher parasitism presented in ATLm and LmAT groups. The presented data suggest that long-term treatment with atorvastatin may alter the resistance to *Leishmania* infection.

Support: CNPq

QT18 - MESIONIC DERIVATIVES DECREASES THE NITRIC OXIDE PRODUCTION BY *LEISHMANIA (L.) AMAZONENSIS* PROMASTIGOTES

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Leishmania is a parasitic protozoa (Kinetoplastida: Trypanosomatidae), which causes different diseases in human including: cutaneous, mucocutaneous and visceral leishmaniasis (TDR, 1999). In previous work, our group demonstrated that the nitric oxide (NO) pathway is involved on *Leishmania (L.) amazonensis*-macrophage interaction (Genestra *et al*., 2003a). Besides, NO production by *L. (L.) amazonensis* promastigotes and axenic amastigotes already was measured and a constitutive nitric oxide synthase (cNOS) was purified and characterized by immunofluorescence and affinity chromatography (Fonseca-Geigel, 2000; Genestra *et al*., 2003b). The significance of the occurrence of NOS in *Leishmania* is not known at the present, but the data up to now showed that NOS is prominent in promastigotes containing a high number of metacyclic forms, suggesting an association with differentiation and infectivity of the parasite. As a part of our research program of chemotherapy against leishmaniasis, we assayed the effect of two salts of mesoionic derivatives. The compounds have an interesting structure feature and presented useful and wide-ranging biological activities. In these work, parasites (MHOM/BR/77/LTB0016 strain) were cultured in Schneider's medium supplemented with 10% of fetal calf serum and pH 7.2/26ºC. The group tests were assayed with 4-phenyl-5-(4'-methoxy or 3'-methoxy cinnamoyl)-1,3,4-thiadiazolium-2-phenylamine chlorides, the most active compounds against *L. (L.) amazonensis* (Silva *et al*., 2002). The IC_{50} used was: 4'OCH_3=0.17 mmol/L, 3'OCH_3=0.04 mmol/L and 0.46 mmol/L for Pentamidine (reference drug). After 24 hours, the parasites were counted, centrifuged and the supernatants were used for the measurement of NO production by the Griess method (Green *et al*., 1982). The results demonstrated that the two compounds tested inhibit significantly (about 60 to 70%/ $p<0.001$) the NO production by the parasites, while Pentamidine inhibited only about 25%. If cNOS-*L. (L.) amazonensis* participates in the establishment of infection within the macrophage and survive in its "adverse" environment, including resistance to the toxic products from the host inducible NOS biosynthetic pathway, the data of the active mesionic class on NO pathway suggest it as possible target within the parasite. Further experiments will be done in order to evaluate the effect of 1,3,4-thiadiazolium-2-amidine mesoionic on NADPH consumption by cNOS purified from *L. (L.) amazonensis* and in NO production by axenic amastigotes of these parasites.

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QT19 - BIOCHEMICAL, ANTIPROLIFERATIVE AND ULTRASTRUCTURAL STUDIES WITH BPQ-OH, A SPECIFIC INHIBITOR OF SQUALENE SYNTHASE, ON PROMASTIGOTE AND AMASTIGOTE FORMS OF *LEISHMANIA (L.) AMAZONENSIS* AND *LEISHMANIA (L.) MAJOR*

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Parasites of the genus *Leishmania* are responsible for leishmaniasis, which affect million of people around the world. Squalene synthase (SQS) is an important enzyme that catalyzes the first committed step in the sterol biosynthesis. Some SQS inhibitors have been studied in mammalian cells with the objective of developing new drugs for the treatment of high cholesterol rates. *Leishmania* is a parasite that require an amount of endogenous sterols for growth and viability and SQS is an essential enzyme for the ergosterol biosynthesis (Urbina *et al.*, Mol. Biochem. Parasitol., 125, 35-45, 2002). In this work we decided to investigate the effect of BPQ-OH, a specific inhibitor of SQS, on promastigote and amastigote forms of *Leishmania (L.) amazonensis* and *Leishmania (L.) major*. The IC_{50} for the promastigote forms of *L. (L.) amazonensis* was 1.03mM. *L. (L.) major* was more resistant, presenting an IC_{50} of 9mM. Total growth arrest and cell lysis was observed both species incubated with 10mM of BPQ. Intracellular amastigote forms of *L. (L.) amazonensis* were 8-fold more sensible than promastigote presenting an IC_{50} of 0.106mM. The presence of BPQ-OH in the culture of both species led to an increase in the expression of the SQS enzyme when compared with the control without treatment. Densitometry results of the Western-Blot showed that *L. (L.) major* presented an increase in the expression when compared with *L. (L.) amazonensis*. This result indicates that the overexpression of SQS in the presence of BPQ-OH is probably related with the resistance presented by *L. (L.) major*. Ultrastructural analysis of the treated parasites revealed intense alterations in the morphology of *L. (L.) amazonensis*. The main ultrastructural change was observed in the plasma membrane, that presented an intense rupture and formation of elaborated structures. Alterations in the mitochondrion-kinetoplast complex was observed, as intense mitochondrial swelling, rupture of their membranes and an abnormal compaction of the kinetoplast. Other alterations include the appearance of multivesicular bodies, myelin-like figures, some changes in the flagellar membrane and the presence of parasites with two or more nuclei and kinetoplast. We conclude that BPQ-OH is a potent inhibitor of the SQS of *L. (L.) amazonensis* and *L. (L.) major*, showing a good IC_{50}, and that SQS is an important enzyme to the control of the ergosterol biosynthesis in members of the Trypanosomatidae family.

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QT20 - PIPERINE AND ITS DERIVATES: POSSIBLE MECHANISMS OF ACTION AGAINST *TRYPANOSOMA CRUZI*. A COMPARATIVE STUDY INVOLVING CHEMICAL STRUCTURE AND BIOLOGICAL ACTIVITY.

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Piperine is a natural alkaloid isolated from *Piper nigrum* and has a wide range of biological activities such as inhibitor of hepatic metabolism and antiinflamatory, analgesic, larvicidal and leishmanicidal effects. Ten (10) derivates have been prepared from the natural product through simple chemical modifications and their toxic effects against *T. cruzi* were evaluated. The results allowed us to get information about the importance of the amide function as well as the length and saturation degree of the central carbon chain for the biological activity. The results assessed in our laboratory with epimastigotes and amastigotes forms have demonstrated the promising activity of the natural product, when compared with its derivatives. The toxic effect of piperine and its derivates on macrophages was evidenced through the phagocytic and trypan blue exclusion test, showing the selectivity of the observed toxicity against the parasites. When epimastigotes were submitted to the treatment with piperine, they showed significant morphologic alterations in the length of the cellular body and flagellum when compared with the control. Treatment of the parasites with intermediate concentrations of the drug interfered with the parasite's citokinesis, promoting the accumulation of cells with 2 nucleus and 2 kinetoplastes but not completely duplicated. Preliminary results have demonstrated that piperine and some derivates were able to inhibit, in a dose dependent way, malate dehydrogenase and glucose 6-phosphate dehydrogenase, respectively NAD^+ and $NADP^+$ dependent enzymes. These data suggest that one of the possible mechanisms of action of piperine is the perturbation of the oxido-reductive balance through a generalized inhibition of dehydrogenases that affects the entire energy metabolism of the parasite.

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QT21 - SYNERGISM BETWEEN STEROL BIOSYNTHESIS INHIBITOR WITH THE CURRENT TREATMENT FOR HUMAN TOXOPLASMOSIS

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Toxoplasma gondii is responsible for chronically infecting approximately 30% of the global human population (1), causing congenital disease, including ocular demages in infants worldwide and is involved in fatal encephalitis in immunocompromised individuals. In human toxoplasmosis, pyrimethamine associated with other folate inhibitors such as sulfadiazine is the most usual treatment, with high efficacy in toxoplasmic encephalitis and chorioretinitis (2). However, this combination is associated with frequent and severe adverse reactions, such as hematologic toxicity due to pyrimethamine and cutaneous rash, leukopenia and thrombocitopenia due to the sulfonamide (3). The antiproliferative effects of these folate inhibitors were potentiated by the simultaneous incubation of tachyzoites of the RH strain with the inhibitor of $D^{24(25)}$ sterol methyltransferase, 22,26-azasterol, as indicated by concave isobolograms and fractional inhibitory concentrations. Monolayers of the epithelial cell LLCMK2 in a 24-well plate were allowed to interact with tachyzoites for 6 hours before the addition of the drugs. After the incubation with the drugs, coverslips were fixed in Bouin's fixative stained with Giemsa and observed in a light microscope. The percentage of infected cells and the parasite proliferation index were determined by examination of at least 400 cells of two different coverslips. Our results using pyrimethamine (0,1mg/ml) and sulphadiazine (25mg/ml) in infected cells for 24 hours of treatment were quite similar to those previously reported (4 and 5) reaching an inhibitory rate of 74% of parasite proliferation. The association of the same concentration of these drugs with 10mM 22,26-azasterol for 24 hours resulted in complete growth arrest of *T. gondii* tachyzoites. Reducing the concentration of folate inhibitors in ten times in combination with 10mM of azasterol produced 80% of inhibition of parasite proliferation, indicating a synergistic effect. The ultrastructural analysis of this material reveled dramatically cytotoxic effect on parasites mitochondria and endodiogeny process.

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QT22 - THERAPEUTIC ACTION OF ATP ON CUTANEOUS LEISHMANIASIS

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The current available therapy of leishmaniasis is painful and presents severe side effects. In this context, our group has been studying the action of ATP in infection. Since the ATP receptor $P2X_7$ is highly expressed on macrophages, and its activation leads to pore formation on the cell membrane, we explored the possibility that $P2X_7$ could be used to favour drug entrance. Peritoneal macrophages were infected and cultured for 48 hours. The cells then were treated with ATP (0, 500, 1000, 2000 and 4000 mM) plus Lucifer Yellow dye, for 10 min at 37ºC, and read at fluorimeter. The infection itself increased cellular permeability, but in presence of ATP, permeabilization was much more notable, sugesting that *L. amazonensis* infection increased $P2X_7$ receptor expression *in vitro*. *In vivo*, infected BALB/C mice were treated for three weeks, two times a week, with PBS (20 ml), Glucantime (3mg in 20ml), Glucantime (3 mg) plus ATP (50 mM, association volume 20 ml), and ATP (20ml at 50 mM) in the foot lesions. The analysis of parasite load showed that ATP did not increase the therapeutic effect of Glucantime, but showed itself an important anti-leishmanial action, reducing the numbers of parasites by 65% (ATP alone), and 71% (ATP plus Glucantime) in relation to PBS controls. To indirectly evaluation of $P2X_7$ expression *in vivo*, infected and uninfected feet were cut off and homogenized in PBS using a tissue grinder. The homogenate was treated with ATP (500mM and 5mM) plus Topro 3 dye, and analyzed by FACS. These preliminary results suggest that the therapeutic effect of ATP *in vivo* may be associated with the increased cell permeability and that ATP may serve as a good adjuvant for the treatment of leishmaniasis.

Acknowledgment CNPq

QT23 - SYNTHESIS OF NOVEL QUINOLINE DERIVATIVES WITH A POTENTIAL ANTILEISHMANIAL ACTIVITY

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Visceral leishmaniasis (VL) is a severe disease associated with infection of the reticuloendothelial system by *Leishmania* species. Recent large scale epidemics of VL in east Africa, India and in a small proportion, in Brazil, and the emergence of a HIV epidemic make VL a priority for the World Health Organization. Pentavalent antimonials have been cornerstone of treatment for the last six decades, but antimonial-resistance strains have been reported. Within the past five years, miltefosine has been demonstrated as the first effective and safe oral treatment against VL, but besides the teratogenic effects, the price of miltefosine is yet to be determined. Based in these circunstances, the development of new drugs is imperious.

In this report, we studied the *in vitro* activity of four novel synthetized quinoline compounds against *L. (L.) chagasi* promastigotes and intracellular amastigotes, and determined the *in vitro* toxicity against mammalian cells by the MTT assay. The 50% Effective Concentration (EC_{50}) showed that compound HGV02 was the most effective against promastigotes, with an EC_{50} value of 0.10 mg/mL. Compunds HGV01, HGV03 and HGV04, showed intermediate values of 5.66 mg/mL, 7.48 mg/mL and 4.87 mg/mL, respectively. Pentamidine was used as standard drug and showed an EC_{50} of 1.15 mg/mL. The intracellular amastigote assay by using compounds at 10 mg/mL, showed lack of antileishmanial activity for compounds HGV 01, HGV 03, HGV 04, but compound HGV02 reduced more than 99% the parasite burden of the infected macrophages. Glucantime was used as control and showed 100% reduction in parasite burden. All compounds demonstrated citotoxicity for peritoneal macrophages, despite of the high Selectivity Index (toxicity for mammalian cells/EC_{50} in promastigotes) of 314 obtained with HGV02. These results confirmed the potential antileishmanial activity of these novel quinoline derivatives, and allow the use of these molecules as models for the design of new synthetic compounds.

This work was supported by FAPESP (Proj. 99/08491-4) and LIMHCFMUSP-49.

QT24 - LETHAL EFFECT OF NEW DERIVED-NAPTHOQUINONE DRUGS AGAINST *CRITHIDIA* AND *HERPETOMONAS*.

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Flagellate protozoa of the Trypanosomatidae family, which includes agents of important parasitic diseases such as leishmaniosis and Chagas' disease, infect a wide range of hosts including animals and plants(Vickerman, 1994). Trypanosomatids belonging to the *Herpetomonas* genus are commonly found in the digestive tract of houseflies and blowflies, usually under the elongated promastigote and opisthomastigote forms. Monoxenous trypanosomatids are usually found in insect hosts and are considered to be not capable to cause parasitic diseases in vertebrates (Wallace, 1966). However, recent data suggested that monoxenous trypanosomatids could be implicated in human infections (Jimenez et al.,1996, Pacheco et al. 1998, Silva JS et al, 2001). This way, we decided to study the effect of new drugs against these trypanosomatids. The biological activities of the naphthoquinones and derivatives a and β-lapachone, extracted from trees of the genus *Tabebuia*, have been greatly studied. The diversity of microbicidal effects, the easily to achieve the quinones in Brazilian forest and the synthetic alternative pathway of obtaining it, led us to consider a-lapachol and β-lapachone as starting points for chemotherapy studies. In the present study, we display the effect of the new semi synthetic substances, napthoquinone derivatives, on *Crithidia* and *Herpetomonas*. Materials and Methods 1- Parasite- *Crithida fasciculate* (CI-IOC-048) and *Herpetomonas samuelpessoai* (CI-IOC-067) were obtained from Dr. M. Auxiliadora Sousa (Trypanosomatids Collection IOC, RJ Brazil) and were kept in liver infusion tryptose (LIT) or BHI-medium. 2-Trypanocidal Assay and substances- A stock solution of substance - α-lapachone (α-lap), β-lapachone (β-lap), Diazo of β-lapachone (6-diazo-β-lap), epoxide of nor-α-lapachone, fenilidrazone-β-lapachone, etil-furano-nor-β-lapachone, lapachol, LAPAc2O (lapachol acetate), bacetone and epoxide of lawsone - was prepared in dimethyl sulfoxide (DMSO), with the final concentration in the experiments of the 0.1%. 3-Analyze of the drugs effect was done after quantification of active parasites on the 1st till the 7th day of the culturing, by counting in a optical microscopy (Olympus BX41). The final concentration of all the drugs tested was 50mM (group control was treated with DMSO 0.1%). Results- The substances α-lap, 6-diazo-β-lap, epoxide of nor-α-lap, 6-fenilidrazone-β-lapachone, etil-furano-nor-β-lapachone, lapachol and bacetone partially inhibited the growth of both, *C. fasciculate* and *H. samuelpessoai,* parasites. However, the substances Alil nor-b-lapachone and epoxilau, were lethal these parasites. These substances were able to kill the

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parasites in a period of 72 hours. Here, we are showing the trypanosomicidal potencial of these drugs on the different kinds of monoxenous trypanosomatids.

Supported by UFF/ FAPERJ.

QT25 - EFFECTS OF SYNTHETIC PEPTIDETEMPORIN A ON *TRYPANOSOMA CRUZI* EPIMASTIGOTES FORMS

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Antimicrobial peptides have been found in animal (vertebrate and invertebrate) and plants kingdom but most of the reports have focused on bacterial than eukariotics cells. They have a considerable structural diversity and consequente aminoacid heterogeneity. Many of them present a potential terapeutic application while others may be useful tools to probe and define important structural function of proteins segments. In this work, flow cytometry, MTT activity and parasites mobility were used to study the effect of one Synthetic peptide from *Rana temporaria* skin (Temporin A) on *T. Cruzi* epimastigotes Cells forms. For flow cytometry, *T. cruzi* epimastigotes forms were grown for 2h with different concentrations of Temporin A (1mM - 1000mM) and after washing ethidium bromide and fluorescein diacetate were addedies and the optical density measured at 600nm and 410nm. *T. cruzi* epimastigote mixture cells containg 700mM/10^7cells of the Temporin A presented 100% uptake of the dyes. These results with the low activity of the mitochondrial desigenasys MTT mensured demonstrated that the *T. cruzi* membranes is a target for lysis induced pore formation. The analysis of other antimicrobial peptides from natural sure is in progress.

Supported by FIOCRUZ and CNPq

QT26 - PROTEASOME AS TARGET OF ANTIMALARIAL DRUGS

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The development of new antimalarial drugs is an urgent priority considering the increasing prevalence of drug-resistant *Plasmodium falciparum* parasites and the absence of effective vaccines or vector control measures. The existence of a *Plasmodium* proteasome has been shown indirectly by using inhibitors. Lactacystin inhibits the *in vitro* development of erythrocytic stages of *P. falciparum* and its inhibitory effect is cell cycle stage-especific and, despite the low therapeutic index of lactacystin that reduced parasitemia in rats, the parasite proteasome remains as a prominsing candidate drug target. Lactacystin was found to inhibit the growth of three different lines of *P. falciparum* at similar molar concentrations, and was consistently more effective against chloroquine-resistent than chloroquine-sensitive-parasite (Li *et al*., 2000, International Journal for Parasitology 30, 729-733). A gene encondig a 20S proteasome b-subunit has been cloned in *P. falciparum*. During erythrocytic stages the parasite undergoes radical morphological changes and many rounds of replication, events that likely require proteasome activity. Proteasomes are major components of the eukaryotic cellular machinery mediating the normal turnover of proteins and the degradation of proteins that have been improperly folded or denatured (Gantt *et al*., 1998, Antimicrobial Agents Chemotherapy 42, 2731-2738). In our laboratory, preliminary results demonstrated that proteasome activity was presented in schizont stage, suggesting that activity is probably relationated with erythrocytic schizogony where occurs highest turnover of proteins and nucleic acid synthesis. We are testing the limonene, nerolidol and lovastatin in cultures of *P. falciparum* (isolated NF 54, clone 3 D7). The values of IC_{50} were: a) nerolidol 760 nM, b) limonene 1,22 mM and c) lovastatin 50 mM. In studies reported in the laboratory have been observated that nerolidol could be interfering in the elongation involved in the biosynthesis of the isoprenic chain attached to benzoquinone ring of coenzyme Q (Macedo *et al*., 2002, FEMS Microbiology Letters 207, 13-20) and, limonene arrested parasite development and inhibits isoprenylation of proteins in *P. falciparum* (Moura *et al.,* 2001, Antimicrobial Agents Chemotherapy 45, 2553-2558). Therefore, we will study how inhibition of isoprenylation of proteins and interference in the elongation of the isoprenic chain attached to benzoquinone ring of coenzyme Q in cultures of the *P. falciparum* could be interfering in the proteasome activity. In human cancer, pro-drug form of lovastatin have been demonstrated to inhibit the proteasome activity (Sharmila Rao *et al*.,1999, Biochemistry 96, 7797-7802) suggesting that lovastatin could be inhibiting this activity in *P. falciparum*.

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QT27 - ORAL DELIVERY OF MEGLUMINE ANTIMONIATE USING β-CYCLODEXTRIN FOR THE TREATMENT OF LEISHMANIASIS

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The need for daily parenteral administration represents one of the most serious limitations in the clinical use of pentavalent antimonials against leishmaniasis. In this work, we investigated the ability of β-cyclodextrin (CD) to enhance the oral absorption of antimony and to promote the oral efficacy of meglumine antimoniate (MA) against experimental cutaneous leishmaniasis. The occurrence of interactions between CD and MA was demonstrated through the changes induced in the spin-lattice relaxation times of both MA and CD protons. When MA was given orally to Swiss mice in either free or CD-complexed forms, antimony plasma levels after MA/CD were found to be about three times higher than after free MA. Anti-leishmanial efficacy was evaluated in BALB/c mice experimentally infected with *Leishmania (L.) amazonensis*. Animals daily treated with oral MA/CD (32 mg Sb/Kg) developed significantly smaller lesions when compared to animals treated with MA (120 mg Sb/Kg) and control animals (treated with saline). The effectiveness of oral MA/CD was equivalent to that of MA given intraperitoneally at a 2-fold higher antimony dose. The anti-leishmanial efficacy of MA/CD was confirmed by the significantly lower parasite load in the lesions of treated animals, when compared to saline controls. This work reports for the first time the effectiveness of an oral formulation for pentavalent antimonials.

This work was supported by grants from CNPq (521010/97-7 and Brazilian Nanobiotechnology Network), CAPES and FAPEMIG.

QT28 - TARGETING *LEISHMANIA (L.) CHAGASI* AMASTIGOTES INTO MACROPHAGES: THE USE OF DRUGS ENTRAPPED INTO LIPOSOMES CONTAINING PHOSPHATIDYLSERINE

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Macrophages, the target cells in the therapy of Leishmaniasis, express a range of surface glycoproteins, named scavenger receptors, able to bind different classes of molecules, including phosphatidylserine, a negatively charged lipid that could be easily included in liposomal formulations, increasing the targeting ability of drugs. In this study, we devised liposome-entrapped antimony (Sb-LP) with the negatively charged lipid phosphatidylserine, in order to improve their targeting to infected macrophages.

By determining the 50% Effective Concentration in *Leishmania (L.) chagasi*-infected macrophages, Sb-LP was 16-fold more effective (EC_{50} = 14.11 mM) than the free drug (EC_{50} = 225.9 mM). Confocal microscopy analysis showed the delivery of DIL-labeled PS-liposomes and ethidium bromide-labeled PS-liposomes to the *L. (L.) chagasi* parasitophorous vacuole (PV), suggesting that fusion between the PV and the liposomal formulation migth have occured, since intracellular amastigotes appeared fluorescent after shorth time incubation. Experimental studies, using BALB/c mice infected with *L. chagasi* amastigotes, showed that pentavalent antimony entrapped into PS-liposomes was at least 133-fold more effective than free antimony, since 100% reduction in the liver parasite burden was only achieved with 100 mg SbV/Kg using free drug, compared to 0,75 mg SbV/Kg by using the PS-liposome entrapped antimony. These results suggest that PS-liposomes could improve the efficacy of old drugs, such as pentavalent antimony, via targeted delivery to *Leishmania*-infected cells.

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QT29 - NATURAL ALTERATION IN THE SUSCEPTIBILITY/ RESISTANCE PATTERN TO BENZNIDAZOLE IN *TRYPANOSOMA CRUZI* POPULATIONS MAINTAINED IN ACUTE OR LONG TERM CHRONIC INFECTIONS IN MICE OR DOGS

Caldas S, Santos FM, Cáu SBA, Caldas IS, Veloso VM, Lana M, Tafuri WL, Bahia MT

Núcleo de Pesquisa em Ciências Biológicas – UFOP

The susceptibility to drugs used in etiologic treatment of Chagas disease is defined by the genetic characteristics of *T. cruzi* strains (Toledo *et al*., *AAC* 47:223, 2003). However, some reports have been published concerning the in vivo induction to drug resistance by pressure of the benznidazole (Bz) in *T. cruzi* strain with partially resistance to this drug (Murta *et al.*, *Parasitology* 116:165, 1998). On the other hand, the natural induction of resistance to Bz in *T. cruzi* populations during the long term chronic phase in dogs was demonstrated recently by Veloso *et al., Mem Inst Oswaldo Cruz*, 96:1005, 2001.

In this study new *T. cruzi* populations of dogs with chronic Chagas disease (2 to 10 years) were isolated. These dogs were previously inoculated with the Berenice-62 and Berenice-78 parental *T. cruzi* strains, that presented 100% of susceptibility to Bz. After isolation, all *T. cruzi* populations that showed resistance to Bz were maintained in Swiss outbreed mice, through successive cycles of treatment (SCT) with Bz. Mice were treated with oral doses of 100 mg of Bz/kg, for 20 consecutive days. Animals were considered cured when parasitological (fresh blood examination, hemoculture), molecular (PCR), and serological tests were negative. After treatment the animals were submitted to immunosuppressive therapy with cyclophosphamide. After reactivation of infection the trypomastigotes were inoculated in mice that were treated again. These SCT were repeated in order observe the stability of Bz resistance/susceptibility phenotype. In this study five *T. cruzi* populations (Be-62 A and B, Be-78 C, D and E) isolated of dogs infected with Be-78 and Be-62 *T. cruzi* parental strains were evaluated. In general, the parasites maintained in treated mice through of SCT showed an altered Bz-resistance: (1) 0% in 1º SCT for Be-62A; (2) 20% (1º SCT) to 100% after the 5º SCT for Be-62B (3) 90% (1º SCT) to 100% in 3º CST, with maintenance of 100% of Bz-resistance until the13º SCT for Be-78C; (4) 90% (1º SCT) to 100% in 6º SCT for Be-78D; (5) 80% (1º SCT) to 14,3% in 4º SCT for Be 78E;. All animals inoculated with Be-78C showed natural reactivation of the parasitemia post-treatment after the 9º treatment cycle.

In this work we demonstrate that *T. cruzi* populations isolated with dogs inoculated with the same *T. cruzi* parental strain present different Bz resistance levels. Besides, the maintenance of these *T. cruzi* populations in successive blood passages in treated mice altered in several ways the Bz resistance level, exemplified by the greater Bz resistance observed in Be-78C and for the decrease of the Bz resistance during the SCT in Be-78E.

Financial support: UFOP, FAPEMIG

QT30 - NATURAL ALTERATION IN THE SUSCEPTIBILITY/ RESISTANCE PATTERN TO BENZNIDAZOL IN *TRYPANOSOMA CRUZI* POPULATIONS MAINTAINED IN ACUTE OR LONG TERM CHRONIC INFECTIONS IN MICE OR DOGS.

Caldas S, Santos FM, Caldas IS, Veloso VM, Martins HR, Lana M, Tafuri WL, Bahia ML

Núcleo de Pesquisa em Ciências Biológicas, UFOP

Natural induction of resistance to Benznidazol (Bz), in *T. cruzi* populations, during the chronic infection of dogs was demonstrated previously by Veloso *et al.* (*Mem Inst Oswaldo Cruz*, 96:1005, 2001).

In this work new *T. cruzi* populations were isolated from dogs with chronic infections (inoculated with Bz-susceptible strains), and susceptibility to Bz was determined after the isolation and during the maintenance these populations through successive blood passages in mice (SBP). Swiss outbreed mice were infected by intraperitoneal route with the Berenice-62 A and B and Berenice-78 C, D, E isolated from different dogs after 2 to 10 years of infection with Be-62 and Be-78 parental *T. cruzi* strains, respectively. The Bz susceptibility was determined in the first blood passage in mice, and to follow the *T. cruzi* populations were maintained in SBP (in the absence of specific treatment). Sixteen mice were inoculated at each five SBP for the evaluation of the infectivity, parasitaemia curves, mortality and susceptibility to Bz. The parasitological cure was determined by fresh blood examination, hemoculture, serological test, PCR and reactivation of the disease by immunosuppressive therapy with cyclophosphamide. After only one blood passage in mice we observed 0%, 20%, 80%, 90% and 80% of resistance to Bz amongst the animals inoculated with isolate Be-62 A and B and Be-78C, D, E respectively, showing a natural induction of Bz resistance during long term chronic infection in dogs. On the other hand, a new change in Bz-susceptibilities was found during the maintenance of the parasite in BSP (acute phase). Diverse variation patterns were observed such as: (1) stability in the Bz resistance in Be-62 A (0 to 20% in 10 SBP), Be-62 B (20 to 40% in 15 SBP) and Be-78 C (80 to 100% in 55 SBP); (2) reduction in the Bz resistance in Be-78 D (90 to 22% in 40 SBP), and Be-78 E (80 to 30% in 10 SBP). The alteration of the Bz resistance degree during the SBP was not accompanied by significant variation in other biological parameters such as the parasitaemia curves pattern, infectivity and mortality

These results are in agreement with the hypothesis that the maintenance form of the *T. cruzi* (long term chronic infections or in successive blood passages -acute phase), can influence the susceptibility/resistance phenotype of benznidazole in the *T. cruzi* populations studied.

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QT31 - EVALUATION OF BENZNIDAZOLE TREATMENT IN MIXED INFECTIONS OF BALB/C MICE WITH CLONES OF *TRYPANOSOMA CRUZI* FROM DIFFERENT GENOTYPES.

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The use of biochemical and molecular markers is useful on the demonstration of *T. cruzi* natural mixed infection in humans as well as vectors and reservoirs. Mixed infections were experimentally demonstrated in vectors (Pinto et al, Parasitol. Res. 84: 348, 1998) and mice (Lana et al, Exp. Parasitol, 96:61, 2000). It was showed that there is not just a juxtaposition of the parasites of the mixture suggesting a stimulation or inhibition from one clone to another, sometimes reciprocal. The goal of this work is to characterize the mixed infection with clones from different genotypes comparing their biological properties and susceptibility to benznidazole with results provided from monoclonal infections obtained for Toledo et al, AAC 47: 223 (2003). Until now the clones used into the mixtures were: two clones from genotype 19, two from genotype 20 (*T. cruzi I*) and two from genotype 39 (*T. cruzi II) (*Tybayrenc & Ayala, Evolution 42: 277, 1988), one more susceptible and other more resistant to BZ (Toledo e cols, AAC 47:223, 2003).Groups with 16 BALB/c mice were inoculated via IP, with 5000 blood trypomastigotes of each clone. After the pre-patent period, 8 animals were treated during the acute phase by oral doses of 100mg/kg weight for 20 consecutives days and evaluated in parallel with 8 not treated control mice. The mortality rates were daily registered until the 120º day and the hemoculture performed 30 days post treatment.

Preliminary results indicate that the infectivity was always 100%, similar or higher than the observed in monoclonal infections when compared with the most infective one. The mortality rates are similar to the observed in monoclonal infections. The analysis of parasitemia showed an apparent stimulator effect between the clones (6/9 = 66% from the experiments) suggesting more than a juxtaposition of individual clones, since the parasitemia level were higher than the theoretical media from the mixture (arithmetic media from monoclonal infections). Only one experiment (Gambacl1 + Cuicacl1), showed parasitemia lower than the theoretical mixture, which suggest an inhibitory effect between the clones. The chemotherapic treatment strongly reduced the parasitemia of all mixtures that remained subpatent after the beginning of the treatment. These results corroborate data of monoclonal infections. However in some cases the reactivation of parasitemia was observed after the end of treatment although with lower level in relation to the control group. The results of hemoculture showed that mixtures with clones partially susceptible to BZ (Gamba + SO3cl5) were 100% positive indicating the permanency of the infection in all animals. Serological results (ELISA and anti-trypomastigotes antibodies –AATV by Flow-Citometry) and PCR that will be used as criterion of cure were not evaluated yet.

Grants: FAPEMIG, CNPq, CAPES e UFOP.

QT31 - BENZNIDAZOLE TREATMENT FOLLOWING ACUTE *TRYPANOSOMA CRUZI* INFECTION TRIGGERS CD8$^+$ T CELL EXPANSION AND PROMOTES RESISTANCE TO REINFECTION

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Many studies have shed light on the mechanisms underlying both immunoprotection and immune dysregulations arising after *Trypanosoma cruzi* infection. However, little is known about the impact of benznidazole (N-benzyl-2-nitroimidazole acetamide), the available drug for the clinical treatment in Brazil, upon the immune response in the infected host. Here we investigate the effect of benznidazole therapy on the lymphoid compartment during the course of experimental *T. cruzi*-infection. Although amelioration of a variety of clinical and parasitological signs was observed in treated mice, recovery from splenocyte expansion was not detected. Interestingly, this sustained splenomegaly observed in benznidazole-treated mice was due to a preferential expansion of CD8$^+$ T lymphocytes. Moreover, although blocking the expansion of recently activated CD4$^+$ and CD8$^+$ T cells seen in infected hosts, benznidazole treatment led to a selective expansion of effector/memory CD8$^+$ T lymphocytes, associated with lower rate of apoptosis. Besides, surviving benznidazole-treated infected animals were protected from death after reinfection, performed in late phases after the primary infection. Together, these data suggest that, in addition to its well-known direct role in blocking parasite replication *in vivo*, benznidazole appears to directly affect immune regulation in *T. cruzi* infected hosts.

Supported by CAPES, IOC and CNPq

QT33 - TARGET ORGANELLES OF BETA-LAPACHONE DERIVATIVES IN *TRYPANOSOMA CRUZI*

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Plants containing naphtoquinones are commonly employed in folk medicine and studies about the anti-tumoral activity of beta-lapachone, inducing apoptosis [1-2] and inhibiting topoisomerases, led to the suggestion of it potential use in the clinics as adjuvant for treatment of certain types of cancer [3]. The effect of this quinone against *Trypanosoma cruzi* is associated to free radicals generation [4]. Naphthoquinones derivatives, isolated from *Tabebuia* ("ipês"), and semi-synthetic derivatives totalizing 60 compounds have been assayed against trypomastigotes of *T. cruzi*, and three with the highest activity against were naphthoimidazoles derived from beta-lapachone, with the aromatic moieties phenyl (N1), 3-indolyl (N2) and *para*-phenyl (N3) linked to the imidazole ring [5-7].

In the present work, we observed effect of these three derivatives against amastigotes of *T. cruzi* interiorised in peritoneal macrophages and heart muscle cells primary cultures, and host cell damage occurred only at concentrations about 20-fold higher than those needed to block intracellular parasite proliferation.

Flow cytometry and ultrastructural studies of epimastigotes and trypomastigotes treated with N1, N2 or N3, showed swelling of the mitochondrion in both parasite forms. It were also observed disorganization of the reservosome morphology in epimastigotes, and damage of the kinetoplast in trypomastigotes. The mitochondrion alterations were associated with decrease of the membrane potential of this organelle, in relation to untreated parasites, detected by rhodamine 123 [8], despite the maintenance of the integrity of the plasma membrane monitored by propidium iodide [9]. Experiments using acridine orange as marker of acidic compartments, such as reservosomes and acidocalcisomes in epimastigotes [10], and acidocalcisomes in trypomastigotes [11] showed a decrease in fluorescence in treated parasites.

Taken together, these data suggest that the mitochondrion and acidic compartments are the first targets of the action of naphthoimidazoles against *T. cruzi*. The potent activity of the naphthoimidazoles on intracellular amastigotes and bloodstream trypomastigotes together with their low cytotoxicity to the

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mammalian cells encourage us to performed *in vivo* experiments with this derivatives of beta-lapachone.

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QT34 - TRYPANOSOMICIDAL EFFECT OF NEW DERIVATIVES, AGAINST *T. CRUZI*, SYNTHESIZED FROM α AND β-LAPACHONE.

*Bourguignon SC[1], Araújo ME[2] & Ferreira VF[3]

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Chagas' disease, caused by the parasite *Trypanosoma cruzi*, is endemic in Latin America. It is a very serious public health problem in several countries, with about 17 million people known to be infected with the parasite, and a further 100 million at risk of infection, either through contact with an insect vector or via blood transfusion. At present, the only available therapeutic agent for Chagas' disease in Latin America is benzanidazole. In this context, an intensive research program has been focused on the search for alternative natural and synthetic drugs. The biological activities of the naphthoquinones and derivatives a and β-lapachone, extracted from trees of the genus *Tabebuia*, have been intensively studied. The diversity of microbicidal effects, the easy access to natural sources of these quinones in Brazilian rain forest and the synthetic alternative routes led us to consider α-lapachol and β-lapachone as starting points for chemotherapy studies. In the present work we describe the effect of the new semi synthetic substances, napthoquinone derivatives, on epimastigote form of *T. cruzi*. Materials and Methods 1- Parasite- *T. cruzi* Dm28c epimastigotes was raised in liver infusion tryptose (LIT) or BHI-medium. 2-Trypanocidal Assay and substances- A stock solution of substance - α-lapachone (α-lap), β-lapachone (β-lap), Diazo of β-lapachone, epoxide of nor-α-lapachone, fenilidrazone, Alil nor-β-lapachone, lapachol, lapachol with zinc and acetic anidrid, bacetone and epoxide of lawsone - was prepared in dimethyl sulfoxide (DMSO), with the final concentration in the experiments of the 0.1%. 3-Analyze of the drugs action- was realized by number of active (alive) parasites on the 1st, 2nd, 3rd, 4th, 5th and 7th days of the culture, using a Neubauer chamber. The final concentration of all drugs was 50mM. and the group control was treated with DMSO 0.1%. Results- The substances α-lap, diazo of β-lap, epoxide of nor-α-lap, fenilidrazone, alil nor-β-lapachone, lapachol and bacetone inhibited the growth partially but they were not lethal for *T. cruzi*. However, the substances β-lap, alil nor-β-lapachone and epoxilau, were lethal for *T. cruzi.* These substances killed the parasites in a period of 72 hours. These results revealed these substances to have good trypanosomicidal activities.

Supported by UFF/ FAPERJ

QT35 - TRYPANOCIDAL EFFECT OF NEW DERIVATIVES, AGAINST *T.CRUZI*, SYNTHESIZED FROM α AND β-LAPACHONE.

Bourguignon SC[1], Araújo ME[2], Neto, M.N.[3], Gomes, T.S.[3] & Ferreira VF[3]

[1]Detpo de Biologia Celular e Molecular Inst. Biologia, UFF. [2]Detpo de Bioquímica e Biologia Molecular, IOC. [3]Depto de Química Orgânica, Instituto de Química da UFF.

Chagas' disease, caused by the parasite *Trypanosoma cruzi*, is endemic in Latin America. It is a very serious public health problem in several countries, with about 17 million people known to be infected with the parasite, and a further 100 million at risk of infection, either through contact with an insect vector or via blood transfusion. At present, the only available therapeutic agent for Chagas' disease in Latin America is benzanidazole. In this context, an intensive research program has been focused on the search for alternative natural and synthetic drugs. The biological activities of the naphthoquinones and derivatives a and β-lapachone, extracted from trees of the genus *Tabebuia*, have been intensively studied. The diversity of microbicidal effects, the easy access to natural sources of these quinones in Brazilian rain forest and the synthetic alternative routes led us to consider lapachol and β-lapachone as starting points for chemotherapy studies. In the present work we describe the effect of the new semi synthetic substances, napthoquinone derivatives, on epimastigote form of *T. cruzi*. Materials and Methods 1- Parasite- *T. cruzi* Dm28c epimastigotes was raised in liver infusion tryptose (LIT) or BHI-medium. 2-Trypanocidal Assay and substances- A stock solution of substance - α-lapachone (α-lap), β-lapachone (b-lap), Diazo of β-lapachone (6-diazo-β-lap), epoxide of nor-α-lapachone, fenilidrazone-β-lapachone, etil-furano-nor-β-lapachone, lapachol, LAPAc2O (lapachol acetate), bacetone and epoxide of lawsone - was prepared in dimethyl sulfoxide (DMSO), with the final concentration in the experiments of the 0.1%. 3-Analyze of the drugs action- was realized by number of active (alive) parasites on the 1st, 2nd, 3rd, 4th, 5th and 7th days of the culture, using a Neubauer chamber. The final concentration of all drugs was 50mM. and the group control was treated with DMSO 0.1%. Results- The substances α-lap, 6-diazo-β-lap, epoxide of nor-α-lap, 6-fenilidrazone-β-lapachone, etil-furano-nor-β-lapachone, lapachol and bacetone inhibited the growth partially but they were not lethal for *T.cruzi*. However, the substances β-lap, etil-furano-nor-β-lapachone and epoxilau, were lethal for *T.cruzi.* These substances killed the parasites in a period of 72 hours. These results revealed these substances to have good trypanosomicidal activities.

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QT36 - A NEW SYNTHETIC CHALCONE WITH INCREASED ACTIVITY AND SELECTIVITY AGAINST MURINE CUTANEOUS LEISHMANIASIS.

Falcão, C. A. B.; Torres-Santos, E. C.; Yunes, R and Rossi-Bergmann, B.

We showed previously the therapeutic effectiveness of the chalcone 2'-6'-dihydroxy-4'-methoxychalcone (DMC), isolated from the plant *Piper aduncum* against murine leishmaniasis (Antim. Ag. Chemot. 43:1234-41,1999). Nineteen analogous were synthesized adding different substitute groups on the original plant molecule to optimize its efficiency. The chalcones were incubated at differents concentrations with fluorescent promastigotes and intracellular amastigotes of *Leishmania (L.) amazonensis*-GFP. The leishmanicidal activity was expressed as fluorescent intensity of the samples. For cytotoxic activity against mammalian cells, the mouse lymphocytes were incubated for 48 hours with different concentrations of the chalcones in the presence of 2.5 mg/ml Con A with and inhibition of cell proliferation measured by incorporation of 3H-thymidine in the last 6 hours. Alternatively, mouse macrophages were incubated for 48 hours with the chalcones and cell viability measured by incorporation of

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propidium iodide. Four chalcones exhibited high leishmanicidal activity and low citotoxicity to mammalian cells. Presence of Cl and Br, as well as the NO_2 group increased the leishmanicidal activity of the original molecule. However, Cl also increases the citotoxicity to lyphocytes and macrophages. Para substitutions in the second ring drastically reduced its antileishmanicidal activity. The NO2-containing CH8 chalcone was the most selectively active molecule, and this was selected for *in vivo* assays. BALB/c mice were infected with $2x10^6$ promastigotes of fluorescent *Leishmania (L.) amazonensis* in the ear and afer 7 days treated s.c. in the lesion with CH8 (3,3 mg) or Pentostan (200 mg) in a volume of 10 ml of saline, twice a week, for four weeks. The CH8 chalcone-treated animals developed significantly smaller lesions as compared with saline controls. The parasite burden was also effectively controlled, and was as low as obtained in animals treated with much higher Pentostan doses.

These resullts show that structure/activity analysis of the synthetic chalcones led to a potential lead compound (CH8) with in vitro and in vivo antileishmanial selective activity.

QT37 - EFFECT OF THE ANTIMALARIAL DRUGS QUINIDINE AND CLOTRIMAZOLE ON HEMOZOIN FORMATION IN THE BLOOD FEEDING INSECT *RHODNIUS PROLIXUS*

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The blood-feeding organisms use hemoglobin as the major food source and its digestion result in heme release. Free heme is a powerful generator of reactive oxygen species which can damage a diversity of macromolecules. A diversity of protective mechanisms have been described in order to avoid the toxic effects of free heme. One of these mechanisms relies in the aggregation of heme into a harmless crystal named hemozoin (Hz), in different hematophagous organisms such as *Plasmodium*, *Schistosoma* and the triatomine insect *Rhodnius prolixus*. Quinoline-derived drugs, such as chloroquine, form complexes with heme blocking Hz production in *Plasmodium* and in *R. prolixus.* In this present work, we investigated the effects of inhibition of heme aggregation by two antimalarial drugs, quinidine and clotrimazole, in *Rhodnius prolixus* midgut. When adult insects were fed with different concentrations of quinidine, there was a dose-dependent inhibition of Hz production *in vivo*. At 500 mM, quinidine inhibited Hz production in more than 99% in the midgut. The azole derivative clotrimazole, also inhibited Hz formation *in vivo* when insects were fed with 50mM of this drug. Moreover, the inhibition of heme aggregation by quinidine results in a dose-dependent increase of heme levels in the hemolymph. Furthermore, a slight reduction in protein content in the hemolymph of quinidine fed insects, was observed. Taken together these results suggest that heme detoxification into Hz is a target of the quinoline and others antimalarial drugs and that this process has an important physiological role to this insect.

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IM1 - INFLAMMATORY MEDIATORS AND MAST CELLS IN HUMAN LOCALIZED CUTANEOUS LEISHMANIASIS LESIONS.

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In localized cutaneous leishmaniasis (LCL), the lesions are described as a chronic granulomatous inflammatory reaction composed of macrophages, T lymphocytes, plasma cells and mast cells. Despite of the T cell and type 1 cytokines are essential for the infection control, other cell types and inflammatory mediators may be important in this process. The aim of this study is to evaluate the expression of inflammatory mediators and mast cells subpopulations in human LCL lesions. The inflammatory infiltrate of LCL lesions caused by *Leishmania (Viannia) braziliensis* was analyzed by immunostaining using monoclonal antibodies that recognize inflammatory mediators (histamine, leukotriene B4/ LTB4 and prostaglandin F2a/PGF2a), mast cell proteases (tryptase and chymase) and iNOs. A total of 16 frozen biopsies obtained from localized cutaneous lesions were studied. The patients, all of them living in endemic areas of Rio de Janeiro, were classified in two groups: 9 with a period of evolution up to three months (early lesions) and 7 patients with more than three months of illness duration (late lesions). The number of cells expressing histamine was significantly elevated in early lesions, when compared to those with more chronic illness duration. Expression of PGF2a was found in both groups, with a slightly elevated amount of positive cells in early lesions. However, the number of LTB4 positive cells tended to increase in late lesions. These results indicate that histamine and lipid mediators could be involved not only in the initial stages of the inflammatory infiltrate development, but also in mechanisms that contribute to the progression of lesions. Both clinical groups presented similar patterns of iNOs expression. In patients with early lesions, the amount of tryptase+ cells was significantly higher than the chymase+ cells. However, in late lesions chymase+ cells were found in greater quantity. These results suggest that changes in mast cells phenotype could be involved in the immunopathogenesis of cutaneous lesions.

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IM2 - MODULATION OF EXPERIMENTAL *LEISHMANIA (L.) MAJOR*-INFECTION IN MICE BY NORMAL POLYSPECIFIC IMMUNOGLOBULIN INTRAVENOUS THERAPY

Nogueira, A.[1], Olivieri, B.P.[2], Montalvao, F.[1], Modesto, H.[1], Kaveri, S.[3], Amaral, V.F.[1], Araújo-Jorge, T.C.[2] & Vasconcellos, R..[1]

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Natural antibodies (NAb) are normally present in the sera of healthy individuals in the absence of a specific antigenic stimulation. Given the knowledge on the important role of NAbs, it is possible to associate deviations of these repertoires to an effective response towards protozoa infections. Indeed, our recent published date showed a nice correlation between NAb repertoire and resistance of mice to experimental *Trypanosoma cruzi* infection. At the present work we associate a skewed autoantibody repertoire to resistance or susceptibility to *Leishmania (L.) major* infection. Using a semi-quantitative immunoblot technique, we demonstrated that BALB/c mice (susceptible) presented an increased IgG autoreactive repertoire late after infection. In contrast, at the same time after infection, C57BL/6 mice (resistant) displayed an increased IgM autorreactive repertoire. We are now directly testing the role of NAb in the modulation of *L. major* infection. For such aim, BALB/c mice will be infected with *L. (L.) major*, submited or not to Intravenous immunoglobulin (IVIg) therapy. IVIg is a pool of normal polyspecific immunoglobulins obtained from 10000 people, representing a normal spectrum of serum IgG reactivities. IVIg has been successfully used on the treatment of autoimmune diseases and in chronic inflammatory processes At our work, we are using the following IVIg therapeutic schemes: (1) Infection and no treatment; (2) IVIg 3 weeks before infection; (3) IVIg 1 week post-infection. (4) IVIg 6 weeks post-infection. These experiments are in course. Infection parameters are being evaluated and serum samples being collected to further analysis of the autoantibody reactive repertoire. Mechanisms involved in the possible protection given by IVIg will be addressed on our studies.

IM3 - EFFECT OF PASSIVE TRANSFER OF ANTI-MSP3 AND ANTI-GLURP ANTIBODIES ON *PLASMODIUM FALCIPARUM* GROWTH *IN VITRO* AND *IN VIVO*

Bianco-Jr, C.[1], Carvalho, L.J.M.[1], Alves, F. A.[2], Luna, T.G.S.[1], Oliveira, S. G.[3], Theisen, M.[4], Muniz, J.A P.C.[2], Daniel-Ribeiro, C.T.[1]

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Introduction: The Merozoite Surface Protein-3 (MSP3) and the Glutamate Rich Protein (GLURP) of *Plasmodium falciparum* were selected as candidate antigens based on epidemiological data and also because of their strong effect in antibody-dependent cellular inhibition (ADCI) assay of *P. falciparum* growth. We have tested 18 different antigen-αdjuvant formulations containing recombinant proteins or peptides derived from these two proteins in *Saimiri* and *Aotus* monkeys, the WHO-recommended primate models for malaria vaccine trials. Most formulations were immunogenic and in some cases able to induce a partial anti-parasite immunity, as verified in challenge experiments with *P. falciparum*. However, it is important to know whether antibodies raised by immunization with recombinant antigens have similar effects to those elicited against the native proteins by infection. In the present work, we aim to study the protective effect of antibodies raised against the native MSP3 and GLURP proteins through passive transfer experiments in *Saimiri* and *Aotu*s monkeys, as well as their effect in ADCI assays. Methods: *Saimiri sciureus* and *Aotus infulatus* monkeys were repeatedly infected (at least four times) with blood stages of the *P. falciparum* FUP or FVO strains, respectively, until becoming refractory to further infections. Sera was repeatedly collected and pooled until obtained a volume of nearly 100ml for each species. This pooled malaria-immune serum will be precipitated with Ammonium Sulphate, dialyzed and passed through Sephadex columns to obtain purified total IgG. The IgG will then be passed through CNBr-Sepharose coupled to a recombinant hybrid MSP3-GLURP protein and properly eluted, allowing the acquisition of purified anti-MSP3 and anti-GLURP antibodies. After extensive dialysis and concentration adjustment, the antibody preparation will be tested in ADCI assays and also passively transferred to naïve monkeys, which will then be challenged with 50,000 *P. falciparum*-parasitized erythrocytes. Parasitemia will be daily evaluated and monkeys treated with Choloroquine or Mefloquine if necessary. Results: we have performed the repeated infections and obtained the pooled immune serum. The antibody purification step is ongoing.

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IM4 - SOME OBSERVATIONS ON THE SUSCEPTIBILITY OF *CEBUS APELLA* (PRIMATES: CEBIDAE) TO THE EXPERIMENTAL INFECTION BY *LEISHMANIA (L.) CHAGASI*.

Carneiro, L.A.; Tabosa, K.F.; Brígido, M.C.O.; Campos, M.B.; Lainson, R. & Silveira, F.T.

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Introduction: The monkey *Cebus apella* has a very large geographical distribution in South America, and in north Brazil this species has been associated with the silvatic cycle of *Leishmania (V.) shawi*, a dermotropic parasite causing cutaneous leishmaniasis of man (Lainson et al.,1988). This monkey has been successfully used as a model for studying cutaneous leishmaniasis (Lainson & Shaw, 1977; Silveira et. al., 1989, 1990, 1997) and, for this reason, we are at present investigating its susceptibility to experimental infection with *Leishmania (L.) chagasi.* Objectives: To determine the susceptibility of *Cebus apella* to experimental infection with *Leishmania (L.) chagasi* and the animal's usefullness as a model for Americans visceral leishmaniasis. Materials & Methods: 10 specimens of *Cebus apella* were used - 4 males and 6 females 8 werw adults and 2 juveniles. All were born and raised in captivity. *Protocol*: 6 monkeys (3 that had previously been used to study the animal's susceptibility to *L. (V.)* shaw, and 3 that had no previous contact with *Leishmania*) were inoculated intradermaly into the base of the tail with 2 x 10^6 promastigotes from stacionary cultures in Difco B45 culture medium. Four others, all having had no previous contact with *Leishmania*, were inoculated with 3 x 10^7 amastigotes from infected hasmters by two routes: two by intravenous injection and two by intraperitoneal inoculation. *Evaluation of infections*: clinical examination, IgG humeral response (IFAT) and a search for amastigotes in Giemsa-stained bone-marrow smears were made monthly. Results: In animals inoculated with promastigotes we have till now found no signs or symptoms of clinical infection 16 month post inoculation: neither have we been able to detect parasites in the bone- marrow or demonstrate IgG antibody against *L. (L.) chagasi*. Among the animals injected with amastigotes, the monkeys inoculated by the intravenous route showed parasites in bone- marrow smears one month later. The two inoculated by the intraperitoneal route have till now shown no parasites in the bone-marrow at one month p.i.. Other indications of infection have not been observed till now, doubtless due to the short period of incubation. Conclusion: It is as yet too early to say if *Cebus apella* can serve as a satisfactory model for American visceral leishmaniasis, but the present results are considered encouraging.

IM5 - OUTCOME OF *LEISHMANIA (V.) BRAZILIENSIS* INFECTION IN THE EAR DERMIS OF MICE

de Oliveira, CI[1], Oliveira, F[1], Novais, FO[1], Barral, A[1,2] and Brodskyn, C[1,2]

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Extensive work with *Leishmania major* has revealed that Th1 T cells and associated cytokines IFN-γ and TNF-α mediate healing while Th2 cells and associated cytokine IL-4 mediate susceptibility. However, little work has been done in *L. (V.) braziliensis*, probably due to the fact that an experimental model is not readily available. Recent reports have shown that parasite inoculation in the ear dermis closely resembles the natural infection, leading to important findings concerning the pathogenesis of disease. We investigated the course of infection with *L. (V.) braziliensis* (MHOM/BR/01/BA788) by injection of 10^5 parasites in the ear dermis of BALB/c mice. Parasite burden was assessed weekly and by day 35 post infection, parasites achieved a 1000-fold expansion. Thereafter, parasites were gradually destroyed so that beyond day 63 they could not be detected at the inoculation site. Histopathological evaluation revealed an intense inflammatory infiltrate at the peak of lesion development (day 35 post infection) composed mainly by infected macrophages and granulocytes and scarce lymphocytes. Accordingly, RT-PCR failed to detect IFN-γ at the inoculation site. On the other hand, TNF-α expression was detected at days 14, 35 and 49 post infection. Concerning the draining lymph nodes, parasites were detected from day 14 to day 125 post infection, although at lower levels. In terms of cytokine production, RT-PCR showed the presence of IFN-γ and TNF-α throughout the infection period. Regarding chemokine expression, we observed, by RT-PCR, the presence of MCP-1, MIP 1a, MIP 1b and RANTES in the draining lymph nodes. Presently, we are evaluating the chemokine expression at the inoculation site by immunohistochemistry. Similar to recently published data, inoculation of *Leishmania* parasites in the ear dermis resembles the natural infection. Our results confirm that BALB/c mice cure an infection with *L. (v.) braziliensis* due to the development of a parasite-specific Th1 response. To our knowledge, this is the first report showing that the activation of the Th1 pathway also occurs after inoculation of *L. (v.) braziliensis* in the ear dermis.

Supported by: FAPESB, CNPq

IM6 - SERA FROM CHRONIC CHAGASIC PATIENTS WITH MUSCARINIC ACTIVITY PROLONGS QT INTERVAL IN ISOLATED RABBIT HEARTS.

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Introduction: Chronic chagasic patients (CChP) with cardiac disease present several arrhythmias that could evolute in sudden death. QT interval parameters are potential prognostic markers of arrhythmogenicity risk, cardiovascular mortality and have been evaluated in chagasic patients (Circulation. 2003, 108(3): 305-12). We previously showed that sera from CChP induced alterations in cardiac electrogenesis and impair atrioventricular conduction in isolated hearts (Circ 1997, 96(6): 2031-7). These effects could be explained by β-adrenergic and muscarinic receptor activation. The aim of our study was analyze the acute effect of CChP sera, previously characterized as having muscarinic activity, on QT interval in isolated rabbit hearts.

Methods and Results: Rabbits (both gender) were killed by cervical dislocation and hearts were immediately cannulated through the aorta, and perfused by modified Langendorff technique with Tyrode solution (in mmol/L; NaCl 137, glucose 9, $NaHCO_3$ 18, KCl 2.7, NaH_2PO_4 1.8, $MgCl_2$ 0.5, $CaCl_2$ 2.7, bubbled with carbogenic mixture 5%CO_2/ 95% O_2). The experimental protocol consisted of three 20 minutes perfusion period (control, serum and washout). In the second period serum from CChP (n=16) and normal blood donors (NBD, n=10) was diluted 1:100 (vol.:vol.) in control solution. The QT interval was measured in all periods (10 representative beats from each period. The QT interval measured in presence of CChP serum (255 ± 6,2; mean ± SEM) was significantly different of control (241,3 ± 6,2; $p<0,01$) and washout periods (233,8 ± 6,5; $p<0,001$). NBD sera had not effect on QT interval; control (271,1±12,1), serum (280±13,3), and washout (277,8±13,1).

Conclusion: Our result suggest that sera from CChP with muscarinic like activity were able to prolong QT interval. This effect can contribute for the genesis of some arrhythmias present in CChP.

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IM7 - IGF-I AFFECTS DIFFERENTLY THE LEISHMANICIDAL REACTIVE OXYGEN- AND NYTROGEN INTERMEDIATE-DEPENDENT MECHANISMS

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Insulin-like growth factors are polypeptides stimulating proliferation and differentiation of a wide variety of cell types, are present in the blood, in many tissues and particularly in macrophages. It is one of the first factors encountered by the *Leishmania* promastigotes as soon as they are injected into the skin, and subsequently after internalization by macrophages. We have previously reported that insulin-like growth factor-I induces proliferation of *Leishmania* promastigotes and amastigotes in vitro and exacerbates the lesion development in cutaneous leishmaniasis in mice (Goto et al. Proc.Natl.Acad.Sci.95:13211,1998). Since leishmanicidal effect of murine macrophages is mediated by reactive oxygen and nitrogen intermediates, effect of IGF-I on exacerbation of lesion development might be related to these leishmanicidal mechanisms. We have previously seen that NO production is decreased in macrophage infected with *Leishmania (L.) amazonensis* upon IGF-I stimulation (Rev.Inst.Med.Trop.S.P Suppl.12 (IM12) 2002). In this study, we searched the effect of IGF-I or IGF-II on induction of H_2O_2 production in *L. (L.) amazonensis*-infected macrophages. BALB/c mouse peritoneal macrophages (2 x 10^5/ well) were infected with stationary phase *Leishmania (L.) amazonensis* (WHOM/BR/00-LTB-0016) promastigotes (*Leishmania*:macrophage=2:1) at 33ºC for 3 hours. Either macrophages or *Leishmania* were pre-incubated for 5 minutes with rIGF-I or rIGF-II (50 ng/ml) or maintained in the culture system, or maintained without IGFs (control). Cultures were set up in sextuplicates and we evaluated the reactive oxygen species produced by macrophages on supernatant by H_2O_2 assay using horse radish peroxidase-dependent oxidation of phenol red. We present here data from one representative experiment from four. H_2O_2 level (nmol/mL) in the control was 5.1±0.9 (mean + standard deviation), with preincubation with IGF-I of macrophages was 4.1±1.0, of *Leishmania* was 7.0±0.7 and when IGF-I was maintained in culture, 4.4±0.6. H_2O_2 level (nmol/mL) with preincubation with IGF-II of macrophages was 4.5±0.9, of *Leishmania*, 6.9±1.2 and when maintained in the system 4.3±0.4. Contrary to the observed decrease of NO production by macrophages in the presence of IGF-I, neither IGF-I nor IGF-II leads to a significant decrease of H_2O_2 production by macrophages.

Supported by: FAPESP, CNPq and LIM-38 (HC-FMUSP).

IM8 - EFFECT OF INSULIN-LIKE GROWTH FACTOR (IGF) II ON THE LESION DEVELOPMENT IN EXPERIMENTAL CUTANEOUS LEISHMANIASIS

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We have previously reported that a inflammation related- and constitutively present growth factor, insulin-like growth factor-I, induces a direct proliferative response on *Leishmania* promastigotes and amastigotes in vitro and exacerbates the lesion development in cutaneous leishmaniasis in mice (Goto et al. – Proc. Natl. Acad. Sci. 95: 13211, 1998). We had not verified any direct effect of IGF-II on *Leishmania* promastigotes and amastigotes therefore the study was not procceeded with IGF-II. However, since IGF-II is a factor with effect on a wide variety of cell types, particularly in macrophages, its effects on host macrophage-parasite interaction is likely. Therefore, here we studied the effect of IGF-II on lesion development in cutaneous leishmaniasis in mice. BALB/c mice were injected in the hind footpad with 10^7 stationary phase *Leishmania (L.) amazonensis* (WHOM/BR/00-LTB-0016) promastigotes, preincubated for 5 min with or without rIGF-II (50 ng/ml). The contralateral footpad of each animal was injected with sterile PBS as a control. The progression of the lesion was evaluated measuring foot thickness with a dial caliper at 14, 28, 42 days post-infection (PI). Lesion size was calculated subctrating from the thickness of the infected foot, the thickness of the contralateral non-infected footpad. The size of the lesion in control *Leishmania*-infected mice was 0.06±0.06 mm (mean + standard error) at 14, 0.92±0.51 at 28, and 2.74±0.71 at 42 days PI while, surprisingly in IGF-II pre-incubated *Leishmania*-infected mice, 0.02± 0.03 at 14, 0.23 ±0.15 at 28, and 1.70± 0.46 at 42 days PI. We evaluated in parallel the in vitro NO production in the supernatant of BALB/c mice peritoneal macrophages infected with *Leishmania*, pre-incubated for 5 min with or without rIGF-II (50 ng/ml). Cultures were set up in sextuplicates and we evaluated the nitrite [that reflects the nitric oxide (NO) production] in the supernatant by Griess method. We have done 3 similar experiments and we present here data from a representative experiment. NO levels (mM) in the promastigote-infected macrophages was in the control without IGF-II 4.5±2.3 (mean ± standard deviation) and 8.0±0.5 with promastigotes preincubated with rIGF-II.

Our results show that the mice injected with *Leishmania* pre-incubated with IGF-II developed a smaller lesion from day 28 PI. Observation of increased NO production by macrophages infected with IGF-II- preincubated *Leishmania* suggests that IGF-II have opposite in vivo and vitro effect compared with IGF-I.

Supported by: FAPESP, CNPq and LIM-38 (HC-FMUSP).

IM9 - EFFECTS OF GLYCOINOSITOLPHOSPHOLIPIDS (GIPL), EXTRACTED FROM *T. CRUZI* IN THE MODULATION OF THE ADAPTIVE IMMUNITY.

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Molecules from the surface of *Trypanosoma cruzi* parasite have been described as playing a role in parasite adhesion, infectivity and also in the inflammatory response due to parasite infection. This initial response, determined by receptors present in phagocytes and APCs, may be very important in guiding the adaptive immunity generated against the parasite. Glycoinositolphospholipid (GIPL) belongs to the GPI family of anchors and is one of the most abundant surface molecule in epimastigotes forms of *T.cruzi* (eGIPL). Evidence suggests the occurrence of GIPLs also in infective trypomastigote and intracellular amastigote forms of *T. cruzi*. Recent work developed in our laboratory has described that eGIPL can induce a proinflammatory response in a Toll like receptor 4 (TLR4)-dependent manner. In the present study we demonstrate that the treatment with eGIPL can enhance the Th2 response in C57BL/10 and BALB/c mice immunized with OVA. Mice injected with eGIPL 24 h prior to the second OVA/alum immunization developed an increase in bone marrow eosinopoiesis, as revealed by bone marrow cultures in the presence of rIL-5. Moreover, an augmentation in the serum levels of OVA-specific IgE is also observed in the eGIPL-treated mice.

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IM10 - LEISHMANIASIS: A DYNAMICAL SYSTEM APPROACH.

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The TH1/TH2 paradigm has been largely used in the interpretation of several diseases, particularly in leishmaniasis. But, so far we are aware, there is not a mathematical description of this model related to leishmaniasis. We have extended and modified a previous published set of equations (Bergmann et al 2002. *Bull Math Biol* 63, 425-446) in order to adapt it to leishmanial disease particularities. The main modifications were: 1) the assumption of a logistic parasite growth curve instead of an exponential one, 2) the assumption of the TH2 arm of the response having a positive action on parasite growth. The set of three simultaneous differential equations describing the TH1 arm, TH2 arm and parasite growth were analysed for conditions of existence and stability of the solutions.

Stability solutions were obtained for the following situations and its possible clinical correlations: 1) TH2 and parasite extinction [TH1 cure], 2) TH1 extinction, TH2 and parasite coexistence [stable TH2 infection], 3) TH2 extinction, TH1 and parasite coexistence [stable TH1 infection] and 4) TH1, TH2 and parasite coexistence [stable TH1/TH2 infection]. Geometrically the situations 1) and 2) were characterised as stable nodes, and situations 3) and 4) as stable spiral focus. In the last situation, a phase plane analysis showed an oscillatory behaviour of TH1, TH2 and parasite in relation to time. Some predictions of the model agree with experimental data: TH1 cure, stable TH1 infection and stable TH1/TH2 infection. Host "death" (TH1 and TH2 extinction with parasite survival), TH2 cure (TH1 and parasite extinction) and TH1/TH2 cure were predicted as possible states (equilibrium points), but they were not attractors (stable conditions) in the model. Less obvious results were: 1) stable TH2 infection, 2) after infection the necessity of parasite presence for stable TH1/TH2 coexistence. The behaviour of the parasite growth is an important element for the stability conditions. For example, in stable TH1/TH2 infection one of the sufficient conditions was expressed by a relation in which inhibitory effects of host carrying capacity predominates over stimulating TH2 action on parasite growth, on the other hand a TH1 destructive action on the parasite, higher than parasite intrinsic growth rate, was important in determining the stability of TH1 cure. The stable TH2 infection is dependent on a decreasing TH1 response and on inhibitory effects of host carrying capacity that predominates over stimulating TH2 action on parasite growth. The system is intrinsically unstable for TH2 cure

IM11 - EFFECT OF ORAL TREATMENT WITH THE ENDOTHELIN RECEPTOR ANTAGONIST BSF-461314 IN THE MYOCARDITIS DURING MURINE EXPERIMENTAL *T. CRUZI* INFECTION

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Endothelins have been shown to participate in leukocyte activation in several models of inflammation and are suggested to participate in pathological and functional damages to the cardiac endothelium during *T. cruzi* infection. In an attempt to investigate the role of endothelins in the acute and chronic *T. cruzi*-driven myocarditis, we infected C57BL/6 mice with 5000 tripomastigotes of the Y *T. cruzi* strain. Mice were treated with BSF-461314 (30 mg/Kg/day, *per os*), an endothelin ET-A receptor antagonist, or PBS, starting on day 1 post-infection (p.i.) until sacrifice on day 10, 15 or 20 p.i. Parasitemia, myocardial histopathology (inflammation and infection scores) and cardiac cytokines measurement by ELISA were assessed at these days. The parasitemic curve in BSF group did not show significant difference when compared with the control group, although inflammation was much more intense in the BSF than PBS group 10 days after infection. Moreover, a higher mortality was found in the BSF group (30% compared to 10%). In regard to cytokine production, the analysis revealed that *T. cruzi* infection induces increased levels of IL-10 and IL-4 (both on day 10 day p.i.) and MCP-1 and TNF-α (throughout the whole experimental period). Treatment with BSF increased IL-10 levels, what could be correlated with more intense myocarditis found in these animals. In conclusion, BSF treatment does not have a strong effect in *T. cruzi*-infected mice. Future *in vitro* studies will assess the role of endothelins in macrophage activation and *T. cruzi* killing.

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IM12 - EFFECT OF POLY(I:C), AN INTERFERON INDUCER, UPON PARASITE BURDEN, SUPEROXIDE AND NITRIC OXIDE PRODUCTION IN *LEISHMANIA*-INFECTED HUMAN AND MURINE MACROPHAGES

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We previously demonstrated an IFN-beta-induced, NO-independent increase in parasite burden in human macrophages infected with *Leishmania (L.) amazonensis in vitro.* In murine macrophages, however, IFN-alpha/beta has been shown to induce iNOS *in vitro* and *in vivo*. To further explore the molecular mechanism of macrophage activation/desactivation by type I IFN *in vitro*, we investigated the effect of poly(I:C), an interferon inducer mimicking viral double stranded RNA, upon parasite burden, superoxide and NO production in both human (monocyte-derived) and murine (bone marrow-derived) macrophages. We found that poly(I:C) was able to induce NO (measured as nitrite) production in both human and murine macrophages, which could be reverted by the addition of an iNOS inhibitor (L-NMMA) or a neutralizing anti-IFN-alpha/beta antibody. Surprisingly, poly(I:C) did not significantly reduce intracellular *Leishmania* amastigotes in human or murine macrophages, indicating that NO production by itself might not be sufficient for a leishmanicidal effect. In contrast, an inhibitor of superoxide dismutase strongly decreased parasite burden in both human and murine macrophages, argueing for a significant participation of superoxide in parasite clearance. A strong superoxide production, as measured by a hydroxylamine-Griess assay, was indeed observed in human macrophages, either spontaneously or after triggering with PMA and poly(I:C). In uninfected as well as *Leishmania*-infected murine macrophages, a low spontaneous superoxide production was observed, which could be modestly triggered by PMA and poly(I:C), but not by LPS. In conclusion, superoxide and nitric oxide production appear to be reciprocally regulated in human and murine macrophages. Endogeneous IFN-alpha/beta production upon poly(I:C) treatment is able to induce NO in both models, without a significant leishmanicidal effect, which could be easily obtained through inhibition of superoxide desmutase.

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IM13 - PARTICIPATION OF IMMUNOGLOBULINS AND CELLS IN THE PATHOGENESIS OF GLOMERULONEPHRITIS IN VISCERAL LEISHMANIASIS.

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Renal involvement in visceral leishmaniasis (VL) is very frequent, but the

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pathogenesis is still unclear. Deposition of immune complexes has been considered the major mechanism of glomerulonephritis (GN) in VL. However, in previous studies in dogs and hamsters with VL, we detected deposit of IgG in glomeruli (Mathias et al., Braz. J. Med. Biol. Res. 2001), but also the presence of $CD4^+$ and $CD8^+$ T cells (Costa et al., Braz. J. Med. Biol. Res. 2000) in the renal lesions. To further study the pathogenesis, we started the characterization of the renal lesions in BALB/c and C57/BL6 strains of mouse infected intraperitoneally with $2x10^7$ purified *Leishmania (L.) chagasi* (MHOM/BR/72/strain 46) amastigotes. We observed focal glomerular hipercellularity, and interstitial focal inflammatory infiltrate that decreased in intensity from four to eight weeks. IgG deposits were detected in glomeruli and its intensity also decreased from four to eight weeks. Since in mouse VL develops a glomerular lesion that is similar to that observed in human, dog and hamster, we procceeded the study of participation of immunoglobulins and cells in the pathogeneisis of renal lesions in VL in BALB/c mice. Naive mice were injected either with *L. (L.) chagasi*-infected hamster serum and/or with splenic cells from *L. (L.) chagasi* antigen immunized BALB/c mice (N = 3 – 4/group). Control mice received serum and cells from naive animals (N = 3/group). Forty eight hours after transfer, the recipient animals were sacrificed and the kidney taken for analysis. We quantified by morphometry the number of cells/glomerulus, analyzing 50 glomeruli/animal. The median number of cells/glomerulus in control animals was 47.0, in recipient of immune splenic cells and serum, 65.6, of infected hamster serum, 60.2, of immune splenic cells, 65.0. The results suggest the participation of both immune serum and cells in the induction of glomerular hipercellularity in VL.

Supported by: CAPES, CNPq, LIM-38 (HC-FMUSP).

IM14 - TGF - B AND T CELL APOPTOSIS AS IMMUNOSUPPRESSIVE MECHANISM OF *LEISHMANIA AMAZONENSIS* ANTIGEN

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Diffuse cutaneous leishmaniasis is a disease normally associated with *Leishmania (L.) amazonensis* and anergy to parasite antigens. We have systematically observed that whole *L. (L.) amazonensis* antigens (LaAg) as used in Leishvacin™, a vaccine currently under clinical trials in Brazil, strongly inhibits specific and mitogenic murine T cell responses. Here we show *in vitro* that TGF-β plays a key role in the anergic mechanism of LaAg since addition of anti-TGF-β to lymph node cells from 7 day-infected BALB/c mice restores their capacity to respond to the antigen with increased proliferation. *In vivo*, we found that i.m. immunization of BALB/c, but not C57Bl/6, with 2 doses of LaAg (25mg/dose) with a 7-day interval increases the production of TGF-β and IL-10 while decreasing TNF-α. The vaccination in BALB/c but not in C57Bl/6 induced an increase in the numbers of $CD4^+$ apoptotic cells in the lymph nodes. Moreover, when animals were infected with $2x10^6$ fluorescent *L. (L.) amazonensis*-GFP seven days after the second vaccine dose, BALB/c but not C57Bl/6 mice developed increased lesion growth and significantly higher parasite loads, as compared with non-vaccinated controls. Contrary to LaAg, vaccination with *L. (V.) braziliensis* Ag was protective. Administration of anti-TGF-β (100mg/dose) during LaAg vaccination promoted a milder infection in BALB/c mice and reduced the parasite loads to levels similar to non-vaccinated controls. Interestingly, the pattern of IFN-γ production was not altered by anti-TGF-b treatment, despite the increased IL-12, TNF-α and NO production. These results, although carried out in rodents, warns that such vaccine may produce a disease-aggravating effect in more susceptible individuals by an apoptotic mechanism possibly mediated by TGF-β.

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IM15 - IL-4 IS NOT A SUSCEPTIBILITY FACTOR IN MICE INFECTED WITH *LEISHMANIA (L.) AMAZONENSIS* BY THE DORSAL SKIN ROUTE

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We report on the influence of IL-4 on the susceptibility and lesion caused by *L. amazonensis* infection in mice infected in the dorsal skin in comparison to footpad infection. Although most experimental studies in cutaneous leishmaniasis used the paw sole as infection site, mammals or man are usually infected in other areas where the skin is much thinner and not under constant friction. Ten million *L. amazonensis* (Josefa strain) stationary phase promastigotes were injected in the footpad or in the dorsum of susceptible BALB/c or resistant C57BL/6 mice and in their respective IL-4-deficient (IL-4 KO) strains. The kinetics of infection, parasite load, cytokine and serum antibody levels were verified. The parasite counts in the draining lymph nodes of the dorsal injection site were similar in IL-4 KO and in wild-type BALB/c mice. Interestingly, no lesion was observed in the dorsum of C57BL/6 IL-4 KO mice, in spite of detectable parasites in the draining lymph nodes. Production of IL-12p40 and IFN-γ by draining lymph nodes from C57BL/6 IL-4 KO mice inoculated in the footpad was elevated. However, when the inoculation site was the dorsal skin, no significant differences were noted in cytokine production levels by lymph node cells. The serum levels of *Leishmania*-specific IgG2a and total IgE were high in the IL-4 KO and in wild-type BALB/c mice, respectively. In conclusion, in our experiments, IL-4 was neither sufficient nor necessary for susceptibility to *L. (L.) amazonensis*, but the route of infection is an important determinant factor that influences the outcome of infection.

Supported by: FAPESP

IM16 - CELLULAR IMMUNE RESPONSE TO *LEISHMANIA (V.) BRAZILIENSIS* AND SUPERANTIGENS IN HUMAN AMERICAN TEGUMENTARY LEISHMANIASIS

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Cellular mediated immune response is essential for the control of human American tegumentary leishmaniasis (ATL). The inflammatory infiltrate is composed of a high number of memory and activated cells, but only a very small percentage is *Leishmania*-specific reactive T cells. Clinical observations have shown that the development of secondary infection by *Staphylococcus aureus* in cutaneous and mucosal lesions is common. Since staphylococcal toxins can induce a massive human T cell stimulation and cytokine production, the aim of this study was to analyze the "in vitro" T cell immune response induced by *Leishmania* and staphylococcal toxins in human ATL. A total of 49 patients with active cutaneous (LCL) or mucosal (ML) disease were studied. Lymphocyte proliferative response (LPR) assays were performed using peripheral blood mononuclear cell cultures stimulated with *L. (V.) braziliensis* total antigens (Lb Ag), mitogen (concanavalin-A) and superantigens derived from *S. aureus* (enterotoxin A/SEA and enterotoxins B/SEB). Supernatants obtained from T cell cultures were tested for IFN-γ and TNF-α production using ELISA assays, and the results were expressed as pg/ml. The LPR was positive for both leishmanial and bacterial stimulus (median SI: Lb Ag=4,0; SEA=1,6 and SEB=5,8). The levels of IFN-γ were elevated when compared to non-stimulated

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cultures, particularly in PBMC stimulated with Lb Ag and SEB (median: Lb Ag=1855,2; SEA=449,8 and SEB=3710). TNF-α production was detected only in cultures stimulated with SEB (median=43,4). Superantigens are able to induce T cell proliferation and cytokine release. Therefore, the development of bacterial co-infection during active disease could lead to the recruitment of non-*Leishmania* specific cells and the increase of type 1 cytokines in the inflammatory infiltrate, suggesting a role for these bacterial superantigens in the immunopathogenesis of human tegumentary leishmaniasis.

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IM17 - RT-PCR DETECTION OF CXCL12 CHEMOKINE IN SPLEEN DURING EXPERIMENTAL *PLASMODIUM CHABAUDI* AJ STRAIN INFECTION IN C57BL/6J MICE.

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The mechanisms of parasitemia clearance in malaria, one of the major parasitic disease in the world, are not well understood, but in the intact host is dependent of the spleen, the site for killing of the intraerythrocytic parasite. During malaria infection, there are a clear increase in spleen volume and cellularity. Recently, we studied the CXCL12 chemokine production in spleen during rodent malaria, both by *P.berghei* and *P.chabaudi* CR strain infected mice, showing that this chemokine presented a specific pattern of synthesis, that induces homing and activation of immune cells, especially those produced by the spleen organizer reticular cell. We studied the production of chemokine, by mRNA detection by RT-PCR, The supplementation of this chemokine in lethal model results in an attempt to the control of parasitemia. *P.berghei* malaria is frequently related to immunopathological disease, as cerebral malaria and exacerbated TNFa production, but *P.chabaudi* AJ malaria, equally lethal, presented less immune response and death due anemia. We decided to study the production of SDF-1a and related chemokines, looking for new aspects of those chemokines in the spleen function in malaria.

We infected groups of three animals that were sacrificed on a CO_2 chamber, with careful dissection of spleen, with 1/3 of the spleen immediately placed in three volumes of Trizol ™ and conserved at –70ºC until mRNA extraction. These procedures were repeated at the 4th, 6th, 7th, 8th and 11th days after infection. Parasitemia was determined daily by tail blood smears. The extracted RNA was submitted to RT-PCR with oligo-dT for cDNA synthesis at the first round, with specific primers in the second round PCR. We detect the specific product of each chemokine in TBE-PAGE and silver staining, with semi quantitative comparison with mRNA β-actin bands using Imaje J. SDF-1a mRNA is present only before the 4th day after infection, when the parasitemia is detected in the blood, but disappeared in the 11th when the infection results in death of almost all mice. Interestingly, the constitutive SDF-1b mRNA was undetected during the evolution of the experimental malaria. These data suggests that the production of SDF-1b is greatly affected during this malaria, aside to the fact that there are no organized production of SDF-1a in this lethal malaria infection by *P.chabaudi* AJ. Those data also suggests that the isoforms of those chemokines could be involved in dichotomic effects in the malaria, both related to immune or hematological response to the disease.

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IM18 - TUMOR NECROSIS FACTOR RECEPTOR II(TNFR II) EXON 6 POLYMORPHISM IN AMERICAN TEGUMENTAR LEISHMANIASIS

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Background: The pathogenesis of infection has been associated with a pro and anti-inflammatory cytokine profile, which can be directly influenced by individual genetic inheritance. In this regard, it has been shown that susceptibility and resistance to intracellular pathogens is often associated with an allelic polymorphism of cytokine-coding genes and theirs receptors, resulting in a differential clinical expression among individuals presenting the same pathology.

American tegumentar leishmaniasis (ATL) is an endemic disease mainly caused by *L. (Viannia) braziliensis* in Brazil. The disease is expressed either by a self-healing localized cutaneous ulcer (LCL) or a destructive inflammation of the oro-nasal mucosae (ML). In both forms there is a mixture of Th1 and Th2 cytokines. However, Th1 cytokines predominate in LCL forms, in contrast to the MCL form, where the expression of IL-4 is four-fold higher than in LCL. In addition, it has been previously demonstrated the association between TNF promoter polymorphisms and ML.

Aims: The aim of the present work was to evaluate the possible association of a single nucleotide polimorphism at the nucleotide 196 within the exon 6 of the gene coding for TNF-α receptor and the severity of ATL.

Patients and Methods: Patients were selected within a particular clinical presentation (30 with LCL and 30 with ML). This group was subdivided according to response to therapy: half presented a good response to Glucantime and in 15, achievement of cure demanded either an additional course of treatment or patients presented reactivation of the lesion within a period of 6 months after the first course of Glucantime. Controls consisted of 28 samples from blood donors, and 32 individuals with no present or past history of cutaneous or mucosal lesion, but with a positive Montenegro's skin test. Genomic DNA was extracted from biopsy of the lesions or peripheral blood, after clearance by the Fiocruz Ethical Committee.

Samples were genotyped by PCR-RFLP using TNF receptor II specific primers. The amplified products were then digested using *Nla*III restriction enzyme.

Results: The allele frequency analysis suggests an association between the presence of the mutant 196G allele and patients presenting a bad therapeutic response. No polymorphism association was observed when LCL was compared to ML patients or controls. These results suggest that this particular polymorphism could have a role in the pathogenesis of the disease, either by hampering the resolution process or by exacerbating the inflammatory reaction. Complementary research with a larger number of samples and their functional analysis of the immune response are necessary to confirm this hypothesis.

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IM19 - FUNCTIONAL AND MORPHOLOGICAL EVALUATION OF PERITONEAL MAST CELLS DURING EXPERIMENTAL ACUTE INFECTION WITH *TRYPANOSOMA CRUZI* IN RATS

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Mast cells are multifunctional cells capable of secreting a wide variety of mediators. Following activation, these cells express mediators such as histamine, serotonine, leukotrienes and prostanoids, as well as proteases and many cytokines and chemokines, all essential to the genesis of an inflammatory response. During *Trypanosoma cruzi* infection, it has been suggested that mast cells could contribute to the control of the parasite by recognizing and killing IgG-opsonized trypomastigotes and through secretion of mediators. Increased numbers of mast cells have been demonstrated in chagasic patients with chronic disease and in experimental models. In the present work, the involvement of mast cells was studied *in vivo* and *in vitro* during the acute experimental infection with *Trypanosoma cruzi* in rats. Female Holtzman rats infected with Y strain of *T. cruzi* (300.000 trypomastigotes, i.p.) were sacrificed in different time points of infection (24h, 48h, 12 and 20 days) for quantification of mast cell numbers and histamine levels in the peritoneum and histopathological analysis of the mesenterium. In parallel, mast cells were co-cultured with bloodstream trypomastigotes and the released and intracellular histamine levels were quantified. Compared to non-infected controls, the acute *T. cruzi* infection did not induce an increase of mast cells neither in peritoneum nor in mesenterium. However, degranulated mast cells, a high number of milk spots and a diffuse mononuclear inflammatory process were observed in the mesenterium, particularly at day 12 of infection, corresponding to the peak of the parasitemia. The *in vivo* intracellular histamine levels measured in the peritoneum was not different from the controls, but when mast cells were co-cultured with trypomastigotes, the intracellular histamine decreased after 30 minutes. Our data suggest that experimental infection with *T. cruzi* induce mast cell degranulation during the acute phase in rats and may participate in pathogenesis mechanisms during acute Chagas disease.

Supported by IOC/FIOCRUZ/RJ and CNPq.

IM20 - CELLULAR IMMUNE RESPONSE IN SHEEP AND CATTLE EXPERIMENTALLY INFECTED WITH *TOXOPLASMA GONDII*

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Toxoplasma gondii is a protozoan parasite that can infect all warm-blooded animals. Sheep and cattle show different susceptibilities to *T. gondii* infection. Primary infection in pregnant sheep can result in abortion or the birth of weak lambs but they are then protected against further challenge by the development of an effective immunity. Cattle on the other hand, can be readily infected, but abortion or perinatal mortality has not been recorded. The evidence suggests that cattle develop a more effective immune response to *T. gondii* infection than sheep. Seronegative calves and lambs were maintained at the Veterinary Hospital/ USP in an environment with minimal risk of *Toxoplasma* infection. Two animals of each species were inoculated subcutaneously (s.c.) with 5 x 10^2 ME 49 *T. gondii* cysts, two other immunized (s.c.) with 10^7 irradiated tachyzoites (3 monthly doses), while one remained as control. Animals that received irradiated tachyzoites were challenged with 5 x 10^2 ME 49 cysts at 150th day. Serology was performed biweekly and cellular immunity evaluated in Ficoll-Hypaque purified lymphocytes, by PHA or antigen proliferation assays, by ^{3}H thymidine uptake. Infected animals, either cattle or lamb, presented a clear elevation in specific antibody serum levels, with usual antigen maturation pattern. Immunized animals, from both species, presented lower antibody titers, but with similar maturation profile. In proliferation assays, there is a clear antigen induced lymphocyte proliferation after the infection, in both species, despite a lower PHA response during infection, that remain for at least 6 months. After immunization, the lymphocyte response was lower than those driven by infection, but was also clearly seen after 6 months and challenge. PCR studies in organs of infected and immunized and challenged animals are in course. Those data show that the infection of ruminants with *T. gondii* resulted in intense immune activation, both humoral and cellular, a fact that could be also induced at lesser extent by immunization with irradiated tachyzoites.

This work was supported by LIMHCFMUSP and CAPES.

IM21 - EVALUATION OF LYMPHOPROLIFERATIVE RESPONSES IN HUMAN VISCERAL LEISHMANIASIS INDUCED BY A RECOMBINANT ANTIGEN FROM *LEISHMANIA (L.) CHAGASI* AMASTIGOTES.

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A straight correlation between the IFN-γ production and resistance to visceralizing *Leishmania* species has been demonstrated in murine model. In humans IFN-γ production and T cell proliferation have been implicated in the control of *L. (L.) chagasi* infection, whereas IL-10 production has been correlated with pathology in *L. (L.) donovani* infections (Carvalho et al., 1992, J. Infect. Dis. 165:535-540; Ghalib et al., 1993, J. Clin. Invest. 92:324-329). In our laboratory an antigen of apparent molecular mass of 30 kDa (p30) was identified in *Leishmania (L.) chagasi* amastigotes and showed to induce lymphoproliferative responses mediated by $CD4^+$ Th1 and a partial protection against challenge with *L. (L.) chagasi* in BALB/c mice (Pinto et al., 2000, Int. J. Parasitol. 30:599-607). The present work evaluates lymphoproliferative responses induced by a recombinant form of *L. (L.) chagasi* p30 in people living in Teresina, the capital of the state of Piauí, Brasil, where a significant number of visceral leishmaniasis (VL) cases has been reported. The recombinant antigen (R30) was used in cultures of lymphocytes purified from human peripheral blood by Ficoll-Hypaque density gradient centrifugation. The recombinant antigen was produced by expression of the gene *Ldccys1* obtained by PCR amplification using genomic DNA from *L. (L.) chagasi* amastigotes and primers corresponding to the ORF of *L. (L.) chagasi Ldccys1* gene previously described (Omara-Opyene and Gedamu, 1997, Mol. Biochem. Parasitol. 90:247-267). A fragment of 1.3 kb was obtained and cloning of this fragment in pHis vector resulted in a recombinant protein of 47 kDa (R30).

The proliferation assays were carried out in lymphocyte cultures of fourteen patients hospitalized at the Infectious Disease Hospital, Teresina, which had a diagnosis of VL confirmed by identification of amastigotes in bone marrow tissue and enzyme-linked immunosorbent assay (ELISA) to detect antibodies to *L. (L.) chagasi*. All patients also presented VL clinical symptoms: fever, splenomegaly, anaemia, cough and wasting. Four patients treated and cured, as well as two uninfected controls were also included in the present study.

Peripheral lymphocytes from thirteen symptomatic patients were stimulated by R30 presenting stimulation indexes (SI) ranging from 4.0 to 16.0. All cured individuals presented SI to R30 ranging from 1.6 to 13.0, whereas normal subjects did not respond to stimulation by the antigen. The evaluation of lymphoproliferative responses to R30 is currently extended to a higher number of patients, and analysis of limphokine profile in the supernatants of lymphocyte cultures from these patients in the presence of R30 is also in progress. Our preliminary results indicate that R30 is recognized and induces T cell proliferation in both symptomatic and cured subjects, opening perspectives to use this antigen in protective immune schedules against *L. (L.) chagasi*.

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IM22 - INVOLVEMENT OF NITRIC OXIDE (NO) AND TNF-A IN THE ANEMIA AND IN THE OXIDATIVE STRESS IN ERYTHROCYTES FROM MICE SUSCEPTIBLE AND RESISTANT TO *TRYPANOSOMA CRUZI* INFECTION

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Trypanosoma cruzi (Y strain)-infected susceptible mice (Swiss and C57BL/6 $iNOS^{-/-}$) and resistant mice (C57BL/6) showed difference in parasitemia levels and survival rates. *T. cruzi* infection is associated with anemia and leukopenia only for C57BL/6 mice. The data obtained indicate that lethality in acute *T. cruzi* infection not necessarily is associated with low number of blood cells. Treatment of both strain with aminoguanidine (AG, 50mg/Kg) and the use of $iNOS^{-/-}$ mice, revealed that the anemia in mice is not reverted in the absence of NO. In addition, we showed that *in vivo* blockade of TNF-α provoked an dramatic increase in the percentage of reticulocytes in C57BL/6 mice infected, but didn't modify the measured values for hemoglobin content (Hb), hematocrit (HMT), and erythrocyte count (RBC) of those animals. Suggesting that the TNF-α produced during *T. cruzi* infection inhibits the erythropoiesis. The evaluation of the oxidative stress after induction by *t*-butyl hydroperoxide (*t*-BHT) revealed that the oxyghemoglobin oxidation was found to be higher in Swiss mice infected with *T. cruzi*. Treatment with AG protected completely against OxyHb oxidation. In the mice C57BL/6 strain, a strong iNOS responsive, the OxyHb oxidation rate increased from 21.6 to 31.9. Treatment with AG was able to reduce to 24.4. The rate of OxyHb oxidation in the mice C57BL/6 $iNOS^{-/-}$ increased from 28.0 to 37.4. Anti-TNF-α treatment did not affect the OxyHb oxidation in mice C57BL/6 infected. The oxygen uptake in Swiss mice erythrocyte was significantly increased after 14 days of infection ($p<0,05$) and reduced below the control levels for AG treatment ($p<0,001$). The C57BL/6 strain showed a significant decrease in oxygen uptake after 14 days of infection. However the AG treatment induced an additional significant decrease in this parameter ($p<0,01$). $iNOS^{-/-}$ mice showed a very significant reduction in the oxygen uptake ($p< 0,01$). Treatment with anti-TNF-α did not revel any difference related to 14 days infected C57BL/6 mice ($p>0,05$). In the mice Swiss, there were not any variations in $T^{ind}$for all groups studied ($p>0,05$). However, for the C57BL/6 strain a significant reduction ($p<0,05$) was observed after 14 days of infection with complete recuperation of the control levels after treatment with AG ($p<0,01$). Also, a significant decrease in T^{ind} was seen for the C57BL/6 $iNOS^{-/-}$ after 14 days of infection ($p< 0,001$) in comparison with normal $iNOS^{-/-}$). The treatment with anti-TNF-α also protected against the reduction of antioxidant capacity of erythrocyte on day 14 pos infection ($p<0,001$).

IM23 - PROFILE OF PLASMATIC CYTOKINES AND AUTOANTIBODIES IN THE MALARIA ASSOCIATED ANEMIA IN INDIVIDUALS FROM THE BRAZILIAN AMAZON

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In Brazil, the malaria associated anemia is recorded in all age groups including adults, differently from the observed in hyperendemic areas where malarial anemia is more frequently observed in children below two years old. The mechanisms of malarial anemia induction are poorly understood, but the participation of cytokines and autoantibodies (AAb) has been considered. In the present work we evaluated the profile of the cytokines TNF-α, IFN-γ, IL-10, IL-12, MIF, the MCP-1 chemokine and the presence of anti-erythrocyte (aERY-AAb) and anti-phospholipids (aPHO-AAb) autoantibodies in the anemia associated to *P. vivax* (Pv) and *P. falciparum* (Pf) infections. Blood samples from 117 (77 Pv, 38 Pf and 2 Pv/Pf) patients with acute malaria assisted at the Instituto Evandro Chagas/IEC/Belém and at the Hospital Municipal de Paragominas, Pará State, as well as a control group (38 individuals with no history of malaria) were assayed for hematological and biochemical parameters. The plasma concentrations of cytokines, chemokine and the presence of autoantibodies were measured by ELISA. Twenty patients with acute malaria were anemic (13 Pv and 7 Pf), being 16 with mild (Hb 10 to 12g/dl), three with moderate (Hb 7 to < 10g/dl) and one with severe (Hb < 7g/dl) anemia. Surprisingly, the seven anemic patients infected by Pf presented mild anemia, whereas those presenting moderate to severe anemia were infected with Pv. A significant increase in the levels of TNF-α, IFN-γ, IL-10, IL-12 and MCP-1 was observed in patients with malaria as compared to the control group, whereas the levels of MIF did not change. There was a positive correlation between the levels of TNF-α, IL-10 and MCP-1 with the parasitemia degree but not with the presence of anemia. The plasmatic levels of IL-12 were significantly higher among patients with different degrees of anemia than in those with malaria without anemia. The presence of aERYAAb and aPHOAAb were significantly more frequent in patients with malaria than in the control group, but there were no correlations between the presence of these AAb and the parasitemia degree or the presence or intensity of the anemia. The analysis of new samples recently collected from patients with malaria at Paragominas is being performed to complete this study.

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IM24 - CENTRAL NERVOUS SYSTEM INVOLVEMENT IN EXPERIMENTAL *LEISHMANIA (L.) AMAZONENSIS* INFECTION

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The main goal of this paper is to describe pathological alterations of the central nervous system observed in experimental tegumentar leishmaniasis. BALB/c and Swiss mice were subcutaneously infected with 10^4 *L. (L.) amazonensis* amastigotes. Animals were sacrificed and the whole brain was removed for histological and immunocytochemical studies. Histological examination revealed that 66,6% of infected mice presented a discrete hyperemia and inflammatory infiltrate in the meninges, composed by mononuclear cells and neutrophils with no detectable parasites. However, parasitized macrophages were detected in the cerebral parenchyma, as well as mast cells, lymphocytes and polymorphonuclear cells. Necrosis in cerebral parenchyma was also observed. Confocal fluorescence microscopy showed that $CD8^+$ T lymphocytes are the major component of the inflammatory infiltrate in CNS. Besides these cells, $CD4^+$, CD11b and dendritic cells are present, in small numbers, in the inflammatory processes of the CNS. In conclusion, *L. amazonensis* is able to cross blood-brain barrier and cause significant pathological changes in the CNS.

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IM25 - MIP 1A AND RANTES IN HUMAN LOCALIZED CUTANEOUS LEISHMANIASIS

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Background: Chemokines are cytokines with chemotatic activity with important role in the selective recruitment of leucocytes into inflammatory areas. Also, these mediators are responsible for the activation of various cells in inflammatory sites. MIP 1a (macrophage inflammatory protein 1a) and RANTES (regulated upon activation normal T cell expressed and secreted), both classified as C-C chemokines, are attractants for lymphocytes, monocytes and eosinophils. These chemokines are produced not only by non-inflammatory resident cells, such as epithelial cells, fibroblasts, keratinocytes and endothelial cells, but also by monocytes and lymphocytes. Previous studies demonstrated that the predominance of Th1 cytokines over Th2 cytokines is correlated to the resistance against *Leishmania* infection. Although, previous works reported the association of RANTES and MIP1a with Th1 response, the role of chemokines in human leishmaniasis still remains to be elucidated.

Objectives: To determine the production of MIP and RANTES in mononuclear cells cultures and their expression "in situ".

Material and Methods: Twenty-three patients with active localized cutaneous leishmaniasis (LCL), all of them living in endemic areas of Rio de Janeiro, were studied. Cultures of mononuclear cells obtained from blood (PBMC) and biopsies (LMC) were stimulated with *Leishmania (V.) braziliensis* promastigote antigens (Ag-Lb). The supernatants were tested for MIP1a (n=16 cases) and RANTES (n=5 cases) using ELISA assays. Immunohistochemistry assays were performed using monoclonal antibodies on frozen sections (n=6 cases).

Results: We observed a significant decrease of MIP1a production in Ag-Lg stimulated LMC cultures (19,8 ± 22,7 ng/ml), when compared to the controls (52,3 ± 40,2 ng/ml). In PBMC, similar levels of MIP1a were observed in both Lb-Ag stimulated (43 ± 43,16 ng/ml) and control cultures (42,6 ± 32,96 ng/ml). RANTES production was detected in PBMC (1665 ± 134 g/ml) and LMC cultures (1028 ± 257 pg/ml). The expression of MIP1a was detected only in three cases and positive focus were mainly localized in the upper dermis. RANTES staining was present in all LCL samples examined. In this case, the majority of chemokine producing cells were basal epidermis keratinocytes, but we also observed few scattered positive cells in the inflammatory infiltrate.

Conclusion: LCL lesions have been considered as a self-healing condition. Our findings indicate that *Leishmania* infection could be correlated with the inhibition of MIP1a production. Futhermore, the synthesis of RANTES, but not MIP1a, might play a role in immmunoregulatory mechanisms associated with the outcome of disease.

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IM26 - IMPORTANCE OF FAS/FAS-L ENGAGEMENT IN THE CARDIAC INFLAMMATORY INFILTRATION INDUCED BY *TRYPANOSOMA CRUZI* INFECTION

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Trypanosoma cruzi is the etiologic agent of Chagas' disease and affects 16-18 million people in Latin America. The infection is characterized by an acute phase with trypomastigote forms circulating in the blood and also intracellular proliferating amastigote forms in different tissues. It is also observed considerable inflammatory infiltration throughout the cardiac tissue leading to severe cardiac alterations and congestive heart failure. This is possibly the result of multiple aggressive mechanisms to the tissue, determined by different cytotoxic molecules, cellular populations and physiological alterations of the organ. One of the most important cytotoxic pathways employed to induce cellular death is based in the engagement of Fas/Fas-L molecules and the goal of this project is to evaluate whether the Fas-based cytotoxic pathway plays a role in the development of *T. cruzi*-induced myocarditis. Our results indicate that the lack of Fas-L (glg/gld mice) disfavor the cellular inflammatory infiltration, in accordance with previous results using Coxsackievirus (1), and the advance of cardiac fibrosis, although the mortality rate is comparable to Balb/c infected mice. The histopathological analysis of cardiac samples taken from gld/gld mice infected with *T. cruzi* Y strain revealed numerous parasite nests distributed throughout the tissue but scarcely associated to inflammatory infiltration. In contrast to Balb/c infected mice, which showed fewer parasites but intense inflammatory infiltration. Enzimatic evaluation of cardiomyocytes destruction based in creatin kinase (CK) activity showed higher cellular death in Balb/c mice. Besides, we found no marked regenerative anemia, but AST and ALT dosage revealed discrete hepatic injury in both mice but with normal levels of urea in the acute phase. Flow cytometry analysis of cardiac inflammatory cells harvested from *in vivo* infected mice showed predominance of $CD8^+$ T cells in Balb/c and gld/gld mice on the 15^{th} day post infection and remarkably more than 90% of the cells were $D32^+$ (FcgRII) T cells. The labeling of intracellular citokines revealed a Th1 pattern, with high levels of IL-2 and IFN-γ in both mice, but also IL-10 in gld/gld mice. We found equivalent levels of perforin, bcl-2 and iNOS in both mice. In this work we also analyzed cardiomyocytes from adult infected mice by flow cytometer and observed the production of IFN-γ and TNF-α but also high levels of iNOS and bcl-2 after infection. These results indicate the importance of Fas/Fas-L pathway in the cardiac inflammatory infiltration triggered by *T. cruzi* infection and demonstrate the adaptation of the cardiac tissue facing the infection.

Refernce

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IM27 - COURSE OF INFECTION AND IMMUNE RESPONSE OF C57BL/6 MICE CHALLENGED WITH STATIONARY PHASE OR METACYCLIC *LEISHMANIA* PROMASTIGOTES

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Natural infection by *Leishmania* parasites is initiated by the inoculation of a few infectious metacyclic parasites into the host skin. Most of the laboratory studies however use stationary phase promastigotes that are a mixture of infective and non-infective promastigotes. In this work we, initially, compared the course of infection and immune response in animals infected with either total stationary phase (total) or purified metacyclic promastigotes of *L. (L.) amazonensis* (PH8 strain). C57BL/6 mice were inoculated with 2 x 10^5 total stationary phase promastigotes or with 1 x 10^5 metacyclic promastigotes. Curiously, lesion size in animals infected with total promastigotes was smaller than in those infected with metacyclic promastigotes, even though tissue parasitism was similar between the two groups, which suggested an increased inflammatory response at the site

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of infection. This was confirmed by histological analysis of the lesions at two weeks after infection, even though no differences were noted thereafter. Measurement of IFN-γ and IL-4 production by lymph node and spleen cells did not show any difference between the two groups. In order to evaluate the inflammatory potential of these two parasite forms, thioglycollate elicited peritoneal macrophages were infected in vitro with total or metacyclic promastigotes and TNF-α production measured after 6 hours. Surprisingly, our results show that metacyclic promastigotes from *L. (L.) amazonensis* induced higher levels of TNF-α production than total stationary phase promastigotes. In summary, our results show that while the presence of non-metacyclic promastigotes in the inoculum affect the inflammatory response to the parasite *in vivo*, this observation cannot be explained by the ability of these parasites to induce TNF-α production *in vitro*.

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IM28 - PAFR -/- MICE PRESENTS DELAYED IFN-γ PRODUCTION AND AUGMENTED SUSCEPTIBILITY TO *LEISHMANIA (L.) AMAZONENSIS*

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Leishmania (L.) amazonensis is an intracellular parasite and the causative agent of cutaneous leishmaniasis in New World. Although some IFN-γ is produced in response to infection in the C57BL/6 mouse strain, these mice are unable to resolve infection completely. The lipid-derived mediator, PAF, has been associated with induction of NO, migration of neutrophils and killing of intracellular *L. (L.) amazonensis in vitro* and *in vivo*. To evaluate the involvement of PAF and its receptor (PAFR) in resistance to *L. (L.) amazonensis*, PAFR deficient mice (PAFR -/-) and its wild type C57BL/6 control (WT) were infected with $1x10^6$ stationary forms of *L. (L.) amazonensis* in hind footpads. The infection was followed for 11 weeks. PAFR KO mice were more susceptible to infection and developed larger and progressive lesions (6 mm at 11th week). In contrast, WT mice developed a lesion that stabilized at around 2 mm. Qualitative histopathological analysis revealed a more severe lesion in PAFR -/- with an intense and extensive necrosis. There did not appear to be a difference in the inflammatory infiltrate. Parasitism was higher in PAFR -/- (around 10 fold increase per mg of tissue) when compared to WT. Interestingly, the concentration of IFN-γ, IL-4 and TNF-α as accessed by ELISA at the lesion site at 4th and at 11th and were similar in both groups. In cultures of lymph node and spleen, the level of IFN-γ was smaller in PAFR -/- mice at 4th week of infection when compared to WT. On the other hand, at 11th week, the IFN-γ level is similar in both groups. IL-4 was not detected in lymph node or spleen cell cultures. Moreover, production of the chemokine MCP-1 was similar in both groups, whereas the concentration of RANTES was different in both time points analyzed. There was a strikingly lower concentration of RANTES in the lesion site of PAFR -/- mice. Recent data have shown the importance of immunoglobulins in the susceptibility to *L. (L.) amazonensis*. IFN-γ, RANTES and PAF can modulate the activation of B cells. In order to investigate the involvement of immunoglobulins in the present model, anti-*L. (L.) amazonensis* antibodies in serum of infected animals were accessed by ELISA and our results shown that PAFR -/- presented a higher titer of anti-*L. (L.) amazonensis* antibodies and an impressive up regulation of IgG1 secretion. Our results point to an important role of PAFR in resistance to *L. (L.) amazonensis,* promoting early activation of IFN-γ production. Moreover, RANTES may also be involved in the resistance to this parasite.

Support: CAPES

IM29 - INFLUENCE OF NORMAL MICROBIOTA ON SOME ASPECTS OF IMMUNE RESPONSE DURING EXPERIMENTAL INFECTION WITH *TRYPANOSOMA CRUZI* IN MICE

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To study the influence of normal associated microbiota on systemic immunological responses during experimental Chagas' disease, germ-free and conventional NIH Swiss mice were infected with Y strain of *Trypanosoma cruzi*. Conventional mice showed a slightly higher survival than the germ-free ones as well as, seven days after the infection, a tendency to a lower parasitemia. Additionally, higher IFN-γ, TNF-α and NO productions ($P < 0.05$) by spleen cell cultures and higher blood levels of specific immunoglobulins of IgG2a isotype ($P < 0.05$) were observed in conventional animals when compared to their germ-free counterparts. On the other hand, germ-free mice showed higher production of IL-10 by spleen cell cultures ($P < 0.05$). In conclusion, the presence of the normal microbiota induces a more efficient Th1 immune response during an experimental infection with *T. cruzi* in mice.

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IM30 - IMPLICATION OF TRANSFORMING GROWTH FACTOR B IN CHAGAS' DISEASE MYOCARDIOPATHY

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Cardiac dysfunction with progressive fibrosis is a prominent feature of chronic Chagas disease. Transforming growth factor beta (TGF beta) is a strong inductor of extracellular matrix synthesis and accumulation. To test if TGF beta could play a role in the pathogenesis of chagasic myocardiopathy, we measured TGF beta levels by ELISA in patients at different stages: asymptomatic indeterminate (IND), cardiac with ECG alterations but no or slight heart dysfunction (Card 1), and cardiac with ECG and echocardiographic alterations indicating moderate or severe heart dysfunction (Card 2). The three groups had significantly higher circulating levels of TGF beta than non-chagasic persons, and 27% of Card 1 patients had higher TGF beta levels than IND patients. We detected an important immune staining for fibronectin and for phosphorylated-Smad 2, a TGF beta transcription factor that reflects activation of the TGF beta signaling pathway, observed respectively in the extracellular matrix and in the nuclei of cardiomyocites from chagasic cardiac patients heart fragments. The higher levels of latent TGF beta observed in patients with chagasic myocardiopathy, together with intracellular activation of the TGF beta pathway and tissue fibrosis, suggest that TGF beta plays an important role in chagasic pathology. TGF beta may then represent a new target for both preventive and curative treatments of Chagas disease.

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IM31 - DIFFERENTIAL PHENOTYPIC AND FUNCTIONAL CHARACTERISTICS OF CD28- AND CD28+ T CELLS BETWEEN CARDIAC AND INDETERMINATE CHAGASIC PATIENTS

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Chronic human Chagas' disease presents as one of several different clinical manifestations ranging from an asymptomatic to a severe cardiac clinical form. The involvement of the host's immune response in the development and maintenance of the chagasic pathology has been demonstrated by several groups. We have shown that activated $CD4^+$ and $CD8^+$ T cells lacking CD28 expression are increased in the peripheral blood of chagasic patients. Since $CD28^-$ cells may not need co-stimulation to exert their effector functions, we hypothesize that $CD28^-$ cells are important in cardiomyocyte damage. Thus, our study focuses on the characterization of T cells with differential expression of CD28, to gain a better understanding of the biology of these important cell populations. We evaluated the expression of Vb-TCR regions 2, 3.1, 5, 8 and 17, as well as the expression of IFN-γ, TNF-α, IL-4 and IL-10 by $CD28^+$ and $CD28^-$ cell subpopulations from polarized indeterminate and cardiac chagasic patients using flow cytometry. The results were compared to those obtained from non-chagasic individuals. Analysis of TCR-Vb usage showed similar frequencies of all regions analyzed when we compared between $CD4^+CD28^-$ and $CD4^+CD28^+$ cells from chagasic patients and non-chagasic individuals. However, $CD8^+CD28^-$ cells from chagasic patients but not from non-chagasic individuals, displayed a reduced frequency of the analyzed Vbs when compared with the $CD8^+CD28^+$ subpopulation. This suggests that $CD8^+CD28^-$ cells from chagasic patients could display preferential expression of other Vbs due to a dominant expansion of these cells in response to an unknown disease related antigen. Evaluating the expression of key immunoregulatory cytokines by circulating mononuclear cells from chagasic patients, we detected a higher frequency of IFN-γ, TNF-α and IL-4 producing cells in the cardiac than in the indeterminate patients, possibly reflecting an inflammatory reaction in the first group. The levels of IL-10 were similar between groups. We also performed correlation analysis between the frequency of cytokines and the frequency of $CD4^+$ or $CD8^+$ T cells with differential expression of CD28. Interestingly, we observed a positive correlation between $CD4^+CD28^-$ T cells and IL-10 expression in indeterminate and cardiac patients. This result suggests that these cells may carry out an important modulatory role in the immune responses of indeterminates and cardiac patients.

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IM32 - PHENOTYPIC AND FUNCTIONAL CHARACTERISTICS OF T CELLS FROM INDETERMINATE AND CARDIAC CHAGASIC PATIENTS AFTER EXPOSURE TO *T. CRUZI*-INFECTED MONOCYTES

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Infection with the protozoa *Trypanosoma cruzi* causes Chagas' disease, an inflammatory illness that affects approximately 18 million people in Latin America. 20-30% of the infected individuals develop specific cardiomiopathy whereas the great majority of the patients remain in the indeterminate clinical form. Several studies have demonstrated that host's immune response is critical in controlling parasitemia but also in leading to tissue pathology. Interaction between *T. cruzi* and monocytes is certainly an important event in regulating cellular reactivity in Chagas' disease since it may affect T cell response during disease. The aim of this work was to study the effects that infection or expose to parasite antigen to monocytes from indeterminate and cardiac patients would have in T-cells from the patients. We purified adherent cells from peripheral blood mononuclear cells from chagasic and non-chagasic individuals and submitted them to parasite infection or exposure to parasite antigen and added T-cells to the culture. After a period of approximately 18 hours we evaluated the expression of co-stimulatory and adhesion molecules, activation markers and immunoregulatory cytokines, using flow cytometry. Our results showed that: (1) T cells from indeterminate patients showed high levels of expression of CTLA-4, mainly in $CD8^+$ T cells, suggesting an important role for this molecule in controlling cellular responses, specially cytotoxic function; (2) cardiac patients showed a reduced expression of CD86, important ligand for CTLA-4, suggesting a decreased immunomodulatory ability through this pathway; (3) lymphocytes from cardiac but not indeterminate patients showed high IFN-γ expression, as compared to non-chagasic individuals, establishing a direct correlation between this inflammatory cytokine and the severe cardiac clinical form; (4) lymphocytes from cardiac patients showed a high expression of IL-4, possibly important for the control of inflammatory response, critical for the long-lasting nature of the disease. These data show important immunological differences between T-cells from indeterminate and cardiac patients upon contact with the parasite and/or its antigens, offering new information concerning the cellular reactivity in human Chagas' disease.

Financial support: WHO, CNPq/PADCT, PRONEX

IM33 - STIMULATION BY FOOD PROTEINS AFFECTS CYTOKINE PROFILE AND RESISTANCE TO *L. (L.) MAJOR* INFECTION IN C57BL/6 MICE.

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The majority of contacts with foreign antigenic materials occurs in the gut mucosa, and are represented by food proteins and the autochthonous microbiota. Approximately 30 kg of food proteins reaches the human intestine per year and 130±190 g of these proteins are absorbed daily in the gut (Brandtzaeg, 1998). In addition, the number of bacteria colonizing the human large intestine can reach 10^{12} microorganisms/g of stool (Macfarlance *et al*, 1997). The presence of a large gut-associated lymphoid tissue (GALT) has been usually attributed to stimulation by bacterial antigens because this tissue is drastically reduced in germ-free animals. Local production of secretory IgA (sIgA), as well as serum levels of IgA and IgG, but not IgM, are also reduced in these animals (Bos *et al*, 1988). Recently, we described an experimental model in mice that was designed to study the immunological effects of food proteins. These mice were fed a diet where the intact dietary proteins were replaced by equivalent amounts of amino acids (Aa) from weaning up to adulthood and investigated its effects on the development of the immune system. Adult animals that were reared on this balanced protein-free diet (Aa-mice) have a poorly developed GALT resembling suckling mice. Levels of secretory IgA and circulating IgG and IgA are also reduced in Aa-mice, whereas IgM levels are normal. *In vitro* cytokine production by cells from several lymphoid organs shows a predominant Th2 profile with a high concentration of IL-10 and IL-4, and a low concentration of IFN-γ (Menezes *et al*, 2003). These parameters also resemble the immunological patterns observed

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in pre-weaned mice suggesting food protein stimulation is required for the full maturation of the immune system. In the present study, we evaluated the effects of Aa diet in C57BL/6 mice infected with *L. (L.) major*. Adult C57BL/6 mice treated with Aa diet show an increase in footpad lesions as compared to control casein-diet treated mice. After 4 weeks of infection, they resemble susceptible BALB/c mice suggesting that a poor IFN-gamma mature immune response to the parasite is triggered. Indeed, analysis of *in vitro* cytokine production in popliteal lymph node cells of Aa-mice show a reduction in IFN-gamma production and an increase in IL-4 production. Our results suggest that the impairment in immunological maturation observed in C57BL/6 Aa- mice alters their cytokine profile and their resistance to *L. (L.) major* infection.

Supported: CAPES, CNPq and FAPEMIG

IM34 - POLIMORPHONUCLEAR NEUTROPHILS AS A SOURCE OF CYTOKINES IN REGULATING OF THE IMMUNE RESPONSE IN HUMAN VISCERAL LEISHMANIASIS

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The clinical forms of visceral leishmaniasis (VL) are critically influenced by the host immune response where the cure or disease progression has been related to the predominance of a type 1 or a type 2 immune response of T cells, respectively. However, the role of neutrophils as source of cytokines during early infection and on the modulation of the adaptative immune response, has not yet been reported. In this context, we have evaluated, at a single cell level, after a short-term *in vitro* stimulation, the cytokine patterns of neutrophils from adults and children with active LV form (ACT), asymptomatic LV form (AS), cured LV individuals (CR) and non-infected individuals (NI), living in endemic area. Our data demonstrate a decrease on the absolute number of TNF-α + cells in NI and an increased on the expression of IL-4 in NI and ACT, both, in adults and children. Additionally, our results demonstrate an increase on the absolute number of IFN-γ^+ and IL-4 $^+$ cells in AS and CR, both, in adults and children. The cytokine pattern of neutrophils, suggest that NI, would be susceptible to the infection and could also explain the disease progression in ACT. Furthermore, the cytokine pattern observed for AS and CR, suggest that in spite of what was observed, a tendency for a type 1 profile, these individuals modulate the response for secrete IL-4, perhaps to control the disease without allowing the host to inflame. Taken together, these findings suggested that the neutrophils could function as immunoregulatory cells by releasing cytokines, that could influence the outcome of the innate immune response and of the subsequent T cell-dependent immune response during early stages of LV.

Supported by: FAPEMIG, FIOCRUZ and CPqRR

IM35 - CYTOKINE EXPRESSION IN NEUTROPHILS ARE IMPORTANT IN DIRECTING THE ADAPTATIVE IMMUNE RESPONSE IN INDIVIDUALS SUSCEPTIBLE AND RESISTANCE TO *LEISHMANIA (V.) BRAZILIENSIS* INFECTION.

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Neutrophils are the first cells recruited when the peripheral tissue is disrupted, and recent studies have demonstrated that neutrophils, once exposed to inflammatory signals, synthesize several cytokines. In this context, we evaluated, at a single cell level, the cytokine patterns of neutrophils, after a short antigen-specific stimulation *in vitro*. In this study, we evaluated four groups of individuals, including patients with active localized cutaneous leishmaniasis (LCL), subjects cured and treated with conventional chemotherapy (Q) and immunochemotherapy (IQ) and an endemic control group (NI). The results are expressed as number of positive cells/mm3 of peripheral blood. Our data demonstrate an increased in the levels of IFN-gamma, TNF-alpha and IL-12$^+$ neutrophils in LCL in comparison to the other groups. And increased level of IFN-gamma and TNF-alpha + neutrophils in IQ and Q in comparison to NI, and a decreased level of type 2 cytokine (IL-10 and IL-4) in cured patients (IQ and Q) in comparison to infected group (LCL) and control group (NI). Our data show that LCL individuals present a type 0 response, with the presence of type 1 cytokines and type 2cytokines$^+$ neutrophlis. Cured individuals present a predominance of type1$^+$ cytokine pattern by neutrophils, usually associated with activation of type 1$^+$ T cells and resistance to infection. Taken together, these findings suggested that neutrophils could function as immunoregulatory cells by expressing cytokines, that could influence the outcome of the innate response and of the subsequent T cell-dependent immune response.

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IM36 - CONCENTRATION OF MACROPHAGES, TIA-1$^+$ CYTOTOXIC LYPHOCYTES AND CD57$^+$ NATURAL KILLER CELLS CORRELATE WITH THE DEVELOPMENT OF CHAGASIC MEGAESOPHAGUS.

da Silveira, A.B.M.[1], Lemos, E.M.[2], Arantes, R.M.E. [3], Linhares, D.C.[1], Correa-Oliveira, R.[4], Adad, S.J.[4], Reis, D.d'A[1].

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Chagasic megaesophagus is characterised by lumenal enlargement and wall thickening. The organ may have different degrees of involvement, from a slight motor disturbance, only detectable by manometric and scintilographic studies, to large dilations that characterise the more advanced forms of chagasic mega syndrome. Histological studies demonstrated that patients with megaesophagus present lesions of the myenteric plexus, associated with inflammatory infiltrates. Despite being very well described in terms of anatomo-pathological findings, very little information is currently available regarding the phenotype of inflammatory cells and their possible role in the development of megaesophagus. We quantified CD57$^+$ *Natural Killer* cells, TIA-1$^+$ cytotoxic lymphocytes and CD68$^+$ macrophages in esophagus of patients bearing severe megaesophagus and also in chagasic patients without megaesophagus. Patients without mega were classified in two groups, as having high or low number of neurons. Both groups with decreased counting of neurons presented *Natural Killer* cells, as well as cytotoxic lymphocytes in the esophagus, whilst increased numbers of both cellular populations were observed in patients bearing megaesophagus. The levels of CD68$^+$ macrophages in the esophageal *muscularis propria* and plexus regions of chagasic patients were also increased in chagasic patients when compared to non-infected controls. Morphometric analysis of sections labelled with anti-CD68 or anti-PGP9.5 showed that, the higher levels of

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macrophages, the lower density of PGP 9.5+ nerve endings. Those data together point to the participation of cytotoxic lymphocytes, *Natural Killer* cells and macrophages in neuronal lesions occurring in the chronic phase of chagasic megaesophagus.

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IM37 - COMPARATIVE STUDY: PRESENCE OF PARASITE KDNA, INFLAMMATION AND COUNTING OF NEURONS IN CHAGASIC PATIENTS WITH AND WITHOUT MEGAESOPHAGUS.

da Silveira, A.B.M.[1], Vago, A.R.[1], Arantes, R.M.E.[2], Adad, S.J.[3], Correa-Oliveira, R.[4], Reis, D.d'A.[1]

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The pathogenic mechanisms in chagasic megaesophagus are are not entirely understood. Neuronal lesions have been considered the hallmark of this syndrome, and they are frequently observed in association with ganglionitis, periganglionitis and fibrosis. It has been suggested that chronic lesions are consequence of parasite-related mechanisms, as well as immune-mediated cytotoxic damage. In this study we analysed the inflammatory process, the counting of neurons and the presence of kDNA parasite in the esophagus of patients with and without megaesophagus. The presence of kDNA parasite was demonstrated in esophagus of patients with digestive disease, as we had previously published. However, sixty percent of the chagasic patients without megaesophagus analysed in this study also presented parasite DNA in the organ. When analysed for neuronal number, this group could be classified in two, as having low or high counting of neurons. Patients without megaesophagus presenting high number of neurons did not show any inflammatory process, but two of them had parasite kDNA in the organ. Interestingly, all patients without megaesophagus presenting low number of neurons had parasite kDNA and also light inflammatory process. These data strongly suggest that chronic lesions in chagasic megaesophagus might be consequence of immune-mediated mechanisms, that last until the chronic phase of infection, and are dependent on the persistence of parasite in the tissue.

Financial support: CAPES and FAPEMIG.

IM38 - ROLE OF TNF-α RECEPTOR 1 (TNFRP55) IN INFLAMMATORY RESPONSES TO *LEISHMANIA (L.) MAJOR*

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Tumor necrosis factor (TNF-α) has an essential role in the activation of infected macrophages to kill *Leishmania major* after activation with IFN-γ. Although TNF-α has two receptors on the surface of most cells, the introduction of nitric oxide (NO) by TNF-α was believed to be mediated by the receptor 1 (TNFRp55). Mice in which this receptor was deleted by homologous recombination (TNFRp55-/-) resolved parasitism in the footpad when infected with *L. (L.) major,* but more slowly than C57BL/6 wildstrain. More interestingly, even after the levels of parasites at the site of infection were undetectable, TNFRp55-/- did not resolve lesions, and an intense inflammatory infiltrate was present after 25 weeks of infection. The aim of this word is to investigate the reason for the permanence of the cellular infiltrate in lesions from TNFRp55-/- mice infected with *L. (L.) major*. Thus, we determined the expression of chemokines by RT-PCR at the site of infection in C57BL/6 and TNFRp55-/- mice. RANTES and MCP-5 expression was upregulated in C57BL/6 wildtype and TNFRp55-/- mice. However, levels of these chemokines were downregulated at 11 weeks of infection in C57BL/6 mice, while there was still a high level of expression of both chemokines in lesions from TNFRp55-/- mice at this time point. In order to investigate the role of the TNFRp55 on the delayed-type hypersensitivity (DTH) in response to formalin-treated *L. (L.) major*, we injected 10^7 dead parasites in the right footpad of mice infected for 2, 4, 6 and 11 weeks in the left footpad. The size of the response was measured 3, 6, 24, 48 and 72 hours. Surprisingly, TNFRp55-/- mice presented no detectable DTH response and C57BL/6 presented a positive DTH from the 4th week of infection. We determined the expression of chemokines by RT-PCR in the right footpad injected with 10^7 dead parasites after 6 weeks of infection. C57BL/6 wildtype express higher levels of RANTES than TNFRp55-/- mice. We also studied the response of C57BL/6 and TNFRp55-/- mice to carrageenan. We injected carrageenan solution in the right footpad and the lesion was measured 3, 6, 12, 24, 48 and 72 hours. C57BL/6 wildtype present larger footpads at 6 hours after injection than TNFRp55-/- mice, however, TNFRp55-/- mice seemed not to resolve the cellular infiltrate 96 hours after injection. Thus, we conclude that the TNFRp55 plays a role in the resolution of a primary cellular infiltrate, maybe by mediating the downregulation of chemokine expression. As to the recall response, TNFRp55 seems to mediate the migration of cells to the site of a secondary antigenic exposure.

Support: CNPq

IM39 - LEVELS OF NITRIC OXIDE IN THE CANINE VISCERAL LEISHMANIASIS

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The canine visceral leishmaniasis (CVL) is an illness of chronic course in which the dog evolved from an unapparent state of the infection (asymptomatic). These do not present clinical evident signals of infection, low levels of IgG and low tecidual parasitic load. These animals can evolve to serious symptomatic picture. Objectives: To quantify the sera levels of nitric oxide and correlate to the tecidual parasitic density (LDU) in dogs with different clinical forms of the CVL. Materials and Methods: It had been used sera from dogs infected and not infected with *Leishmania (L.) chagasi*, carrying different clinical forms of the infection. The dogs were clinically classified and grouped in: Not Infected (NI=20), Asymptomatic (AS=12), Oligosymptomatic (OS=12) and Symptomatic (SY=16). These dogs are proceeding from the Zoonotic Center Control from Belo Horizonte (CCZ/PBH). The dogs of groups AS, OS and SY presented positive serologic test in at least two of three serologic tests (RIFI, ELISA, ELISA-rK39). The quantification of nitric oxide was carried out indirect through the measure of nitrite and nitrate using the Griess method and the results expressed in ìM. Resulted and conclusions: A gradual fall in the levels of NO was observed with severe clinical form of the CVL. The animals from group AS had presented higher levels of NO in relation to NI and SY group. These data seem to point out an association of high levels of NO on asymptomatic dogs and minor LDU in the spleen, skin and popliteal lymph node. Support: UNIVALE

IM40 - IFN-γ AND IL-10 ARE PRODUCED BY NATURALLY INFECTED DOGS WITH VISCERAL LEISHMANIASIS.

Lima, V.M.F., Dossi A.C.S., Neto, JLJR

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Zoonotic visceral leishmaniasis, caused by both *Leishmania (L.) infantum* and *L. (L.) chagasi* represent 20% of human visceral leishmaniasis in the world and its incidence is growing in urban and periurban areas of the tropics. In Brazil, visceral leishmaniasis (VL) is caused by *Leishmania (Leishmania) chagasi*. Dogs constitute the main domestic reservoir of these parasites transmitted to humans by phlebotomine sandflies. Most of the infected animals are susceptible and develop active disease, which is characterized by high anti-Leishmania antibody titers and depressed lymphoproliferative abilities. The immune response Th1 is correlated with resistance to the pathogen, but the high level of antibodies suggests that the immune response Th2 is associated with active disease. In this study, naturally infected dogs with clinical signs of the disease and positive ELISA test in sera were analyzed. After intravenous injection of 25mg/kg of thiopental (Thionembutal 12.5%) to each animal, 30 ml of blood was collected, lymphocytes were isolated with ficoll-paque-PLUS and RNA was isolated. Interferon-gamma and interleukin–10 profiles were detected by RT-PCR using specific primers. In all animals analized (20 / 20) interferon-gamma expression was detected, but only few animals (5 / 14) showed interleukin–10. The predominance of interferon-gamma production associated with active disease suggests that other cytokines rather than interleukin-10 must be produced to collaborate in the immune response Th2 observed in animals with active disease. The cytokines IL-4 and TGF-beta are being investigated.

Supported by FAPESP

IM41 - PHENOTIPIC PROFILE OF PERIPHERAL BLOOD LEUKOCYTES AND SPLENOCYTES IN CANINE VISCERAL LEISHMANIASIS

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Canine Visceral Leishmaniasis (CVL) can manifest itself in a broad clinical spectrum ranging from asymptomatic to patent hepatosplenic disease. However, the role of different cell populations on the development and/or maintenance of CVL are still unclear. Considering that CVL causes a systemic impairment, it becomes relevant to study other cell compartment like the spleen – the major lymphoid organ interfacing the systemic circulation – where monocyte/macrophage homing and also antigenic stimulation can occur during CVL. We evaluated the immune status of 40 CVL dogs naturally infected with *L. (L.) chagasi* as well 20 healthy controls. The dogs were classified as asymptomatic (n=12), oligosymptomatic (n=12) and symptomatic dogs (n=16) based on their clinical and laboratory records. The phenotypic profile of both, peripheral blood leukocytes and splenocytes was analyzed by flow cytometry. Spleen biopsies were used to access the parasitism ("Leishman Donovan Units" – LDU). Our data demonstrated that symptomatic dogs presented decreased levels of circulating T-cells, including both $CD4^+$ and $CD8^+$ subpopulations, with a lower CD4/CD8 ratio in comparison to the control group. On the other hand, asymptomatic dogs presented high absolute number of $CD4^+$ and $CD8^+$ T-cells when compared to the other groups. Dogs with clinical diseases, including oligosymptomatic and symptomatic, presented a decreased number of $CD21^+$ cells and a progressive reduction of $CD14^+$ cells linked with the severity of the disease. Phenotypic analysis of splenocytes demonstrated a progressive increase in T-cell, mainly $CD8^+$, in all infected dogs in comparison to the control group, which lead to a decreased CD4/CD8 ratio in CVL dogs. A decreased percentage of B-cells, similarly observed in the PB, were observed with the development of patent disease. LDU was higher in the symptomatic animals in comparison to the other infected dogs. Our data pointed out that the maintenance of a stable chronic infection by *L. (L.) chagasi* in dogs involves an increase in T cell subpopulation, mainly $CD8^+$ cells, in both PB and spleen, re-emphasizing that this cell population may be evolved in the mechanisms of protective immunity in CVL. Dogs with symptomatic disease, presenting a reduced potential an antigen presenting cells - evidenced by the lower number of circulating monocytes and B-cells as well as a decreased percentage of B-splenocytes - showed lower results in the *in vitro* proliferate response and were more prone to develop patent disease.

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IM42 - *TRYPANOSOMA CRUZI* CARRYING A TARGETED DELETION OF A TC52 PROTEIN-ENCODING ALLELE ELICITS ATTENUATED CHAGAS' DISEASE IN MICE

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The intracellular protozoan parasite *Trypanosoma cruzi* is the etiological agent of Chagas' disease. We have previously characterized a *T. cruzi* virulence factor named Tc52 sharing structural and functional properties with the thioredoxin and glutaredoxin protein family. Single mutant parasite clones ($Tc52^{+/-}$) exhibiting low virulence *in vitro* and *in vivo* were obtained by targeted *tc*52 gene replacement. In this report, we have extended our study to analyze the immune response and the disease phenotype in $Tc52^{+/-}$- infected BALB/c mice, during the acute and chronic phases of the disease. Significantly lower parasitemia were found in $Tc52^{+/-}$-infected mice, as compared to wild-type parasite (WT)-infected ones. However, the expansion of all classes of lymphocytes and macrophages was similar for both clones. Furthermore, except for IgG2b levels which were higher in the case of WT-infected mice, all classes of Ig presented no significant difference for WT and $Tc52^{+/-}$-infected animals. Interestingly, a lack of suppression of IL-2 production and of T-cell proliferation inhibition was observed in the case of spleen cells from $Tc52^{+/-}$-infected mice. Finally, the pattern of inflammation process was different and characterized as diffused in the case of $Tc52^{+/-}$-infected mice, or presenting numerous foci in the case of WT-infected mice. Localization of the Tc52 protein in tissue sections and infected heart cell primary cultures by immunofluorescence and immunogold labeling, respectively, revealed the presence of Tc52 at the amastigote surface and associated to aggregates within host cell vesicles. Taken together, these results reinforce the notion of Tc52 being a virulence factor playing a role in the phenotype of the immune response associated to the infection and on the course of the disease.

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IM43 - T CELL INDEPENDENT IMMUNITY DURING EARLY STAGES OF HUMAN INFECTION WITH *TRYPANOSOMA CRUZI*

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In this study, a cross-sectional flow cytometric analysis of peripheral blood mononuclear cells (PBMC) was performed to evaluate the immunological status of *T. cruzi*-infected children, aiming to make known the immune response triggered during the early stages of Chagas disease. Fourty-two infected children were classified into four groups, based on their serological features, including early-acute phase (EA), late-acute phase (LA), recent chronic phase (RC) and not infected (NI). Our results demonstrated that three well-characterized major immunological changes could be identified according to the proposed phases of disease. EA was accompanied by expansion of conventional B-cells and up-regulation of CD54 molecule on monocytes, associated with neither monocyte activation phenotypes nor changes of NK or T-cell compartments. LA was characterized by a selective expansion of a distinct lineage of NK cells ($CD16^+CD56^-$) besides a persistent high frequency of B-cells. RC was still accompanied by a high levels of B-cells, leading to a reduced T/B ratio, mainly triggered by the expansion of B1 cells subset, besides a delayed expansion of HLA-DR bright+ monocytes. These findings reinforce the hypothesis that T. cruzi-derived antigens are able to activate NK-cells, before the development of T-cell-mediated immunity. Moreover, our data supported, previous remarks of increased levels of B1-lymphocytes during chronic human Chagas disease and evidenced that this event is already present in initial stages of chronic infection. We discussed that T-cell-mediated immunity during early stages of *T. cruzi* infection may stand for a phenomenon restricted to the cardiac and lymph node compartments, not detectable on the peripheral blood.

IM44 - *IN VIVO* NEUTRALIZATION OF *PLASMODIUM GALLINACEUM* SPOROZOITE INFECTIVITY USING SERA OF *P. FALCIPARUM* PATIENTS

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Plasmodium falciparum (Pf) is the most prevalent species causing malaria worldwide. Practical difficulties, such as, high human infectivity and absence of experimental vectors in Brazil, hampers its use in research. Our group has been using *P. gallinaceum* (Pg), non-infectious to humans, as an alternative model to study malaria, particularly in the identification of protein as marker of acquired immunity in human Pf. We show that Pg sporozoites, react by indirect immunofluorescence (IIF) with sera from subjects exposed to Pf; sera from *P. vivax* subjects or normal sera were negative. Highest scores of positive sera (73-90%) with Pg sporozoites were in subjects denying malaria in the last 5 years or more. These sera also recognized several Pg proteins by immunoblot analysis mainly the circumsporozoite protein (CSP). In this study, we analyze the ability of crossreactive antibodies present in sera of immune Pf patients to neutralize the infectivity of Pg sporozoites. Fresh-isolated sporozoites were incubated with sera from Pf patients who described more than 10 malaria acute episodes. Two immune sera were able to partly neutralize the Pg sporozoite infectivity to chicks, its natural host, in relation to non-immune sera. Monoclonal antibodies anti-PgCSP which totally abrogate the Pg sporozoite infectivity in vivo and in vitro, recognized mainly the C-terminal of the CSP (CSC). Studies with human sera are now undertaken in an attempt to verify whether such CSC protein identifies protective antibodies in ELISA.

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IM45 - MAPPING CD4 T-CELL EPITOPES WITHIN THE AMASTIGOTE SURFACE PROTEIN-2 OF *TRYPANOSOMA CRUZI*.

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Recently, independent groups studied the immunogenic properties of plasmids containing genes encoding the Amastigote Surface Protein-2 (ASP-2). Genetic immunization with *asp-2* gene generated immune responses mediated by antibodies, $CD4^+$ and $CD8^+$ T cells. Most relevant, DNA-vaccinated mice displayed remarkable protective immunity, surviving lethal infection with *T. cruzi* (Garg & Tarleton, 2002, Infect. Immun. 70:5547, Boscardin *et al*., 2003, Infect. Immun. 71:2744 and Fralish & Tarleton, 2003, Vaccine 21:3070). To map a CD4 T-cell epitope in the ASP-2 antigen, 8 recombinant proteins were generated as GST-fusion or His-tag polypeptides. These recombinant proteins representing the different domains of ASP-2 antigen were purified and tested in their ability to be recognized by polyclonal antibodies or to stimulate *in vitro* spleen cells of BALB/c or A/Sn mice immunized with a plasmid containing the *asp-2* gene. In preliminary experiments, we determined that spleen cells from DNA-immunized BALB/c or A/Sn mice secreted interferon-gamma when re-stimulated *in vitro* with recombinant proteins representing the amino acids 67-260 or 261-500 of ASP-2, respectively. We are currently pursuing further the epitope mapping of these T cell determinants using the recombinant proteins.

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IM46 - $CD4^+CD25^+$HIGH REGULATORY CELLS IN PERIPHERAL BLOOD OF CHAGAS DISEASE PATIENTS

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Despite the identification of two major $CD4^+$ T-cells providing the insights that distinct Type 1 and Type 2 cytokine patterns may regulate immune responses, it is clear that populations of T-cells could also mediate immune responses by cell contact in the absence of cytokine secretion. Experiments demonstrating that $CD4^+CD25^+$ T-cell function as key regulatory effectors in mice have provided

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important information about a specific cell population that performs immune regulation through suppression of self response. Whereas the entire population of $CD4^+CD25^+$ T cells exhibit regulatory function in mouse, only the $CD4^+CD25high^+$ population displays a similarly strong regulatory function in humans. The critical importance of identifying the involvement of these regulatory T-cells as a bridge for immune regulation of pathology during chronic human infection by *T cruzi* prompt us to search for a differential frequency of $CD4^+CD24high^+$ T-cells in peripheral blood of chagasic patients presenting different clinical forms of the disease. Here, we report that asymptomatic indeterminate patients showed higher levels of $CD4^+CD25^+$high T-cells in comparison to those symptomatic patients presenting cardiac or digestive forms of disease. Thus, regulatory T-cells expressing high levels of the IL-2 receptor seems to be associated with immunoprotective mechanisms, providing the opportunity to determine whether alterations on these regulatory T-cells are involved in the induction of pathological mechanisms of human Chagas disease.

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IM47 - CHARACTERIZATION OF $CD4^+$ T CELL HYBRIDOMAS SPECIFIC FOR *TRYPANOSOMA CRUZI TRANS*-SIALIDASE.

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BALB/c mice immunized with a DNA plasmid encoding the *Trypanosoma cruzi Trans*-sialidase (TS) developed $CD4^+$ Th1, $CD8^+$ Tc1 and protective immune response against infection[1]. From DNA-vaccinated mice, we obtained $CD4^+$ Th1, which displayed remarkable anti-parasitic activity *in vitro*[2]. The aim of the present study was to obtain T cell hybridomas specific for TS protein that would allow us to further characterize the specificity and function of the $CD4^+$ Th1 cells. For that purpose, lymph node cells from BALB/c mice immunized with the recombinant TS protein was fused to cells AKR thymoma BW 1100.129.237. Twenty-five hybridomas were obtained. Twelve of them were antigen specific, secreting more than 1 ng/ml of IFN-γ when re-stimulated with recombinant TS protein. None of them secreted either IL-4 or IL-10 upon *in vitro* re-stimulation. By flow cytometry analysis, all hybridomas were positives for CD3 and eight of them were highly positive for CD4 T cell marker. Four of five hybridomas tested were also positive for the TCRb chain. One hybridoma positive for the TCRb chain was choused and RNA from the hybridoma clone 09 (H09) was isolated. We employed the nonpalindromic adaptor-PCR (NPA-PCR) method to amplify T-cell receptor (TCR) alpha- and beta-chain transcripts described by Lin WL *at al*., 1998. The NPA-PCR method has been specifically designed for the amplification of transcripts with variable or unknown 5' ends, such as TCRs and immunoglobulins (Ig). The amplifications of products were cloned in *pMOSblue* vector and were sequenced. The variable alfa and beta chains of T cell receptor (TCR) were identified as TCRAV18S2 and TCRBV6S1, respectively. The specific primers to variable alpha and beta chain were constructed. These primers were used to amplification reactions using genomic DNA of H09 as template. The presence of introns in sequences of TCR is important to expression of TCR. The amplified products were cloned in *pMOSblue* vector and were sequenced. The next step the alfa and beta chain will be cloned in pTalfacass and pTbetacass vectors[4], which will be used to express rearranged T cell receptor genes in transgenic mice.

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IM48 - IDENTIFICATION OF CTL EPITOPES IN KMP11 PROTEIN FROM *TRYPANOSOMA CRUZI*

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The Kinetoplastid membrane protein-11(KMP11) is found in several parasites belonging to the *Leishmania* and *Trypanosoma* family. This protein seems to contain epitopes that induce immune cellular responses (Tolson, et al 1994), and it is thought to have a role in protective immunity (Mukhopadhyay, et al 1999). Recently, it was demonstrated that the *T. cruzi* KMP11 is located mainly in the parasite´s flagellar pocket where it is associated with the cytoskeleton. Therefore, its function is critical for the parasite mobility and for its attachment to the host cells (Thomas, et al 2001). Experimental *T. cruzi* infection using the murine model demonstrated that $CD8^+$ T lymphocytes were essential in controlling parasite dissemination. These cells presented cytotoxic activity specific for *T. cruzi* antigens. CD8 T lymphocytes specific for *T. cruzi* antigens were also found in peripheral blood of Chaga´s patients (Wizel, et al 1998). Current data indicates that these cells could play an important role in protection (Reis, et al 1997). In the present study, it was assessed if *T. cruzi* KMP11 could induce CD8 T lymphocytes responses. The aim of this study was to determine the presence of cells specific for the KMP11 synthetic peptide (tleefsakl) in HLA-A2 chagasic patients using γ-IFN production measured by ELISPOT assay. PBMC were isolated using density gradients and cells were typed using specific HLA-A2 monoclonal antibody. Five patients out 17 studied were HLA-A2 positive (29,4%). CD8 T lymphocytes of those patients were purified using magnetic beads and plated for γ-IFN ELISPOT in the presence of peptide pulsed antigen presenting cells. Responses to the well defined HLA-A*0201 restricted peptide from the influenza matrix protein (flu 58-66) were found in 4 out 5 patients with average of frequency of 167.5 $x10^6$ cells. Two of the HLA-$A2^+$ *T.cruzi*-infected individuals tested showed a $CD8^+$ specific response to the peptide with a relative frequency of 110 and 230 $x10^6$ cells. Our dates indicate that KMP11 acts as a T-cell immunogen during Chagas disease but its role in protection has to be defined.

IM49 - EARLY MECHANISM OF COMPLEMENT-MEDIATED LYSIS ARE DIFFERENT AMONG *T. CRUZI* LINEAGE

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Trypanosoma cruzi, the etiological agent of Chagas' disease, is a protozoan that infect about 18 million people in Latin America (WHO, 2003). It has been demonstrated through molecular and biochemical markers (Miles et al, 1978; Zingales et al, 1996) that these parasites can be divided in two main lineage, lineage 1 with domestic life cycle, and lineage 2 with a sylvatic life cycle (Fernandes et al, 1998). Mechanisms of innate immunity can be different among *T.cruzi* strains, and this would strengthen the hypothesis of the different origins proposed by Briones and Zingales, 1998. To infect host cells, *T.cruzi* needs to resist the complement mechanisms, which can be activated by classical or alternative pathway. The time of complement activation and susceptibility to

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lyse of parasites in contact with Normal Human Serun (NHS) could be associated with the capacity of parasite evasion in the host immune system. With the objective to determine the differences among *T.cruzi* lineage related to the resistance and activation of the complement system, we have characterized four *T.cruzi* strains, two of lineage 1 (Y and CL Brener) and two of lineage 2 (Colombiana and Dm28c). We determinated: i) the limite dilution; minimun concentration of NHS capable to lyse 50% of the parasites; ii) and the time necessary for the activation of the classic and alternative pathway, through kinetic with different times (30 seconds, 1 minute, 2,5 min, 5 min, 10 min. e 30 minutes). The limite dilution was similar for Y and CL Brener strain (25% of NHS) while Colombiana and Dm28c strains were more sensible (around 6,25% and 12,5%). The complement-mediated lysis activated by classic and alternative pathway resulted in 70% and 71% of alive parasites to Y and CL Brener strain respectively, at 2,5 minutes, while Colombiana and Dm28c, 11% and 35% respectively. When the classic pathway was blocked with EGTA, it was determined that the parasites were equally resistant to alternative pathway, and only its action was not enough to lyse *T.cruzi* strain, with 63% and 35% of alive parasites at 10 minutes. These datas show that lyse is mediated by a sinergic action of classic and alternative pathway. The alternative pathway is not enough to lyse parasite in a short time and this fact is relevant considerating the rapid process of cell invasion. Moreover, the complement-mediated lyse could be a marker to diferenciate the lineage of differents *T.cruzi* strains. *T. cruzi* Y and CL Brener strains are more resistant than Colombiana and Dm28c strains, and this fate could be related with the different enviroment where lineage 1 parasite had evolved. The understading of these mechanisms could contribute to new approaches to control the disease.

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IM52 - VACCINATION OF BALB/C MICE WITH DNA ENCODING A CYSTEINE PROTEINASE OF *L. (L.) CHAGASI* AMASTIGOTES

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An antigen of apparent molecular mass of 30 kDa (p30) was identified in *L. (L.) chagasi* amastigotes and showed to induce lymphoproliferative responses mediated by $CD4^+$ Th1 and a partial protection against challenge with *Leishmania (L.) chagasi* in BALB/c mice (Pinto et al., 2000, Int. J. Parasitol. 30:599-607). One antigen of 30 kDa identified in *L. (L.) amazonensis* amastigotes was also implicated in protective responses against homologous infection and characterized as a cysteine proteinase (Beyrodt et al., 1997). Although biochemical characterization of *L. (L.) chagasi* p30 revealed that it is deprived of proteolytic activity, two cysteine proteinase genes, *Ldccys1* and *Ldccys2*, were identified in *L. (L.) chagasi* and promastigotes transfected with these genes expressed proteolytic activity of apparent molecular masses of 30 and 43kDa (Omara-Opyene and Gedamu, 1997). Previously, we have isolated the *Ldccys1* gene by PCR amplification using genomic DNA from *L. (L.) chagasi* amastigotes and primers corresponding to the ORF sequence of the *L. (L.) chagasi Ldccys1* gene published in GeneBank. Cloning of this gene in pHis vector resulted in a recombinant protein of 47 kDa which was recognized by a monoclonal antibody directed to p30 from *L. (L.) amazonensis* (MoAb 2E5D3). This result and previous data showing that the MoAb 2E5D3 reacts with p30 from *L. (L.) chagasi* indicate that the 47 kDa recombinant protein corresponds to the p30 antigen from *L. (L.) chagasi*. The aim of the present work was the cloning of p30 gene from *L. (L.) chagasi* in a mammal constitutive expression vector and characterization of the immune responses triggered after the immunization of BALB/c mice with the recombinant DNA. The *Ldccys1* gene was subcloned in pcDNA3 vector and BALB/c mice were immunized with three doses with 14 days interval of 100 µg of either pcDNA3 or plasmid encoding the p30 antigen (pcDNA3-p30) in the quadriceps. Two weeks after the third dose, sera from all animals were tested by ELISA using the recombinant p30 and Immunobloting of *L. (L.) chagasi* amastigotes and bacterium extracts. Only mice immunized with pcDNA3-p30 presented high antibody titles against proteins of molecular weights of 30 and 47 kDa from parasite and bacterium extracts, respectively. Preliminary results showed that the recombinant p30 and amastigote extract induce secretion of IFN-γ in supernatants of lymphocyte cultures from BALB/c mice previously immunized with pcDNA3-p30. Active immunization assays of BALB/c mice by use of the recombinant *Ldccys1* gene and p30, as well as the characterization of protective immune responses induced by these immunization schedules are currently in progress.

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IM53 - A VACCINATION PROTOCOL USING BCG, CYCLOPHOSPHAMIDE AND MICROSOMAL FRACTION PROTECTS MICE AGAINST *LEISHMANIA (L.) AMAZONENSIS*

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We have developed a vaccination protocol using BCG, cyclophosphamide and microsomal fraction of *Leishmania (L.) amazonensis* that is able to protect mice against an infection by virulent amastigotes or promastigotes. Protection was evidenced by a reduction of the lesion of vaccinated mice when compared to non-vaccinated control ones. The imunological mechanisms involved in this protection are being studied. We have demonstrated that vaccinated mice present a strong T-cell immunity, as it was shown by a high DTH response. Immunohistochemistry was performed in order to characterize the cellular population in the lesion and draining lymphnode. Immune serum was used to mark amastigotes while monoclonal antibodies were used to highlight $CD4^+$, $CD8^+$ and macrophages. Confocal analysis reveled a huge amount of both $CD4^+$ and $CD8^+$ in the lesion and lymphnode of vaccinated mice. On the other hand, lesions of control mice presented macrophages, but rare $CD8^+$ and $CD4^+$ cells. The amount of these lymphocytes in the lymphnode was smaller than in vaccinated animals. Antibody titration was performed in mice sera. Vaccinated animals presented lower titers of IgG when compared with control ones. Cytokine titration has demonstrated that vaccinated mice present an early IFN-gamma peak, which may be contributing to the control of the parasite. T-cell immunoblotting essays are being carried out in order to study which Fmic proteins are responsible for the protection obtained. When promastigotes are used to infect mice this protection is even more remarkable, than when amastigotes are used.

Apoio financeiro: IOC

IM54 - INTRAMUSCULAR AND SUBCUTANEOUS IMMUNIZATIONS WITH P36(LACK) DNA VACCINE INDUCE A TH1 RESPONSE BUT DO NOT PROTECT AGAINST *L. (L.) CHAGASI* INTRAVENOUS CHALLENGE

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American Visceral Leishmaniasis in Brazil is a zooanthroponosis formerly restricted to rural and peri-urban areas. With the emergence of new foci in urban areas, this disease has assumed an important role in public health and vaccination is an important mechanism of protection. Several vaccination protocols have been tested for different kinds of leishmaniasis and the use of DNA vaccines is an interesting approach due to its capability to induce a long-term cellular immunity. Furthermore, these vaccines stimulate humoral, helper and citotoxic responses, being the last two very important to induce a protective response in murine models of visceral leishmaniasis. In our study, we tested a vaccination protocol where BALB/c mice were primed and boosted via subcutaneous or intramuscular route with PBS, pCI-neo plasmid or pCI-neo-p36(LACK) DNA vaccine (100 mg/dose and 100 mg/dose in 25% of sucrose solution for subcutaneous and intramuscular immunizations, respectively) and challenged intravenously with 1 x 10^7 *L. chagasi* promastigotes. LACK (*Leishmania* homologue of receptors for activated C kinase) is a 36 kDa protein highly conserved between different life cycle stages and species of *Leishmania* and was previously shown to protect against cutaneous infection by *L. (L.) major*, by redirecting pathogenic Th2 to protective Th1 response when administered as a 24 kDa truncated version. Initially, we tested the capability of the vaccine to induce immune response by measuring the production of IFN-gamma and IL-4 by spleen cells stimulated with *L. (L.) chagasi* antigen (50, 100 and 150 µg/mL of soluble antigen and 50 µg/mL of freeze-thawed antigen) or p36(LACK) protein (25 µg/mL) - measured by ELISA - without challenge. In addition, we tested the vaccine capability of protection through determination of parasite burden in spleen and liver by quantitative limiting-dilution culture, four weeks after challenge (time related with the peak of liver parasite burden). Our results show that intramuscular vaccination with p36(LACK) DNA induces a Th1 response (IFN-gamma but not IL-4 production) when spleen cells are stimulated with 50, 100 or 150 µg/mL of *L. (L.) chagasi* soluble antigen, 50 µg/mL of freeze-thawed promastigotes or 25 µg/mL of p36(LACK) protein. In addition, subcutaneous immunization with p36(LACK) only induces Th1 response if spleen cells are stimulated with either 150 µg/mL of *L. (L.) chagasi* soluble antigen or 25 µg/mL p36(LACK) protein. However, this vaccination does not protect against intravenous challenge with 1 x 10^7 *L. (L.) chagasi* promastigotes at 30 days or 12 weeks after booster.

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IM55 - NASAL VACCINATION WITH HSP65 DNA-LOADED MICROSPHERES INDUCES IFN-G AND PROTECTS MICE AGAINST LEISHMANIASIS.

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Leishmaniasis shares various pathological and immunological features with tuberculosis. Especially by the fact that both are caused by intracellular pathogens, and that the protection for both diseases would be provided by cellular immune response. The highly conserved *M.leprae* 65-kDa heat shock protein (HSP65) codified by plasmid DNA (HSP65-DNA) has been shown to effectively control tuberculosis in mice when administered by the intramuscular route. The DNA vaccine was further ameliorated by association with the adjuvant trehalose dimycolate (TDM) followed by entrapment in biodegradable poly-DL-lactide-co-glycolide (PLGA) microspheres. In this work we evaluated the effectiveness of the HSP65-DNA/TDM/PLGA to protect BALB/c mice against cutaneous leishmaniasis using the mucosal (nasal) route of vaccination. The results showed that animals nasally vaccinated with HSP65-DNA/TDM/PLGA microspheres significantly controlled lesion development throughout the infection with fluorescent *L. (L.) amazonensis*, while none of the controls were effective. The parasite loads measured by tissue fluorescence were also significantly smaller in the vaccinated animals. Nasal HSP65-DNA/TDM/PLGA did not affect the basal production of IFN-γ in the mucosa-draining lymph nodes, but significantly increased IL-10 production. Protection was accompanied by a 7-fold enhancement in IFN-γ production in the lesion-draining lymph nodes as compared with non-vaccinated animals. This work showed that nasally instilled DNA codifying *M. leprae* HSP65 entrapped in DMT/PLGA microspheres could effectively protect BALB/c mice against *L. (L.) amazonensis* infection. These preliminary results highlight the feasibility of developing a nasal DNA vaccination against cutaneous leishmaniasis.

Support by: Brazilian Network of Nanobiotechnology (CNPq), REDE-TB (CNPq), FAPERJ and FAPESP.

IM56 - VACCINATION OF SWISS ALBINO MICE AGAINST KALA-AZAR WITH THE FML-VACCINE. USING A SAPOGENIN ADJUVANT

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The FML-vaccine using Riedel De Haen saponin as adjuvant, developed a significant protective effect in the isogenic CB hamster (87.7%, $p<0.01$) and the Balb/c (84.4%, $p<0.001$) models. The FML+Quil-A saponin vaccine also induced protection against infection with *L. (L.) donovani,* in the canine experimental model. Although the adjuvant activity of saponins seems to be extensively proved, an undesirable hemolytic effect has been pointed out as the main restriction to their use in human vaccination. Removal of the glicidic moiety of several saponins abolished their hemolytic effect. In the present work we isolated the Riedel de Haen total sapogenin fraction through acid hydrolysis (2 N H2SO4 at 100 °C for 1h) and filtration. The residue was subjected to column chromatography on silica gel (10 g, 0.6 × 10 cm), eluted with chloroform-methanol (97.5 : 2.5, v/v) (5 ml each eluent). The fractions 40-45 ml showed similar thin layer chromatography profiles in the above described solvent system (R_f 0.40). The product (96 mg) was identified as quillaic acid by spectroscopic methods. The chemical removal of saponin glicidic moieties gave rise to its sapogenin fraction. Its ^{1}H NMR spectrum showed the presence of two signals (d 9.226 and 9.236). The intensity of the signals suggested two conformational isomers of sapogenin R in the ratio 53% of equatorial aldehyde group to 47% of axial aldehyde group. We immunized Swiss females with three doses of 150 mg FML + 100 mg Riedel sapogenin, through the sc route. Control animals received saline or sapogenin R. Thirteen days after the third doses, sera were collected and analyzed for the presence of anti-FML antibodies by the FML-ELISA assay. The levels of anti-FML specific antibodies are expressed as $\log_2$ titers as follows:

Treatment	Total IgG	IgG2a	IgG2b
SALINE	7	5	8
SAPOGENIN R	12	10	15
FML+SAPOGENIN R	18	14	18

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Significant and specific increases in antibody levels (more than two dilutions) were detected in animals vaccinated with FML+ sapogenin in all subclasses of immunoglobulins. Both saponin and sapogenin potentialyzed the synthesis of both IgG2a and IgG2b immunoglobulins related to complement fixation and protection against infection. The IDR to promastygote lysate was slightly higher in FML treated animals than in controls, although non significant differences were founded between the groups. The chemical treatment abolished the saponin slight *in vivo* toxicity, did not affect their aldehyde contents, but gave rise to an enriched axial aldehyde-containing sapogenin R with enhanced potential on antibody humoral response.

Support: CNPQ-UNIVERSAL, FINEP, CEPG-UFRJ, FUJB-UFRJ, FAPERJ.

IM57 - SAPONIN FRACTIONS IN IMMUNOTHERAPY AGAINST EXPERIMENTAL VISCERAL LEISHMANIASIS WITH THE FML-VACCINE.

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The chemotherapy used in control of the human leishmaniasis doesn't guarantee the cure of canine disease. A vaccine therapy against the disease might be an alternative treatment, avoiding dog sacrifice and reducing the reservoir of *L. (L.) donovani* (LD1S) infection. Besides, it could give rise to data about an alternative therapy for human disease. In a previous work, was demonstrated the potential immunoprotective effect of the FML-saponin vaccine, in the isogenic CB hamster (87.7%, $p<0.01$), in the Balb/c (84.4%, $p<0.001$) and Swiss albino mice (85%, $p<0.01$) models. In this work we analized the possible immunotherapeutic effect of the FML- vaccine using purified saponin fractions on Swiss Albino mice infected with *L. (L.) chagasi.* 2-4 months old females were infected with 2 x 10^8 *L. (L.) chagasi* amastigotes by the i.v. route. Fifteen days after infection, animals were treated with three weekly s.c. doses of FML antigen (150 µg) combined with Riedel De Haën saponin or its fractions (100ug each) obtained through silica gel-column chromatography, using $CHCl_3$:MeOH: CH_3COOH:H_2O (15:9:1:2): F1 (tubes 1-2), F2 (tubes 3-14) and F3 (tubes 15-21). F1 (apolar) and F3 (polar) are composed of one different saponin each. Apparently, F2 contains the former two saponins. Fifteen days after the third dose, the delayed type of hypersensitivity (DTH) against *L. (L.) donovani* f/t promastigote lysate antigen was evaluated. The DTH response was significantly higher in mice vaccinated with FML + Riedel De Haën saponin over the saline control (0.42-0.35mm), both at 24h ($p<0.005$) and 48h ($p<0.005$) after injection. Mice treated with FML + F2 fraction showed an even higher response (0.58mm) at 24h post injection ($p<0,005$) suggesting that F2 contains the main active component of Riedel De Haen saponin. and the LDU in liver are in process.

Support: CNPQ-UNIVERSAL, FINEP, CEPG-UFRJ, FUJB-UFRJ, FAPERJ.

IM58 - EFFECTIVE IMMUNOTHERAPY AGAINST CANINE VISCERAL LEISHMANIASIS WITH THE FML-VACCINE

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American visceral leishmaniasis, is an important canine zoonosis against which there is not efficient treatment. Seropositive infected dogs are sacrificed for epidemiological control. Previous studies with the FML-vaccine showed protection against experimental and field kala-azar. Recently we demostred the potential effect of the FML-QuilA saponin vaccine on immunotherapy in five mongrel dogs experimentally infected with *L. (L.) donovani*. A strong protective effect was obtained in 3/5 immunotherapy treated dogs that remained asymptomatic, IDR positive, parasite free and with normal proportions of CD4 and CD21 lymphocytes. CD8 proportions were significantly increased as expected for a *Quillaja* saponin vaccine treatment. In the present work we analized the immunochemotherapy effect of FML-QuilA saponin vaccine in two symptomatics naturally infected Rotweiller dogs that were previously treated with glucantime and allopurinol. The clinical signs only disappeared after the complete vaccination. The immunochemotherapy treated dogs also remained asymptomatic, IDR positive and parasite free, up to 3 years after vaccination and with a significantly reduced ($p<0.05$) time for cure and intensity of symptoms when compared with immunotherapy treated group. Furthermore, the FML-saponin R was used on 21 naturally infected dogs when seropositive to FML but completely asymptomatic. They showed stable anti-FML IgG1 levels, increasing IgG2 levels and 79-95% of positive DTH response, during the whole experiment. Twenty-two months after complete vaccination, 90% of these dogs were still asymptomatic, healthy and parasite free, indicating that the FML-saponin R formulation was effective in the immunotherapy against visceral leishmaniasis of asymptomatic infected dogs.

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IM59 - SWISS ALBINO MICE VACCINATION WITH THE RECOMBINANT NH36 NUCLEOSIDE HYDROLASE OF *LEISHMANIA (L.) DONOVANI*.

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The 36-kDa glycoprotein fraction (GP36) of FML is recognized specifically by sera from human kala-azar patients and by monoclonal antibodies from mice immunized with FML. It protects Balb/c mice from experimental kala-azar (68%). The gene that encodes an antigenic protein component of GP36 was cloned on the basis of a partial peptide sequence. Based on the predicted open reading frame, the single-copy gene was identified as a nucleoside hydrolase (*Ld*NH) with significant similarity to family members identified from other kinetoplastids. NH enzymes play an important role in the acquisition of preformed purines from host sources. The NH36 gene PCR product was inserted downstream from the *malE* gene of *E. coli*, which encodes maltose-binding protein (MBP), resulting in the expression of an MBP fusion protein. In previous work we showed that NH36 protein (5,10 or 16 µg) in combination with Riedel De Haen saponin (100µg) induces a significant enhancement of the IgG2a and IgG2b subtype of antibodies, the *in vitro* proliferative and the DTH responses suggesting the triggering of a protective immune response. In the present work. We immunized Swiss females with three doses of 32 mg of P70 fusion protein (composed of both NH36 and the P40-Maltose Binding Protein MBP) in combination with 100 mg Riedel saponin, through the sc. route. Control animals received either saline, or saponin, or P40-MBP in combination with saponin or P40-MBP alone.

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Thirteen days after the third dose, sera were collected and analyzed for the presence of anti-FML antibodies by the FML-ELISA assay. The levels of anti-FML specific antibodies are expressed as log_2 titers as follows:

Treatment	IgG		IgG2a	
	Title	Log_2	Title	Log_2
Pre immune	1/16	4	1/16	4
Saline	1/32	5	1/32	5
P40	1/16	4	1/64	6
P70 + saponin	1/16384	14	1/512	9
P40 + saponin	1/2048	11	1/64	6

As expected from previous experiments with purified NH36, significant and specific increases in antibody levels were detected in animals vaccinated with P70 + saponin in IgG and IgG2a. immunoglobulins. However, the P40-MBP saponin control itself induces an immunogenic response indicating a degree of cross reactivity between the leishmanial antigen and the MBP control. An infective challenge with 2x 10^8 amastigotes of *L.(L.) chagasi* was performed. The evaluation of the possible vaccine effect on the reduction of parasitic load is under progress.

Support: CNPQ-UNIVERSAL, FINEP, CEPG-UFRJ, FUJB-UFRJ, FAPERJ.

IM60 - SERUM AND STOOL HUMORAL RESPONSE AND ANTIGEN-DRIVEN PEYER´S PATCHES LYMPHOCYTE PROLIFERATION IN MICE AFTER ORAL IMMUNIZATION WITH IRRADIATED *TOXOPLASMA GONDII* TACHYZOITES.

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Toxoplasma gondii is a protozoan that infects mammalians and birds, causing usually benign disease in humans, except in intrauterine fetal infection or in immune deficient patients. The infection is acquired by ingestion of water and food contaminated with oocysts of feline feces or raw meat contaminated with tissue cysts. Thus, the study of the intestinal immunity in this disease is crucial for the vaccine production. In this work, RH strain tachyzoites of *T. gondii* were irradiated with 255 Gy in the 60-cobalt. Mice were immunized with 10^7 tachyzoites, for oral route, with three doses, suspended in milk (anti-peptic) or aluminum hydroxide (anti-acid) or both, for parasite preservation in the stomach. Specific ELISA for IgA and IgG detection was performed in collected feces and blood samples at weekly interval. Proliferation of Peyer´s patch lymphocytes from C57Bl/6j mice immunized with 255 Gy irradiated tachyzoites was compared to splenocytes from same mice, using infected mice with 10 cysts of Me49 strain as controls. After 15 days of the last oral dose, Peyer´s patches or spleen lymphocytes were suspended in RPMI 1640 medium supplemented with 10% FBS and antibiotics, and 2 x 10^5 cells/well were plated in 96 well plates and stimulated with *T. gondii* antigen (10 µmg/ml). After 48 h of incubation at 37ºC under 5% CO_2, cultures were pulsed for 18 h with [^{3}H] thymidine, harvested and their DNA radioactivity determined. There are few differences between the three challenged groups according to the preservative medium. The production of IgA and IgG in the serum was clearly seen after the first oral dose, increasing thereafter, more intense in the IgG response, but immunized mice produced smaller levels of serum antibodies as compared to chronically infected mice. Fecal IgG production was absent after immunization but IgA production was higher than controls during the first doses but decays after this, suggesting tolerance, with chronically infected mice presented higher levels of IgA in feces. However, studies in cell proliferation of Peyer´s patches lymphocytes shows a great response in immunized mice, specially at the 1st and 2nd doses, similar or higher than chronically infected mice, but, after the 3rd dose, this proliferation subsides. Challenging studies are in progress. Those data shows clearly that the development of mucosal immunity could be attained after oral immunization with sterile parasites, but more improvements must be attained before a complete understanding of mucosal immunity in toxoplasmosis. Our approach would be a good tool for studies in vaccine development in this model, especially for the first scrutiny of oral vaccines that could be used in field for free-living or stray cats, one of the main sources of *Toxoplasma* infection of ruminants.

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IM61 - DETECTION OF ANTI-LACK ANTIBODIES IN VISCERAL LEISHMANIASIS CANINE SERA

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Current serologic tests for the diagnosis of leishmaniasis, such as direct agglutination test (DAT), immunofluorescent-antibody test (IFAT) and the enzyme-linked immunosorbent assay (ELISA) use crude antigen preparations and are limited in terms of both specificity and assay reproducibility. Thus, emphasis has been placed on the characterization of *Leishmania* antigenic components as a tool for obtaining specific diagnosis. The LACK (*Leishmania* homolog of receptors for activated C kinase) antigen is a 36 KDa protein highly conserved and expressed in promastigote and amastigote forms of different *Leishmania* species. Here, we have investigated the presence of anti-LACK antibodies in a panel of canine American Visceral Leishmaniasis (VL) sera. A PCR product spanning the *L. (L.) chagasi* LACK coding region was cloned into the pPROEX vector for expression in *Escherichia coli* and the LACK protein containing a tag of six histidin residues (LACK-HIS) was purified by Nickel affinity chromatography. IFAT Negative sera from asymptomatic dogs were used as negative controls and to establish cut-off values. Anti-LACK antibodies were detected by ELISA in a high proportion of sera of symptomatic, IFAT positive dogs, that have also tested positive in the parasitological evaluation. Anti-LACK antibodies were also detected in sera of asymptomatic dogs, but that tested positive in the parasitological evaluation. Our preliminary findings suggest that LACK protein is a potential tool for the serological diagnosis of VL in the New World.

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IM62 - DNA VACCINATION WITH A PLASMID CONTAINING THE GP72 GENE OF *TRYPANOSOMA CRUZI*.

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The GP72 is a glicoprotein of *Trypanosoma cruzi* involved in the adhesion of the flagellum to the parasite's body. The deletion of the gp72 gene produces atypical forms of reduced infectivity in mice. Here we tested the immunogenicity of a gp72 containing-plasmid as a DNA vaccine against the *T. cruzi* infection. Female Balb/c mice were immunized with four doses of 100 ug of the plasmid. The control group received same doses with the empty vector plasmid pcDNA3.

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After the vaccination schedule, mice's sera were taken in order to perform ELISA assays. The delayed type hypersensibility (DTH) reactions were recorded and the mice were challenged with Tulahuén blood-trypomastigotes. The DTH reactions of the immunized mice were increased ($p<0.05$) compared with the control's reactions, although the optical densities of the sera tested by ELISA were not different between the groups. After the challenge, the immunized mice displayed a slightly lower level of parasitemia than the control group. On the basis of the present results we will attempt to improve the vaccine's immunogenicity employing the gp72-containing plasmid, testing different immunization schedules or multi-component vaccines.

This work was supported by CONICET and FAPESP.

IM63 - IMMUNIZATION WITH THE A2 ANTIGEN AGAINST *LEISHMANIA (L.) AMAZONENSIS* INFECTION: COMPARISON OF THE PROTECTION INDUCED BY PROTEIN OR DNA VACCINATION.

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Successful host protection in experimental leishmaniasis involves a complex Th1 cell-dependent, multicytokine–mediated mechanism. This response, that is probably initiated by IL-12 and then driven primarily by IFN-γ, culminates with the macrophage activation and intracellular killing of parasites. Vaccination with plasmid DNA encoding a specific leishmanial antigen has been shown to be more effective than vaccination with leishmanial protein plus IL-12 in sustaining the Th1 responses required for long-term protection. Besides, recombinant proteins are more expensive and time consuming to produce in relationship to plasmid DNA. Here, we report experiments comparing the efficacy of vaccination with A2 protein and A2 DNA against *L. (L.) amazonensis* infection. The A2 gene was cloned into the pET-16b and pCDNA3 vectors, for expression in *Escherichia coli* and eukariotic cells, respectively. Groups of female BALB/c mice were immunized *subcutaneously* with 2 doses, at 3 weeks intervals, with A2-HIS protein (50 µg) alone or in combination with rIL-12 (1 µg) or 250 µg of *Corynebacterium parvum*. For DNA immunization, animals received the A2-pCDNA3 plasmid (100 µg) alone or in combination with the IL-12-pCI plasmid (100 µg) by *intramuscular* injection in the tibia muscle. Mice were infected 4 weeks after the last dose with $1x10^5$ stationary phase promastigotes of *L. (L.) amazonensis.* Our findings demonstrated that A2–HIS protein induced a parasite specific Th1 immune response, characterized by the high levels of IFN-γ and low levels of IL-4 or IL-10 and provided protection against challenge infection only in the presence of Th1 adjuvants, including *C. parvum.* A2 DNA vaccination induced a specific Th1 immune response and provided protection against challenge infection with *L. (L.) amazonensis* in the absence of IL-12 DNA.

Support: CAPES, PRPq/UFMG.

IM64 - IMMUNE RESPONSES INDUCED BY *LEISHMANIA (L.) DONOVANI* A2, BUT NOT BY LACK ANTIGEN, ARE PROTECTIVE AGAINST EXPERIMENTAL *LEISHMANIA (L.) AMAZONENSIS* INFECTION

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Leishmania (L.) amazonensis is one of the major etiologic agents of a broad spectrum of clinical forms of leishmaniasis and has a wide geographical distribution in Americas, which overlaps with the areas of transmission of many other *Leishmania* species. The LACK and A2 antigens are shared by various *Leishmania* species. A2 was previously shown to induce a potent Th1 immune response and protection against *L. (L.) donovani* infection in BALB/c mice. LACK is effective against *L. major* infection, but no significant protection was observed against *L. (L.) donovani* infection, in spite of the induction of a potent Th1 immune response. In an attempt to select candidate antigen for American leishmaniasis vaccine, we investigated the protective effect of these recombinant antigens (rLACK and rA2) and interleukin 12 (rIL-12) against *L. (L.) amazonensis* infection in BALB/c mice. As expected, immunization with either rA2/IL-12 or rLACK/IL-12 was able to induce a robust Th1 response prior to infection. However, only the BALB/c mice immunized with rA2/IL-12 were protected against infection. A sustained IFN-γ production, high levels of anti-A2 antibodies and low levels of antiparasite specific antibodies were detected in these mice, after infection. In contrast, mice immunized with rLACK/IL-12 displayed decreased levels of IFN-γ and high levels of both anti-LACK and parasite specific antibodies. Curiously, the association between rA2 and rLACK antigens in the same vaccine completely abrogated the rA2 specific IFN-γ and humoral responses and, consequently, the protective effect of the rA2 antigen against *L. (L.) amazonensis* infection. We concluded that A2, but not LACK fits the requirements to compose a safe vaccine against American leishmaniasis.

Support: CAPES, PRPq/UFMG.

IM65 - PROTECTIVE IMMUNITY AGAINST *TRYPANOSOMA CRUZI* INFECTION IN A HIGHLY SUSCEPTIBLE MOUSE STRAIN FOLLOWING SIMULTANEOUS ADMINISTRATION OF PLASMIDS CONTAINING GENES EXPRESSED IN DIFFERENT DEVELOPMENTAL STAGES.

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Genetic immunization of BALB/c or C57BLl/6 mice with plasmids containing a single *Trypanosoma cruzi* gene induced protective immunity against lethal parasitic infection. In contrast, similar immunization of the highly susceptible mouse strain A/Sn did not promote mouse survival after challenge. Here, we tested whether the protective efficacy of genetic vaccination in this mouse strain could be improved by combining two different plasmids in the same immunization regimen. We used plasmids containing genes encoding the catalytic domain of *T. cruzi trans*-sialidase or the Amastigote Surface Protein-2. These antigens are expressed on the surface of trypomastigote or amastigote forms, respectively. In spite of the fact that these two antigens share some structural identity, we observed that immune responses were specific for the homologous recombinant antigen. After challenge with trypomastigotes of the Y strain, a significant proportion (>85%) of A/Sn mice immunized simultaneously with both plasmids survived the infection. Mice immunized with individual plasmids had a variable survival and 100% of control mice died. Histological

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studies performed in the surviving mice 100 days after challenge revealed a reduced inflammatory response in most tissues with the exception of the liver.

Our results provided evidence that a significant proportion of the highly susceptible A/Sn mice can develop protective immunity against lethal infection if immunized simultaneously with plasmids harboring genes expressed in different mammalian developmental stages of the parasite.

Supported by FAPESP and CNPq.

IM66 - RESPONSE OF IL-12P40$^{-/-}$ C57BL/6 AFTER VACCINE-INDUCED IMMUNITY AGAINST *LEISHMANIA (L.) AMAZONENSIS* INFECTION

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Protozoa of the genus *Leishmania* are intracellular parasites and have been identified from patients with diverse clinical forms, including cutaneous leishmaniasis, diffuse cutaneous leishmaniasis (DCL), and visceral leishmaniasis. Infection of mice with *L. (L.) major* leads to the development of either a Th1 or a Th2 response. While activation of cells secreting IL-4 promotes a Th2 response, production of IL-12 induces secretion of IFN-γ by natural killer cells and T cells and favors Th1 cell differentiation and proliferation. A Th1 response plays a key role in controlling the infection. In agreement with these findings, adoptive or vaccine-induced protection against leishmaniasis is largely dependent on cell-mediated immunity, Th 1 lymphocytes and IFN-γ. Induction of a Th1 response is dependent on the presence of IL-12 whilst lymphocytes are activated. This study was aimed at evaluating the immunogenicity of a vaccine composed of killed *Leishmania (L.) amazonensis* promastigotes (Leishvacin®), produced by Biobrás, since the role of Th1 cytokines in vaccine-induced immunity with Leishvacin® in experimental models or in humans is not yet elucidated. To evaluate the role of IL-12 in the vaccine-induced immunity against *L. amazonensis* infection, C57BL/6 Interleukin-12-Deficient Mice (IL-12p40$^{-/-}$ C57BL/6) and wild type controls (WT) were vaccinated in the base of the tail. Each animal received two inoculations at an interval of seven days, each dose containing 100 µg of protein vaccine plus 250 µg of *Corynebacterium parvum*. Twenty-eight days after the second dose, the animals received a further 10 µg of vaccine, without adjuvant. Seven days after this booster, the animals were challenged with *L. (L.) amazonensis* in the left hind footpad. Lesion size was measured for 10 weeks. As described previously, this protocol of vaccination protected C57BL/6 mice against infection: these mice showed smaller lesions and smaller parasite numbers than non-vaccinated controls. IL-12p40$^{-/-}$ mice were more susceptible to infection than WT, developing large and progressive lesion. Comparison between vaccinated and non-vaccinated IL12p40$^{-/-}$ mice showed a statistically significant difference from 3 to 10 weeks post infection. Lymph node and spleen cell cultures from C57BL/6 vaccinated mice presented higher levels of IFN-γ when compared to non-vaccinated mice. Cells from IL-12p40$^{-/-}$ mice produced practically undetectable levels of IFN-γ *in vitro*, regardless of vaccination. IL-4 was not detected in supernatants from lymph node or spleen cell cultures. Our results show that the early control of de *L. (L.) amazonensis* infection conferred by vaccination is independent of IL-12. It is possible that IFN-γ from CD8^{+} cells is playing a role in controlling parasite growth in vaccinated IL-12p40$^{-/-}$ mice.

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IM67 - ISOLATION, SEQUENCING AND EXPRESSION OF GENES FROM *L. (VIANNIA) BRAZILIENSIS* ENCONDING PROTECTIVE ANTIGENS AGAINST CUTANEOUS LEISHMANIASIS.

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IMmunization with genes encoding antigens *Leishmania* Activated C Kinase (*lack*), Thiol-Specific Antioxidant (*tsa*), *Leishmania* Elongation Initiation Factor (*leif*) and *Leishmania (L.) major* Stress Inducible Protein 1 (*lmsti1*) from *Leishmania (Leishmania) major* induces potent immune response mediated by CD4 Th1 cells and protective immunity against *L. (L.) major* infection in highly susceptible BALB/c mice (reviewed by Reed & Campos-Neto, 2003, Curr Opin Immunol. 15:456). The aim of the present study was to isolate, sequence and express these genes from *L. (V.) braziliensis*, the specie widely distributed in Brazil and implicated with cutaneous and mucocutaneous leishmaniasis. The genes were isolated by PCR amplification using genomic DNA from *L. (V.) braziliensis* strain M2903 and primers corresponding to the ORF of each gene, cloned in *pMOS* vector, and sequenced. The predict amino acid sequence analysis of *lack*, *tsa*, *leif*, and *lmsti1* showed, respectively, 96%, 83%, 98%, and 92% of identity to that described in *L. (L.) major*. Northern blot analysis showed that these genes hybridize with transcripts present in both promastigotes and amastigotes of *L. (V.) braziliensis.* The genes were also subcloned in prokaryotic expression vector PET-22b (+) or the eukaryotic expression vector pcDNA3 in fusion with the nucleotide sequence encoding the signal peptide of the mouse Ig kappa chain. The bacterial recombinant proteins were successfully purified. The plasmids harboring these genes isolated from of *L. (V.) braziliensis* are currently been tested in their ability to induce immune responses in mice.

Supported by FAPESP, CNPq and CAPES.

IM68 - *TOXOPLASMA* ENVIRONMENTAL SPREAD EVALUATED BY STRAY DOG SEROLOGY IN COASTAL AREAS FROM SOUTHERN BRAZIL.

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In São Paulo, nearly 60% of adults were infected by *Toxoplasma gondii*, a protozoan that can cause human disease in minor segments of the infected people, specially when congenital infection or immune disease coexists with the infection. The disease is transmitted orally by ingestion of cysts in raw meat or oocysts from cat stools, with spreading to the environment. The detection of those infective forms are difficult and depends on sample size, as the agent had an exquisitely highly efficient transmission. In domestic animals, like dogs, toxoplasmosis was usually asymptomatic, despite some reports of occasionally severe disease, more frequent in younger dogs. There are few reports of an ocular involvement associated to the toxoplasmosis in dogs. Both *Toxoplasma* infective forms infected dogs, usually omnivorous and carnivorous. Recently, there are reports of the seroprevalence of this infection in stray dogs, inferring that this measure could represent the environmental risk of *Toxoplasma* contamination. Based on this meaning, we studied 151 blood samples colleted from stray dogs from Caraguatatuba, at northern coast of São Paulo State in Southern Brazil, that were captured usually to control other zoonotic diseases, as rabies or leptospirosis. The sero-prevalence of *T. gondii* infection was determined by a specific IgG ELISA,

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with inconclusive results confirmed by Western-blot strips from *T.gondii* saline extracts. We found a prevalence of 55.1% (47.0 – 63.0% 95%CI) in those dogs, similar to those reported in other costal areas as Trinidad & Tobago and also in urban areas of São Paulo State. Those data suggests that there is a high environmental contamination in those areas, especially when the incidence was corrected to the short life span of dogs, resulting in a almost 8-10% year conversion. Those data provides an indirect but useful tool that could help in the evaluation of environmental measures to reduce toxoplasmosis incidence in risk areas.

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IM69 - USE OF A CYSTEINE PROTEINASE FROM *LEISHMANIA (L.) CHAGASI* IN SERODIAGNOSIS OF AMERICAN VISCERAL LEISHMANIASIS

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Visceral leishmaniasis (VL) is a parasitic disease associated with high mortality, caused by *Leishmania (L.) donovani*, *L.(L.) infantum* and *L. (L.) chagasi*. Parasitological diagnosis, characterized by demonstration of parasites in tissue samples, is extremely limited by low sensibility and reproducibility (Singh et al., 2003). Serological tests, currently used in VL diagnosis, are based on different methods of antibody detection which include indirect immunofluorescence (IFI) (Pappas et al., 1983), direct agglutination test (DAT) (Harith et al., 1986), immunoblot analysis (Hoerouf et al., 1992), and enzyme-linked immunosorbent assay (ELISA) (Badaró et al., 1996). However, the use of whole parasite extract limits the test specificity due to the ocurrence of cross-reaction with other patogens such as *Trypanosoma cruzi*, *Echinococcus granuloses* and mycobacteria (Badaró et al.,1986; Chiller et al., 1990; Reed et al,1987), leading to the misinterpretation of the serological assays. Thus, the use of recombinant purified antigens has been emphasized as a tool for obtaining more specific serological tests. In the present work we evaluated the use of a recombinant cysteine proteinase from *L. (L.) chagasi* amastigotes (R30) in ELISA for diagnosis of American visceral leishmaniasis. The gene encoding the cysteine proteinase was previously isolated by PCR amplification, cloned in pHis vector and the resulting recombinant antigen was purified by affinity chromatografy in a His-tag column. ELISA was performed with serum samples from Brazilian areas endemic for kalazar and our preliminary data showed a sensibility of 71% when R30 was used as antigen. The test specificity was evaluated by use of serum samples from patients with tuberculosis, cutaneous leishmaniasis and Chagas' disease and showed a specificity of 92% with R30 as antigen, in contrast to that displayed with *L. (L.) chagasi* whole amastigote and promastigote extracts which was of 42 and 31%, respectively. These data suggest that the *L. (L.) chagasi* recombinant cysteine proteinase is an attractive antigen for diagnosis of VL. The evaluation of the sensibility and specificity of the *L. (L.) chagasi* recombinant protein as antigen in ELISA is currently extended to a higher number of kalazar samples, as well as with sera from patients with other diseases.

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IM70 - WESTERN BLOT IDENTIFICATION OF *PHYTOMONAS SERPENS* ANTIGENS RECOGNIZED BY HUMAN CHAGASIC SERA.

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Chagas' disease or American trypanosomiasis affects about 16 million people in Latin America and have great interest on account of the intense cross reactivity of Chagas' disease patients sera with sera from patients with other diseases, mainly caused by other flagellates, in the serologic diagnosis.The search of cheaper, safer and more efficient antigens for the discrimination of Chagas' disease patients has been the objective of many research projects, mainly with recombinant antigens. The immune cross reactivity of *Trypanosoma cruzi* with other Trypanosomatidae family members was early largely demonstrated and in the present work some antigens of fruit flagellates (*Phytomonas serpens*) was demonstrated to be involved in immune cross reactions with *T. cruzi*. *P. serpens* proteic bands recognized by antibodies present in Chagasic and American tegumentary leishmaniasis patients sera was determined by Western blot technique.The results showed the bands of 250, 30 and 26 Kda as the most frequently recognized proteic bands of *P. serpens* by chagasic huma sera and 62, 53 and 43 kDa the most frequently recognized by leishmaniotic human sera. The same reaction was done with *T. cruzi* antigens and human chagasic and leishmaniotic sera.The results also showed strong cross reaction between these Trypanosomatids.The intense cross reactivity among Trypanosomatids and the possibility of induction of immunity by oral ingestion of plant flagellates suggest the possible participation of antigens of *P. serpens* in the evolution of Chagas' disease immunity.

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IM71 - *TRYPANOSOMA RANGELI* AND *TRYPANOSOMA CRUZI*: COMPARATIVE STUDY OF SEROLOGICAL CROSS-REACTIVITY OF IMMUNE MICE SERUM AGAINST EPIMASTIGOTE AND TRYPOMASTIGOTE FORMS

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Trypanosoma rangeli and *Trypanosoma cruzi*, the etiological agent of Chagas disease, are hemoflagellate protozoan parasites which infects a variety of mammals, including humans, in both Central and South America. Despite the non-pathogenic nature of *T. rangeli* to mammal hosts, this parasite plays an important role in Chagas disease epidemiology since triatomine vectors and mammalian reservoirs are shared in a wide geographical area. Comparative studies of the antigenic composition of culture epimastigotes of *T. cruzi* and *T. rangeli* revealed that these parasite species shares ate least 60% of their soluble antigenic composition. Thus, the antigenic overlap and the sympatry of these parasites have major influence on serological diagnosis of Chagas disease. The aim of this study was to evaluate the serological cross reactivity of immune mice serum infected with *T. cruzi* (Y strain) or *T. rangeli* (SC-58 and Choachi strains) against homologous and heterologous antigens (culture epimastigotes and trypomastigotes) by indirect immunofluorescence assays (IFA). Experiments were performed in duplicate using a anti-Mouse IgG conjugate labelled with FITC (Sigma). Our results showed that anti-*T. cruzi* serum was able to recognize both forms of either homologous and heterologous antigens. Anti-*T. rangeli* serum of both studied strains strongly reacted with homologous trypomastigotes and epimastigotes but weakly recognized heterologous trypomastigote forms. Moreover, anti-*T. rangeli* serum showed negative results when assayed with *T. cruzi* epimastigotes. These results suggest that infection by *T. rangeli* produces antibodies with higher specificity when compared with antibodies produced in response to *T. cruzi* infection. Also, the herein reported differences may be

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attributed to antigenic variation of the studied strains and forms. Further Western blot assays will be performed in order to compare the antigenic profiles of both epimastigote and trypomastigote forms of each parasite species.

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IM72 - CANINE VISCERAL LEISHMANIASIS IN ARAÇATUBA (SP): PARASITOLOGICAL, IMMUNOLOGICAL AND MOLECULAR DIAGNOSIS.

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Aiming the improvement of the diagnosis of canine visceral leishmaniasis (CVL) in an endemic area at the northwest of São Paulo state – Brazil, the efficacy of parasitological, immunological and molecular diagnosis methods were studied. Animals, with and without clinical signs of the disease and with direct search of parasites positive in lymph nodes smears and/or antibodies detection by ELISA were selected for the study. According to the clinical signs of the disease, 89 dogs attended in the Veterinary Hospital of UNESP in Araçatuba (SP) were divided into three groups: symptomatic, oligosymptomatic and asymptomatic. Twenty-six dogs from non-endemic area of visceral leishmaniasis were used as negative controls. Fine-needle aspiration biopsy (FNA) of popliteal lymph node was collected, stained by Diff-Quick®, direct immunofluorescence and immunohistochemistry, as well as parasite DNA were amplified and detected by PCR in those samples. The dogs were classified as: symptomatic, 35%; oligosymptomatic, 22%, asymptomatic, 20%; and negative control, 23%. The sensitivity was 75.61% for direct search of parasite in FNA stained by Diff-Quick®, 92.68% for direct immunofluorescence, 92.68% for immunohistochemistry and 100% for PCR in the symptomatic group; 32%, 60%, 76% and 96% for the oligosymptomatic and 39.13%, 73.91%, 100% and 95.65% for the asymptomatic group, respectively. The control group, dogs from non-endemic area of visceral leishmaniasis, presented negative results in all the studied methods, reflecting a specificity of 100% of the methods used in the present study. The correlation between different clinical groups for each methodology showed the direct search of parasites and direct immunofluorescence as the best method for diagnosis of symptomatic cases. ELISA and the immunohistochemistry in FNA showed best results in the diagnosis of the asymptomatic cases. The results showed high sensitivity when immunolabelling techniques were used in relation to the direct search of parasites and the immunohistochemistry had higher sensitivity than the immunofluorescence. PCR presented the highest sensitivity among all the methods used, however, the method detects the parasite DNA and not the parasite itself and it does not reflect the stage of the infection and/or the disease.

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IM73 - CROSS-REACTION BETWEEN *LEISHMANIA (L.) CHAGASI* WHOLE ANTIGEN AND ANTIBODIES OF DOGS FROM NON ENDEMIC AREA INFECTED WITH *EHRLICHIA CANIS*, *TOXOPLASMA GONDII*, *BABESIA CANIS AND DIROPHILARIA IMMITIS* IN ELISA ASSAY USING PROTEIN A.

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Leishmaniasis is a disease caused by a *Leishmania* protozoa. This disease has great importance in public health because dogs are the domestic reservoirs. These protozoa can cause self-healing diseases and lethal visceral leishmaniasis. Infected dogs may develop a severe syndrome characterized by chronic evolution of viscerocutaneous signs, which result from *Leishmania* multiplication in macrophages of spleen, liver, bone marrow, lymph nodes and skin. ELISA is a useful method of diagnosis because of its high sensitivity and specificity. However, this method presents limitations due to cross-reaction with *Ehrlichia canis,* and *Babesia canis*. Serum from symptomatic mixed breed dogs from a region of high incidence of visceral leishmaniasis in Brazil were examined for the presence of antibodies using peroxidase conjugate of either protein A or anti-dog IgG in ELISA assay. The presence of cross-reaction between *L. (L.) chagasi* whole antigen and serum from dogs from areas non-endemic for leishmaniasis and infected with *Toxoplasma gondii* (6), *Ehrlichia canis* (15) e *Babesia canis* (10) and *Dirophilaria immitis* (19) was also investigated in both systems. The results showed that protein A ELISA system is more sensitive than anti-IgG to detect positive animals. In direct comparison with anti-immunoglobulin conjugate, enzyme-linked protein A resulted in higher absorbance values for positive sera without a corresponding increase in absorbance values for sera from non infected dogs. The effect was an increase in the distance between positive and negative values, which aided in the interpretation of the results. In addition, no cross-reaction occurred between *L. (L.) chagasi* whole antigen and serum tested. The ELISA optical density values of the tested serum were similar to those found in healthy dogs from non-endemic areas. These results are important and indicate that ELISA assay using *L. (L.) chagasi* whole antigen from promastigotes associated with protein A can be useful to serological diagnosis of visceral leishmaniasis in dogs.

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IM74 - THE USE OF THE *TRYPANOSOMA CRUZI* RECOMBINANT COMPLEMENT REGULATORY PROTEIN TO EVALUATE THERAPEUTIC EFFICACY FOLLOWING TREATMENT OF CHRONIC CHAGASIC PATIENTS

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One of the greatest concerns in Chagas' disease is the absence of reliable methods for the evaluation of chemotherapy efficacy in treated patients. The available tests, which are able to evaluate cure after the specific treatment, are the complement mediated lysis (CoML) and flow cytometry, but they are not feasible for routine clinical use. In this study, we evaluated an ELISA test based on the recombinant *Trypanosoma cruzi* complement regulatory protein (rCRP) as a method to determine parasite clearance in comparison to the CoML and other methods such conventional serology, hemoculture and PCR in sera samples of 31 patients collected before and after the treatment, followed for an average of 27.7 months after chemotherapy. The results showed that the percentage of patient samples that were positive by rCRP ELISA was reduced from 100% to 70.3%, 62.5%, 71.4% and 33.4% in the first, second, third and fourth years after treatment respectively, while the samples positive by CoML were reduced to 85.2%, 81.2%, 71.4% and 33.4% during the same period, demonstrating the same significant tendency in the reduction of positive samples. On the other hand, the conventional serology (CS) tests did not present this reduction. The percentage of samples positive by PCR was initially 77.4%, and decreased to 55.5%, 68.7%, 47.7% and 50.0% at the fourth year after treatment, confirming

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the drastic clearance of circulating parasites after treatment. Our results strongly suggest that the rCRP ELISA was capable of detecting the early therapeutic efficacy in treated patients, and confirmed its superiority over the CS tests and parasitologic methods.

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IM75 - THE CLINICAL SPECTRUM IN CANINE VISCERAL LEISHMANIASIS AND THE CHANGES IN THE ISOTYPES PATTERNS OF IMMUNOGLOBULINS

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Dogs are considered the most important vertebrate reservoir of the disease due to its greater prevalence and the frequency of *Leishmania* amastigotes in the skin of these animals. In CVL, the immunological mechanism underlying the susceptibility or resistance to severe disease remains for less defined. Policlonal activation of B-cells leading to high titers of circulating antibodies are found in the course of *L. (L.) chagasi* infection and the detection IgG ant-*Leismania* antigens is an important diagnostic to in identifying case of CVL. In the present study were evaluated 40 naturally infected dogs by *Leishmania (L.) chagasi* with different clinical features and 20 non-infected dogs as a control group. The infected animals were classified according to their clinical symptoms in three groups: asymptomatic, oligosymptomatic and symptomatic dogs. These animals were submitted for a detail analysis of serological parameters by Enzyme Linked Immunosorbent test (ELISA). Serum samples were tested from a 1:80 until 1:327,000 dilutions to determine the title limit of each sera by a display of specific monoclonal anti-canine isotype antibodies (IgA, IgM, IgE, IgG, IgG1 and IgG2), employing a soluble *L. (L.) chagasi* antigen. The results show an association between higher levels of IgG1 with asymptomatic animals. In the other hand, higher levels of IgG, IgG2, IgA and IgE were observed in oligosymptomatic and symptomatic dogs. Our results emphasize that progression of disease in dogs is characterized by appearance of specifics isotypes of immunoglobulins (mainly IgG2), which may contribute to aggravation of the clinical status of the infected dog. Furthermore, the high production of IgG2, IgA and mainly IgE in oligosymptomatic and symptomatic group, might suggest an association of this clinical feature with a type 2 immune response.

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IM76 - AVALIAÇÃO DE DESEMPENHO DA PESQUISA DE ANTICORPOS IGG ANTI-PROMASTIGOTAS VIVAS DE *LEISHMANIA (V.) BRAZILIENSIS,* DETECTADOS POR CITOMETRIA DE FLUXO, PARA A IDENTIFICAÇÃO DE LEISHMANIOSE TEGUMENTAR AMERICANA ATIVA.

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Recentemente desenvolvemos um método de detecção de anticorpos IgG anti-formas promastigotas vivas (AAPV-IgG) de *Leishmania (V.) braziliensis* por citometria de fluxo. Para avaliar a aplicabilidade do novo método em identificar LTA em atividade clínica, foram submetidos à AAPV-IgG e à reação de imunofluorescência indireta (RIFI), soros de 145 indivíduos de área endêmica, classificados em dois grupos quanto a ausência/presença de lesão (L-, n=67; L+, n=78). Os resultados da AAPV-IgG foram expressos sob a forma de percentual de parasitos fluorescentes positivos (PPFP) na diluição do soro 1:1024, no intervalo de 0-100%. Os resultados da RIFI foram expressos em títulos de IgG, considerados como positivos títulos iguais ou superiores a 1:40.. O desempenho de ambos os testes, foram avaliados segundo diferentes índices, incluindo sensibilidade, especificidade, valores preditivos, acurácia, índice J de Youden, "receiver operating characteristic curve" (curva ROC) e a razão de verossimilhança (RV). Na análise do desempenho da AAPV-IgG, três pontos de corte foram selecionados, incluindo PPFP de 20%, 50%, 60%. O limiar de PPFP £20% mostrou-se de grande valor diagnóstico, uma vez que exclui a possibilidade de LTA ativa (RV=0,07). O ponto de corte de 50% distinguiu 92% (72/78) dos pacientes L+ como positivos e 81% (54/67) dos indivíduos L- como negativos. No entanto, a análise da curva ROC indicou o estabelecimento de 60% de PPFP como o ponto de corte, no qual a AAPV-IgG apresentou um ganho real em relação à RIFI ³1:40, incluindo sensibilidade (88/92%), especificidade (57/87%), valor preditivo positivo (70/89%) e negativo (81/91%), probabilidade de doença pós-teste negativo (19/9%), acurácia (74/90%) e índice J de Youden (51/80%). Além disto, valores de PPFP>60% (RV=7,98) demonstrou que a AAPV-IgG pode contribuir para o esclarecimento diagnóstico de LTA ativa. Por outro lado, o RV=2,41 para a RIFI foi desprovido de valor diagnóstico. Em conjunto, nossos resultados demonstraram que a AAPV-IgG, por citometria de fluxo, apresenta-se como um novo instrumento para o diagnóstico sorológico da LTA.

IM77 - THE IMPORTANCE OF USING DIFFERENT SEROLOGICAL APPROCHES IN CLINICAL STUDIES AFTER ETIOLOGIC TREATMENT OF CHAGAS' DISEASE.

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Objectives: Aiming to evaluate the performance of different serological approaches to identify post-therapeutic cure during chronic Chagas'disease, we have analyzed the serological status of Chagas' disease patients after specific etiologic treatment during chronic infection, using conventional and alternative methods.

Methods: Sera samples from 60 chronic Chagasic patients living in Berilo/ MG, who have been previously submitted to specific etiologic treatment, were comparatively screened for their IgG reactivity by conventional serology (CS) by RIFI and ELISA – EIE (both from BioManguinhos - FIOCRUZ), besides three alternative tests, including recombinant EIE – EIEr (BioManguinhos - FIOCRUZ), PAGIA (DIAMED) and by flow cytometry analysis of anti–fixed epimastigotes antibodies (FC-AFEA), described by Cordeiro et al, 2001, referred as non-conventional methods (NCS).

Results and Conclusions: Using the positivity on both RIFI and EIE as the criterion for classic serological diagnosis of Chagas'disease, we observed that 87% (52/60) of the treated patients remained positive after treatment; whereas 8%(5/60) and 5% (3/60) of them became serologically indeterminate or negative, respectively. Comparative analysis confirmed that individuals with negative (3/ 3) or indeterminate (5/5) results by CS presented negative results by NCS. Interestingly, 25% (13/52) and 2% (1/52) of individual with positive CS showed negative results on EIEr and FC-AFEA, respectively. Taken together, the EIEr results reached 31.5% (18/57) of sero-dissociation between positive and indeterminate CS. These data showed post-therapeutic serological status

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consistent with those previously reported by Krettli and Brener 1976 for chronic Chagas' disease, with serological cure observed in 5% of treated patients besides 30% of sero-dissociation conventional serology and the alternative methods. Future studies are currently under investigation in our laboratory in order to validate the EIEr findings with those previously presented by Martins-Filho et al 1995 using anti-live trypomastigotas antibodies for cure assessment after treatment of chronic Chagas'disease. Financial Support: PIBIC/FIOCRUZ.

IM78 - CANINE VISCERAL LEISHMANIASIS: HISTOPATHOLOGICAL AND IMMUNOHISTOCHEMICAL STUDY OF EAR SKIN BIOPSIES

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Canine visceral leishmaniasis is an endemic disease in Brazil and it has been increased dramatically in the suburban areas of large cities. Naturally infected dogs with *Leishmania (Leishmania) chagasi* were obtained from Santo Agostinho Clinical Veterinary, Belo Horizonte, MG, Brazil. Forty-four naturally infected animals were clinical classified in asymptomatic, oligosymptomatic and symptomatic. Serological exams of all animals (IFAT, Complement Fixation and ELISA) were positive. Skin fragments of ears of all animals were collected during the clinical exams by veterinarian biopsies (punch of 4mm). All the ear skin tissues were fixed in a solution of buffered formalin 10%. Then, these tissues were analyzed for histopathological and immunohistochemical studies. The main lesion observed in the skin of all infected animals was a diffuse chronic inflammatory reaction in the upper dermis and in focus around vessels and/or glands in the deep dermis. The cellular exudate was composed by mainly macrophages, plasmocytes and lymphocytes. The histopathological picture observed was similar among all cases and it was independently considering the clinical aspects. The streptoavidin-peroxidase technique was carried out to detect intracellular amastigotes forms of *Leishmania* in the paraffin tissues. Our results have indicated an increasing positivism when we consider the immunohistochemical results. In fact, 15,9% positive cases observed in conventional histology (H&E) increased to 34% of positive cases after immunohistochemical observation.

Our histological and immunohistochemical previous results (Xavier et al., 2001) showed asymptomatic and oligosymptomatic dogs with similar ear skin tissue parasitism as the symptomatic dogs. Moreover, *Leishmania* intracellular amastigotes forms were observed independently of the intensity of the cellular exudate. In this work we have observed the similar results. Also, the immunohistochemistry technique has been confirmed an useful tool for epidemiological, clinical, and histopathological studies.

Supported by FAPEMIG, CNPq and UFMG

IM79 - AN ALTERNATIVE IMMUNOHISTOCHEMICAL METHOD TO DETECT *LEISHMANIA* AMASTIGOTES IN PARAFFIN-EMBEDDED CANINE TISSUES

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Canine visceral leishmaniasis (CVL) is a zoonosis and a chronic systemic disease of the dog caused by a protozoan of the genus *Leishmania infantum* in the Old World and *Leishmania chagasi* in the New World. Several methods are currently employed for diagnosis of CVL, including microscopic detection of the parasite in bone marrow and lymph node aspirates, demonstration of specific antibodies anti-*Leishmania* in sera from infected animals, isolation of the parasite by "in vitro" culture or by laboratory animal inoculation. However, a definitive diagnosis is based on the actual detection of the parasite, which is conventionally achieved by examining Giemsa stained smears or histopathological sections stained by hematoxilin and eosin. These methods have a low sensitivity and therefore they are often inconclusive. This is particularly true in canine organs that have a low level of parasitism such as kidneys, lungs and guts or in some cases, in the skin. The technique for immunohistochemical detection of leishmanial amastigotes in canine tissues has been reported previously and it is proven to be undoubtedly efficient for the diagnosis. In this work we describe an ease and inexpensive immunohistochemical approach for *Leishmania* detection in formalin-fixed paraffin-embedded canine tissues. Amastigotes forms of *Leishmania* are easily observed within macrophages in several organs from naturally infected dogs using the streptoavidin-biotin immunohistochemical method with canine hyper immune serum as primary antibody. In addition, the second antibody used was not specific to canine immunoglobulin characterizing a cross immune reaction.

The immunoperoxidase protocol employed in this study, which is based on the use of serum from naturally infected dogs, is inexpensive and readily available, when compared to monoclonal or polyclonal anti-*Leishmania* antibodies (Bourdoiseau et al., 1997; Livini et al., 1983). Although the secondary antibody (LSAB+ Kit, Dako) is not specific to the dog serum ("crusade immunoreaction"), this method proved to be as specific as the use of monoclonal or polyclonal anti-*Leishmania* antibodies. Furthermore, the use of canine serum resulted in a low or absent background staining, and no staining was observed when the canine serum was replaced by PBS or serum from an uninfected dog, clearly indicating that the secondary antibody reacts to the canine serum from an infected dog used in this study as a primary antibody. Our results indicate that this technique could be an useful tool for epidemiological, clinical, and histopathological studies.

Apoio Financeiro: FAPEMIG, CNPq, UFMG

IM80 - RECOGNITION OF *PLASMODIUM VIVAX* VARIANT ANTIGENS (VIR) BY HUMAN ANTIBODIES INDUCED DURING NATURAL INFECTION.

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Variant antigens in *Plasmodium vivax* are encoded by members of the multicopy *vir* gene family. *vir* genes can be subdivided into different sub-families based sequence similarities (del Portillo et al. 2001. Nature, 410:839). We expressed seven glutathione S-transferase fusion proteins corresponding the four *vir* sub-families (A, B, C, and E) obtained from parasites of a single patient from the Amazon Region. Recombinant proteins were purified by affinity chromatography and used in ELISA assays to analyze the naturally acquired antibody responses of individuals during patent *P. vivax* infections. Sera were collected from individuals living in different endemic areas from the north of

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Brazil pertaining to the States of Pará and Rondônia. These same sera were also tested in their ability to recognize two recombinant proteins representing two merozoite surface antigens of *P. vivax*: the merozoite surface protein 1 (MSP1) and the apical membrane antigen 1 (AMA-1). Anti-VIR antibodies were detected against each recombinant protein tested and yet the prevalence of such antibodies was significantly lower than the prevalence of antibodies against MSP1 and AMA1. Studies are now in progress to evaluate the presence of cross-reactive epitopes among the antigens encoded by the different *vir* sub-families using sera of mice immunized with each recombinant protein.

Supported by FAPESP and CNPq.

IM81 - COMPARISON OF THE REACTIVITY BETWEEN ANTIGENS OF *LEISHMANIA (L.) CHAGASI*, *L.(L.) AMAZONENSIS* AND *LEISHMANIA SP.* (BIO-MANGUINHOS) IN THE SERO-DIAGNOSIS OF VISCERAL LEISHMANIASIS BY THE INDIRECT IMMUNOFLUORESCENCEC TEST.

Lima, L. V. R.; De Souza, A. A. A.; Jennings, Y. L.; Corrêa, Z. C. de J.; de Jesus, R. C. S.; Everdosa, D. R.; Ayres, M.;. Silveira, F. T.

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Introduction: antigenic specificity still represents a controversial point of discussion with regards the standardization of an antigen for use in the serodiagnosis of human visceral leishmaniasis (HVL) by the indirect immnofluorescence antibody test (IFAT). For this reason we have sought to compare the reactivity between antigens of *L.(L.) chagasi* (amastigotes and promastigotes), *L.(L.) amazonensis* (amastigotes) and a *Leishmania sp.* (promastigotes) from Bio-Manguinhos. Objective: to standardise an antigen for the diagnosis of HVL by the IFAT. Material and methods: *Sera*: 90 serum samples from patients with a previous serological diagnosis of HVL were randomly selected, together with 30 samples from individuals resident in Belém, Pará, with no previous evidence of infectious.Antigens were prepared from the promastigotes of *L.(L.) chagasi* (strain MCAO/BR/1998/M18011, Imperatriz, Maranhão State), amastigotes of *L.(L.) amazonensis* (strain IFLA/BR/1966/PH8, Belém, Pará), and promastigotes of the *Leishmania sp.* from Bio-Manguinhos. The antigens of amastigotes were made on IFAT slides by dab-smears of pieces of liver, spleen and skin of hamsters infected with the respective parasites. The promastigote antigen of *L.(L.) chagasi* was prepared from stationary phase cultures in Difco B45 medium, with a suspension of 3 x 10^6 parasites/ml. The 3 antigens were distributed on IFAT slides, fixed with acetone, and preserved at -20 ºC. The Bio-Manguinhos antigen was used following the maker's instructions. *Serological test*: the IFAT was carried out using anti-IgG (Bio-Manguinhos) for the 4 antigens, with positive sera considered to be those with a titre equal or above 80. *Statistical analysis*: we used the screening-test and curve ROC (IC 95%, of the programme Bio-Estat 2.0) to evaluate the sensibility and specificity, and the Dunnett (ANOVA) ($p < 0,01$) to evaluate differences between the averages of the antigen titres. Results: the amastigote antigen of *L.(L.) chagasi* atained a 100% sensibility and specificity level. That of *L.(L.) amazonensis* amastigotes achieved a 87% sensibility efficiency and a 93% specificity efficiency. The *Leishmania sp.* (Bio-Manguinhos) antigen gave sensibility and specificity efficiencies of 88% and 90%, respectively. ROC curve values were d=0,00 for amastigotes of *L.(L.) chagasi*; d=0,17 for promastigotes of the same parasite; d=0,15 for amastigotes of *L.(L.) amazonensis*; and d=0,16 for promastigotes of the *Leishmania sp.* (Bio-Manguinhos). With regards differences between averages of the reacting sera, it may be noted that the amastigote antigen of *L.(L.) chagasi* (6.366) was significantly ($p < 0,01$) better than the promastigote antigen of the same parasite (3.712); that of *Leishmania sp.* Bio-Manguinhos was 1.299; and that of amastigotes of *L.(L.) amazonensis* 1.070. Conclusion: the results show that the *L.(L.) chagasi* amastigote antigen is the antigen of choice for the sero-diagnosis of HVL and for monitoring chemotherapy of this disease.

IM82 - EVALUATION OF IGG SUBCLASSES ANTI-*LEISHMANIA* BY ELISA IN DOGS WITH AMERICAN VISCERAL LEISHMANIASIS (AVL) IN RIO DE JANEIRO/RJ.

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Introduction: The dog is the main reservoir of the AVL in urban areas. High levels of IgG are detected in canine AVL, and also in asyntomatic animals with high degree of parasitism in the health skin and viscerals. Deplazes et al. (1995), suggest that the titles of IgG1 and IgG2 are safer indicators for the status of the illness than the IgG. In this work we investigate, through ELISA, the seroprevalence of IgG and subclasses IgG1 and IgG2 anti-*Leishmania* in dogs of AVL endemic area, evaluating their importance for the diagnosis of the illness. Methodology: It was used in ELISA a partially soluble antigen of promastigotas forms of *L. (L.) chagasi*. For this study the samples of serum had been classified in the following groups: group I - 20 serum of dogs with positive parasitologic diagnosis (8 of symptomatic dogs and 12 of asyntomatics); group II -16 dogs without parasitologic diagnosis, with positive serologic; group III- 3 dogs with negative parasitologic and group IV - control group of healthy animals. Results: In group I the seroprevalence in ELISA for the asyntomatics animals was 8.3% (1/12) for IgG1 and 100% (12/12) for IgG2 and for the symptomatic animals was 12,5%(1/8) and 100% (8/8) respectively; in group II the seroprevalence for IgG1 was 43,7% (7/16) and 100% (16/16) for IgG2; in groups I and II the seroprevalence for IgG was 100%; in groups III and IV all the sera had been not reactors for IgG and its subclasses. Conclusions: The IgG2 was prevalent and detected in high levels in dogs with AVL, however, in these same animals, IgG1 was detected in low levels. On the contrary to many authors, significant difference was not observed between the levels of IgG1 and IgG2 when correlating these subclasses with the presence or absence of clinical signals. The sensibility and specificity of ELISA for IgG2 detection were higher than IgG detection, and the agreement with the indirect immunofluorescense, seems to strengthen the safe use of this test for the diagnosis of the canine AVL.

Support: Secretaria de Vigilância em Saúde

IM83 - IMMUNE RESPONSES AND PROTECTION INDUCED BY A COMBINED LACK AND *MYCOBACTERIUM* HSP65 DNA VACCINE AGAINST *LEISHMANIA (L.) MAJOR* INFECTION

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The LACK (*Leishmania* homolog of receptors for activated C kinase) antigen is a 36 KDa protein highly conserved and expressed in promastigote and amastigote forms of *Leishmania.* Immunization of BALB/c mice with a truncated (24-kDa) version of LACK, protein or DNA, confers strong protection against *L. (L.) major* infection. Here, we compared the protective effect of na encapsulated LACK DNA vaccine against *L. (L.) major* and *L. (L.) amazonensis* infection in BALB/c mice. Development of Th1 immune responses are essential for protection against *Leishmania* infection. *Mycobacterium* HSP65 shares high homology with *Leishmania* HSP proteins and is able to induce high levels of IFN-γ, when tested as a DNA vaccine against *Mycobacterium* infection. Thus, *M. leprae*

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HSP65 and interleukin 12 (IL-12) genes were evaluated as adjuvants. Eukariotic cells expression vectors contaning the LACK, HSP or *IL-12* coding regions were encapsulated in microspheres of lactic and poliglicolic acids (PLGA) by the method of multiple emulsions in a ratio of 6 ug of DNA/mg of polimer. Groups of eight BALB/c mice were immunized with two intramuscular injections (30 days interval) of 2,5 mg of microspheres and challenged 30 days latter with with 10^6 metacyclic promastigotes of either *L. (L.) major* or *L. (L.) amazonensis* in the hind footpad. Control groups were immunized with vector DNA or IL-12 DNA. Mice immunized with either LACK, HSP or a combination of these two DNA vaccines and challenged with *L. (L.) major* were significantly protected as indicated by reduction of edema in the infected footpad as compared with control groups. No significant differences were observed among groups immunized with LACK or HSP DNA and those immunized with LACK/IL-12 or LACK/HSP combined DNA vaccines. No significant protection was observed in mice immunized with IL-12 or vector DNA alone or in mice challenged with *L. amazonensis*. Protection was accompanied by significant reduction of parasite loads in the infected footpad and increased specific IgG2a antibody levels in sera of mice. Splenocytes of protected mice produced increased levels of IFN-γ and TNF-α and decreased levels of IL-4, as compared with mice immunized with IL-12 or vector DNA. We also observed that low doses of encapsulated DNA are required for induction of protection and specific Th1 immune responses. In addition, we demonstrated a significant cross-protection against *L. (L.) major* infection by immunization with *M. leprae* HSP65 DNA vaccine.

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IM84 - VISCERAL LEISHMANIASIS NEW IMMUNODIAGNOSTIC ALTERNATIVE.

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Visceral leishmaniasis (VL) is a severe world public health problem with 12 millions people infected, an incidence of 500000 and annual mortality of 80000 cases (WHO, World Health Report, 1998). Although multidisciplinary efforts carried out to eradicate this disease it remain without effective treatment making indispensable the rapid diagnosis of disease to increase the probability of successful cure. Nowadays there are not experimental immunoassay or commercial kit for VL diagnostic to be reliable, with low cost and easy application. In this sense the present study consist of Standardization of dot blot-ELISA technique for Visceral leishmaniasis immunodiagnostic. The antigen was a pool of surface glycoproteins ($gp_{27,}$ gp_{50} and $gp_{67)}$ purified from promastigotes of *Leishmania (L.) mexicana*, NR strain. Serum of patients with the different clinical manifestations of leishmaniasis treated or no treated with Glucantime®, Chagas' disease or malaria from endemic areas of Sucre state located at northeastern of Venezuela were assayed. We found between 11 serum of VL patients without treatment that 9 of them were positives whilst only 3 of 13 serum of VL patients treated with antimonial pentavalent were positives. These results are in agreement with the pointed out by Da Matta *et al*, J. Clin. Lab. Anal.,2000,14(1):5-12 who reported that type and subtypes antibodies levels decreasing after administered treatment. There were not cross reactivity with Chagas' disease and Malaria. Efficacy of immunodiagnostic test employed in present study using criterions of Pozo, Med.Clin.,1998,90:779-85, showed sensibility of 82% and specificity of 83%. In summary these findings suggest that dot blot-ELISA assay using the surface glycoproteins antigen of *L. (L.) mexicana* promastigotes is a method of high sensitivity, specificity and reproducibility, which will be implemented for visceral leishmaniasis diagnostic, regarding of course, clinical complementary information of the patient. Furthermore is an easy application method useful for field epidemiological study and valuable tool for rapid diagnostic and appropriate treatment.

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IM85 - PROFILE OF ANTI-*TOXOPLASMA GONDII* IGG AVIDITY IN EXPERIMENTALLY INFECTED RABBITS

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Toxoplasmosis is one of the more common parasitic zoonoses worldwide. The causative agent *Toxoplasma gondii* infects most warm-blooded animals including man, with significant veterinary and medical importance, because it may cause abortion or congenital disease in its intermediate hosts. As well as other mammals, the rabbits are also susceptible to *T. gondii* and fatal disease has been reported in this specie especially in wild life. The main objective of this work was to standardize and to evaluate the prognostic efficiency of the ELISA avidity in the temporal diagnosis of rabbit toxoplasmosis. We screened New Zealand rabbits with approximately 45 days of age and negative specific IgG by ELISA. These animals were inoculated subcutaneously (s.c.) with 10^7 irradiated tachyzoites of the RH strain of *T. gondii*. The animals were maintained at the Institute of Tropical Medicine/USP, receiving commercial food and water *ad libitum* and observed by 6 months, a period during which it did not present any clinical signs of the infection, with normal growth. Blood was collected biweekly and analyzed for anti-*T. gondii* IgG by ELISA and avidity determination using a 6 M urea washing. Antibody titer increased during the first weeks of infection achieving a plateau after 60^{th} day of infection, but avidity maturation (100%) was only achieved at day 150^{th} of infection, presenting a linear correlation with infection time until this time. The ELISA avidity was confirmed as a useful tool in the diagnosis of acute *T. gondii* infection in rabbits and could be predictive of the stage of infection and used in epidemiological studies. IgG avidity maturation appears to be a universal phenomenon in mammals.

This work was supported by LIMHCFMUSP and CAPES.

IM86 - STANDARDIZATION AND EVALUATION OF THE IGG AVIDITY IN THE DIAGNOSIS OF TOXOPLASMOSIS IN CATS AND ITS RELATIONSHIP WITH OOCYST EXCRETION

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Felids play a major role in the epidemiology of toxoplasmosis, since they are the definitive hosts of *Toxoplasma gondii*. Cats are also the only domestic animals in which the parasite completes the sexual stage of the life cycle, producing oocysts that are excreted in the feces and infect other hosts. As cats are the key element in the transmission of *T. gondii* to animals and humans, we decide to study the anti-*T. gondii* IgG maturation in this specie, looking for markers of environment contamination by oocysts, that are mainly excreted in the acute stage of this infection. We screened young cats breed in captivity, selecting those negative for specific IgG by ELISA. These animals were feed with brain from experimentally infected mice containing 1200 cysts of the AS-28 strain *T. gondii*. The animals

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were maintained at the Veterinary Hospital/UEL, receiving commercial food and water *ad libitum* and observed by at least 3 months. Blood was collected weekly and analyzed for anti-*T. gondii* IgG by ELISA and avidity determined using a 6 M urea washing. The feces were collected daily and analyzed qualitatively and quantitatively for oocysts, which were excreted during the first few weeks after infection. Antibody titer increased during the infection and IgG avidity achieved 50% only after the day 60th of infection, presenting a linear correlation with infection evolution. During oocyst shedding, serum samples presented low IgG avidity (<50%). The avidity maturation of IgG antibodies in cats was similar to other mammals and this test could be used to discriminate acute infections in those animals, allowing both the determination of incidence of this disease in cats and estimation of oocysts shedding in feces.

This work was supported by LIMHCFMUSP and CAPES.

IM87 - AMERICAN CUTANEOUS LEISHMANIASIS IN A RURAL AREA OF GOIÁS STATE, BRASIL.

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Increasing of leishmaniasis incidence around the world represents a serious problem of public health. Current available data indicate that 1.5 million cases of leishmaniasis are diagnosed every year and more than 80% of the total 12 million cases live in development countries, particularly Iran, Afghanistan, and Brazil. Over the last three years (2000-2002) autochthonous cases of American cutaneous leishmaniasis (ACL) have been identified in an area of rural workers belonging to the landless movement (MST) in Goiás state, Brazil. These workes have occupied forest areas near to riverheads which represent a very suitable habitat for several species of *Lutzomyia*, the leishmaniasis vector, widely distributed in those areas. In Goiás state two *Leishmania* species, *Leishmania (L.) amazonensis* and *Leishmania (V.) braziliensis*, have been incriminated in diagnosed cases of cutaneous leishmaniasis. The aim of the present work was to characterize *Leishmania* species isolated from MST people with ACL as well as to follow up these patients after treatment. The present report describes twenty two cases of patients with ACL which were attended in the outpatient department for leishmaniasis of Anuar Auad Hospital in Goiânia, the capital of Goiás state. Diagnostic procedures included clinical symptoms evaluation, exposure history, direct microscopy visualization of lesion biopsies, growth of transforming promastigotes in axenic medium cultured with material isolated from lesions, hystopathological analysis of lesion thin sections, immunological methods such as the Montenegro skin test, detection of antibodies against *Leishmania* by IFA and ELISA, and detection of *Leishmania* DNA. Fifteen isolates obtained by punch biopsy were processed and the *Leishmania* species were characterized by polymerase chain-reaction (PCR). Amplification was achieved by use of total DNA extracted from biopsies and primers from kinetoplast minicircles of *Leishmania* published in GeneBank. The *Leishmania* species isolated from 93.3% patients was identified as *Leishmania (V.) braziliensis*, whereas 6.3% were infected with *L. (L.) amazonensis*. These results show that the PCR technique by use of kDNA is useful for identification of *Leishmania* species and represents a very good support for the other methods currently used for *Leishmania* characterization. Treatment data showed that 93.3% of ACL patients responded to Meglumine antimoniate (Glucantime), whereas one patient which did not respond to Glucantime was succesfully treated with Amphoterecin deoxycholate. Extension of diagnosis by PCR amplification to a higher number of patients with ACL from the same area is now in progress, however our preliminary results described in the present work permit us to conclude that unprojected settlements represent a very relevant and serious risk of increasing human cases of ACL, leading to a significant change in the epidemiology of *Leishmania (V.) braziliensis*, the causal agent of disfiguring mucocutaneous leishmaniasis in Brazil.

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IM88 - SURVEY OF *PLASMODIA* SEROEPIDEMIOLOGY AND DETECTION OF ASYMPTOMATIC MALARIA CASES AMONG RESIDENTS OF ATLANTIC FOREST AREAS IN ESPÍRITO SANTO STATE, BRAZIL.

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Several malaria cases have been notified during the last few years in regions surrounded by the Atlantic Forest in Espírito Santo State. The aim of this study was to determine the exposure of human subjects to *Plasmodium* parasites (sexual and asexual stages) by serological tests and to determine the asymptomatic malaria profile in areas with low endemicity. Results of a seroepidemiological survey and the detection of asymptomatic malaria cases in the Espírito Santo State are reported here. Aliquots of total blood were obtained from 1.600 subjects from the following Municipalities: Santa Tereza, Santa Maria de Jetibá, Domingos Martins, São Roque do Canaã, Santa Leopoldina and Alfredo Chaves. The prevalence of IgM and IgG antibodies against malaria asexual stages was evaluated by IIF (Indirect Immunofluorescence) with crude *P. vivax and P. malariae* antigens. ELISA tests using synthetic peptides corresponding to the CSP (circunsporozoite protein) of *P.vivax* complex and *P. malariae* were used to detect IgG antibodies against the sexual stages of the parasite. PCR reactions, for detection of *P. vivax*, *P. falciparum* and *P. malariae* specific DNA, were carried out using multiplex primers. IIF for the detection of anti *P. malariae* antibodies was proceeded only for sera that gave positive results in ELISA and PCR. The IgM/IgG seroprevalence for *P. vivax* and *P. malariae* antigens were 29.1%/44,4% and 1.7%/14.7% respectively. The seroprevalence of antibodies against the CSP synthetic peptides were 30.7% (88/286) (classic *P. vivax*); 7.1% (20/280) (*P.vivax* VK247); 14.1% (39/276) (*P.vivax* like) and 9.1% (26/285) (*P. malariae*). Asymptomatic *P. vivax* and *P. malariae* infections were detected in 3 and 9 individuals respectively and a mixed *P. malariae/P. falciparum* infection was detected in one sample. These studies were carried out with the purpose of evaluate malaria transmission in a unique ecological niche (Atlantic Forest, Espírito Santo State). Our results indicate that a more comprehensive study is necessary and may be of great help in the design of effective intervention strategies for the control of the disease.

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IM89 - CHARACTERIZATION OF BRAZILIAN HUMAN *GIARDIA DUODENALIS* ISOLATES BY USING IMMUNOLOGICAL AND BIOCHEMICALPARAMETERS

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Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) of the trophozoites extracts, evaluation of the immune humoral response in rabbits, cross-reactivity of the immune sera to the homologous and heterologous antigens by using the immunofluorescent test (IFT) and western blotting were used to characterize three Brazilian human isolates of *Giardia duodenalis* (syn: *Giardia lamblia*, *Giardia intestinalis*) and its clones. The Portland-1 strain (ATCC 30888) was included in the study as a reference pattern.

The Brazilian isolates were axenized from cysts obtained from the feces of patients showing different clinical characteristics. The BHRA93 was isolated from a symptomatic patient while the BHRF92 was isolated from an asymptomatic one. The BHLF93 was isolated from an asymptomatic patient who presented a persistent *G. duodenalis* infection despite the different anti-giardial therapies. The reference pattern was the Portland-1 strain (ATCC 30888), axenized in 1971 from the duodenal aspirate of a symptomatic woman in the United States of America. One clone of each isolate was included in the study.

Antigenic differences among the isolates were observed by SDS-PAGE, western blotting and IFT. Greater antigenic heterogeneity was observed between Portland-1 and Brazilian isolates. Several protein bands ranging from 15 to 200 kDa were identified in the SDS-PAGE. All the isolates induced the production of anti-*Giardia* specific antibodies in rabbits immunized with whole antigenic extract of the trophozoites. Cross-reactivity of the anti-sera was greater with the homologous antigen than with the heterologous ones. Portland-1 and BHRA93, both symptomatic isolates induced higher titers of antibodies sera.

Little difference was detected between the parental isolates and the clones.

These data represent a significant advance on the current knowledge about *G. duodenalis* in Brazil.

Supported by: FAPEMIG and CNPq

IM90 - OCCULAR TOXOPLASMOSIS: ROLE OF RETINAL PIGMENT EPITHELIUM MIGRATION IN INFECTION

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We used a murine experimental model of ocular toxoplasmosis. Our aim was to study the migration of retinal pigmented epithelium (RPE) into the retinal layer during infection of C57BL/6 mice with *Toxoplasma gondii*. Eyes from infected and non-infected animals were analyzed on the 60th day of infection by light and transmission electron microscopy. Non-infected showed the typical normal morphology. In contrast, in infected eyes we observed free parasites in the retinal vasculature, presence of mononuclear inflammatory infiltrate (MNII) and parasites in the vasculature of choroids. No inflammatory infiltrate was observed; RPE cells were identified near the MNII in nuclear and plexiforme layers. RPE cells were also found on ganglionar and in outer segments of the photoreceptor. The morphology showed that RPE cells caused a discontinuity of the nuclear and plexiforme layers. Clusters of parasites were found surrounded by RPE cells and MNII in the inner plexiforme layers. Ultrastructural analysis showed that RPE cells migrated through the epithelium into the inner retinal layers. We did not observe Toxoplasma cysts in many eyes in which pathological changes were detected. Only 8.3% of the animals presented Toxoplasma cysts in the inner nuclear layer in the absence of inflammatory cells. Migration of RPE cells can be triggered by disruption of the RPE monolayer or injury to the neural retina as in the case of toxoplasmosis.

Apoio financeiro: IOC

IM91 - CARACTERIZATION OF CLINICAL PARAMETERS OF VISCERAL LEISHMANIASIS IN DOGS NATURALLY INFECTED WITH *L. (L.) CHAGASI.*

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Dogs are the domestic reservoirs of zoonotic visceral leishmaniasis caused by *Leishmania (L.) chagasi*. Have been considered to be most important than human infection due to its greater prevalence and the frequency of *Leishmania* amastigotes in the skin of these animals. In this study two groups of adult mongrel dogs were used. The first group with 40 naturally infected animals (immunofluorescence test-IFAT positive and parasite isolation) each classified according to their clinical symptoms (asymptomatic, oligosymptomatic and symptomatic); the second group (20 dogs) was the control, serologically and parasitologically negative for *L. chagasi*. The dogs were submitted to detailed serological, parasitological and biochemical-hematological parameters. The study on IgG antibodies was performed by IFAT and ELISA which was detected increase of levels and titles of IgG according to clinical evolution of CVL. In the parasitological studies (mieloculture, impression smears of skin biopsies and sternal bone marrow puncture for evaluation of parasite burden by "Leishman Donovan Units" – LDU) was detected *Leishmania* amastigotes in 90% symptomatic dogs in different tissues showing a positive correlation in to the disease progression. The parasitism in the bone marrow and skin was higher in the symptomatic animals in comparison to the infected dogs. Analysis of biochemical parameters showed hypergammaglobulinemia with reversal of the Albumin/Globulin ratio in oligosymptomatic and symptomatic animals. Hematological changes were observed in symptomatic dogs with anemia and leukopenia (lymphopenia, monocytopenia and eosipenia). Our data suggest that changes in laboratorial parameters (serological, parasitological and biochemical-hematological) might play an important role in the accompaniment of CVL porgnostic, can be used in the evaluation of clinical trials, since specific changes occur in each clinical symptoms groups.

Support: CNPq, FAPEMIG, FBB, UFMG, FIOCRUZ (CPqRR).

IM92 - CANINE VISCERAL LEISHMANIASIS: A REMARKABLE HISTOPATHOLOGICAL PICTURE OF ONE ASYMPTOMATIC ANIMAL REPORTED FROM BELO HORIZONTE, MINAS GERAIS, BRAZIL.

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Human and canine visceral leishmaniasis in the New World are caused by *Leishmania (Leishmania) chagasi* which is transmitted by the phlebotomine *Lutzomyia (Lutzomyia) longipalpis*. *Leishmania* is an intracellular protozoan parasite that is delivered to its vertebrate host by the bite of an infected sandfly. Following injection into the skin, the extracellular promastigote form of the parasite must rapidly enter its host cell, the macrophage, and later transform into the intracellular amastigote form. Visceral leishmaniasis (VL) remains a serious public health problem in the world and dogs (*Canis familaris*) are the

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peridomestic reservoir host (Anderson, 1980; Grimaldi et al., 1989; Tesh, 1995). In Brazil, VL is highly endemic in the semiarid northeastern poor states of Ceará, Bahia, Maranhão, Piauí, Pernambuco, Rio Grande do Norte and Paraíba. In all states there is an association with infected dogs and abundant *L. longipalpis* (Chagas et al., 1938; Deane & Deane, 1962; Guedes et al., 1978). Canine VL appears to be spreading further in Brazil and outbreaks have recently been reported in regions as City of Belo Horizonte (MG), (Genaro et al., 1988; Michalick et al., 1993), Teresina (PI), São Luiz (MA), Fortaleza, (CE), Rio de Janeiro (RJ) (Marzochi et al., 1994) and Bahia (BA) (Cunha et al., 1995; Carvalho et al., 1996; Ashford et al., 1998). Serological exams complement fixation reaction (CFR), indirect imunofluorescense (IFAT) and enzyme-linked immunosorbent assay (ELISA) were carried out in ICB/UFMG. The parasitism of all organs, expect skin tissues, was evaluated by the *Leishmania Donovani* Units (LDU) indices. An asymptomatic dog was sacrificed with a lethal dose of 33% Thiopentalâ (intravenous). After the necropsy samples of liver, spleen, cervical, axillary and popliteal lymph nodes, and skin (ear, nose and abdomen) were collected and fixed in 10% neutral buffer formalin solution. All tissues samples were dehydrated, cleared, embedded in paraffin, cut (4-5mm thickness) and stained by Hematoxylin and Eosin (H&E) and immunocytochemistry to detected parasites in paraffined tissues (Tafuri et al., 2003). Immunolabelled parasites were quantified by morphometrical analysis (KS300 software - Zeiss). Under optic microscopical observation all organs showed an intense parasitism associated to a severe pathological picture. All lymph nodes had conspicuous histological architecture alterations. Lymphocytes were substituted for macrophages stuffed with an intense number of amastigotes forms of *Leishmania*. The lymphoid nodules (without germinal centers) and the mantle zones in the cortex that surround the follicles were markedly attenuated. Livers showed small intralobular granulomas composed by macrophages loaded with amastigotes. Spleens had an intense depression of the white polp whereas the lymphocytes were replaced by parasitized macrophages. All fragments of different anatomical region showed a chronic inflammation characterized by plasmocytes, macrophages and lymphocyte. Intracellular parasites were ease found in macrophages in the dermis. Taken together of the morphometrical analysis data of all organs we observed higher numbers than others asymptomatic dogs that we have been analyzed in our laboratories. This asymptomatic dog had average of 16,86 to 33,49 μm^2 parasites/field. In contrast, others asymptomatic dogs had shown averages of 0,55μm^2 to 9,41$\mu m2$ parasites/field. These parasitological results and general histopathological features of this asymptomatic animal indicated an anergic immune response. However, we did not observe any clinical sign of the disease at the necropsy time.

IM93 - INFLUENCE OF SALIVA FROM *LUTZOMYIA LONGIPALPIS* IN INITIAL EVENTS OF INFECTION BY *LEISHMANIA (L.) CHAGASI* USING AN *IN VITRO* PRIMING SYSTEM.

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Infection by *Leishmania (L.) chagas*i and the initial immune responses against this parasite are not completely understood. Interactions between the parasite, APC and lymphocytes may determine the outcome of the infection. Furthermore salivary components from *Lutzomyia longipalpis* can influence immune responses against the parasite. We evaluated the initial immune response against *Leishmania (L.) chagasi* (MHOM/BR2000/MER2) from PBMC of normal donors by using an *in vitro* priming system (lVP). Brief1y, PBMC were separated by ficoll gradient and cultured in 24 well plates at 5 x 10^6 cells/mL and subsequently infected with *Leishmania (L.) chagasi* promastigotes stationary phase (1x10^6 parasites/mL) plus 2 pair/mL of *Lutzoymia longipalpis* salivary gland sonicate (SGS). Macrophages served as APC for second stimulation and were infected 24 hours before the incubation with previously stimulated lymphocytes. Supernatants from the first round of stimulation were harvested in order to perform cytokines quantification. Blast cells were adjusted to 10^6/mL and used in the second stimulation and for flow citometry analysis. After 72 hours, supernatants from the second stimulation were harvested for ELISA and cytokine quantification. This system al1owed us to detected cytokines and surface markers, which can be modulated by the parasite and salivary gland sonicate. We tested the capacity of the *Leishmania (L.) chagasi* alone or simultaneously with SGS to stimulate the cel1s in the IVP. We found that the *Leishmania chagas*i plus SGS stimulated weak IFN-gamma production on the 1st stimulation, whereas in the second stimulation a higher production of IFN-γ was observed. Expression of $CD4^+CD25^+$: 12,5% ± 3,2 and 16,5% ±2,5 and $CD8^+CD25^+$: 8,3 ±1,5 and 10,42 ±2,3 after 1st and 2nd stimulation respectively. Actually we are looking for different markers of activation and trying to identify the influence of saliva in the immune response against *Leishmania (L.) chagasi.* The phlebotomine saliva components seem to affect the functions of the main cells involved in this response, altering cytokine profile and the expression of costimulatory molecules which are important to drive cell differentiation, and in this sense a protective immune response against *Leishmania*.

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IM94 - THE INFECTIVITY OF *LEISHMANIA (VIANNIA)* SPP. TO CULTURED MACROPHAGES OF BALB/C MICE

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Introduction: the infectivity of *Leishmania* may vary within the same species or strain of parasite (Pearson & Souza, 1996), and in the Old World this variation has been demonstrated for *L. (L.) major* and *L. (L.) infantum* (Liew *et al*., 1990; Mendez *et al*., 1996). Up till now, however, there have been few observations regarding such variation for New World species, in particular those of the Amazon Region where seven species have been shown to be the etiological agents of cutaneous leishmaniasis. Objective: to determine, *in vitro*, the infectivity of strains of *Leishmania (Viannia)* in cultures of peritoneal macrophages of BALB/c mice, using infection-rate as the parameter. Materials and methods: 18 strains of the following 5 species of *Leishmania (Viannia)* were studied: *L. (V.) shawi, L. (V.) braziliensis* (cutaneous and mucosal leishmaniasis), *L. (V.) lainsoni, L. (V.) naiffi and L. (V.) guyanensis*. Parasites used were stationary phase promastigotes from NNN (Difco B45) cultures. The inoculum into the cell cultures was in the proportion of 4 parasites/per macrophage. The cultures were incubated at 35^{0}C with 5% CO_2 and, 24 hours following inoculation, the slides were stained by Giemsa's method to determine the infection-rate (percentage of macrophage infected x number of parasites per macrophage). Results: the infection-rates of the species *L. (V.) shawi* (300,8); *L. (V.) braziliensis* (358,5) from cases of mucosal leishmaniasis and *L.(V.) naiffi* (289,6) were similar, and greater than the infection-rates of *L. (V.) guyanensis* (224,1), *L. (V.) lainsoni* (229,2) and the strains of *L. (V.) braziliensis* (167,1) from cases of localized cutaneous leishmaniasis The strains of *L. (V.) braziliensis* from cases of mucosal leishmaniasis were more infective ($p< 0,05$) than those from localized cutaneous leishmaniasis caused by the same parasite, and the other species, with exception of *L. (V.) shawi*. Conclusion: the difference of infection-rate among strains of *L. (V.) braziliensis* from patients with localized cutaneous leishmaniasis may have been influenced by the fact that this parasite develops slowly in culture compared with other species. In this way, the population of promastigotes used for the inoculation of the macrophage cultures was only at the beginning of the stationary phase and therefore with less infective forms than the other species which have more rapid

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growth. With regards to the difference seen between the strains of *L. (V.) braziliensis* from localized cutaneous and mucosal leishmaniasis, we suggest that this is due to a greater virulence of strains from patients with the latter disease.

IM95 - THE EVASION MECHANISM OF *TOXOPLASMA GONDII* FROM THE MICROBICIDAL ACTIVITY OF ACTIVATED MACROPHAGES IS BASED ON PHOSPHATIDYLSERINE EXPRESSION THAT INDUCES SECRETING OF TRANSFORMING GROWTH FACTOR-β CAUSING AN AUTOCRINE EFFECT.

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Toxoplasma gondii is an obligate intracellular protozoan, which infects different cell types. Activated macrophages express inducible nitric oxide (NO) synthase (iNOS) that produce NO, a microbicidal agent that controls *T. gondii* growth. However, active invasion of *T. gondii* inhibits NO production. To understand how this parasite inhibits this microbicidal agent, mice peritoneal macrophages were seeded over cover slips, cultured in Dulbecco's Modified Eagle's Medium (DMEM) with 5% fetal bovine serum and activated with interferon-γ (IFN-γ) and lipopolysaccharide (LPS). Tachyzoites, RH strain, were obtained by peritoneal washes of infected mice. Activated macrophages were infected with a 10 to 1 tachyzoite macrophage ratio and some were treated with anti- transforming growth factor-α (TGF-β) IgY (6 µg/ml) during the 2h interaction period and until 48 h. Culture supernatants were collected after 24 and 48 h for nitrite evaluation by the Griess reagent. For localization of iNOS, filamentous actin and Smad 2 and 3 (intracellular mediators of TGF-β signaling), macrophages were labeled with anti-iNOS, phalloidin and ant-Smad, respectively. PS expression of tachyzoites was analyzed in a flow cytometer after annexin-V-FITC labeling; parasites were also treatment with annexin V after interaction. Infected macrophages presented reduced expression of iNOS and filamentous actin. This result was reverted after treatment with anti-TGF-β. Further evidence for TGF-β involvement was the increase of phosphorylated Smad 2 and 3 in infected macrophages. Tachyzoite presented 50% of its population expressing PS. Furthermore, PS blockage by annexin-V abolished NO production inhibition and parasite survival. These results indicate that the evasion mechanism of *T. gondii* is based on the surface expression of PS that induces in infected macrophages secretion of TGF-α. This factor causes an autocrine effect that induces actin filament depolymerisation, iNOS degradation and NO production inhibition as a consequence. This evasion mechanism mimics the anti-inflammatory state caused by the uptake of apoptotic cells and is similar to the mechanism used by *Leishmania* (de Freitas Balanco *et al*. Curr. Biol. 11, 1870-1873, 2001) both based on PS expression leading to TGF-β secretion.

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IM96 - MODULATION OF EFECTOR FUNCTION OF MURINE MACROPHAGES BY GLUTATHIONE

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Leishmania are obligate intracellular protozoan parasites that infect host macrophages. The murine model of *L. (L.) major* infection has been extensively used for investigation of the mechanisms controlling disease development. It is well documented that the control of the infection requires the induction of an immune response capable of activating macrophages to a microbicidal state, which depends mainly on the production of nitric oxide and killing of the parasites living within macrophages. Promastigotes bind to specific receptors on macrophages and are internalized by receptor-mediated phagocitosis. This initial macrophage-parasite interaction is crucial for the establishment of host cell infection.The leukocyte integrin Mac-1 or CD11b is one important molecule for host cell invasion. We have recently observed that the host response to *L. major* infection can be significantly improved by increasing *in vivo* glutathione (GSH) levels. When *L. (L.) major* infected BALB/c mice are treated with N-acetyl-cystein (NAC), a GSH precursor, the histopathologic outcome of disease is greatly improved, characterized by less intense tissue vacuolization and reduced parasite load (Rocha-Vieira *et al*., Immunol., 2003). Considering that macrophages are the main effector cells controlling parasite replication at the site of infection, we have investigated whether GSH modulation can increase the leishmanicidal activity of macrophages. To approach this question, murine macrophages were stimulated *in vitro* with IFN-γ (60U/mL) and LPS (10ng/mL) in the presence of two glutathione modulating agents: NAC, a GSH precursor, and diethyl-maleate (DEM), a GSH depleting agent. The effects of GSH modulation on macrophage-parasite interaction through CD11b and the nitric oxide production were evaluated. Our data indicate that the macrophage functions studied can be improved or impaired by GSH modulation. Modulation of macrophage function by GSH could be a useful pathway to improve the host response to *Leishmania* infection.

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IM97 - EVIDENCES OF APOPTOSIS IN MACROPHAGES INFECTED WITH *LEISHMANIA (L.) AMAZONENSIS* AND *LEISHMANIA (V.) GUYANENSIS*

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Leishmania is a gender of intracelular protozoan parasites of vertebrate animals, including man. It replicates inside macrophages, which end up rupturing and releasing the parasites, infective for neighboring cells. Being an amplifying step, host cell death may be a key point in the development of diseases. In the murine model, BALB/c is susceptible to most species of *Leishmania (L.) amazonensis*, for instance, cause a growing lesion at the site of infection and, eventually the death of the host. However, *L. (V.) guyanensis* produce no lesion following infection. Using MTT assay, we have observed a sudden reduction in the viability of peritoneal BALB/c macrophages infected with *L. (V.) guyanensis*, but not in cells infected with *L. (L.) amazonensis*. To investigate whether *L. guyanensis*-infected macrophages were dying from apoptosis, we examined the DNA fragmentation through agarose gels and TUNEL technique. In agarose gels, we have observed in both *L. (L.) amazonensis* and *L. (V.) guyanensis*-infected cells the fragmentation of DNA that appeared as a ladder pattern with fragment sizes multiples of ~200 bp, typical of apoptotic cells. Using the TUNEL technique, we have also observed that both *L. (L.) amazonensis* and *L. (V.) guyanensis*-infected cells had their nuclei labeled. The percentage of apopotic nuclei was higher in macrophages infected with *L. (V.) guyanensis* than in cells infected with *L. (L.) amazonensis*. These results suggests that programmed cell death occurs in macrophages infected with *Leishmania*. It remais to be investigated whether macrophage death through apoptosis contributes to a less severe outcome of the disease.

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IM98 - ELIMINATION OF *LEISHMANIA (V.) GUYANENSIS* BY MURINE MACROPHAGES: POSSIBLE INVOLVEMENT OF REACTIVE OXYGEN INTERMEDIATES IN APOPTOTIC DEATH OF THE PARASITES

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Murine leishmaniasis have different outcomes determined by either the species of *Leishmania* or the mouse strain. In the present work, we show that BALB/c mice, incapable of healing *L. (L.) major* or *L. (L.) amazonensis* lesions, do not develop lesion when infected with *L. (V.) guyanensis*. Accordingly, the percentage of macrophages infected *in vitro* with *L. (V.) guyanensis*, but not with *L. (L.) amazonensis*, decreases significantly in 72 h reaching almost null values in 96 h. Using the TUNEL technique, we have shown that, in 24 hs after infection, 25% of *L. (V.) guyanensis*-infected macrophages had parasites with DNA fragmentation, whereas 8% of *L. (L.) amazonensis*-infected cells had stained parasites. In 100 macrophages observed, around 35 positive *L. (V.) guyanensis* amastigotes were found as oposed to 9 positive *L. (L.) amazonensis* amastigotes. These results suggest that *L. (V.) guyanensis* amastigotes die through apoptosis inside the macrophage. Since *L. (V.) guyanensis*-infected macrophages do not produce detectable levels of NO, we have looked at the ability of *L. guyanensis* to induce the respiratory burst. We have found that *L. (V.) guyanensis*, but not *L. (L.) amazonensis*, induces the respiratory burst of BALB/c macrophages, as determined by chemoluminescence. Inhibition of respiratory burst impaired the capacity of BALB/c macrophages to eliminate *L. (V.) guyanensis*. We have also shown that H_2O_2 is able to kill *L. (V.) guyanensis* in a dose-dependent manner. Our results suggest that the elimination of *L. (V.) guyanensis* by BALB/c macrophages is probably through apoptosis, which may be due to reactive oxygen intermediates generated during infection.

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IM99 - PRODUCTION OF OXIGEN REACTIVE SPECIES BY MACROPHAGES INFECTED WITH *LEISHMANIA (L.) MAJOR*

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Leishmania (L.) major is an intracellular parasite of human macrophages that also infects mice under experimental conditions. Natural resistance to this parasite is characterized by the development of small lesions at the site of infection that spontaneously heal, although parasitological cure does not occur naturally. Resistance, in the murine model, is characterized by production of high levels of interferon-gamma (IFN-γ), which activates macrophages to produce nitric oxide (NO). This radical is the ejector molecule that kills the parasite inside the macrophage. Several studies have dismissed other oxygen reactive species as having any effect on *L. (L.) major*. However, we have observed that IFN-γ knockout mice are more susceptible to infection with *L. (L.) major* than mice that lack the inducible nitric oxide synthase (iNOS). Hence, it is possible that in the absence of NO, macrophages resort to a different control mechanism that is still dependent on IFN-γ. Here, we investigate if oxygen reactive species are produced by macrophages from wild-type, IFN-γ knockout or iNOS knockout mice in response to infection with *L. (L.) major*. Macrophages were infected in vitro with *L. (L.) major* in the presence or absence of IFN-γ or phorbol miristate acetate (PMA). We found that both knockout mice produced oxygen reactive species (as detected by reaction with luminol) at a much higher level than the wild type mice, in the absence of any stimulus. When stimulated with *L. major*, wild-type mice produced very low levels or oxygen reactive species, while both iNOS and IFN-γ knockout mice produced 10 times as much. The same profiles were obtained when macrophages from knockout and wild-type mice were stimulated with PMA or zymosan. iNOS knockout macrophages were able to control growth of *L. (L.) major* when stimulated with IFN-γ and PMA. We suggest that, in the absence of NO production, macrophages resort to production of high levels of reactive oxygen species, which are capable of some control of parasite growth.

IM100 - NITRIC OXIDE PATHWAY FROM *L. (L.) AMAZONENSIS* PARTICIPATES ON PARASITE-MACROPHAGE INTERATION

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Analysis of the interaction of *Leishmania* promastigotes with the target host cell suggests that both parasite and host molecules are involved in cell adhesion. The main function of macrophages is to destroy intracellular pathogens, but the manner in which *Leishmania* and other intracellular parasites are able to survive and replicate within this ostensibly hostile intracellular milieu is an important question in cell biochemistry and immunology (Bogdan *et al*., 1996). Nitric oxide (NO) a free radical derived from molecular oxygen and the guanidine nitrogen of L-arginine, is involved in a variety of biological functions in different cells, and is an important anti-microbial effector molecule in macrophages against intra- and extra cellular pathogens (Moncada & Higgs, 1995). The present work provide evidence of the participation of NO pathway from *L. (L.) amazonensis* in infection mechanism, through assays realized with the L-aginine analogs L-NAME (Nw-nitro-L-arginine methyl esther) in order to verify the importance of L-arginine metabolism to macrophage-parasite interaction. *L. (L.) amazonensis* (MHOM/BR/77/LTB0016 strain), were maintained in Schneider's Insect Medium supplemented with 10% of heat inactivated FCS at 26^{0}C and pH 7.2. Murine resident peritoneal macrophages obtained from BALB/c mice were collected in cold serum free RPMI 1640 medium and incubated for 2 hours at 37°C in an atmosphere containing 5% CO_2. The cells ($5x10^5$ cells/well) were maintained in the same culture conditions for 24 hours before the infection. *L. (L.) amazonensis* promastigotes were pre-incubated with 0.5mmol/L L-NAME for 24 hours and parasites were harvested fro the medium in the log late phase of growth. Parasites were incubated overnight with peritoneal adherent cells (10 parasites/cell, $5x10^6$ parasites/well), in a CO_2 incubator at 37°C. Infected macrophages were maintained in RPMI 1640 with 5% HIFCS for 24 and 48 hours. Coverlids were fixed in methanol and stained with Giemsa's solution and culture supernatants were collected until the assay for measurement of nitrite by the Griess method (Green *et al*., 1982). It was possible to observe the interference of L-NAME on metabolic NO pathway of parasites, at the point to interfere on macrophages infection. The infection range of the murine macrophages by *L. (L.) amazonensis* pre-cultured with L-NAME decreases significantly from 93,3% to 61% in 24 hours and was still lower (19%) at 48 h post infection. The NO production also was seriously affected. These facts pointed strongly that the promastigotes-NO pathway exerts a fundamental role to establishment of the infection; and this data corroborated with studies of Ballestieri *et al.* (2002) such stated out that the increase of the parasites number on macrophage phagolysosom, in these times evaluated, takes to downregulation of $NO^{\cdot}$ production by iNOS.

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IM101 - LEISHMANICIDAL ACTIVITY AND NITRIC OXIDE SYNTHASE EXPRESSION BY HUMAN MACROPHAGES ARE DEPENDENT ON INTRACELLULAR GSH LEVELS.

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Glutathione (GSH) is the major intracellular redox buffer. It plays an essential role in protecting cells against oxidant damage and modulates the expression of several genes. Moreover, it is critical for T cell proliferative response to mitogens and can modulate the pattern of cytokine secretion during cellular immune responses. We are interested in how gluthathione modulation could be employed to improve immune responses. For this purpose we evaluated the response of human macrophages to *Leishmania (V.) braziliensis* infection and IFN-γ and LPS stimulation in the presence of glutathione modulating agents. Reducing intracellular GSH levels in macrophages with dethyl-maleate (DEM) led to an increased frequency of infected macrophages, which was further correlated with a reduced expression of nitric oxide synthase, in response to IFN-γ and LPS stimulation. These findings indicate that reduced GSH levels are detrimental to the nitric oxide dependent leishmanicidal activity of human macrophages. We are know investigating whether increasing macrophage GSH levels could be used as a strategy to improve the host response to leishmania infection.

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IM102 - IFN-γ PRODUCTION IN MUCOSAL LEISHMANIASIS PATIENTS IS NOT ASSOCIATED TO A+874T POLYMORPHISM IN A PILOT STUDY

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Interferon-gamma (IFN-γ) is a cytokine, which is believed to play a key role in controlling the intracellular pathogens but can also trigger inflammatory complications of many infectious diseases. High levels of IFN-γ and TNF-α are implicated in the development of tissue damage in mucosal leishmaniasis (ML) (Da-Cruz et al. 1996, Bacelar et al. 2002, Amato et al. 2003). Recent results of our group pointed that two subgroups of IFN-γ producers are found among long-term cured ML patients: high and low responders although those differences were not significant at that time. These results raised the hypothesis that those two IFN-γ profiles could be associated with the beneficial clinical evolution or reactivation of the disease (Da-Cruz et al., 2002). Our aim was to investigate if single nucleotide polymorphisms (SNPs) A+874T of IFN-γ gene (that have been previously associated with low/high production of the cytokine in tuberculosis) are associated to the clinical course of ML and the levels of IFN-γ production *in vitro*. Methodology and patients: Twenty-six ML and 15 cutaneous leishmaniasis patients were studied. The cellular immune responses were analyzed *in vivo* by Montenegro skin test (MST). For *in vitro* studies, lymphoproliferative response assays (LPR) of peripheral blood mononuclear cells (PBMC) stimulated with *Leishmania (V.) braziliensis* antigens (Lb-Ag) were performed. The culture supernatants were tested for IFN-γ production by ELISA tests. The genomic DNA was obtained from frozen PBMC and the detection of genotype for IFN-γ polymorphism by polymerase chain reaction-amplified refractory mutational system (PCR-ARMS). Results: As expected, ML patients produce higher levels of IFN-γ than patients from the cutaneous form. The production of IFN-γ was directly related to the period of illness (r=0,65, p=0.002), but not to the MST diameter. We have observed TT genotype (12,1%), that was normally accounted for high IFN-γ producer, only in ML but the number of patients did not allow statistical analysis. The levels of IFN-γ in culture supernatants were not statistically significant (p=0.43) among the different genotypes either in ML (AA mean= 8.216±7.377,5 pg/ml; TA mean=10.550±8.801pg/ml; TT mean=3.167±3.665pg/ml) or cutaneous leishmaniasis (AA mean= 993±1.655 pg/ml and TA mean= 2.517±2.353 pg/ml). Interestingly, IFN-γ secretion among TT patients was lower than in the other genotypic groups. Conclusions: The preliminary results suggest that IFN-γ genotype could influence the outcome of ML, but once the status ML vs. CL is achieved other levels of regulation might be at place to define IFN-γ secretion in leishmaniasis patients.

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BM1 - PROMOTER REGIONS OF *PLASMODIUM VIVAX* ARE UNABLE TO RECRUIT THE TRANSCRIPTIONAL MACHINERY OF P. FALCIPARUM.

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Stable and transient transfection in *Plasmodium* has been use to study gene function and gene regulation, becoming a powerful tool to better understand the biology of this parasite. This technique has helped to elucidate the mechanisms involved in virulence, imune evasion, drug resistance, cell invasion, stage differentiation and others. *Plasmodium vivax*, the parasite responsible for about 80% of Brazilian malaria cases, and which causes great socio-economical burden for this region cannot be cultured continuously *in vitro* precluding the use of transfection technology to carry functional studies of this human malaria parasite. To functionally characterize promoters and elements responsible for the control of gene expression in *P. vivax*, we constructed vectors having the luciferase reporter gene under the control of the promoter regions of the *P. vivax msp1* and *dhfr* genes and used them in heterologous transient transfections in *P. falciparum*. Strikingly, none of the constructs were able to drive expression of the luciferase gene unlike positive controls which included promoter regions from *P. berghei* and *P. chabaudi*. These data seem to indicate that unlike other *Plasmodia spp*, *P. vivax* promoters are unable to recruit the transcriptional machinery of *P. falciparum*. As the AT-content of the *pvmsp1* and *pvdhfr* promoter regions is GC-rich, present efforts are guided to construct a luciferase reporter plasmids with an AT-rich promoter region from *P. vivax* which closer resembles promoter regions of *P. falciparum*.

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BM2 - *PLASMODIUM VIVAX*: PRIMARY STRUCTURE AND ALLELE POLYMORPHISM OF THE *PVMDR1* GENE AMONG ISOLATES FROM BRAZIL, PAPUA AND MONKEY-ADAPTED STRAINS.

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Despite intensive efforts at eradication and control, malaria remains a major public health problem. Yearly, it is estimated that 300-500 million people worldwide are afflicted with a death toll of 1.7-2.4 millions mostly in children below five years old. The emergence of *Plasmodium falciparum* resistance to widely used antimalarial drugs such as chloroquine as worsened this scenario and made malaria control and treatment even more difficult. Drug resistance is also now emerging in *Plasmodium vivax*, the most widely distributed human malaria and responsible for 70-80 million clinical yearly cases. Multidrug resistance genes (mdr) from *Plasmodium* are proposed to be involved in certain forms of drug resistance, including resistance to mefloquine, chloroquine and quinine. These genes have been reported and analyze for *P. falciparum* but not for *P. vivax*.

To identify *P. vivax* mdr genes, degenerate oligonucleotides were used in PCR amplifications of *P. vivax* DNA and a unique 4.5 kB fragment cloned and sequenced. *In silico* analysis revealed that this sequence shared 70% similarity with the pfmdr1 gene and that it displayed conserved domains with two highly conserved ATP bidding cassette (ABC) sites and two conserved transmembrane domains. This same fragment was amplified cloned and sequenced from different chloroquine sensitive and resistant isolates including 4 patients from the Brazilian Amazon, 4 from Papua and 2 from *P. vivax* monkey-adapted strains. Similarity and dendrogram analyses revealed that sequences could be grouped according to their geographical origin and that within each geographical group resistant samples branched independently. Interestingly, all samples from Papua and the monkey-adapted chloroquine resistant strain revealed the presence of an in frame stop codon that resulted in a truncated MDR protein. The identification and characterization of the *P. vivax mdr1* gene opens new avenues to further studies of drug resistance in this human malaria parasite.

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BM3 - STUDIES ON THE POLYMORPHISM OF CYTOADHERENT DOMAINS OF VARIANT SURFACE ANTIGENS IN FIELD ISOLATES OF *PLASMODIUM FALCIPARUM* FROM THE BRAZILIAN AMAZON.

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The infection of humans with the apicomplexan parasite *Plasmodium falciparum* causes the most serious form of Malaria. An important virulence factor is a variant surface antigen, located on the surface of the infected red blood cell (iRBC), and named *Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP1). Different allelic forms of this protein mediate the cytoadherence to several different endothelial receptors. PfEMP1s are encoded by the large multigene family named *var*. Recent studies indicate that the majority of PfEMP1s show cytoadherence to the CD36 receptor. The responsible CD36 binding domains are highly conserved in their three-dimensional structure, and can be used as components of an anti-adherent vaccine. Apparently the same occurs for cytoadherence of PfEMP1/s to CSA, a major complication in primigravid women. In the current study, *P. falciparum* field samples are being collected and selected *in vitro* for cytoadherence to the ICAM1 receptor, implied by different authors to be one of the most important receptors for cytoadherence of iRBC in brain capillaries, which itself possibly results in cerebral malaria. The corresponding *var* gene transcripts are being characterized from *P. falciparum* cultures selected for ICAM1 cytoadherence. The repertoire of *var* gene transcripts associated to ICAM1-cytoadherence in different *P. falciparum* isolates will provide data on the viability of an anti-adherence vaccine, by determining similarities or differences between the corresponding transcribed *var* genes. This is the first study concerning the repertoire of ICAM1-cytoadherent domains of PfEMP1s in field isolates. The first *var* gene sequences from 5 different ICAM1-cytoadherent field samples will be presented.

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BM4 - *PLASMODIUM FALCIPARUM VAR* GENES TRANSCRIPTION AND SILENCING IN A CENTRAL *VAR* GENE CLUSTER DEPENDS ON THE COMBINATION OF SPECIFIC *VAR* INTRON AND PROMOTER SEQUENCES.

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Text: The *Plasmodium falciparum* erythrocyte membrane protein-1 (PfEMP-1), encoded by the multigene family named *var*, is responsible for the cytoadherence of infected erythrocytes in malarial infections. Approximately 50 *var* genes exist per parasite genome, which are mostly located in subtelomeric

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regions of all chromosomes, but are also found as clusters in central chromosomal regions. It was shown that almost all *var* transcripts are detectable in ring stage whereas in trophozoite stage one or only a few genes are transcribed while the rest of the family remains transcriptionally downregulated. Recent data published by Deitsch et al. indicate that *var* gene silencing requires the presence of a *var* intron and elements within it and an upstream element in the promoter. In the present study we selected a parasite adhesion phenotype by multiple panning procedures on E-selectin and identified a transcribed *var* gene in a centromeric/ central cluster of 4 *var* genes and 1 *rif* gene on chromosome 4. In order to describe factors, which were important for *var* transcription of one *var* gene or silencing of an adjacent one in the cluster, we compared the promoter and intron sequences and the *var* gene sequences itself, and tested the presence of transcripts. Interestingly, an adjacent 5' localized *var* gene containing exactly the same promoter sequence, but a different intron, appeared not to be transcribed. The same was true for a copy of another *var* gene localized in 3´-postion of the transcribed *var* gene, which contained the same intron, but a different promoter compared to the transcribed *var* gene. All promoters localized in the cluster were of the previously described 5B1-type. Our data indicate that the combination of *var* promoters and *var* introns are critical for silencing and transcription of the respective *var* genes.

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BM5 - SEQUENCE DIVERSITY, ANTIBODY RECOGNITION AND EVOLUTION OF THE MALARIA VACCINE CANDIDATE ANTIGEN MEROZOITE SURFACE PROTEIN-2 (MSP-2) OF *PLASMODIUM FALCIPARUM*

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The malaria-vaccine candidate Ag merozoite surface protein-2 (MSP-2) of *Plasmodium falciparum* comprises polymorphic central repeats flanked by dimorphic domains that define the allelic families FC27 and IC1. We have examined temporal patterns of sequence variation in the *MSP-2* gene of *P. falciparum* populations in Brazil and its impact on MSP-2 Ab recognition by local patients. DNA sequence analysis of 61 isolates revealed only 25 unique *MSP-2* alleles, many of them differing by single-nucleotide replacements and insertion/deletion events. Identical *MSP-2* alleles were found in genetically unrelated parasites collected 6-13 years apart, indicating their independent origin by homoplasy in the absence of major selective pressure exerted by variant-specific immunity. To examine Ab cross-reactivity patterns by ELISA, recombinant Ags derived from both locally prevalent and foreign MSP-2 variants were used. Ab recognition of FC27-type Ags and local IC1-type variants correlated with cumulative malaria exposure, but was frequently IgM-restricted. Foreign IC1-type variants, such as 3D7 (included in an experimental vaccine currently under field trial), were poorly recognized. Abs were able to discriminate between local and foreign IC1-type variants, but cross-recognized structurally different IC1-type Ags that were prevalent in local parasites. In contrast, cross-reactive Abs to local and foreign FC27-type variants were more frequently found. The IgG1:IgG3 subclass balance of MSP-2 Abs was exposure-dependent, but no clear-cut bias towards IgG3 was found in heavily exposed patients. We suggest that consensus or ancestral *MSP-2* sequences derived from evolutionary models could be used in vaccine prototypes to minimize the genetic difference between local parasites and vaccine Ags.

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BM6 - *PLASMODIUM FALCIPARUM:* GENETIC DIVERSITY OF THE MEROZOITE SURFACE PROTEIN-2 (MSP-2) IN ISOLATES FROM ENDEMIC AREAS OF PARÁ AND RONDÔNIA STATES, BRAZIL

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Plasmodium falciparum had been shown to present an extensive genetic polymorphism, and a given isolate can be constituted by different clonal populations. In the present work, we have evaluated the genetic polymorphism of the MSP-2 antigen in eighteen *P. falciparum* isolates from an endemic area of Pará (Paragominas) and seventy-eight from Rondônia (Porto Velho), Brazil. Forty fragments from Pará and one hundred nineteen from Rondônia isolates were amplified by nested-PCR using specific primers for the central variable region of MSP-2. The PCR fragments were analyzed in a 2% agarose gel. In Pará, seven different alleles were detected: 600bp (12.5%), 570bp (12.5%), 550bp (42.5%), 490bp (2.5%), 470bp (2.5%), 450bp (25%) and 400bp (2.5%); in Rondônia, ten different alleles were detected: 620bp (1%), 600bp(1%), 550bp (43%), 520bp (4%), 500bp (2%), 490bp (1%), 470bp (2%), 450bp (38%), 400bp (5%) and 340bp (3%). In order to detect sequence microheterogenities, thirty-four fragments from Pará were also analyzed by the SSCP (Single Strand Conformational Polymorphism) technique. Each fragment was digested with *RsaI* restriction enzyme, denatured and analyzed in 10% silver stained polyacrilamide gel. In this case, no sequence polymorphism was observed in any of the analyzed fragments, showing a sequence similarity between these alleles. We concluded that the isolates from Pará and Rondônia presented a limited genetic polymorphism when compared to those observed in areas of high endemicity. The allelic typing and sequencing of the samples will provide a better evaluation of MSP-2 polymorphism in the studied areas.

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BM7 - PROKARIOTIC EXPRESSION AND PURIFICATION OF *PLASMODIUM GALLINACEUM* CS N-TERMINAL AND C-TERMINAL AND PRELIMINARY ANALYSIS OF CROSS-REACTIVITY WITH SERA OF *P. FALCIPARUM* INFECTED PATIENTS

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The circumsporozoite (CS) protein is the predominant surface antigen of *Plasmodium* sporozoites and the major target recognized by the host immune system. CS proteins have been characterized from many *Plasmodium* and contain a secretory signal sequence, a N-terminal region and a C-terminal region, regions of charged amino acids, and an anchor sequence. Phylogenetic analysis using CS gene suggested that the *P. gallinaceum* is more closely related to the *P. falciparum* than other species of human malaria *Plasmodium*. Therefore, the *P. gallinaceum* antigens showed a strong cross-reactivity with antibodies against *Plasmodium falciparum*. Thus, the *P. gallinaceum* has been used as a model to study the immunity against *P. falciparum* infections. In this study, the N-terminal (CSA) and C-terminal (CSC) regions of *P. gallinaceum* CS protein were subcloned in the vector pET32a (Novagen). The expressions of CSA and CSC proteins were induced by addition of 1mM IPTG and recombinant proteins purified using Ni-NTA resin and electroelution. The proteins were used as antigens to coat microplates and specific antibodies were search in sera

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of patients infected with *P. falciparum* in ELISA. Significant levels of antibodies anti-CSA were detected in sera of *P. falciparum* infected patients. The antibody levels were detected independent of the number of malaria infections and the last malaria was *P. falciparum* or *P. vivax*.

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BM8 - EVALUATION OF POLYMORPHISM OF THE P126 *P. FALCIPARUM* PROTEIN AND ITS POSSIBLE ROLE IN DEVELOPMENT OF SPECIFIC IMMUNE RESPONSE

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The *Plasmodium falciparum* P126 antigen is one of a number of erythrocytic-stage proteins being studied as potential malaria vaccine components. The P126 amino-terminal portion, containing 6-octamer repeat, has been shown to be involved in the induction of protection against *P. falciparum* challenge in monkeys. However, a polymophism present in some isolates that contained 5- instead of 6-octamer repeats was observed. In this study we evaluated the genetic polymorphism of N-terminal region of the P126 protein in *P. falciparum* isolates and its possible role in development of specific immune response in individuals living in Brazilian endemic areas. The frequency of polymorphism was verified by SSCP-PCR in 83 isolates from Porto Velho (RO) and 92 isolates from Peixoto de Azevedo (MT). The humoral immune response was analyzed by ELISA using the synthetic peptide Nt47, corresponding the N-terminal region of the protein. Only two different allelic fragments were detected in each area studied: I (199pb) and II (175pb). In Porto Velho, the allele I was detected in a higher frequency (92%) than allele II (8%). In Peixoto de Azevedo the alleles I and II were observed in similar frequencies, 59% and 41%, respectively. Analysis by SSCP does not revealed microheterogeneities of sequences between fragments with same size and only one SSCP pattern was observed for each fragment identified. It was not observed associations between allelic fragments and the humoral immune response against Nt47. However, a positive correlation between cytophylic response (IgG1+IgG3) and the presence of fragment I in individuals living in Porto Velho was verified. The data here presented showed a limited genetic polymorphism of the P126 in *P. falciparum* obtained from infected individuals living in Porto Velho and Peixoto de Azevedo. This allelic polymorphism seem does not influence the development of specific humoral immune response.

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BM9 - EXPLORING THE GENOME OF *TRYPANOSOMA VIVAX*: TOWARDS A COMPARATIVE GENOMICS APPROACH

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Trypanosoma vivax is a hemoparasite affecting livestock industry in South America and Africa. Despite the high economic relevance of the disease caused by *T. vivax*, few researches on its molecular characterization has been done to date compared with human trypanosomes as *T. brucei* spp and *T. cruzi*. The main reason is the difficulty to grow the parasite into laboratory rodents and "in vitro". These characteristics have limited the research on *T. vivax* during the last decades, consequently very few markers have been described for its molecular characterization. A search in Genbank showed that there are only 22 entries for *T. vivax* confronted with nearly 98319, 38686, 23586 for *T. brucei*, *T. cruzi* and *Leishmania*, respectively. *T. vivax* (molecular) biology is also little understood, even considering major differences as mechanical transmission in South America and both cyclical and mechanical transmission in Africa. In order to identify new molecular markers for *T. vivax*, a small-insert genomic library was constructed into the BamHI site of pUC18 using the cloned stock ILDat2160. The libray was semi-normalized by hybridization with known repetitive regions (rDNA, satellite DNA, mini-exon,etc). Negative colonies were selected, then inserts ranging from 1.5 – 3 Kb chosen for sequencing. From the 501 chromatograms obtained, quality evaluation, vector removing and assembly were performed using the Phred/Phrap/Consed package. The resulting 288 sequences were used as queries for the Standalone Blast software (NCBI) using TblastX, BlastN and BlastX in combination with the following databases: Kineto (EBI), NR, NT (NCBI), RepBase (GIRI), *T. vivax* and *T. congolense* (Sanger). Several scripts in Perl and Bioperl were written to obtain frequencies of hits and parse the Blast results. The Blast survey shows that our data has 39.58%, 60.76, 48.61% and 86.81% of "no hits" in Kineto, Repbase, NT and *T. congolense* databases using TblastX. Moreover, using BlastN, our data has 82,64%, 45.14%, 76.38% and 93.06% of "no hits" in RepBase, *T. vivax*, NT and *T. congolense* databases. The BlastX search with the NR database showed 77.78% of "no hits". The most abundant hits were those presenting high similarity (e-values better than 1e-8) to Histone H4, Dynein, Protein kinase, Actin-like protein, Phosphoglycerate mutase, INGI retroelement, *T. brucei* – MVAT, protein kinase, *T. brucei* - GRESAG, ubiquitin and P-glycoprotein. While we have identified a number of new markers for *T. vivax*, in general most of our data represent new genomic regions that should explored as species-specific markers, especially those with "no hits" in the databases. Finally, all regions presenting high similarity to kinetoplastid databases can be further explored in a comparative genomics approach.

Supported by IOC-Fiocruz, CIRAD and IFS

BM10 - PROT-OGIM: A NOVEL SOFTWARE TO IDENTIFY MOTIFS IN ORPHAN GENES FROM PROTOZOAN GENOMES PROGRAM.

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Parasite genome programs has been showing a high percent of unknown genes when submit to GeneBank Database. It was notified in recents reports from *P. falciparum*, *C. elegans* and others organism a big amount of unknown genes(higher than 30%). These genes called orphan genes represent a challenger to basic research to find functions and to discover the involvement of different genes in cellular mechanism. Results obtained from data-mining analyses in genome program follow general rules and is necessary to dissect the information for specialists.

Trypanosomatidae is a family with a very complex gene regulation and cell biology. Orphan genes could be a putative chimioterapic target specially when associated with protozoan organell not present in mamalian cell. Using mathematics approach with regular expression including especific protozoan and others eucariotic motifs and domains, we have develop a new software, PROT-OGIM (orphan gene identification motifs from protozoan) able to renamed orphan genes from all informations available in tripanosomatids genomics program. We are defining new genes to analise function through heterologous and homologous transfection in order to understand the cell biology of the parasite and to elucidate new stool as vaccine and drug candidates to control the disease.

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BM11 - A SURVEY OF THE *LEISHMANIA (V.) BRAZILIENSIS* GENOME

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The current status of genome projects of various organisms is encouraging. Such progresses strongly support research efforts on comparative analysis of content and organisation of the genomes from different organisms.

To expand the available information for *Leishmania* ssp., our aim is to generate sequences and proceed comparative and functional analyses on *L. braziliensis* genome. For this purpose, two different strategies have been pursued. The first one is centered on the development of a systematic gene trapping strategy using a Tn5 transposon system associated with the functional study of delimited regions of the genome. Clones from a *L. (V.) braziliensis* genomic library (into cosmids) corresponding to the chromosomes 2, 5, 14 and 35 were chosen as target for the transposition reactions. After transfection into *Leishmania* parasites, the selected clones will allow to localize and conduct functional studies of trapped genes. The second strategy aims to generate a suitable coverage of *L. (V.) braziliensis* genome and has been carried out using a shotgun genomic library. Eleven thousand and two hundred recombinant clones (about 2 kb inserts) were sequenced corresponding to 5 Mb, approximately 14% of the *L. (V.) braziliensis* haploid genome. Database comparisons showed that 81.02% of the genome survey sequences (GSSs) lacked high score similarity to any protein sequences in GenBank. Nineteen percent of the sequences matched with deposited genes in public databank and were classified according to functional groups such as metabolism, signal transduction, information pathways and electron transport, among others. It is intriguing the low level of similarity found between *L. (V.) braziliensis* GSSs and the *L. (L.) major* databank (54.8%). We are currently investigating the meaning of this finding.

(Supported by FAPESP)

BM12 - GENEDB: A KINETOPLASTID DATA RESOURCE

Christopher Peacock

Wellcome Trust Sanger Institute

GeneDB is a generic database resource for prokaryotic and eukaryotic organisms. The resource is designed to display genomic and post-genomic data integrated with information from scientific literature and community sources. GeneDB has an easy to use, intuitive interface that is consistent between organisms. For some organisms, including some Kinetoplastid and *Plasmodium* species, full time biological staff not only maintain and curate the database but act as a means of interaction between the community and the genome projects. This will improve not only the service to the user but also facilitate the contribution of unpublished information to GeneDB. Facilities currently available include simple querying, complex querying using Boolean operators, browsable lists, chromosome maps, sequence searching using BLAST and OMNIBLAST, motif searching, bulk data downloads, graphical gene and protein feature maps, sequence genome browser and comprehensive links to and from other data resources. Consistent annotation, use of controlled vocabulary and complex querying across species, will greatly improve comparative studies between related organisms. Currently, the datasets in GeneDB include three Kinetoplastid organisms (*Leishmania (L.) major, Trypanosoma brucei* and *Trypanosoma cruzi*) with a further three planned for addition in the near future (*Trypanosoma vivax, Trypanososma congolensi* and *Leishmania (L.) infantum*).

BM13 - EFFECT OF GAMMA RADIATION ON *TRYPANOSOMA CRUZI* IS ASSOCIATED WITH PARTIAL LOSS OF CHROMOSOMES AND INDUCTION OF RAD51 GENE EXPRESSION

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Trypanosoma cruzi is the human parasite of Chagas' disease. Genes involved in DNA repair have been described in this species, however the DNA breaks repair by homologous recombination has not been depicted yet. The *Rad51* gene product is one of the major proteins involved in homologous recombination processes in eukaryotic cells. Here, we have isolated and characterized the *Rad51* gene from *T. cruzi* (*TcRad51*). Using PCR strategy, we cloned the *TcRad51* open reading frame. Remarkably, this gene is present in two copies in the *T. cruzi* genome as revealed by Southern blot. However, analysis of eleven *T. cruzi* strains by 5´-terminal sequencing of the two *TcRad51* copies demonstrated a high degree of conservation in this gene. To investigate whether conservation of *TcRAD51* gene could be associated with the importance of DNA breaks repair by homologous recombination for parasite survival, we submitted three strains from distantly related lineages to gamma irradiation. These strains showed the same high radiation resistance, since motility of the parasite was not abolished by dosages as high as 1 KGy. Additionally, an enhancement in the *TcRAD51* gene expression was observed after irradiation. Also, one of these strains, Cl Brener, showed a different chromosome pattern in a "Pulse Field Gel Electrophoresis" (PFGE) after irradiation, suggesting a likely recombination event or a possible loss of an unessential chromosome. The high gamma radiation resistance of *T. cruzi* is a highly unexpected phenomenon for which we provided the first molecular evidences of its association with a DNA repair gene.

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BM14 - ISOLATION AND CHARACTERIZATION OF POLYMORPHIC MICROSATELLITES OF TRINUCLEOTIDE REPEATS FROM *TRYPANOSOMA CRUZI* GENOME

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The genetic variability in the nature populations of *T. cruzi* has been demonstrated by a great number of biochemical and molecular techniques. In 1998, a new aproach to characterize *T. cruzi* samples emerged with the discovery of polymorphic microsatellites of CA repeats in the *T. cruzi* genome. We now characterize new polymorphic microsatellite loci for *T. cruzi* consisting of trinucleotide repeats to avoid the problem with inaccurate size alleles due to *Taq* DNA polymerase slippage observed for dinucleotide repeats. Using the software Tandem Repeats Finder, we identified 634 trinucleotide microsatellite loci with the number of repeats greater or equal to 7 on total of 32, 607 *T. cruzi* sequences deposited on GenBank release 128. A most frequent repeats found were $(AAT)_n$ where 7 £ n £ 39 (44%) and $(AAC)_n$ where 7 £ n £ 16 (22%). We selected 9 markers for primer design using the program Oligos v.9.5. In addition to the search for repetitive motifs in DNA databases, we constructed an genomic library enriched 3 times for $(AAC)_n$ repeats. We obtained 36 recombinant colonies and after hybrization with the $(AAC)_n$ probe, 24 colonies (67%) were positive

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and all of them were selected for sequencing. Eleven colonies (45%) showed 8 different $(AAC)_n$ repeats with 5 £ n £ 10 and four of them were selected for PCR amplification. To test the variability of the thirteen selected microsatellite loci, we performed a rough screening to evaluate the degree of polymorphism. We typed four genetically distant *T. cruzi* isolates that have been previously characterized by rRNA and $(CA)_n$ microsatellites. Six loci showed accentuated size polymorphism (ACC8, GTT8, AAC16, AAT18, AAT21 and CAA737), three loci displayed discrete size polymorphism (CAA739, CAA9P and CAA9IM), two loci were apparently monomorphic (CTC7 and AAC10) and finally two loci amplified fragments with size different from expected (AGC19 and TTA25).

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BM14 - EXTENSIVE HETEROZYGOSITY IN TWO HYBRID GROUPS OF *TRYPANOSOMA CRUZI*

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Trypanosoma cruzi had been divided previously into two major lineages, or three zymodemes. The species was further subdivided into six groups; I, IIa, IIb, IIc, IId, and IIe by Brisse, et al. (2000). We sought to clarify the evolutionary relationships among these subgroups through the use of molecular markers. Here we provide evidence of group-specific single nucleotide polymorphisms in protein coding genes and intergenic regions between single-copy and tandemly-arrayed multicopy genes. Hybrid groups IId and IIe display heterozygosity at nine loci on at least six different pairs of chromosomes. Markers analyzed include the intergenic regions of the tandemly-arrayed, multicopy genes histone H1, histone H3, histone H2B, histone H2A, and heat shock protein 60. Single nucleotide polymorphisms in protein-coding genes of TcMSH2 (a DNA mismatch repair gene) and glucose phosphate isomerase and the intergenic region between the single copy genes tcp17 (an unidentified ORF) and tcpgp2 (an ATP binding cassette transporter) were identified. Group-specific polymorphisms were identified by sequencing representative strains from each group. Genotyping of all strains was performed by PCR-RFLP. Previous analysis of rRNA and protein coding genes suggested that groups IIa and IIc were hybrids of *T. cruzi* I and *T. cruzi* II. Groups IId and IIe were also shown to be hybrids, but their position relative to the other *T. cruzi* II subgroups was unclear due to the presence of two genotypes at several loci. Our phylogenetic analyses suggest that strains from groups IIb and IIc were the parental lines that contributed genetic material to create groups IId and IIe. The two genotypes present in these hybrid strains may represent two distinct gene loci, or two alleles of the same gene. Our real-world observations are consistent with the study by Gaunt et al. (2003) which found that laboratory-generated hybrid *T. cruzi* strains retain both of the parental genotypes. Furthermore, some of the loci in specific IId and IIe strains have undergone further recombination between the two parental sequences and now display “mosaic” genotypes. The sequence variation supports distinction of four clades of *T. cruzi.* The evidence implies that mechanistic assortment or loss of chromosomes in formation of hybrids is not a random process. *T. cruzi* IId and IIe may represent examples of heterozygote advantage.

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BM15 - A NEW SPECIES OF TRYPANOSOMATID HARBORING BACTERIAL ENDOSYMBIONT: MORPHOLOGY AND PHYLOGENETIC RELATIONSHIPS WITH OTHER SYMBIONT-CONTAINING TRYPANOSOMATIDS.

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Trypanosomatids are parasites of insects, plants and several species vertebrates and invertebrates and also species that are hosts for intracellular bacterial and viral symbionts. Only 5 species harboring bacteria-like endosymbionts are available in culture, all isolated from insects: *C. deanei*, *C. oncopelti*, *C. desouzai*, *H. roitmani* and *B. culicis*. Trypanosomatids isolated from other insect species and from fruit (*Morus* sp) showing endosymbionts revealed to be very similar to *C. deanei* by several molecular markers. Despite isolated from different hosts and distributed into three genera, trypanosomatids harboring bacterial symbionts shared several morphological, biochemical and molecular features. Among these species, only *B. culicis* presented epimastigotes whereas all others presented choanomastigote forms. In this study, we characterized a symbiont-containing trypanosomatid (TryCC219) isolated from a phlebotomine in the Mato Grosso do Sul State, Brazil. Giemsa-stained culture cells of this isolate also show choanomastigotes, although larger than that of other related-species, and also presented choanomastigotes with postnuclear-located kinetoplast called opisthomorphs, typical of symbiont-containing choanomastigotes. Transmission electron microscopy of TryCC219 showed morphological characteristics shared exclusively by trypanosomatids harboring bacterial-symbionts as the absence of paraxial rod and opposition of mitochondrial branches to plasma membrane portions lacking subpellicular microtubules. However, TryCC219 symbionts differed from the commonly described in trypanosomatids, presenting longer and more irregular shape. Moreover, its kDNA can be arranged either in a looser, as in other species, or in a tightly network. As for all other species, the presence of symbiont in the isolate TryCC219 enlarged the biosynthetic capability of the trypanosomatid-host, which could be easily cultivated in very simple culture media, with no source of hemin.

Several studies about the phylogenetic relationships inferred among these species showed that they form a monophyletic group, distant from *Crithidia*, *Blastocrithidia* and *Herpetomonas* species lacking symbionts, thus requiring a taxonomic revision and deserving separated status. However, despite the small number of trypanosomatids (3-5) included in these studies, there is a significant heterogeneity within this group, which need to be better evaluated. With this purpose, we are comparing 12 endosymbiont-harboring trypanosomatids by several approaches. All new isolates included in our study were clustered in this group by SSUrDNA sequence analysis, whereas no endosymbiont-free trypanosomatids were positioned in this group despite we have analyzed several new species/isolates of *Crithidia* (40) and *Herpetomonas* (13). However, among the new isolates with symbionts, only TryCC219 showed high sequence divergence from all other previously described species when we compared RAPD-patterns, classes of kDNA-minicircles, SSUrDNA sequences and length of ITSrDNA. Moreover, ribosomal sequences of the TryCC219-symbiont although close-related to those from other trypanosomatid-symbionts showed significant ITS sequence divergence. Therefore, all data permitted to classify the isolate TryCC219 as a new species of trypanosomatid harboring bacterium-endosymbiont.

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BM16 - COULD *TRYPANOSOMA CRUZI* CYCLES OF TRANSMISSION BE INFLUENCED BY HOSTS' PREVIOUS NATURAL EXPOSURE TO LOW INFECTIVE LINEAGES?

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For the two past decades our laboratory has been working on immunization studies using an attenuated *Trypanosoma cruzi* strain as immunogen against virulent parasites in different animal models (mice, guinea pigs and dogs). We have proved different degrees of protection in laboratory experiments and field studies (Basombrío et al.: Infect Immun 1982, 342-350; J Parasitol, 1987, 73, 1, 236-238; Am J Trop Med Hyg, 1987, 37, 1, 57-62; Am J Trop Med Hyg, 1993, 49, 1, 143-151). Our actual knowledge on *T. cruzi* phylogeny allows a re-interpretation of previous experiments. Now, we present the results of the characterization of these strains by Multilocus Enzyme Electrophoresis Analysis (MLEE) at 15 loci and Random Amplified Polymorphic DNA (RAPD) with five primers, using *T. cruzi* I, IIa, IId and IIe reference strains as control. We found that the attenuated strain belongs to *T. cruzi* I lineage, and the virulent strain most frequently used belongs to *T. cruzi* IIe. Moreover, in this work we show an extensive cross-resistance in hosts immunized with *T. cruzi* I and challenged with *T. cruzi* II lineage. Simultaneously, in a geographically restricted area in Argentina -located in the same region where field immunization studies in dogs were carried out by us- we have evidence that different lineages circulate in domestic cycle (*T. cruzi* I, *T. cruzi* IId and *T. cruzi* IIe). There we found evidence of natural selection on distinct lineages by different mammal species, including humans, suggesting some degree of specificity of particular lineages for specific hosts (Diosque et al., Int J Parasitol, in press). Low virulence had been associated with *T. cruzi* I by different authors, while natural resistance to certain strains in some mammals had been demonstrated by others. In the epidemiolgy of Chagas' disease both -genetic characteristics of parasites and hosts- have been implicated in infectivity and, therefore, in the determination of transmission patterns. We hypothesize that interactions among different parasite lineages of *T. cruzi* by natural non-infective first contact with some hosts, could play a protective role in the transmission dynamic of the parasite in areas where different lineages circulate. We think that these possible "natural immunizations" could be considered, in addition to host and parasite genotype and others epidemiological features, as another variable that could play an important role in the transmission dynamic of Chagas' disease.

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BM17 - *TRYPANOSOMA RANGELI* STRAINS REVEALED LIMITED SEQUENCE VARIABILITY IN BOTH INTERNAL TRANSCRIBED SPACERS (ITS) FLANKING THE 5.8S RRNA GENE

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Trypanosoma rangeli is a hemoflagellate protozoan parasite of a variety of mammalian species, including humans, in a wide geographical area in both Central and South America. Despite the apparently non-pathogenic nature of *T. rangeli*, this parasite infection allows the occurrence of serological cross-reactivity with *T. cruzi*, turning difficult the specific diagnosis of Chagas disease. Studies on biological and molecular aspects of these parasites, such as evolution and behavior in mammalian hosts and triatomine vectors, revealed important intra and inter-specific heterogeneity. In this study, we have comparatively evaluated the ITS-1 and ITS-2 spacers flanking the 5' and 3' ends of the 5.8S subunit of the ribosomal RNA gene (rRNA) among *T. rangeli* strains isolated from distinct hosts and geographical origins. Confirming the PCR-RFLP patterns obtained for ITS-1, cloning and sequencing of the whole ITS repeat (ITS-1/5.8S rRNA gene/ITS-2) revealed a low-level variability of both spacers among the studied strains and the presence of single nucleotide polymorphisms (SNP's) with variable motifs and length within the 5.8S subunit of the rRNA gene. Despite the detected variability, no correlation with epidemiological inferences such as hosts or geographical origins was possible. Intra-specific sequence analyses showed that ITS-1 spacer is less polymorphic than ITS-2 spacer. The inclusion of homologous sequences from *T. cruzi* and *Leishmania* spp. strains obtained from the GenBank allowed a clear inter-specific differentiation, confirming the feasibility of the ITS repeat (ITS-1/5.8S rRNA gene/ITS-2) as a marker for pathogenic trypanosomatids differentiation. The SNP's contained within the 5.8S rRNA gene of distinct strains are under study.

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BM18 - COMPARATIVE STUDIES OF AMASTIN GENE SEQUENCES IN DIFFERENT *TRYPANOSOMA CRUZI* STRAINS

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The amastin gene occurs in multiple copies in the *T. cruzi* genome and codifies a 174 aminoacids, highly hydrophobic membrane protein. In spite of the constitutive transcription, the steady state levels of amastin genes are 60-fold higher in amastigotes compared to epimastigote and trypomastigote forms. Several molecular markers corroborate the existence of two major lineages for *Trypanosoma cruzi*, denominated *T. cruzi* I and *T. cruzi* II, which are associated with the domestic (*T. cruzi* II) and sylvatic (*T. cruzi* I) life cycle of the parasite. More recently, based on the sequence of the MSH2 gene, microsatellite markers and mitochondrial sequences analyses, a classification of strains into three groups termed haplogroups denominated A, B and C have been proposed. The aim of this work is to extend these studies by analyzing sequence variability of the amastin gene among *T.cruzi* strains. We selected two strains representative from each haplogroup, which are: Colombiana and D7 (haplogroup A); JG and 239 (C); Cl-Brener, 167 and 182 (B). To investigate genotypic variation, amastin genes were amplified from DNA isolated from these strains, and the 760 nucleotides PCR products were cloned into the TOPO-TA vector (Invitrogen). Sequencing of the plasmid inserts were performed with the Mega BACE sequencer and analyzed using these programs: Multialin (http://prodes.toulouse. inra.fr/multalin/multalin.html), Blast (http://www.ncbi.nlm.nih.gov/BLAST/), Bioedit (http://www.mbio.ncsu.edu/BioEdit/bioedit.html) and Boxshade (http://www.ch. embnet.org/software/BOX_form.html). A filogenetic tree was constructed using program Mega based on the obtained data. The results suggest the amastin sequences can be divided into three haplogroups and also that increased variability of the amastin gene are found within strains belonging to haplogroups C and B (*T. cruzi* II) when compared to strains from haplogroup A (*T. cruzi* I).

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BM20 - CHROMOSOMAL SIZE VARIATION IN *TRYPANOSOMA CRUZI* ISOLATES AND EVOLUTIONARY INFORMATION ON PUTATIVE HYBRID STRAINS

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Trypanosoma cruzi is divided into two major phylogenetic lineages, named as *T. cruzi* I and *T. cruzi* II. An Expert Committee recommended that the classification of isolates typed as Zymodeme 3, rDNA group 1/2 and isoenzyme group 39 should await further studies. Despite the prevalence of clonal evolution in *T. cruzi*, convergent lines of evidence indicate that some *T. cruzi* genotypes are the result of ancient hybridization events. Among the putative hybrid strains CL Brener and rDNA group 1/2 isolates are included. We have evaluated whether chromosome size polymorphism would give evolutionary information on *T. cruzi* hybrid isolates. For this purpose, we have mapped 23 probes on chromosomes of four isolates representative of heterozygotic genotypes (CL Brener and three clones of rDNA group 1/2); three isolates of *T. cruzi* I and two, of *T. cruzi* II groups. These genetic markers cover almost all CL Brener chromosomes ranging from 600 to 3,300 kb. The relationships among the stocks were calculated by the aCSDI formula for the analysis of chromosomal size variation (Dujardin et al., 1995) and compared with those obtained from RFLP data originated from the hybridization of genomic DNA with the same probes. Both phenetic analyses identified three clusters, corresponding, respectively, to *T. cruzi* I, *T. cruzi* II and rDNA group 1/2 strains. Depending on the nature of the analyzed probes, CL Brener was included either in *T. cruzi* II or group 1/2 clusters. Recently, we have started to analyze by the by aCSDI method the phylogenetic relationship of four isolates classified as Zymodeme 3 (kindly provided by Prof. J.R. Coura and Dr. O. Fernandes, FIOCRUZ). Preliminary aCSDI analysis from 12 probes indicates that three stocks of Zymodeme 3 are closer to *T. cruzi* II group and one stock is included in the same branch that clusters CL Brener and group 1/2 strains. A larger number of genetic markers will be analyzed to elucidate the evolutionary association among Zymodeme 3 isolates and stocks from Bambuí (Minas Gerais), which belong to rDNA group 1/2 (kindly provided by Drb. E. Chiari, UFMG).

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BM21 - IDENTIFICATION OF *T. CRUZI* GENOTYPES FROM SYLVATIC CYCLES AND GENETIC DIVERSITY REVEALED BY RAPD ANALYSIS.

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The genetic variability of *T. cruzi* has been widely explored. Considerably heterogeneity has been demonstrated among isolates from both the domestic and sylvatic cycles, although contrasting to domestic cycles, little is known about the genetic polymorphism among *T. cruzi* isolates in sylvatic cycles. *T. cruzi* transmission cycles can be broadly classed as enzootic-cycles. To analyze the genetic diversity and population structure of Brazilian *T. cruzi* isolates from sylvatic mammals from areas of different transmission cycles we compared isolates from: a) Amazon Basin, where *T. cruzi* is enzootic, with few human cases of Chaga's Disease despite widespread sylvatic cycle involving several sylvatic mammal and triatomine species, b) São Paulo, a former endemic area where intradomiciliary transmission is presently considered controlled whereas the sylvatic cycle is very active, specially in areas of Atlantic Coast Rainforest.

Isolates from Amazon Basin (15) and São Paulo (17) were obtained from marsupials, non-human primates, bats and rodents. All new isolates (obtained harmocultures or xenodiagnosis) were classified as *T. cruzi* by tradicional criteria of morphology, growth features, infectivity for mice, behavior in triatomines and development in mammalian cultures cells. Behavior in mice (parasitemia and virulence) and% of metacyclic forms in cultures disclosed high heterogeneity among the isolates.

All isolates were molecularly diagnosed to separate *T. cruzi* from *T. rangeli* and *T. cruzi*-like and mini-exon typed into the three major lineages using human and triatomine isolates previously typed as *T. cruzi* I (TC1), *T. cruzi* II (TC2) or Z3 as contrals. In the Amazon Region it was found TC1 and Z3-lineages, as previously reported for human, sylvatic mammal and triatomine isolates. In this region, while these both lineages were recovered from non human primates, enlarged the sylvatic mammalian order host-range of Z3-lineage so far restricted mainly to armadillos, all isolates from marsupials and bats were TC1. Isolates from São Paulo were typed as TC1 or TC2. All TC2 isolates are from marsupials and rodents of Atlantic Coast, where people live close to forest whereas TC1 are from a former endemic area that is characterized by human cases associated to TC2 and domestic cycle. Thus, typing of isolates from these two areas is in agreement to their transmission cycles. RAPD analyses revealed high genetic polymorphism among isolates of different lineages and significant intra-lineages heterogeneity. Mini-exon typing and RAPD-dendogram branching-pattern suggested some association of lineages and/or sub-lineages with geographic region and mammalian species. A phylogenetic relationships among isolates from sylvatic mammals and triatomines of distinct transmission cycles are being inferred using SSUrDNA sequences.

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BM22 - CHARACTERIZATION OF TWO STRAINS OF *TRYPANOSOMA CRUZI* CHAGAS, 1909 (KINETOPLASTIDA, TRYPANOSOMATIDAE) ISOLATED FROM SPECIMENS OF *TRIATOMA RUBROVARIA* (BLANCHARD, 1843) (HEMIPTERA, REDUVIIDAE, TRIATOMINAE) COLLECTED IN WILD ENVIRONMENT.

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The flagellate protozoan, *T. cruzi* is the etiological agent of Chagas's disease, a serious medical and social problem affecting approximately 18 million people in Latin America. The large heterogeneity of the parasite that circulate among human, different species of Triatominae and sylvatic and domestic animals it makes with that it presents great diversity of behavior, leading the different forms of clinical presentation of the disease in the man. Two *T. cruzi* strains, denominated QG1 and QG2, isolated from faeces of fifth instar nymphs of *Triatoma rubrovaria* collected in the Rio Grande do Sul state have been studied. In order to the characterization of *T. cruzi* strains maintened in Swiss mice, morphology of the bloodstream forms, the curve parasitemic, pre patent period, histiotropism, and the mortality rate in relation to the infection of Swiss mice were recorded. The strains have been maintenance in LIT medium. The molecular characterization was carried out by the technique of amplification of 24Sá rRNA gene sequences. The two strains, QG1 and QG2, have been classified as Type or Biodeme III and Lineage 2 indicating that both belong to the now defined group *T. cruzi* I.

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BM23 - ANURAN TRYPANOSOMES: MORPHOLOGICAL, BIOLOGICAL AND MOLECULAR CHARACTERIZATION OF BRAZILIAN ISOLATES.

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Several species of Anura belonging to distinct families have been described harboring trypanosomes around the world. Most descriptions of anuran trypanosomes have been based on bloodstream forms, few isolates are available in culture and there are no cultivable isolates from South America. There are few data about development of trypanosomes in both anurans and vectors (leeches and dipterans). There are more than 60 trypanosome species described in anurans. Species classification is based on morphology and morphometry of blood trypomastigotes, host-species and geographic origin. The high polymorphism of trypanosomes in blood and the existence of morphologically similar flagellates in different host-species and geographic regions proved that these parameters are not suitable for taxonomy. There are no studies about anuran trypanosomes in Brazil. Isoenzymes, riboprinting and RAPD patterns distinguish the anuran species. Phylogenetic studies based on SSUrDNA clustered together all anuran trypanosomes from North America, Europe and Africa. To evaluate the prevalence, morphology, diversity and phylogenetic relationships among trypanosomes from Brazilian anurans we examined 237 specimens, of 49 species of Anura, from different ecosystems: Amazon; Pantanal; Atlantic Forest and Cerrado.

Blood trypanosomes were investigated by microhaematocrit, revealing 42% of infected animals (138 specimens) and by haemocultures, resulting in 43% of cultures (from 237 animals). A total of 107 anuran specimens were found harboring trypanosomes, ranging from 27% in Atlantic Forest to 69% in Pantanal (average of 48,5% in all regions). From 101 positive haemocultures, 70 isolates from 21 anuran species were established in culture. Morphological analysis of Giemsa-stained blood smears revealed a high polymorphism of size and shape among trypanosomes from distinct hosts and even within the same animal. It was observed very large trypomastigotes, long or rounded, showing a well-developed undulating membrane and small kinetoplast. Although epimastigotes from culture were more homogeneous than blood forms, inter and intra-isolate polymorphisms were detected, showing three major morphological patterns.

Analysis of polymorphisms of kDNA minicircle classes, length of ITS of rDNA and RAPD patterns showed high genetic variability among Brazilian isolates. These analyses distinguished isolates from different host-species as well as isolates of the same host-species. On the other hand, high homogeneity was observed among some isolates from different genera and families of Anura. In addition, isolates from distinct geographic regions showed similar patterns of molecular markers. Therefore, morphology, host-species and geographic origin are not enough to classify anuran trypanosomes.

Despite the high polymorphism, the association of molecular markers distributed the isolates in several groups. However, these grouping patterns could not be associated to either, host species or geographic origin. Phylogenetic analysis based on SSUrDNA is being inferred to define the taxonomic position of these groups as well as the phylogenetic relationships among them and with anuran trypanosomes from other countries.

Supported by FAPESP

BM24 - GENES ENCODING CATHEPSIN-B LIKE CYSTEINE PROTEASES OF TRYPANOSOMES: ISOLATION, CHARACTERIZATION AND PHYLOGENETIC ANALYSIS.

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Cysteine-proteases are one of the most important families of enzymes that includes cathepsin L (cat-L) and B (cat-B), involved in a wide spectrum of biological processes and implicated in host-parasite relationship and in pathogenicity of parasites. These enzymes have been used as target for drugs, vaccines and diagnosis. In contrast to cat-L, there are few studies about cat-B of trypanosomatids and they are restricted to *Leishmania* and to the pathogenic trypanosomes *T. brucei* and *T. cruzi*. Characterization and inference of relationships of cat-B genes from trypanosomes of diverged phylogenetic groups, patoghenic or not, and with different biological cycles and behavior in their vertebrate and invertebrate are important for studying molecular evolution of both cysteine-proteases and trypanosomes themselves. Moreover, these data could provide a basis to further understanding the mechanism of enzyme action in a variety of organisms and to compare gene structure and function.

With this purpose we decided to compare sequences of cat-B genes of the non-pathogenic species *T. lewisi* (plus the allied species *T. rabinowitschae*) and *T. rangeli* with those from pathogenic species of *T. cruzi* and *T. brucei.* DNA fragments containing the catalytic domain of cat-B genes (~612bp) were isolated by PCR using degenerated primers, cloned and sequenced. Cat-B sequences differed among most trypanosome species on RFLP patterns of genomic DNA through cross-hybridization analysis using the amplified cat-B sequences as probes. Results suggested that *T. lewisi* (and probably *T. brucei*) has more than one copy of cat-B gene whereas *T. rangeli* apparently has a single copy similarly to described for *T. cruzi* and *Leishmania* spp. Northern blot analysis revealed transcripts of cat-B variable in size and sequence according to species of trypanosomes. To infer phylogenetic relationship among cat-B genes within *Trypanosoma* we constructed gene trees based on aligned sequences from the following species: *T. lewisi, T. rabinowitschae,* two isolates of *T. rangeli* belonging to distinct genetic groups (plus one sequence of an isolate of another group from GeneBank), 3 isolates of *T. cruzi* typed as *T. cruzi* 1, *T. cruzi* 2 and Z3 and *T. brucei* (sequence obtained from *T. brucei* genome data base). Analysis of sequence divergence revealed high genetic variability among cat-B from trypanosomes. Exception was *T. lewisi* and *T. rabinowitschae* that shared very similar cat-B genes. In contrast to the low sequence divergence among *T. cruzi* isolates significant intraespecific polymorphism was detected among isolates of *T. rangeli*. The branching patterns of the inferred trees constructed using nucleotides or predicted amino acids sequences were not identical, although, in both analysis *T. brucei* was positioned in a separated branch and *Leishmania* spp. were always positioned as outgroup of *Trypanosoma*. The Cat-L derived phylogenetic trees were compared with those obtained using cat-L and SSUrRNA sequences.

Supported by FAPESP and Capes.

BM25 - ISOLATION AND SEQUENCING OF CATEPSINA L–LIKE CISTEINE-PROTEINASE GENES OF *TRYPANOSOMA VIVAX* STOCKS REVEALED HIGH POLYMORPHISM AND A NEW MARKER FOR DIAGNOSIS

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T. vivax, T. congolense and *T. brucei* are the major pathogens of cattle in Africa. *T. vivax* is also widespread in Central and South America. In contrast to African stocks, which are cyclically transmitted by tsetse flies, American stocks of *T. vivax* are mechanically transmitted by biting flies. Stocks from different geographical regions differ in morphology, pathogenicity, zymodemes and DNA sequences, separating stocks from West Africa and South America (mild/assymptomatic infections) from East African stocks (severe disease).

Cathepsin L-like (cat-L) cysteine proteases have been implicated with development in vector insects and with pathogenicity of *T. cruzi*, *T. brucei* and *T. congolense.* Cat-L genes are grouped in arrays of tandem repeats consisting

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of conserved catalytic domain and sequences variable according to species, stocks, and genes within the same isolate. Multiple copies and different degrees of conservation makes this gene excellent for diagnosis and evaluation of genetic relatedness within African trypanosomes and among *T. vivax* stocks differing in pathogenicity, as well as to compare stocks mechanically and cyclically transmitted.

We characterized cat-L gene sequences of *T. vivax* stocks from South America (Brazil), West Africa (Nigeria) and East Africa (Kenya). Sequences containing the catalytic domain were amplified by PCR, cloned, sequenced and nucleotide and amino acids sequences were aligned and compared to other trypanosomes. The high degree of similarity among sequences from all members of the subgenus *Trypanozoon* (*T. evansi, T. equiperdum* and *T. brucei* ssp.) contrasted with the high divergence detected among stocks of *T. vivax*. In addition, significant polymorphisms were observed among sequences of the same *T. vivax* stock, suggesting the existence of isoforms of cat-L genes, as described for *T. cruzi* and *T. congolense.* Phylogenetic analysis showed similar topologies of cat-L gene trees using either nucleotides or amino acid sequences clearly segregating sequences according to trypanosome species: a) sequences from *Trypanozoon* (Trypanopains) tightly clustered together; b) despite high genetic distance sequences among *T. vivax* stocks (Vivaxpain) they always clustered; c) sequences from *T. congolense* (Congopain 1 and 2) were separated in another clade. Cat-L genes from *T. cruzi* (Cruzipain 1 and 2) were positioned as outgroup of African species.

We developed a *T. vivax* specific PCR based on cat-L gene sequence able to detect all tested stocks, from South America, West and East Africa. No amplified products were generated for *T. congolense*, *T. theileri*, *T. evansi* and *T. b. brucei*. This method proved to be enough sensitive to detect cryptic *T. vivax* infection using crude preparations of field-collected blood samples. A multiplex PCR assays based on cat-L sequences are being currently improved to simultaneous detection of all cattle trypanosomes.

Supported by FAPESP and Capes.

BM26 - BIOLOGICAL AND MORPHOLOGICAL CHARACTERIZATION AND PHYLOGENETIC RELATIONSHIP OF BAT TRYPANOSOMES

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Trypanosomes have been recorded from numerous species of bats throughout world. Four subgenera are recognizing as occurring in bats: *Schizotrypanum, Megatrypanum, Herpetosoma* and *Trypanozoon*. Chiroptera are the only hosts of *Schizotrypanum* in the Old World, excepting *T. cruzi*, which is restricted to Americas and infects all mammalian orders. Despite *T.c.cruzi*, all other species classified in this subgenus are *T. cruzi*-like species restricted to bats: *T. cruzi marinkellei* (Central and South America), *T. dionisii* and *T. vespertilionis* (New and Old Word). Data about bat trypanosomes are mostly restricted to morphology of blood forms. Most cultivable species are of *Schizotrypanum* and showed significant genetic heterogeneity. Due to misclassification and to the lack of large number of isolates from distinct bat species and geographic regions, classification and phylogeny of *Schizotrypanum* are still controversy and the validity of several species is questionable.

To evaluate the prevalence and the genetic diversity among trypanosomes from different bat species and geographic regions of Brazil we investigated trypanosomes in bats from different and distant Brazilian ecosystems, Amazonia, Pantanal and Atlantic Forest, including bats belonging to 37 distinct species. From 460 bats examined, blood trypanosomes were detected in 129 by microhematocrit. Blood trypanosomes revealed significant polymorphism, although most were trypomastigotes typical of *Schizotrypanum*. It was obtained 57 cultures from Amazonia, Pantanal and Atlantic Forest, with no significant differences in prevalence of bat trypanosome infection in the studied regions. Isolates were obtained from 13 species of bats: 9 from insectivorous, 2 from frugivorous and 2 from hematophagous. Infectivity for mice and triatomines were used to distinguish *T.c.cruzi* from all other species. Most isolates multiply as amastigotes within mammalian cells in vitro. Differences in growth and morphological features (light and TEM) were also detected. Together, biological and morphological data suggested four major groups of isolates.

To investigate the taxonomic position of Brazilian bat trypanosomes, phylogenetic relationships among 46 isolates were inferred based on SSUrDNA sequences. Phylogenetic trees showed that isolates from Brazilian bats is polyphyletic and segregated into two distant branches. Most isolates were clustered into a major monophyletyc branch containing all *Schizotrypanum* species and were distributed into three heterogeneous groups: A) *T. c. marinkellei*, lacking infectivity for mice and only transiently infecting triatomines; B) *T. dionisii*, incapable to infect mice and triatomines; C) *T. cruzi,* infective for mice and triatomines, which members were segregated into two branches (*T. cruzi* 1 or Z3). Group D were positioned far from *Schizotrypanum,* differ in growth and morphology from all other groups and lacks infectivity for mice, triatomines and cell cultures. The grouping of isolates are in agreement with biological and morphological data and are independently of host-species and geographical origin, except the group D which was constituted only by isolates of *Pteronotus parnelli* from Amazon Region.

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BM27 - BRAZILIAN ISOLATES OF *TRYPANOSOMA* (*MEGATRYPANUM*) *THEILERI*: DIAGNOSIS AND DIFFERENTIATION OF ISOLATES FROM CATTLE AND WATER BUFFALO BASED ON BIOLOGICAL CHARACTERISTICS AND RANDOMLY-AMPLIFIED DNA SEQUENCES

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Trypanosoma (*Megatrypanum*) *theileri* is the type-species of the subgenus *Megatrypanum* and is considered a cosmopolitan parasite of cattle with a worldwide distribution. In contrast to *T. theileri* in cattle, there are few records of *T. theileri*-like in buffaloes. More recently, *T. theileri*-like was recorded in water buffaloes from Brazil. Despite the ubiquity of *T. theileri* and in contrast to pathogenic salivarian trypanosomes of bovids (*T. vivax*, *T. brucei brucei*, *T. evansi* and *T. congolense*), few data exist about the vertebrate cycle, pathogenicity, biochemical and molecular characteristics of *T. theileri*. Moreover, there are no studies comparing morphological, growth and molecular features of isolates from cattle, buffaloes and other species of Bovidae. So far, no convincing data are available to tell whether *T. theileri*-like from distinct species of bovids belongs to only one species or whether trypanosomes from these hosts constitute different species. Thus, classification of these organisms at specific and infraspecific level needs additional taxonomic parameters.

In this study we detected and cultivated isolates of *Trypanosoma* (*Megatrypanum*) *theileri* from cattle and water buffaloes in São Paulo State, Southeastern Brazil, which were characterized by comparing morphological, growth and molecular features. Although isolates from cattle and water buffalo were morphologically indistinguishable, differences in growth characteristics separated them. Our RAPD analysis showed that, although *T. theileri* isolates were all from the same geographic region and always clustered together, organisms from this cluster were segregated into two subclusters according to their host species. These data demonstrated that *T. theileri* trypanosomes from cattle and water buffalo are not identical, supporting the idea of the host specificity of

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Megatrypanum spp. The trypanosomes from water buffalo reported here are the first *T. theileri*-like isolates from the Asiatic buffalo (*Bubalus bubalis*) to be continuously cultured and biologically and molecularly compared with cattle isolates. In our RAPD analysis, *Megatrypanum* from wild mammals were segregated into different branches. The sequence of a synapomorphic RAPD-derived DNA fragment, which was shared by all *T. theileri*-trypanosomes from cattle and buffalo but not detected in any of 13 other trypanosome species, was used as target for a conventional *T. theileri*-specific PCR assay. We also defined RAPD fragments that permitted to distinguish cattle from buffalo isolates. Therefore, distinct growth features and genetic variability distinguished between isolates from cattle and water buffaloes of the same geographic origin, suggesting an association of these isolates with their host-species. However, these results did not exclude the existence of genetic variability among isolates of the same host species from different geographic regions. We are currently carrying out further studies to better evaluate the diversity among isolates from bovids from other areas of Brazil.

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BM28 - REPTILIAN TRYPANOSOMES: MORPHOLOGICAL, BIOLOGICAL AND MOLECULAR CHARACTERIZATION OF BRAZILIAN TRYPANOSOMES FROM LIZARDS, SNAKES AND ALLIGATORS

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Reptiles can be infected by species of *Leishmania* in the Old World and by species of *Trypanosoma* in New and Old World. Although it was described several species of trypanosomes in lizards, chelonians, crocodilians and snakes, most data are restricted to morphology of blood forms and many species must be synonyms as well as new species can be underestimated. The only adopted taxonomic parameters for species classification are morphology, host-species and geographic origin. Leeches, ticks and dipterans can transmit reptilian trypanosomes. Although there are few data about life cycle of these trypanosomes in both vertebrate and invertebrate, they are considered non-pathogenic from their hosts and most are reported as highly species-restricted. Few isolates from reptiles are available in culture and there are only inconclusive data about prevalence, genetic diversity and phylogenetic relationships. Studies based on few isolates showed partition of isolates from lizards into two distant clades, together with isolates from fishes, anuran and chelonians (aquatic clade) or with avian isolates. There are no molecular studies regarding South American trypanosomes of reptiles.

Aiming to isolate in culture and to study the genetic diversity among Brazilian trypanosomes from reptiles we examined blood samples from lizards and snakes (several species) and from alligators. Few blood samples of lizards (2,85% of 140 animals) and snakes (2,75% of 400 animals) were positive for trypanosomes, in contrast to 32% of 37 alligators examined by microhaematocrit. Morphological analysis of Giemsa-stained smears revealed a high polymorphism of size and shape among blood trypomastigotes from distinct hosts. Most forms were large trypomastigotes, showing a well-developed undulating membrane and a very small kinetoplast. It was obtained 14 isolates by haemocultures: 4 from lizards (*Tropidurus plica* from Amazonian Region); 7 from snakes (*Crotalus durissus terrificus* from Southern and Southeast Brazil) and 3 from alligators (*Caiman yacare* from Pantanal Region). Different growth and morphological features were observed among the isolates. When cultivated at 25°C, most culture forms were large epi- and tripomastigotas, differing according to host-species.

Comparative analysis showed large size variability of kDNA minicircles among the reptilian trypanosomes, with patterns shared for isolates of the same host-species. Genetic polymorphism evaluated by analysis of ITS of rDNA amplified by PCR disclosed length variability. In addition, cross-hybridization analysis of ITS indicated significant sequence heterogeneity. These data permitted to separate the isolates into three major groups, according to their host species, despite small heterogeneity among alligator isolates. Analysis of ssUrDNA genes, amplified by PCR, cloned and sequenced, also revealed high polymorphism among the reptilian isolates. We are currently inferring phylogenetic trees based on ssUrDNA among our isolates aligned with those from reptiles (GeneBank) and other hosts in order to define their taxonomic position and phylogenetic relationships.

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BM29 - MOLECULAR DIFFERENTIAL DIAGNOSIS BASED ON ITS1 RDNA: OPTIMIZATION OF THE PCR ASSAY FOR FIELD STUDIES.

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Trypanosomosis is one of the most intractable protozoan diseases of livestock caused by more than five species of trypanosomes. There is a general lack of species-specific PCR-based assays for livestock trypanosome infection since there are reports showing that some *T. vivax* infection were not detected by PCR in West Africa and Latin America. More recently Dávila (2002) developed a PCR assay based on the ITS1 rDNA (Tryps-B primers) to detect several livestock trypanosome infections in a single reaction using only one pair of primers. The assay was shown to be efficient and will be tested in the field in the following enzootic countries: Bolivia, Brazil, Burkina Faso, Uganda and South Africa, within a consortium funded by IAEA. Before the assay is tested in the field, some extra optimization was needed in order to identify the best PCR cocktail able to detect as many trypanosome species as possible. PCR cocktails with different concentrations of dNTP's, $MgCl_2$, KCl and adjuncts were tested in combination with two "DNA mix" as templates in 25µl reactions. DNA Mix A: *T. vivax, T. evansi, T. congolense* Savanna, *T. congolense* Kilifi, and *T. simiae,* DNA Mix B: *T. vivax, T. evansi, T. congolense* Savanna, *T. congolense* Kilifi. dNTPs were tested in the following final concentrations: 72.5mM, 108.75mM, 145mM, 181.25mM and 217.5mM. $MgCl_2$ was tested in the following final concentrations 0.5mM, 1mM, 1.5mM, 2mM and 2.5mM. KCl was tested in the following final concentrations: 25mM, 50mM and 75mM. The following adjuncts were also tested according to the manufacturer indications of the PCR Optimization kit II â Sigma: Formamide, Ammonium sulfate, Betaine, BSA, Glycerol and DMSO. The size of the products obtained individually were: *T. vivax* - 230bp*, T. evansi*-550bp*, T. congolense* Savanna -710bp and *T. congolense* Kilifi - 650bp, and *T. simiae* - 400pb. Our results show that 2 PCR cocktails containing higher concentrations of dNTPs and KCl worked better amplifying several trypanosome species. Cocktail K: 2mM MgCl2, 50mM KCl, 217.5mM dNTPs, 45µg/ml BSA. Cocktail L: MgCl2 2mM, KCl 75mM, 217.5 mM dNTPs, 5% DMSO. With either DNA mix A or B, only 4 different species of trypanosomes were amplified. We do not consider this a limitation for the assay, as when an animal is found infected with any of the four species, it will have to be treated anyway and this will help to avoid the use and costs of 4 different reactions for each animal. Based on these results the assay based on ITS1 rDNA showed to be ready to be tested in the field. The optimization of the assay was useful to establish the best PCR cocktail in order to detect unequivocally as many species as possible.

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BM30 - ANALYSIS OF *TRYPANOSOMA RANGELI* RDNA LOCUS: SEQUENCE POLYMORPHISM OF THE INTERGENIC SPACER IN DIFFERENT GEOGRAPHICAL STRAINS

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Trypanosoma cruzi and *Trypanosoma rangeli* are the only two known American trypanosomes of man. In the insect vector both trypanosomes divide and develop infective forms but *T. rangeli* affects feeding mechanism and survival. Although both parasites occur in the same mammalian hosts and share Triatomine vectors, *T. cruzi* causes Chagas' disease and *T. rangeli* is considered non pathogenic to humans This overlapping complicates the diagnosis and epidemiology of the Chagas disease, because both parasites have similar surface antigens. We have undertaken the development of *T. rangeli* species specific PCR assays based on the rDNA locus. The analysis of the rDNA intergenic spacer (IGS) by long PCR amplification of previously typed *T. rangeli* strains from different geographical locations in Colombia and Venezuela revealed size polymorphisms. Sequencing of four cloned IGS from *T. rangeli* San Agustin (Colombia) demonstrated a high sequence conservation within this isolate. Based on the IGS San Agustin sequences we have designed an amplification assay with a 500 bp product. When five *T. rangeli* DNAs from different geographical areas of Venezuela were tested, this PCR assay resulted in the specific amplification of only the homologous San Agustin strain. This data suggests high heterogeneity within the *T. rangeli* strains tested as judged by this criteria introducing a new aspect to consider in the already complex epidemiology of mixed infections with *T. cruzi*. We are currently testing an increasing number of *T. rangeli* strains from Central America to Brazil. Supported by FONACIT grant S1-98002681 to GP. AMT has a fellowship from FONACIT and UNU/BIOLAC.

BM31 - CHAGAS' DISEASE MOLECULAR SEROEPIDEMIOLOGY IN TWO RURAL POPULATIONS AT SUCRE STATE, VENEZUELA

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Chagas' disease is a serious public health problem in Latin America (WHO, World Health Report, 1998). In Sucre state located at Northeastern of Venezuela, we selected two rurals populations: Ribero municipality with high endemicity records in 1982 of *Rhodnius prolixus* peridomestic infection of 49.3% and domestic 7.2% with *T. cruzi* parasite 10.8% and 4.9% respectively, and Arismendi municipality with low endemicity records of *Rhodnius prolixus* peridomestic infection of 1.2% and domestic 0.1% both without *T. cruzi* parasite (source: Sucre Rurals Endemics Division dead files). However, 2000 latest official report by the Chagas' Disease Venezuelan Control Programme (CDVCP) showed no seroprevalence at Sucre state, which is surprising regarding the gradual state of abandon of epidemiological control and surveillance imposed by economics limitations. In this sense, by active search we carried out a molecular seroepidemiologic study at Catuaro village, Ribero municipality (10°24'00'' LN and 63°29'50''LW) and San Juan de las Galdonas village (10°42'46'' LN and 62°50'20'' LW) with the propose to determine Chagas' disease seroprevalence. We studied 239 random selected individuals during 2000 june-october. Inmunodiagnostic was realized using quantitative ELISA IgG anti *T. cruzi* (Bioschile, IGSA) and direct agglutination test (DAT) with crude antigen of *T. cruzi*, YBM strain, HOM zimodem. PCR standardization was carried out with S35/S36 primers on serum samples of seropositives individuals confirmed by the immunological tests before mentioned. DNA purification were made by two methods: conventional procedure with Guanidine HCl/EDTA and Wizard commercial kit (Promega). Seroprevalence of Catuaro was 21.3% (2.7 higher in relation to national prevalence, CDVCP, 2000). San Juan de las Galdonas showed similar national results (7.3% vs. 8.1%), highlighting between 20 years old individuals, 50.0% of them were seropositives suggesting active transmission of the Chagas' disease. By PCR, the sensibility was 100% with DNA purified by Wizard kit showing direct correlation between the ethydium bromide bands intensity of the amplified DNA fragments and the optics densities of ELISA, whilst the conventional method sensibility was 40%. These findings showed the highest seroprevalence observed in Venezuela likely associated with the abandonment of control, prevention and epidemiologic surveillance at Sucre state. In addition is important pointing out that purification of DNA serum samples with Wizard kit represent a new valuable molecular tool for detection of *T. cruzi* in seroepidemiological studies.

Financial support: Universidad de Oriente: CI-5-1901-0848/99 and CI-5-1005-1055/01

BM32 - MULTICLONAL *LEISHMANIA (V.) BRAZILIENSIS* POPULATION STRUCTURE AND ITS CLINICAL IMPLICATION IN A REGION OF ENDEMIC AMERICAN TEGUMENTARY LEISHMANIASIS (ATL).

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In Corte de Pedra (CP), northeastern Brazil, *Leishmania braziliensis* causes three distinct forms of American tegumentary leishmaniasis (ATL). To test the hypothesis that strain polymorphism may be involved in this disease spectrum and accurately characterize the parasite population structure in CP, we compared one *L. (L.) major*, two non CP *L. (V.) braziliensis*, one CP *L. (L.) amazonensis* and 45 CP *L. (V.) braziliensis* isolates, obtained over a ten-year period from localized cutaneous, mucosal and disseminated leishmaniasis patients, using randomly amplified polymorphic DNA (RAPD). Electrophorectic profiles were mostly unique across species. All typing protocols revealed polymorphism among the 45 CP *L. (V.) braziliensis* isolates, which displayed 8 different RAPD patterns and greater than 80% overall fingerprint identity. The UPGMA dendrogram based on the sum of RAPD profiles of each isolate unveiled nine discrete typing units clustered into five clades. Global positioning showed extensive overlap of these clades in CP, precluding geographic sequestration as the mechanism of the observed structuralization. Finally, all forms of ATL presented a statistically significant difference in their frequencies among the clades, suggesting that *L. (V.) braziliensis* genotypes may be accompanied by specific disease manifestation after infection.

BM33 - A NEW ENZYMATIC VARIANT OF *LEISHMANIA (VIANNIA) GUYANENSIS* FOUND IN ISOLATES FROM PATIENTS IN SANTARÉM, PARÁ STATE, BRAZIL

Jennings, Yara L. L.; Ishikawa, Edna A. Y.; de Souza, A.A. Adelson; Lainson, Ralph; Silveira, Fernando T.

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Introduction: Among the various dermotropic species of *Leishmania* in the Amazon region, *Leishmania (V.) guyanensis* is essentially silvatic and predominantly distributed north of the river Amazonas. Antigenic variants in populations of the parasite have been found, related to its reaction with the monoclonal antibody B19 (Grimaldi *et al*., 1991; Romero *et al.*, 2002). Till now, enzyme electrophoresis, however, has indicated that strains from north Brazil are represented by populations homogenous and identical to the reference-strain of *L.(V.)guyanensis* (MHOM/BR/1975/M4147 – Monte Dourado, Pará). This communication records the first finding of intraspecific variation observed by enzyme electrophoretic characterization among isolates of this parasite. Objetives: To study the phenotypic characters of strains of *L.(V.) guyanensis* by their monoclonal antibody and enzyme electrophoretic profiles. Materials and methods: Thirteen samples of *Leishmania* isolated from patients from three municipalities of the lower amazon region (Santarém: 9; Óbidos: 3; Almerim: 1) in the west of Pará State were studied by a panel of 23 monoclonal antibodies (biotine-avidine system) and enzymatic electrophoresis in starch-gel with the six enzymes 6PGDH, G6PD, PGM, MPI, ASAT and ALAT. Profiles of the samples were compared with the reference-strains of all the know *Leishmania* species from the Amazon region. Results: All thirteen isolates reacted with the monoclonal antibody B19, which is species-specific for *L.(V.) guyanensis*, and thus showed a reaction comparable with that of serodeme II recorded by Shaw *et al*. (1984). With regards enzyme electrophoresis, two different profiles were detected: one (6 samples) agreeing with that of the reference-strain of *L.(V.) guyanensis* and another (7 samples) differing in the motility of the enzymes 6PGDH and PGM. Conclusions: The present study records, for the first time, the presence of *L.(V.) guyanensis* south of the Amazon river, in an area of the Santarém municipality, Pará. This extends the recorded distribution in Amazonian Brazil, although it is geographically very close to the major endemic area, north of the Amazon river. On the other hand, while all thirteen isolates showed the same serodeme profile as that of the reference-strain of *L.(V.) guyanensis*, seven showed a enzymatic variant of the reference-strain in mobility of the enzymes 6PGDH and PGM. This contrasts with the results of Romero *et al.* (2002) who indicated the presence of a different serodeme in a population of *L.(V.) guyanensis* from Manaus, Amazonas State, whereas the enzyme electrophoretic profiles were compatible with that of the reference-strain of that parasite.

BM34 - AUTOCHTHONOUS VISCERAL LEISHMANIASIS IN DOGS OF EMBU-DAS-ARTES, SÃO PAULO.

Savani, E.S.M.M.[1]; Neves, E.[2]; D'Áuria, S.R.N.[1]; Zampieri, R.A.[3]; Ishikawa, E.[4]; Camargo, M.C.G.O.[1]; Shaw, J.J[3]. & Floeter-Winter, L.M.[3]

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Visceral leishmaniasis is caused by *Leishmania (Leishmania) chagasi* and the geographical distribution of its vector, *Lutzomyia longipalpis*, is considered as a major limiting factor for the dispersion of the disease. During recent years we have witnessed an increase in the distribution of both the disease and the vector. Classically the disease is endemic in north-eastern Brazil and parts of Amazônia but there has been a steady increase in the number of recorded cases outside these areas in such States as São Paulo. Cases of canine visceral leishmaniasis were recorded in the northern region of São Paulo state in 1998 and in 1999 autochthonous human cases of visceral leishmaniasis were diagnosed in the city of Araçatuba which is situated in this same region.

A serological survey was initiated in dogs of Embu das Artes, a region near to the capital of São Paulo State. 462 serum samples were examined at the Centro de Controle de Zoonoses de São Paulo, using the indirect immunofluorescent reaction, between February and May 2003. Of these 412 were non-reactive, although 8 had titers of 1:20 dilution they were considered as negative. The other 42 samples has titers ranging from 1:40 to 1:1280. Fragments of spleen, liver, bone marrow aspirate and lymph nodes were taken from 13 serologically positive animals that had been sacrificed. Amastigotes were seen in Giemsa stained tissues smears of 7 animals. Promastigotes were isolated in culture from two samples of dogs in which parasites had not been seen in Giemsa smears. In another sacrificed dog, amastigotes were seen in cytological preparations of spleen, liver, bone marrow aspirate and skin lesion. Promastigotes were obtained from culture of fragments of liver, bone marrow and skin lesion. Serology was not performed on this animal. Promastigotes of three isolates were identified as *L. (L.) chagasi* by monoclonal antibody typing and/or an SSU-based PCR test.

This is the first time that this species of *Leishmania* has been detected in dogs in a region that is so close to the city of São Paulo. In the absence of records of human cases from this region the present findings of *L. (L.) chagasi* is a complete surprise. An entomological survey is under way but so far no specimens of L. longipalpis have been found. Further studies in neighbouring counties are needed to assess the potential risk of visceral leishmaniasis becoming endemic in Latin Americas largest city, São Paulo.

Financial support; FAPESP, FUNASA and CNPq

BM35 - THE FIRST RECORD IN THE AMERICAS OF AN AUTOCHTHONOUS CASE OF *LEISHMANIA* (*LEISHMANIA*) *INFANTUM CHAGASI* IN A DOMESTIC CAT (*FELIS CATUS*) FROM COTIA COUNTY, SÃO PAULO STATE, BRAZIL

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Leishmaniasis in domestic cats (*Felis catus*) has been reported sporadically in various parts of the world. These observations suggest that the cat is an accidental host, but its true role as a reservoir of the disease is unknown. The majority of cases in the literature refer to cutaneous lesions and rarely related visceral infections. The species incriminated in these cases, when identification was performed, was either *Leishmania* (*Leishmania*) *infantum* or a *Leishmania*(*Viannia*) species.

In July 2000, a two year old domestic male cat that had been born and reared in Cotia Municipality, São Paulo State, Brazil, was taken to a local veterinary clinic because of a nodular lesion on its nose. The animal had lost both weight and musculature, was severely dehydrated and had enlarged lymphatic ganglion.

The indirect fluorescence test for leishmaniasis of a serum sample was positive at a 1:80 dilution. Serology for feline immunodeficiency virus (FIV) and feline leukemia virus (FeLV) was negative, but a positive result (1:400) was obtained for feline infectious peritonitis (FIP). So, apparently, the animal was not immunodepressed because a viral infection.

Both intra and extracellular amastigotes were abundant in Giemsa stained smears of the cutaneous nodule. The animal was sacrificed and Giemsa stained cytological preparations were made from both the spleen and liver. Attempts to isolate the parasite from tissue samples in blood agar base culture medium failed. DNA extracted from a spleen fragment was used in a rDNA based PCR assay and resulted in the amplification of a 520 bp fragment. This fragment was purified, cloned and sequenced. A comparison of the obtained nucleotide sequence with those described by Uliana *et al.*(1994), detected presence of a C in position 1714 and a G in position 1721, identifying the parasite as *Leishmania (L.) chagasi*.

The present finding is the first record of a natural infection of *L. (L.) i.*

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chagasi in the domestic cat (*F. catus*) in Brazil and in the Americas. The cases of feline visceral leishmaniasis described in the literature are all from cats living in endemic visceral leishmaniasis areas of the Old World. What is even more surprising, in respect to the present report, is that the cat was a native of an area where so far there have been no reports of either autochthonous human or canine visceral leishmaniasis.

Financial support: FAPESP, CNPq and FUNASA.

BM36 - FURTHER EVIDENCE SUPPORTING THE WATER RAT, *NECTOMYS SQUAMIPES*, AS A PRIMARY RESERVOIR OF *LEISHMANIA (VIANNIA) BRAZILIENSIS* IN AN ENDEMIC REGION OF CUTANEOUS LEISHMANIASIS IN PERNAMBUCO STATE, BRAZIL.

Brandão-Filho, S. P.; Carvalho, F.G.; Brito, M.E.F.; Alves, I.C.; Ishikawa, E.; Cupolillo, E.; Floeter-Winter, L.M. & Shaw, J.J.

Centro de Pesquisas Aggeu Magalhães/FIOCRUZ, Recife; Instituto Evandro Chagas, Belém; Instituto Oswaldo Cruz/FIOCRUZ, Rio de Janeiro; Instituto de Ciências Biomédicas/USP, São Paulo.

There is an accentuated incidence of cutaneous leishmaniasis (CL) in the State of Pernambuco, especially in the region that corresponds to that of the Atlantic rainforest ("Zona da Mata") which accounts for 60% of the recorded cases. A multidisciplinary study of the ecoepidemiology of CL has been developed in the Amaraji, locality that is situated in the "Zona da Mata", some 90Km from the state capital, Recife. One of the principal objectives has been to find natural infections of *Leishmania*, especially *Leishmania (Viannia) braziliensis* in both wild and sinanthropic mammals. Monthly collections of small wild mammals were made using Tomahawk wire traps.

A *Leishmania* (MNEC/BR/2003/CPqAM191) was isolated from a hamster in July 2003 that had been inoculated with the skin and spleen cells collected from a water rat, *Nectomys squamipes*. The animal had been captured in October 2001 in an area of swamp land surrounded by sugar cane and pasture. The isolation was made in Blood Agar base (Difco B45) and the strain was identified as *L. (V.) braziliensis* using the specific G6PD PCR, according the method described by Castilho *et al*., 2003, *J. Clin. Microbiol*. *41*: 540-546. It was also identified as *L. (V.) braziliensis* serodeme 1, using a panel of monoclonal antibodies. The present result adds further weight to the hypothesis that rodents are the principal sylvatic reservoirs of *L. (V.) braziliensis* (Brandão-Filho *et al*., 2003, *Trans. R. Soc. Trop. Med. Hyg. 97*). The brown rat, *Rattus rattus*, is perhaps a reservoir in the peridomestic habitat. *Lutzomyia whitmani* is the principal vector in the zoonotic cycle and other species such as *Psychodopygus complexus (Lu. complexa)* a possibly involved in the enzootic cycle.

Financial Support: CPqAM/ FIOCRUZ, FAPESP, FACEPE and CNPq.

BM37 - MOLECULAR CHARACTERIZATION OF *LEISHMANIA* ISOLATED FROM THE CUTANEOUS LESIONS OF PATIENTS FROM RONDÔNIA-BRAZIL

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The leishmaniases are caused by many species of *Leishmania* that are respectively classified as belonging to either the subgenera *L.* (*Leishmania*) or *L.* (*Viannia*). Different markers have been used in taxonomic studies, such as rDNA, kDNA, isoenzymes and monoclonal. In the present study, 10 *Leishmania* isolates of patients from Rondônia-Brazil were analyzed by amplification and sequencing using distinct targets, such as the small subunit ribosomal RNA (SSU rRNA) and the glucose-6-phosphate dehydrogenase coding gene (*G6PD*). G6PD presents eight isoforms and is widely used in multilocus enzyme electrophoresis to identify *Leishmania*.

In the PCR assays, one set of paired primers were used in the SSU rRNA based assay and three pairs for G6PD based assay (Uliana *et al.,* 1994 and Castilho *et al.*, 2003). The amplified fragments were cloned and sequenced.

Comparisons of SSU rDNA variable regions sequences of the isolates with *L. (L.) amazonensis, L. (L.) mexicana, L. (L.) donovani, L. (L.) chagasi, L. (L.) major, L. (V.) braziliensis* and *L. (V.) guyanensis* grouped all 10 isolated into subgenera *L.* (*Viannia*).

The alignment of the deduced G6PD amino acid sequence of the isolates showed a similarity of 94% to 100%, when compared to particular G6PD N-terminal region of the named species of the subgenera *L. (Viannia)*. Isolate MHOM/BR/1998/M16726 was the most divergent having a similarity of 96% with *L. (V.) guyanensis, L (V.) panamensis, L. (V.) shawi* and *L. (V.) lainsoni*, a similarity of 94% with *L. (V.) braziliensis* and a similarity of around 61% with *L.* (*Leishmania*) isolates. These results, associated with the analysis of other markers will contribute to determining the species diversity of the *Leishmania* of this geographical region of Brazil.

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BM38 - EXTRACTION AND AMPLIFICATION OF *LEISHMANIA* DNA FROM ARCHIVED GIEMSA STAINED SLIDES (1965-2000) FOR THE DIAGNOSIS OF AMERICAN CUTANEOUS LEISHMANIASIS (ACL) IN BRAZIL

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Centro de Pesquisas René Rachou/FIOCRUZ – MG, UFMG, UFJF e UFOP

In the present work, we have used the polymerase chain reaction coupled to restriction fragment length polymorphism of the conserved region of minicircles kDNA from *Leishmania* (PCR-RFLP mkDNA) for specific *Leishmania* identification, directly from Giemsa stained slides containing biopsy imprints from ACL human lesions. The slides had been prepared for routine parasitological examination. This material allowed to perform a 35 years retrospective study in the ACL endemic region of Vale do Rio Doce – MG. This area has been regarded as an ACL endemic region since the 60s and *L. (V.) braziliensis* and *L. (L.) amazonensis* were described as the aethiological agents of leishmaniasis there. 475 out of 3,652 Giemsa stained slides from ACL patients were selected. An area of two imprints was scrapped from each slide, resuspended in water and the DNA extracted by heating. The preparation was centrifuged and the supernatant used as DNA source for PCR. Firstly, a specific PCR amplification was performed using primers that amplified the conserved region of the *Leishmania* kDNA minicircles. Then, RFLP was done using the endonucleases HaeIII and ApaLI. 395 (83.2%) out of 475 slides, were PCR-positive, showing the expected DNA amplified fragment of 120 bp. All 395 PCR-positive samples were digested by the enzymes and they showed RFLP profiles identical to those from reference strains of *L. (V.) braziliensis*. PCR-RFLP mkDNA was able to identify *L. (V.) braziliensis* as the only causative agent of ACL in Vale do Rio Doce-MG, in samples of slides archived from 1965 to 2000. The predominance of phlebotomine sand flies of the species *Lutzomyia whitmani* suggests this diptera as the likely vector of *L. (V.) braziliensis* in that region and in the State of Minas Gerais. On the other hand, the information on the absence of *Lu. flaviscutellata* the vector of *(L.) (L.) amazonensis* and cases of diffuse cutaneous leishmaniasis in the region are coincident. Our results show

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that archived slides are useful for both DNA extraction and species identification of the aethiological agent of ACL.

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BM39 - A POLYMERASE CHAIN REACTION MULTIPLEX ASSAY IN THE DIAGNOSIS OF NATURALLY INFECTED *LUTZOMYIA* PHLEBOTOMINES BY *LEISHMANIA* SPP.

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It is known that *Lutzomyia* transmits leishmaniasis in endemic areas of the New World and the rate of natural infection of these sandflies is low. Insects infected with *Leishmania* can be diagnosed by microscopy, culture and the polymerase chain reaction (PCR) test. Herein, we propose a PCR-multiplex assay for a better performance of the molecular diagnosis of natural infections of *Lutzomyia sp* with *Leishmania* in an endemic area of the Rio de Janeiro State. We have developed a PCR test that is able to amplify concomitantly the minicircle kDNA sequences from *Leishmania sp* -120 bp (Passos et al., 1996) and the *Lutzomyia sp* cacophony gene - 220bp (Lins et al., 2002). The insects captured in peri-domiciliar area were transported to the laboratory for taxonomic identification of male and female. Between May/2002 and July/2004 we have got predominantely *Lu. intermedia* (2498♂, 808♀)*, Lu. longipalpis (*74♂, 18♀*), Lu. migonei* (114♂, 75♀)*, Lu. fischeri* (0♂, 57♀), *Lu. firmataoi* (21♂, 5♀), *Lu. schreiberi* (10♂, 0♀), *Lu. quinquefer* (0♂, 2♀) and *Lu. pelloni* (2♂, 0♀). Some of these species were pooled in sets of 10 insects in 100 mL of buffer 10 mM Tris-HCl pH 9.2 containing 10 mM EDTA. These pools were treated with 1mg/mL of proteinase K and the DNA recovered by using the phenol-chloroform extraction method. The expected amplification products occur in 11 out of the 24 female pools assayed, accordingly to the *Lutzomyia* spp.: *Lu. intermedia* (n=6)*, Lu. migonei* (n=4) and *Lu. fischeri* (n=1). Curiously, we have not detected *Leishmania* parasites in a previous analysis of individual female sandflies by using the mid-gut dissection method. Furthermore, the hybridization of these amplicons with a specie specific probe will be necessary in order to check which *Leishmania* spp. is infecting those phlebotomines. The sandfly male pools were included in the study as negative controls of the *Leishmania* PCR assay detection method and also as positive controls for *Phlebotomus* genes. In this last case, the assay is able to detect only a single 220bp band. Our strategy help us in the identification of true negative samples for *Leishmania*, avoiding false negative diagnostic results that may occur by the presence of PCR inhibitors during the DNA sample processing. We also demonstrate that PCR-multiplex is a suitable approach to the establishment of the *Leishmania*-sandfly relationship and for epidemiological studies of leishmaniasis.

	Lu. intermedia	*Lu. migonei*	*Lu. fischeri*	*Lu. longipalpis*	*Lu. firmatoi*	*Lu. schreiberi*	*Lu. quinquefer*	*Lu. pelloni*	Total
Areas	♂/♀	♂/♀	♂/♀	♂/♀	♂/♀	♂/♀	♂/♀	♂/♀	♂/♀
Piabas	2/4	32/6	0/0	0/0	21/5	3/0	0/0	0/0	58/15
Colônia	1563/606	8/0	0/0	0/0	0/0	1/0	0/0	0/0	1572/606
P. Fome	402/97	56/7	0/18	74/18	0/0	6/0	0/0	0/0	538/140
V.Grande	191/23	3/0	0/0	0/0	0/0	0/0	0/0	2/0	196/23
V.Pequena	338/55	63/8	0/6	0/0	0/0	0/0	0/0	0/0	401/69
R.Bonito	2/2	4/0	0/0	0/0	0/0	0/0	0/0	0/0	6/2
G.Funda	0/0	0/0	0/0	0/0	0/0	0/0	0/2	0/0	0/2
Grumari	0/21	1/0	0/0	0/0	0/0	0/0	0/0	0/0	1/21
Total	2498/808	167/21	0/24	74/18	21/5	10/0	0/2	2/0	2772/878

Consolidated of colleted Phlebotomies in Jacarepaguá area: May/2002 until July/2004

Supported by CNPq and Centro de Referência de Leishmaniose

BM40 - PCR STANDARDIZATION TO IDENTIFY CYSTS OF *ENTAMOEBA HISTOLYTICA* AND *E. DISPAR* FROM FECAL SPECIMENS

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The correct diagnosis of the infection caused by the protozoan *Entamoeba* has critical significance for treatment due to the morphologic similarities between cysts and trofozoítos of *Entamoeba histolytica* and the nonpathogenic one *E. dispar*. The examination of fecal samples by optic microscope is not able to differentiate these two species. It reaches about 60% sensitivity in detention of cysts and can give false-positives since it can not distinguish trofozoítos of other species of *Entamoeba*. The PCR in the diagnosis of ameba reveals, therefore, as an important tool for the differentiation between *E. histolytica* and *E. dispar*. Some protocols had been described and used for the detention of the two species, amount them the amplification of fragments of genes of rDNA that have shown more sensible than the methods of antigen detention. However, in all the protocols the DNA is obtained through laborious and delayed techniques that usually results in low yield. Good yield and quality of DNA from human feces are obtained through kits that are very expensive. In this work we present a new method for the extration of DNA from human feces. This method showed to be so sensible as the commercial kit DNA Stool minikit QIAamp® and was used to extract DNA from fecal samples of individuals with and without amebiasis. The extracted DNA was used to standardize a specific PCR with primers for the two species of *Entamoeba*. It were used fecal samples from the Amazon region and southeastern of Brazil. Samples were fixed in formalin 10%, concentrated by the sedimentation method in formol–ether, examined by optic microscope and later the DNA was extracted through a modified alkaline lise protocol. The PCR was carried out using specific primers able to amplify a different ribossomal DNA fragment of 310 bp from each ameba. The PCR products were loaded in a 4% gel polyacrylamide, submitted to electrophoresis and visualized through silver nitrate. Of 150 analyzed samples, 93 were positive being 27 specific for *E. histolytica* and 66 for *E. dispar*. The DNA extration method demonstrated to be simple, effective and of low cost and the diagnosis through the PCR showed also to be sensible, fast and specific for the differentiation of *E. histolytica* from the non-pathogenic one *E. dispar*.

Finnancial support: Fapemig

BM41 - A SINGLE STEP DUPLEX PCR TO DISTINGUISH *ENTAMOEBA HISTOLYTICA* FROM *ENTAMOEBA DISPAR*

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In this study, a single step duplex polymerase chain reaction procedure was

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developed for a rapid, specific and sensitive identification of *E. histolytica* and for its diagnostic differentiation from *E. dispar.*

Two gene sequences were targeted simultaneously in this PCR procedure. One comprises an internal segment of 242 bp of the cysteine proteinase 5 (EhCP5) gene (Bruchhaus et al., 1996), present only in *E. histolytica* (EhCP5), and the oligonucleotide primers designed were EhCP6F, and the other with 300 bp sequence within the actin gene, and the oligonucleotide primers designed were Act3, common to both *E. histolytica* and *E. dispar* (Edman, Meza & Agabian, 1987; Huber *et al*. 1987), in order that two amplicons will identify the first and one amplicon identifies the last one, respectively.The PCR products were submitted to electrophoresis in 4% polyacrylamide gel and amplicons visualized by silver staining. The PCR developed in the present work was specific and efficient to identify and differentiate these parasites from each other in either cultured or from stool material.

Good correlation between zymodeme and PCR methods was observed. Additionally, the results showed that this duplex PCR approach may be applied also for stool samples confirming either positives or negatives as reported by the optical microscopy technique with sensitivity to detect just one cyst (wells 12 and 13). In conclusion, these results altogether might facilitate now to distinguish these two parasites in positive samples and bring about a tool for important diagnostical differentiation.

Finnancial support: Fapemig

BM42 - GENETIC VARIABILITY OF *TOXOPLASMA GONDII* STRAINS FROM BRAZIL DETECTED BY RANDOM AMPLIFIED POLYMORPHIC DNA – POLIMERASE CHAIN REACTION (RAPD-PCR) AND SIMPLE SEQUENCE REPEAT ANCHORED – PCR (SSR-PCR)

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Toxoplasma gondii is currently considered to be the only species in the genus *Toxoplasma*. However, strains of this parasite have been subdivided into two or three major groups based on their virulence or lethality for mice during acute infections. Genetic diversity among *T. gondii* strains from different parts of the world, mainly Europe and North America, has been demonstrated by several molecular techniques. Our aim was to study the genetic variability of 19 *T. gondii* strains isolated from humans and animals in Brazil using two methods for analyzing DNA polymorphism: Random Amplified Polymorphic DNA-PCR (RAPD-PCR) and Simple Sequence Repeat anchored-PCR (SSR-PCR). It is the first time that Brazilian *T. gondii* strains are studying under RAPD and SSR, PCR-based methods. Two reference strains, RH (highly virulent) and ME49 (avirulent), were submitted to both assays. *Besnoitia* sp, *Plasmodium falciparum* and *Babesia bigemina* were used as out-groups. Purified *T. gondii* tachyzoites were obtained from peritoneal cavities of Swiss or C57BL/6 INF-g knockout mice, depending on the growth rate. DNA was prepared by proteinase K digestion followed by phenol/chloroform/isoamyl alcohol extraction and ethanol precipitation. RAPD-PCR analysis was performed using the kit "Ready To Go" Amersham Pharmacia Biotech-Uppsala, Sweden with seven different primers and SSR-PCR with $(CA)_8RY$ and $CAA(CT)_6$ primers. The amplified DNA fragments were separated on 5% polyacrilamide gels, which were silver stained and photographed. RAPD-PCR and SSR-PCR profiles were used for building phenetic trees by Unweighted Pair Group Method using Arithmetic Averages (UPGMA). Phenograms built with computer software TREECONW showed great similarity in the topology of the trees. Both phenograms presented two major clusters that grouped *T. gondii* strains according to their murine virulence. The strains AS28, BV and N, which are highly virulent for BALB/c mice, were clustered with RH reference strain, the most commonly studied highly virulent strain of *T. gondii*. The other group showed that the strains which presented a level of virulence more similar to that of ME49 reference strain (avirulent) also presented a closer genetic relationship. The genetic variation within each lineage was significantly lower (P<0,05) than that between the lineages. Regarding out-groups, *Besnoitia* sp presented the closest relationship to *T. gondii* while *Plasmodium falciparum* the most distant. The results presented here demonstrate that intra-specific genetic variability separate Brazilian *T. gondii* strains in two groups that correlate with murine virulence phenotype, as showed for genus *Toxoplasma*.

This research was sponsored by: CNPq, FAPEMIG and FAPEBIO

BM43 - LACK OF TECHNICAL SPECIFICITY IN THE MOLECULAR DIAGNOSIS OF TOXOPLASMOSIS

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The accurate diagnosis of toxoplasmosis in immunocompetent patients or pregnant women with possible risk of *Toxoplasma gondii* transmission via placenta, is extremely important and need to be investigated. Routine serological diagnosis provides high sensitivity, but not high specificity. In this scenario, methods involving parasite detection should be implemented. Several approaches based on polymerase chain reaction (PCR) directed to distinct protozoan genomic targets have been developed and the most used is the B1 gene. Herein, we report the molecular detection standardization of *T. gondii* using PCR targeted to the B1 gene, coupled to a non-isotopic hybridization step with a probe derived from a PCR positive control performed with purified parasite DNA. Using this procedure, we were able to detect 93 fg of protozoan DNA (one parasite corresponds to 100 fg of DNA). Thirty-four individuals with suspicion of toxoplasmosis by serological diagnosis were submitted to our PCR-hybridization assay. Eight seronegative individuals were also included in the study. The presence of circulating protozoan was detected, in: (i) 50% of 10 acute phase patients presenting positive IgM anti-*T.gondii* independently of the IgG results; (ii) 12.5% of 24 chronically infected patients with positive IgG and negative IgM; (iii) 0% of 8 seronegative individuals. The data showed that in some cases, PCR products with the expected molecular size of the target sequence (194 bp) could be evidenced in the visual inspection on agarose gels, but did not hybridize with the B1 gene probe. Those amplified fragments were direct sequenced and correspond to scattered human sequences from chromosomes 2 and 10, co-amplified under the same experimental conditions, showing an homology of 88 and 89%, respectively. The PCR positive control fragment used as our molecular probe, revealed 96% of similarity with the B1 gene sequence. The results demonstrate that PCR amplification of these gene of *T. gondii*, with primers previously described in the literature, promotes the co-amplification of human sequences. These spurious products were revealed with the use of molecular approaches other than the mere visualization of the amplified products.

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BM44 - THE IDENTIFICATION OF MINICIRCLES IN *TRYPANOSOMA VIVAX*

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Kinetoplast DNA (kDNA) minicircles are in most cases heterogeneous within a given parasite and almost completely different among trypanosome species. They are organized into one to four conserved regions (CR) representing approximately 10% of the molecule and an equal number of variable regions, depending on the species. Each CR contains three conserved sequence blocks (CSB) identified in different trypanosomatid species. The minicircles of *Trypanosoma vivax* were previously investigated by Borst et al (1985), however their sequences were not determined and are not available in the databases. Our main objective was the identification of new molecular markers for *T. vivax,* then, a semi-normalized genomic library was constructed and 400 GSS sequenced and analyzed "in silico". From 501 reads, 8 different minicircles were identified using the standalone version of Blast with the KINETO and NR databases of EBI and NCBI, respectively. Usually, best hits presented e-values worth than 0.01, nevertheless similarities were always with the conserved region of trypanosomatid minicircles. When multiple aligments were done using the ClustalX software, only 1 CR was found, containing the CSB-1, CSB-2 and CSB-3. When *T. congolense*, *T. evansi*, *T. brucei* and *T. cruzi* minicircles were included in the alignment, we confirmed that CSB-3 (GGGGTTGGTGTAA) is highly conserved to their homologs described in other trypanosomatids, and is thought to be origin of replication of minicircles. CSB-2 is less conserved among *T. vivax* minicircles and of the eight nucleotides that compose this sequence, the last five are identical. We also observed that CSB-1 sequence is approximately 85 nucleotides upstream of the CSB-3 sequence, while CSB-2 is approximately 48 nucleotides upstream of CSB-3. This organization was observed in the 8 minicircles analyzed. The size of the minicircle is ~480 bp, which would be in accordance with the 465 bp reported by Borst et al (1985). The exact size of minicircles will be determined in further experiments. Our next steps are the identification of more minicircles with a probe specific to CSB-3, and the use of those minicircles for the design of species-specific PCR-based diagnosis and typing tools. The putative gRNAs genes encoded by the minicircles will be also studied.

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BM45 - *PLASMODIUM* SPP. INFECTION IN WILD BIRDS FROM MATA ATLÂNTICA, MINAS GERAIS STATE: EVALUATION BY THIN BLOOD SMEARS AND PCR

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The standard method for avian malaria diagnosis is the microscopic examination of thin blood smear stained with Giemsa. However, this method presents some limitations and low sensitivity. As a result, the thin blood smears-based prevalence and distribution of avian plasmodia might be underestimated. Alternative diagnostic methods based on parasite DNA detection, such as the Polymerase Chain Reaction (PCR), have been used for identification of malaria species Our present goal is evaluate the *Plasmodium* infection in 137 wild birds from Mata Atlântica (Reserva Particular do Patrimônio Natural Mata do Sossego), Minas Gerais State. The microscopic observation allowed to detect 7.3% of positive birds while a PCR protocol of 18 SSUrRNA gene showed a rate of 34.3% of *Plasmodium* infection. Correlation between both methods employed was not observed. Seventy three birds were captured in a fragmented area and 64 in non-fragmented area of Mata do Sossego. None correlation was verified between parasitism frequency and fragmentation, considering the two techniques used. Birds biological characteristics such gender, age, diet, nest and others were evaluated according to the presence of *Plasmodium* sp. Interestingly, only the diet was correlated with the parasite infection. The birds which main diet was based on insects were statistically more infected than others ones. This result indicate that the alimentary habits could be involved in efficacy of avian malaria transmission, reinforcing the importance of parasite-host interaction in ecological studies.

Financial support: FAPEMIG, CNPq

BM46 - COMPARISON OF DNA EXTRACTION PROCEDURES FOR *LEISHMANIA* PCR FROM STORED SANDFLIES.

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Leishmaniases are endemic South American diseases transmitted by sand flies. In these insects, after they bite an infected mammal reservoir, the flagellated parasite access and grows in the sand fly gut, until its regurgitation in the next insect meal, closing the transmission cycle. The prevalence of natural infection by flagellates in sand fly populations could be an index of the environmental risk for the transmission of that diseases. The natural infection by flagellates rate is usually very low and its identification by taxonomic and microscopic exams demands a lot of work. The detection of infection in large numbers of insects, with determination of flagellate species would be attaining with PCR reactions. These reactions had been standardized for biopsy or scraping material, aside to flagellate culture, usually to repeated genes sequences, as mini-exon, kDNA or ribossomal genes, but most of them were tested only against mammalian DNA. In this work, we tested several ways to obtain the intestinal content of phlebotomine and their DNA, looking for interference in the PCR for leishmania detection using primers directed to ribossomal (SSUrRNA) genes. Several approaches were tested in order to obtain phlebotomine DNA, as crude total homogenate, centrifugation against pinhole using insect chitin as sieve or the classical phenol chloroform extraction. Usually, for each reaction ten laboratory breed insects (*Lutzomyia longipalpis)* and leishmania (free or that were seed with known numbers of promastigotes of *Leishmania (L.) amazonesis*, strain IFLA/BR/67/PH8) were added to individual tubes, aside of phlebotomine DNA mass extracted, to introduce a competitive DNA confounding factor.

The Leishmania PCR reaction was completely functional in the presence of added total phlebotomine DNA at 1 mg/tube. Leishmania PCR was capable to detect until 1 promastigote/sand fly. Any attempt to separate intestinal contents by centrifugation fail to maintain stable material for PCR detection, probably due extensive DNA destruction. PCR also fail in phenol-chloroform extraction of intact whole parasite, which results in sequestration of the DNA by the chitin exoskeleton.

In order to assure that the intestinal promastigote DNA was present in the material after the extraction procedure, usually by homogenizing the whole insect with destruction of chitin exoskeleton, the extraction of phlebotomine DNA must be cautiously performed.

The use of PCR, especially with Poisson distribution and extreme care in extraction, could be a powerful tool for measurement of the natural infection rate by leishmania in sand flies from endemic areas.

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BM47 - *LEISHMANIA* SPECIES-SPECIFIC PCR IN PARAFFIN EMBEDDED SKIN BIOPSIES FROM CUTANEOUS LEISHMANIASIS FROM THE ESPÍRITO SANTO STATE, AS COMPARED TO OTHER MOLECULAR BIOLOGY APPROACHES.

Paiva, B.R[1]; Passos, L.N[2]; Falqueto, A[2]., Volpini A.C.[3], dos Santos S.L.[3], Pagliari C[4], Romanha, A.J.[3] & Andrade Jr, H.F[1].

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The diagnosis of the infecting species in cutaneous leishmaniasis is a crucial step for adequate therapy, as the prognosis and mucosal involvement is dependent of the infecting species. Usually, the diagnosis of suspected lesions is performed by stained imprints morphology, which precludes the identification of the species of the agent. Moreover, the skin biopsies are often paraffin embedded for histology. Recently, molecular approaches had allowed the diagnosis based on PCR amplification of repeated genes present in parasite genomes, that are usually performed in fresh or culture amplified samples. We devised a new set of primers looking for amplification of mini-exon genes, and tested in 58 paraffin embedded samples from cutaneous leishmaniasis patients collected at Espírito Santo, Brazil. Consecutive sections of 5 mm from the blocks were obtained and stored, aside to process for Leishmania immunohistochemistry. Isolated sections were placed in microfuge tubes, de-waxed by xylene, and their DNA extracted with phenol chloroform proteinase K treatment. The devised sequences were performed using Primer 3 software, with a 177bp product specific to L. (V.) braziliensis, using DNA from standard promastigotes from the South American leishmanias. When isolated section were tested with the same primers, 62.1%(36/58) were clearly positive. There was no correlation between the PCR and the semi-quantitative immunohistochemistry analysis. Formol stored tissues from patients from the same area were also tested and gave a PCR positivity of 46.1%(6/13). The paraffin tissue sections were also tested by PCR- RFLP. All 58 tested samples were positive for *Leishmania* and identified as *L. (V.) braziliensis*. We concluded that different molecular approaches for the diagnosis of species of *Leishmania* in cutaneous leishmaniasis patients could be performed in paraffin-stored material. Although the methods presented different sensitivities, they showed very clear results, suggesting their inclusion as a quick tool in the management of cutaneous leishmaniasis, especially after the histological diagnosis.

This work is supported by LIMHCFMUSP and CAPES.

BM48 - DNA MICROARRAYS: LEVELS OF RNA TRANSCRIPTS DURING GROWTH OF CL BRENER AND CLUSTERIZATION OF AMASTIGOTE ESTS

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The differential gene expression during growth of CL Brener epimastigotes (doubling time = 17.7 hours) was investigated with a microarray slide containing 710 ESTs of CL Brener and 20 previously characterized genes of various *T. cruzi* strains. These targets represent 665 unique sequences. The ESTs were derived from non-normalized and normalized cDNA libraries of CL Brener epimastigotes (Urmenyi et al., 1999) and were kindly provided by Dr. W. Degrave and Dr. A. Brandão (FIOCRUZ, Rio de Janeiro, Brazil). Search for similarity with BLASTN and BLASTX programs indicates that 75% of the ESTs have no matches in protein and DNA databases. Total RNA was isolated from epimastigotes during exponential growth (48 h) and in the stationary phase (336 h). cDNAs were labeled with Cy3 and Cy 5, mixed and hybridized with the slide. To prevent dye bias, normalization of the data was achieved by dye swap. The images were analyzed with ScanAlyze 2.44 program and a M-A plot was obtained (i.e. base-2-logarithm of swap normalized ratios (M) versus base-2-logarithm of intensities (A)). We concluded that 47 targets (6.4%) exhibited up-regulation by 1.7 to 2.6-fold in mid-log phase epimastigotes as compared to stationary phase parasites. To confirm these findings, some sequences were used as probes in Northern blots of total RNA of the two populations. Hybridization with a GAPDH sequence was used for normalization. The radioactive images were collected in phosphor screens and scanned with the Storm System (Molecular Dynamics). The hybridization signals were quantified by densitometric analysis using the ImageQuant Molecular Dynamics Program. In all the cases Northern blots gave higher hybridization ratios when compared with the microarray ratios. Aiming at further construction of a more representative microarray slide, 1,233 ESTs from CL Brener amastigotes (a kind gift of Dr. A. Gonzalez, CSIC, Granada, Spain) were clustered by CAP3 program. Initial analyses were performed by Dr. F. Agüero (San Martin, Un. San Martin, Buenos Aires, Argentina), and subsequently in our laboratory. The sequences were distributed in 285 clusters (with 2 to 20 sequences/cluster) and in 300 singletons (total of 585 unique sequences). Clustering of CL Brener amastigote and epimastigote ESTs (see above) makes-up 1,162 unique sequences. We have further added 44 ESTs from Tulahuen amastigotes (kindly provided by Dr. S. Teixeira, UFMG, Belo Horizonte, Brazil), and a total of 1187 unigenes was obtained. These targets will be deposited in the microarray slide for future investigation of differential gene expression in *T. cruzi* strains displaying particular biological characteristics.

Support: FAPESP and CNPq.

BM49 - GENE EXPRESSION PROFILE OF CARDIOMYOCITES INFECTED WITH DIFFERENT FORMS OF *T. CRUZI* TRYPOMASTIGOTES

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Trypanosoma cruzi, the causative agent of Chagas' disease, exhibits complex developmental programs during life cycle transitions in the infected host and insect vector. The infective form circulating in the blood stream of the mammalian host (blood trypomastigote) is originated from the amastigotes and the metacyclic trypomastigotes are originated from the cellular differentiation of epimastigotes in the insect gut. Both trypomastigote forms can infect mammalian cells; however they are adapted to interact with different hosts reinforcing the assumption that these infective forms are indeed different. We are using microarrays to study the gene expression profiles of cardiomyocites infected with the two distinct trypomastigotes in order to evaluate comparatively the host cell response. Eighteen-day murine embryos cardiomyocites were infected with Vero cell culture derived (VERO) and *in vitro* derived metacyclic (TAU) trypomastigotes and samples were taken at 1h, 2h, 4h and 6h. They were hybridized to Genechip® arrays and images were analyzed with the RMA package (Bioconductor project).

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Differential expression was assessed using an F-test with correction to multiple testing (SAM software). The host cell responds quickly to the infection, increasing and decreasing genes in both infection processes but the overall result is that infection leads to more down-regulated genes. Comparison of infected cells showed that some genes are equally regulated in TAU and VERO infection (e.g, beta actin, kinesin). There are few qualitative differences between VERO and TAU infected cells, involving specially immune response genes (chemokines, NFK-α, CSF) and most of the differences are quantitative, as the tendency is the same, but the magnitude is different. These results suggest that TAU and VERO infections have different kinetics related to differences in terms of infection capability in both trypomastigotes forms.

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BM50 - SEQUENCE VARIABILITY OF A *T. CRUZI* RNA BINDING PROTEIN AND ITS RECOGNITION BY HUMAN CHAGASIC SERA.

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We have described a new *T. cruzi* antigen, called TcRBP48, identified by immunoscreening an amastigote cDNA library of the Tulahuen strain with human chagasic sera. TcRBP48 is encoded by a multi-copy gene family and is expressed at similar levels in all life stages of the parasite. Sequence analyses showed that TcRBP48 is the homologue of the *T. brucei* RNA binding proteins p34 and p37, which interact with 5S rRNA. To better characterize the chagasic immune response against this antigen, we expressed and purified this protein as a recombinant GST fusion protein. The GST::TcRBP48 purified fraction tested on ELISA assays showed that it is specifically recognized by human chagasic sera (n=45), but not by non-infected individuals or patients with leishmaniosis (n= 17), toxoplasmosis (n= 10) and malaria (n= 9). Levels of reactivity against TcRBP48 were not statistically different when chagasic sera from patients with severe, moderate and mild chagasic cardiomyopathy as well as asymptomatic patients were compared. Regardless the clinical manifestations, individual patients showed different levels of reactivity with recombinant TcRBP48. In light of the studies showing two or three major lineages in the *T. cruzi* population, we proposed to investigate the existence of sequence variability in the TcRBP48 genes from different *T. cruzi* strains and to test if that may cause different levels of the humoral immune response against this antigen. A 440 bp fragment corresponding to the N-terminal region of the TcRBP48 protein was amplified from the genome of various *T. cruzi* strains. Amplified fragments from 35 strains were submitted to RFLP analyses, and the sequences corresponding to the various copies of the gene present in 10 strains were determined. Comparative sequence analyses showed that the TcRBP48 gene is variable and that the patterns of amino acid substitutions can separate *T. cruzi* strains in three groups. The corresponding nucleotide changes were also detected by RFLP analyses of PCR fragments after digestion with *Hha* I.. Fragments corresponding to the genes present in the Colombiana and 1005 strains were cloned into pGEX vectors and the recombinant GST-fusions of these two isoforms were expressed and purified from *E. coli* cultures. In spite of the amino acid changes, the humoral response against these two isoforms was not different when sera from 13 patients were tested on ELISA assays. Our results suggest that the TcRBP48 is an important target of the humoral immune response in Chagas' disease and may constitute an antigen component that can be used to improve the specificity of serological tests, in spite of its variable sequence composition. This antigen can also be used for typing *T. cruzi* strains by PCR-RFLP.

Supported by grants from WHO and CNPq.

BM51 - CLONAL-HISTOTROPIC MODEL FOR CHAGAS DISEASE: A NEW STUDY STRATEGY BASED ON GENETICALLY MODIFIED *TRYPANOSOMA CRUZI* STRAINS

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Using LSSP-PCR we have demonstrated the occurrence of differential tissue distribution of distinct of *T. cruzi* populations in mice. For instance, when BALB/c mice were co-infected with *T. cruzi* JG strain and the Col.1.7G2 clone, a clear predominance of the DNA of Col.1.7.G2 was observed in the rectum, diaphragm, esophagus and blood, while JG strain DNA was detected in the heart of these same animals (Andrade et al., 1999). The presence of specific DNA patterns were used an indicators for the presence of parasites in each analyzed tissue. In the present work we developed an alternative strategy based on genetically modified *T. cruzi* (expressing Green Fluorescent Protein – GFP- or Red Fluorescent Protein – RFP) to identify the parasites directly in the infected tissues. Initially we transfected *T. cruzi* epimastigotes with pTREXGFP and pTREXRFP transient expression vectors (Vazquez et al, 1999) and obtained JG strain and Col1.7G2 clone expressing GFP and RFP. However the number of fluorescent parasites decreased with time even under selective drug pressure. Therefore, we utilized the integrative expression vector pROCKNeo (Da Rocha and cols, submitted) that allows the integration of exogeneous genes into the parasite genome by homologous recombination in the b-tubulin locus. Parasites stably expressing GFP and RFP were obtained. The transfected parasites present no significant differences in their infectivity for VERO cells when compared with the wild type populations. Pictures obtained by confocal microscopy showed intense green fluorescence in the intracellular forms of transfected parasites within VERO cells for both JG and Col1.7G2 populations.

Financial Support: CAPES, CNPq, FAPEMIG and WHO

BM52 - GENETIC ANALYSIS OF AN ANIMAL MODEL (*HOLTZMAN* RATS) EXTENSIVELY USED IN THE STUDY OF EXPERIMENTAL CHAGAS DISEASE.

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Chagas disease caused by the intracellular parasite *Trypanosoma cruzi*, is an infection of endemic nature in 21 countries of Latin America, with 16 to 18 million infected people. The biological, biochemist and genetic behavior of *T. cruzi* strains have been extensively studied in the experimental model at the Laboratório de Neurobiologia, ICB, UFMG. Three distinct profiles of parasitemia (low, moderate and high), have been observed in *Holtzman* rats inoculated with 10.000 trypomastigotes of CL-Brener clone (isolated from the parental CL strain) ABC and Y strains of the parasite. A recent study, developped in our laboratory investigating the organs distribution and histopatological alterations caused by mixture (10.000 + 10.000, 10.000 + 1.000, 1.000 + 1.000) of two subpopulations of *T.cruzi* (JG and CL-Brener clone) demonstrated that after 120 days of double infection JG strain was found in the heart and organs of the majority of the analyzed animals. Only the group inoculated with 10.000 trypomastigotes of each population showed the presence of CL-Brener clone in the esophagus, solium and diaphragm. The observed histotropism could be related such to the

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genetic variability of the infectant population as to the genetic background of the host. The main aim of this work was to investigate the degree of genetic polymorphism between animals (*Holtzman* rats) which have been used as model to the experimental Chagas disease. The RAPD technique was used for analysing DNA samples isolated from the blood of 20 animals from an "out-bred" colony from the Center of Bioterism of the ICB, UFMG. Six primers of arbitrary sequence were used (OPA 1, 2, 3, 8, 9 and ëGT11), which were able to produce profiles composed by multiple amplicons (5 to 14). The analysis of the profiles obtained by RAPD demonstrated a low degree of genetic variability between the analyzed animals, suggesting that the variability previously observed in the virulence and pathogenicity of subpopulations of *T.cruzi* when inoculated in *Holtzman* rats, seems to be mainly associated with factors related to the strains of the parasite.

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BM53 - PCR DETECTION OF *TRYPANOSOMA CRUZI* KDNA DIRECTLY FROM COLON SAMPLES OBTAINED FROM PATIENTS WITH CHAGASIC MEGACOLON BY USING A SENSITIVE HOT START PROTOCOL

[1]Aguiar, A. F, [2]Adad, S. J., [1]Reis, D. d´A., [3]Oliveira, E. C., [3]Luqueti, A. O., [1]Vago, A. R.

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The pathogenesis of Chagas disease is not completly understood. Due to the difficulty in detecting parasite in tissue samples from chronic chagasic patients the role of *T. cruzy* on the development of tissue lesions observed at chronic phase was considered controversial by many years. However, recent studies in heart and esophageal tissues obtained from chronic chagasic patients using more sensitive techniques as PCR, have shown a correlation between the detection of parasite DNA and the presence of inflamation. Chagasic megacolon is clinically characterized by symptoms as obstipation, dyskinesia, meteorism and achalasia of the internal anal sphincter. The main macroscopic alterations of megacolon are dilatation and hipertrofy of the organ. Microscopically this process is characterized by presence of inflammatory infiltrates, degeneration of myocites and nervous cells. Studies on the examination of colon samples from patients with megacolon by histologycal techniques have failed to demonstrate a close correlation between the megacolon pathogenesis and the presence of *T. cruzi*. At the present study we have analysed colon samples surgically obtained from 20 chagasic patients with megacolon belonging to two distinct groups: the first group consisted of samples obtained from 11 individuals provenient from an endemic region of Goiás state, while the second one, was formed by samples from 3 different anatomic regions (sigmoid-rectum transition, sigmoid´s media portion and descendant transition of sigmoid) of 9 individuals from the region of "Triangulo Mineiro", at Minas Gerais state. By using a hot-start protocol PCR to amplify a 330 bp fragment from the variable kDNA region of parasite we detected *T. cruzi* kDNA in samples from 13 patients (65%) of the two groups. In the second group, kDNA parasite was detected in 7 of 9 patients analysed, and interestingly, from the 7 positive cases, 5 demonstrated kDNA T. cruzi only in the sigmoid-rectum transition.

Financial suport: FAPEMIG and CNPq.

BM54 - MOLECULAR TYPING OF *TRYPANOSOMA CRUZI* DIRECTLY FROM TISSUES OF CHRONIC PATIENTS WITH CHAGASIC MEGACOLON BY USING LSSP-PCR

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Chagas'disease has a variable clinical course, ranging from symptomless infection to severe chronic disease with cardiovascular or gastrointestinal involvment. The factors influencing this clinical variability have not been elucidated, but most likely genetic variation of both the host and parasite are important. Thus, researchs into intraspecific genetic polymorphisms of *T. cruzi* exploring a variability of both nuclear and kinetoplast DNA (kDNA) have failed to demonstrate a significant correlation between the genetic of parasite and the clinical course of the disease. This fact can be in part explained, because these techniques worked with parasites isolated from vectors and from the blood of patients. To unravel the molecular epidemiology of Chagas disease at a fine level, we need to be able to study parasite variability directly from clinical tissues. In this work we used LSSP-PCR (Low-Stringency Single Specific Primer PCR) technique for analysing the kDNA region of the parasite to genetically type *T. cruzy* directly from colon samples obtained from 13 chagasic patients with chagasic megacolon, wichs were proceeding from distinct endemic areas of Ghagas'disease, Goiás and Minas Gerais states. Informative genetic signatures were obtained to each analysed sample and although we cold not demonstrate strong correlation betwen the profiles obtained.

Financial suport: FAPEMIG and CNPq.

BM55 - ANALYSIS OF DIFFERENTIALLY EXPRESSED GENES AMONG *TRYPANOSOMOMA CRUZI* TRIPOMASTIGOTES

Pavoni, D.P.[1]; Probst, C.M.[1]; Poersch, C.O.; Cardoso, J.[1]; Correa, A.[1]; Arauco, P.R.C.; Ávila, A.R.[1]; Monteiro-Góes, V.[1]; Goldenberg, S.[1,2;] Krieger, M.A.[1,2]

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The differentiation of epimastigotes into metacyclic trypomastigotes (metacyclogenesis) involves the transformation of a replicative, non-infectious form of *Trypanosoma cruzi* into a non-replicative, infectious one. The functional and morphological changes occurring during this process result from important changes in the parasite gene expression program. To improve our knowledge about *T. cruzi* differentiation and particularly, of the metacyclogenesis process, we have done a systematic analysis of differentially expressed genes using microarray technology. The *T. cruzi* microarray utilized was made at our laboratory, using EST sequences (from public databases and from an internal transcriptome project). The newest version of the biochip has more than 5,000 different contigs in triplicate in a 17K spots biochip. The major technical problem we face is that some cellular forms yield tiny amounts of RNA, insufficient to perform our experiments. In order to bypass this limitation, we used a method to amplify the RNA. The amplification was made linearly, using a T7 promoter to transcribe the cDNA derived from the RNA. The promoter was linked to an oligo-dT primer; consequently the RNAs generated were complementary to the original RNA (antisense RNA – aRNA). Our results demonstrated that this RNA amplification protocol did not introduce distortions that could compromise the analysis of the

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microarrays. Hence, *in vitro* transcription is an adequate way to amplify the RNA of *T. cruzi*. RNA samples, extracted from *T. cruzi* epimastigotes and metacyclic trypomastigotes, were analyzed in hybridization experiments performed on the microarray slide. With this method we were able to select several metacyclic trypomastigote expressed genes. In addition we are also starting the characterization of the gene expression programs in both trypomastigotes (Metacyclic trypomastigotes and Cell Derived trypomastigotes) in order to select common and specific sets of genes.

Financial support from PRONEX, CNPq, Fiocruz

BM56 - SELECTION AND FUNCTIONAL CARACTERIZATION OF EXPRESSION REGULATED GENES OF DIFFERENTIATING EPIMASTIGOTES DURING THE METACYCLOGENESIS PROCESS

Giese, V.L.[1]; Dallagiovanna, B.[1]; Ávila, A.R.[1]; Monteiro-Góes, VS[1]; Probst, C.M.[1]; Pavoni, D.P.[1]; Krieger, M.A.[1,2] & Goldenberg, S.[1,2].

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The differentiation of *T. cruzi* non-infective forms (epimastigotes) into infective forms (metacyclic trypomastigotes) is known as metacyclogenesis. This process is of great interest because it involves differential gene expression associated with virulence of the parasite to the mammalian hosts. The development of reproducible axenic culture conditions has made possible the study of this process *in vitro*. Differentiating epimastigotes adhere to a substrate and are released from it upon transformation into metacyclic trypomastigotes.

In order to gain further insight into the mechanism of gene expression regulation in *T. cruzi*, we are using micro-array analysis to identify genes specifically expressed in the course of metacyclogenesis process. The regulation of gene expression Trypanosomatids has unusual mechanisms as compared to other eukaryotes. The transcription in Trypanosomatids is polycistronic and it is assumed that most of the regulation of mRNA levels is determined at the post-transcriptional level. We have used a *T.cruzi* home-made micro-array in order to compare, using a competitive hybridization assay, total and polysomal RNA fractions isolated from parasites at 24h of differentiation. Eight ESTs which are differentially mobilized to the polysomal RNA fraction at 24h of differentiation were selected to further analysis. Three strategies were used to obtain the complete sequences of these 8 ESTs. Seven sequences were homologous to sequences already deposited in the GeneBank and two of them are being studied in further details, encoding a calpain-like protein and Sec-31 protein. We are presently identifying the coding sequences of these genes in *T.cruzi* Dm28c in order to express the recombinant proteins and to raise specific polyclonal antibodies. The purified recombinant proteins and the antibodies will be use in biochemical and microscopy analysis to investigate the function of these genes during *T. cruzi* metacyclogenesis.

Financial support from PADCT, PRONEX and CNPq.

BM57 - DIFFERENTIAL GENE EXPRESSION DURING THE ONSET OF *TRYPANOSOMA CRUZI* METACYCLOGENESIS.

Sotomaior, VS[1]; Dallagiovanna, B[1]; Monteiro-Góes, VS[1]; Giese, VL[1]; Nardelli, SC[1]; Stange, F[1]; Santos, LBR[1]; Pavoni, DP[1]; Picchi, G[1]; Probst, CM[1]; Goldenberg, S[12]; Krieger, MA[12].

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The mechanisms involved in triggering metacyclogenesis which is driven by remarkable changes in gene expression remain unclear. *T. cruzi* metacyclogenesis can be mimicked in vitro by using chemically defined conditions that allows the selection of parasite samples at distinct time-points during the differentiation process. To address this issue mRNA differential display and *T. cruzi* DNA microarray were used to select genes differentially expressed during the onset of the metacyclogenesis process, which consists of a 2hrs nutritional stress prior to incubation in the differentiating medium (TAU3AAG). The mRNA differential display experiments resulted, after cluster analysis in 5 contigs and 8 singletons whereas the competitive hybridization experiments unraveled at least a hundred differentially expressed sequences. Thirty of these genes were selected for further characterization and were used as probes to screen a shotgun library. Newly derived sequences were compared with gene bank databases searching for similarities. It was possible to indicate putative function to 18 of these 30 selected genes. For instance, one of the sequences selected by mRNA differential display as a stress-specific gene is similar to a SWI/SNF related, matrix associated, actin dependent regulator of chromatin (gb|AAK53826.1). Another example is a gene whit expression increased during nutritional stress and is similar to a probable ubiquitin-conjugating enzyme variant from *Leishmania major* (emb|CAC14533.1). The expression patterns of the selected genes are being confirmed by Northern-blot and Real Time RT-PCR. The approach here reported will be useful in unraveling the genes that are differentially expressed during the onset of metacyclogenisis.

Financial support from PRONEX, CNPq.FIOCRUZ

BM58 - ANALYSIS OF GENE EXPRESSION CHANGES IN RESPONSE TO DIFFERENT STRESSES: SELECTION OF GENES INVOLVED IN TRIGGERING *TRYPANOSOMA CRUZI* METACYCLOGENESIS.

Monteiro-Góes, V.[1]; Sotomaior, V.S.[1]; Correa, A.D.; Santos, L.B.R.[1]; Probst, C.M.[1]; Pavoni, D.P.[1]; Goldenberg, S.[1,2]; Krieger, M.A.[1,2]

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The differentiation of epimastigotes into metacyclic trypomastigotes (metacyclogenesis) involves the transformation of a replicative, non-infectious form of *Trypanosoma cruzi* into a non-replicative, infectious stage. An essential event in metacyclogenesis is the nutritional stress at which the parasites are naturally exposed in the mid-gut of the insect host. Important alterations in the gene expression program occur during this process and they might play an important role in the physiological and morphological changes observed during the metacyclogenesis. However, little is known about the ability of other types of stresses in triggering metacyclogenesis and about the genes involved in the regulation of these responses. Our approach consists in the comparative analysis of different stress conditions in triggering metacyclogenesis *in vitro* and the analysis of the expressed genes using microarray technology. We are now selecting sets of genes specifically expressed for each kind of stress. The different stress modalities were temperature increase, pH decrease and cell population density increase. Preliminary results of the comparison among the different stresses showed that temperature and pH stress present a greater efficiency than the nutritional stress in promoting parasite differentiation.

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BM59 - CHARACTERIZATION AND GENE PROFILING OF *TRYPANOSOMA CRUZI* AMASTIGOTES OBTAINED *IN VITRO*.

Correa, A. [1]; Ávila, A.R.1; Manhães, L. [1]; Motta, M.C.M. [1,2*]; Pavoni, D. [1]; Probst, C.1; Cardoso, J. [1]; Claure Arauco, P.R. [1]; Corrêa-da-Silva, M.S. [2]; Contreras, V.T. Krieger, M.A. [1,4]; Goldenberg, S[1,2,4]

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Trypanosoma cruzi life cycle involves at least three distinct developmental stages: epimastigotes, trypomastigotes and amastigotes. The epimastigote forms replicate in the midgut of the insect host and develop into non-replicative metacyclic trypomastigote forms by the process of metacyclogenesis. Metacyclic trypomastigote differentiates, within the host cells, into the replicative amastigote form (amastigogenesis), which in turn differentiates into the infective bloodstream trypomastigotes. Amastigogenesis remains poorly understood; however, the development of culture media and appropriate growth conditions has allowed mimicking most of the life cycle *in vitro* paving the way to a better understanding of this process. Nowadays, *in vitro* culturing conditions render possible obtaining metacyclic trypomastigotes from epimastigotes and amastigotes from metacyclic trypomastigotes.

This work focuses on determining: first, if the amastigotes obtained *in vitro* are comparable to amastigotes obtained *in vivo* or from infected cell culture, and second, which genes are specifically expressed by amastigotes.

To accomplish the first goal, amastigotes obtained *in vitro* have been examined by electron microscopy. The ultrastructural analysis of amastigogenesis in axenic conditions, after 72 h of induction, showed that most of the parasites presented the amastigote form and many cells were actively dividing. Transmission electron microscopy showed that most parasites presented a barrel-shaped kinetoplast, which is characteristic of amastigotes and the nucleus presented a less compact chromatin displaying an evident nucleolus. We are currently characterizing this form by molecular methods, including western blot analyses using the antiserum against the amastigote specific antigen Ssp4.

To address the second goal, homemade DNA microarrays containing approximately 5000 ORFs are being used to identify amastigote either specific or non-specific genes. Firstly, competitive hybridization will be performed to compare the two replicative forms of the parasite life cycle, namely amastigotes and epimastigotes. Secondly, an *in vitro* time course experiment will be carried out starting from epimastigotes up to amastigotes, passing through the metacyclic trypomastigote form. This approach will allow us to characterize each developmental stage with a catalogue of stage-specific expressed genes and, to look for developmental regulators that could be putative targets for developing better ways to control *T. cruzi* and Chagas disease.

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BM60 - A MEMBER OF THE WD40 GENE FAMILY IS DIFFERENTIALLY EXPRESSED IN *T. CRUZI* METACYCLIC TRYPOMASTIGOTES.

Nardelli, S.C.[1], Reis, A.[1], Ávila, A.[1], Dallagiovanna, B.[1], [1,2] Krieger, M.A. [1,2] Goldenberg,S. [1,2]

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Metacyclic trypomastigotes are the infective forms of *Trypanosoma cruzi*. These forms are found in the posterior intestine of the invertebrate host and are released through the insect excreta during feeding in the vertebrate host. Once in the blood stream of the mammalian host they can infect different cells. The study of genes specifically expressed during this stage is important in order to identify genes involved in the infection process and that might act as virulence factors. In addition, the studies might lead to the identification of targets to be used in future attempts of rational drug design against Chagas' disease. Using the technique of Differential Display we have isolated a fragment of 901 nucleotides, which was named C20. Northern blot analysis showed that this gene is specifically expressed by metacyclic forms. The sequence of C20 showed significant homology to sequences present in gene data bases, encoding proteins that display the conserved domain WD40. Proteins of this family have essential roles in several cellular processes such as signal transduction, apoptosis and ribosomal RNA processing, among others. The complete sequence of this gene in *T. cruzi* Dm28c was obtained (2.247 nucleotides) with an open reading frame of 1.335 nucleotides. In order to express this protein in *E.coli* the complete coding sequence was cloned in the expression vector pQE31. The recombinant protein fused to a histidine-tag was purified by affinity chromatography and rabbits have been immunized to obtain a specific polyclonal antibody.

Financial support from PADCT, PRONEX and CNPq.

BM61 - EVALUATION USING MICROARRAY TECHNOLOGY OF POLYSOMAL MOBILIZATION IN GENE EXPRESSION REGULATION DURING THE *T. CRUZI* METACYCLOGENESIS

Avila, A. [1], Pavoni, D. P.[1,] Monteiro-Góes, V.[1], Probst, C. M.[1], Dallagiovanna, B.[1], Correa, A. C.[1], Claure Arauco, P. R.[1] Cardoso, J.[1], Picchi, G. [1], Sotomaior, V.[1], Reis, A. C. dos [1], Bitencur, L.[1], Stange, F.[1], Nardelli, S. [1], Fragoso, S. P[1], Nunes, L.[2], Oliveira, R. C.[2], Alves, J. [3], Ibachi, N. [3], Wen, L. [3], Manque, P. A.[3], Ozaki, L. S.[3], Serrano, M. G.[3], Puiu, D. [3], Mallonee, D. [3], Xu, P. [3], Zwierzynski, T.A.[3], Carvalho, M. R.[3], Buck, G. A.[3], Krieger, M.A.[1], Goldenberg, S.[1]

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The regulation of gene expression in trypanosomatids is exerted mainly at the post-transcriptional level. The fact that most trypanosomatid mRNAs result from processing of polycistronic transcripts suggests that stage specific genes must have their expression regulated either through selective transport to the cytoplasm, or through specific degradation/stabilization pathways or, alternatively, through selection of the sequences to be translated in the polysomes. In the case of *Trypanosoma cruzi*, modulation of gene expression is of great importance because the parasite faces different environments (hosts) during its life-cycle, alternating replicative and non-infective stages with non-replicative and infective stages. The development of a chemically defined medium that supports *T. cruzi* metacyclogenesis has rendered possible obtaining parasites at various stages of the differentiation process. In order to gain further insight into the mechanisms involved in *T. cruzi* gene expression regulation, we have constructed a *T. cruzi* biochip and compared, total cytoplasmic and polysomal RNAs from parasites at various time points of the differentiation process. The results indicate that specific sets of mRNAs are mobilized to the polysomes whereas other mRNA sequences remain in the cytoplasm in a translation repressed form. These results were further corroborated by northern blot and quantitative RT-PCR analysis.

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BM62 - MOLECULAR CHARACTERIZATION OF GENES INVOLVED IN CAMP SIGNAL TRANSDUCTION PATHWAY IN *TRYPANOSOMA CRUZI*

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The cellular differentiation from *Trypanosoma cruzi* epimastigotes to metacyclic trypomastigotes as well as the characterization of some genes and factors involved in this process, have been studied by our group on the last twenty years, including the role of cAMP as an important secondary messenger in inducing metacyclogenesis. To select and clone genes expressed at early stages of the metacyclogenesis process, we have performed the RDE (Representation of Differential Expression) technique comparing mRNA extracted from parasites at 24 h of the differentiation process with epimastigote mRNA. One gene selected using RDE was similar to a regulatory subunit of cAMP (Cyclic 3'-5' Adenosine Monophosphate) dependent protein kinase (PKA). The holoenzyme occurs naturally as a 4-membered quaternary structure, with two regulatory (R) and two catalytic (C) subunits. We are presently searching genes that encode the other subunits of PKA to be able to perform the molecular characterization of this important component of cAMP signal transduction pathway. The involvement of this pathway in metacyclogenesis has been studied by expression analysis of the different genes using microarray and real time RT-PCR methods. In addition we are making specific antibodies against recombinant proteins to corroborate the expression analysis including the characterization of the cellular localization of its components.

Financial support: PRONEX, PADCT/CNPq, Fiocruz.

BM63 - DIFFERENTIAL GENE EXPRESSION IN *TRYPANOSOMA CRUZI* STRAINS

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Trypanosoma cruzi is not a homogeneous population but is rather composed by a pool of strains which circulate in both the domestic and sylvatic cycles involving humans, vectors and animal reservoirs of the parasite. Studies of isolated *T. cruzi* populations from different origins demonstrated the presence of a large range of strains with distinct characteristics. This intriguingly intraspecific variation has been extensively investigated by characterizing the morphology of blood forms, parasitemic curves, virulence, tissue tropism, pathogenicity and sensitivity to drugs. These phenotypic differences between strains of the same species are determined by differential gene expression. At least two distinct groups of *T. cruzi* were identified by a number of molecular markers and named group I and II. The differential expression of genes in these groups was analyzed by suppression subtractive hybridization (SSH). Two *T. cruzi* strains, TA and Bol-SB belonging to group I and II, respectively, were cultured in LIT-Medium and, after 3 passages, RNA from epimastigotes was extracted at the log phase. The cDNA was synthesized from mRNA and SSH Analysis was performed using the cDNA subtraction kit (BD Clontech Laboratories). Two reactions, were performed, the forward reaction with TA strain as tester and Bol-SB as driver, and the reverse reaction. Subtracted cDNA fragments from both reactions were directly cloned and 40 clones were sequenced. Sequence alignments and homology searches identified several cDNA clones that should be differentially expressed in these two *T. cruzi* strains. A couple of clones have been identified which did not reveal homology with any known *T. cruzi* gene but shared similarity with either *Leishmania, Plasmodium* or *T. brucei* genes. According to the *T. cruzi* genome project, so far the gene products of several genes are still unknown and may lead to the characterization of new proteins with interesting functions. Experiments are currently under way in order to analyze whether the genes identified from SSH analysis are also differentially expressed in other *T. cruzi* strains, and whether there is a correlation of the expression pattern with parasitemic curves, pathogenicity, morphology and virulence.

BM64 - PRELIMINARY CHARACTERIZATION OF TRANSCRIPTS ABUNDANT IN AMASTIGOTE FORMS OF *LEISHMANIA (L.) MAJOR*

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Previous work conducted in our laboratory (Iribar, PhD thesis, 2001) to characterize a *Leishmania (L.) major* gene with uncommon features led to a preliminary study of its genomic region. This analysis revealed two transcripts present in higher levels in amastigotes when compared to promastigotes. Our aim is the characterization of both transcripts, named EB4.4 and EE3.4, present in a 4.4 kb *Eco*RI-*Bgl*II and in a 3.4 kb *Eco*RI-*Eco*RI genomic fragments, respectively. A subclone from EB4.4 has been generated (pUCEB4.4) and sequenced and it bears two putative genes; a class I Fructose-biphosphate aldolase and a RNA-dependent helicase (Rev.Inst.Med.Trop.S.Paulo 44-suppl.12, 2002). We are currently characterizing the second clone, pUCEE3.4. It has been fully sequenced using a transposon-based strategy (Tosi & Beverley, NAR, 28:784, 2000). The transposon GFPKAN-mariner was used for primer-island sequencing. This process was successful and the entire fragment was sequenced to completion. The assembly and generation of the consensus sequence was accomplished with Phred/Phrap/Consed (Wilson et al, Nature, 368: 32, 1994). The 'in silico' analysis of the sequence data revealed that EE3.4 is 3441bp long, its AT content is 40.5%; and two Open Reading Frames (ORFs) were predicted to be present and coded by the same strand. The similarity search in public databank (http://www.ncbi.nlm.nih.gov) indicates that one of the ORFs from EE3.4 did not match any previously deposited known gene and the second ORF seems to be a ribosomal protein coding gene. The levels of transcripts present in pUCEB4.4 and pUCEE3.4 are being analysed in Northern experiments to determine which of them are expressed preferentially in amastigotes. These experiments will lead to further functional analyses that will involve overexpression of individual genes in the parasite and the use of insertion mutagenesis. The insertion of a selectable marker for *Leishmania* within one ORF from pUCEB4.4 or pUCEE3.4 will allow the overexpression of their second putative gene in the parasite. The generation of mutagenized versions of both recombinants is underway using a transposition system (NeoKO-mariner).

Supported by FAPESP.

BM65 - IDENTIFICATION AND ANALYSIS OF *LEISHMANIA* EXPRESSED SEQUENCE TAGS CHARACTERISTIC OF NON-CODING RNAS

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Sequencing of the *Leishmania* genome has led to the identification of genes based on the predicted proteins they encode. However, sequences that lack open reading frames and encode RNAs as their final product might be overlooked during the annotation process. Accumulating evidence indicates that such non-coding RNAs (ncRNAs) can play critical roles in gene expression regulation as microRNAs (miRNAs) described in many organisms from worm to man. We are currently investigating five putative *Leishmania* ncRNAs genes whose secondary structure presents hairpin-like motif resembling those found in miRNAs. For instance, *ODD1* has a conserved hairpin in its 3´ region that presents homology to an *Arabidopsis thaliana* precursor miRNA.

Northern analyses were carried out to investigate whether these transcripts have a regulatory function. Total RNA extracted from several *Leishmania* species, in different developmental stages, was blotted on to nylon membranes and probed with antisense oligonucleotides based on the hairpin structures found in genes *ODD2* and *ODD3*. *ODD2* showed a hybridization pattern that could be related to the detection of its precursor and the processed transcript. *ODD3* presented a more complex pattern of hybridization that varied according to the species tested. Parasite transfectants carrying cosmids that contain either *ODD2* or *ODD3* were subjected to increasing levels of Hygromicin B (up to 160mg/ml) in order to overexpress these genes. Northern analyses of these transfectants suggested that at least one of the transcripts detected by *ODD2* could be a RNA target. Also, the transfectant overexpressing *ODD3* presented a cytostatic phenotype and morphological differences when compared to the parental strain. These are preliminar data and work on progress is focused in understanding the functional role of these genes.

BM66 - COHESINS AND CONDENSINS: STUDIES ON THE STRUCTURAL MAINTENANCE OF THE CHROMOSOME COMPLEXES OF *TRYPANOSOMA CRUZI*

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The factors involved in the chromosome organization, spacial distribution and condensation during the *T. cruzi* life cycle are unknown. Distinctly from the most eukaryotic cells, the chromosomes of *T. cruzi* do not condense at mitosis and are difficult to be distinguished. Moreover, little is known about the factors that play a role in the maintenance of the different levels of chromatin condensation observed along the life cycle of *T. cruzi*. Chromatin condensation may be an important mechanism for the regulation of gene expression in this parasite, since the chromatin in a highly condensed state may reduce the levels of RNA transcription, as observed in the metacyclic and bloodstream trypomastigote stages. In order to shed light into these issues we have initiated the characterization of *T. cruzi* genes encoding for cohesin and condensin complexes, that are involved in the structural maintenance of the chromosomes (SMC) in other eukaryotic cells. Cohesin is a complex necessary to maintain sister chromatid cohesion from chromosome duplication until the onset of anaphase. This protein complex contains four core subunits, two SMC subunits (SMC1 and 3) and two non-SMC proteins (Scc1/Mcd1/RAD21 and Scc3/SA). By the other hand, the condensin complex is required for the establishment and maintenance of chromosome condensation. The holocomplex of condensin is composed of two SMC subunits (SMC2/CAP-E and SMC4/CAP-C) and three non-SMC subunits (CAPD2, CAP-G and CAP-H). We have characterized nine genes of *T. cruzi* encoding for putative cohesins and condensins, which were collectively named *T. cruzi* SMC genes.The search for homology showed that the deduced amino acid sequences from *T. cruzi* SMC genes share about 45% of similarity with their eukariotic counterparts. Antisera were raised in mice either against peptides synthesized from the deduced amino acid sequences of *T. cruzi* SMC proteins or recombinant *T. cruzi* SMC proteins expressed in *E. coli*, in order to immunolocalize and study the expression pattern of these proteins through *T. cruzi* life cycle. In addition, we have launched the analysis of the expression of these genes by Northern blot and the study of their organization by PFGE and Southern blot.

Financial support: PRONEX, CNPq

BM67 - TELOMERE AND SUBTELOMERE OF *TRYPANOSOMA CRUZI* CHROMOSOME ARE ENRICHED IN PSEUDOGENES AND RETROTRANSPOSON-LIKE ELEMENTS ASSOCIATED TO HOT-SPOTS OF RECOMBINATION

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The sequencing of a ~30 kb telomeric recombinant cosmid (C6) (Chiurillo et al., *Mol. Biochem. Parasitol*. 100:173, 1999) from the pathogen protozoan *Trypanosoma cruzi* provided us with useful landmarks to establish the general features of telomeres and subtelomeres of this parasite. Our findings can be summarized as follows: we confirmed the presence of hexameric repeats and a 189-bp species specific junction at the chromosomal end; subtelomeric region appeared to be enriched in retrotransposon-like sequences and pseudogenes of the TS (Trans-sialidase)-like family; and an open reading frame corresponding to putative surface protein DGF-1. *T. cruzi* subtelomeric region also contains sequences related to the RHS (Retrotransposon Hot Spot) multigene family of *T. brucei* which present a hot spot for non-LTR retrotransposon insertion. Analysis of sequences related to Ts-like family encoding surface proteins suggests that the chromosomal ends could have been the site of generation of new GP85 variants, an important adhesin molecule involved in the invasion of mammalian cells by trypomastigote forms.

Supported by FAPESP and CNPq (JFSF); FONACIT (Venezuela) (JLR).

BM68 - CHARACTERIZATION OF A NOVEL SUBTELOMERIC REPETITIVE ELEMENT FROM *LEISHMANIA*.

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Telomeres are the extreme regions of chromosome ends and are crucial for cell stability and viability. The telomeric and subtelomeric regions contain repetitive elements potentially involved in events of recombination between non-homologous chromosomes. Sequencing of one end of *Leishmania major* chromosome 20 revealed the presence of a novel repetitive element, named LST-R378 (Pedrosa et al, MBP 114: 71, 2001). PCR and Southern blot analyses were performed and revealed the presence of LST-R378 in the genomic DNA from different *Leishmania* species from Old and New World. Curiously, a region of 81 nucleotides from LST-R378 is 83% identical to a fragment of a P-type

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ATPase gene from *L. (L.) donovani*. Amplification reactions revealed a potential polymorphism of LST-R378 in different species; in a *L. (L.) mexicana* strain a single band of about 400bp is amplified, in *L. (L.) amazonensis* two fragments were observed and in different strains of *L. (V.) braziliensis* size-polymorphic fragments were amplified. The amplification reaction conducted on *L. (V.) braziliensis* 2904 genomic DNA revealed three fragments ranging from 300 to 600 nucleotides, all of them contain the same ATPase fragment as shown by their complete sequences. *In silico* search for the intact ATPase coding gene found it on chromosome 16. Its genomic region is currently being recovered to study its flanking sequences and to investigate the presence of a repetitive element similar to LST-R378.

Supported by FAPESP.

BM69 - HOMOLOGUES OF RPA-1 AND RBP38 ARE THE PROTEIN COMPONENTS OF TWO *LEISHMANIA (LEISHMANIA) AMAZONENSIS* G-RICH TELOMERIC (LATG) COMPLEXES

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Until the present report there were no descriptions of the components of telomeric chromatin in *Leishmania* spp. Species of this genus are the ethiological agents of leishmaniasis, a worldwide spread disease of significant medical and economic importance. The chromosomal ends of the Brazilian pathogen *Leishmania* (*L.*) *amazonensis* contain conserved TTAGGG telomeric repeated sequences. Protein com-plexes that associate to these sequences (LaTG1, LaTG2 and LaTG3) were identified and characterized by gel shift assay and UV cross-linking using anion exchange purified fractions from S100 and nuclear extracts. Different physical-chemical properties of the complexes were determined. All complexes are formed at 4 °C, although LaTG2 and LaTG3 are also formed at temperatures up to 20 °C. All three complexes are stable at high salt concentrations. Size-estimation of the protein-forming complexes was achieved by in situ UV cross-linking. These proteins do not interact with double-stranded DNA and the C-rich telomeric strand, but they can associate with an RNA oligonucleotide cognate to the G-rich telomeric sequence. All three complexes were not formed i) in competition assays using specific telomeric oligonucleotides and ii) when the extracts were pre-treated with proteinase K, indicating that they are protein-forming complexes. The protein components of LaTG2 and LaTG3 were purified by affinity chromatography using a 5´-biotinilated G-rich telomeric oligonucleotide and were eluted by KCl step gradient. The column fractions were separated by SDS-PAGE and Coomassie stained to determine their protein contents. The estimated dissociation constants (K_d) for LaTG2 and LaTG3 complexed to DNA and RNA sequences are in nM range. After purification and renaturation experiments they were identified as the ~35 kDa and the ~52 kDa protein bands, respectively. These purified protein bands were in gel digested with trypsin and the resulting peptides were subjected to MALDI-TOF MS fingerprinting mass spectrometry. The peptide fingerprint analysis showed that the ~52 kDa component of LaTG2 is similar to the subunit 1 of the conserved single-stranded binding protein, replication protein A (RPA-1) and the ~35 kDa protein component of LaTG3 is a homologue of the conserved trypanosomatid RBP38 RNA binding protein.

Financial support: FAPESP, WHO/TDR – UNDP Bank.

BM70 - GENOMIC ORGANIZATION OF TELOMERIC AND SUBTELOMERIC SEQUENCES (TAS – "TELOMERE ASSOCIATED SEQUENCES") OF *LEISHMANIA (LEISHMANIA) AMAZONENSIS*

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Telomeres are protein-DNA complexes that protect linear chromosomes from degradation and end fusions ensuring genomic stability. The telomeric repetitive sequence is guanine rich and protrudes towards the end of the chromosome as a 3'-G overhang structure.

Sequences adjacent to telomeres constitute the subtelomeric region, which, in *Leishmania* spp., was characterized as "*Leishmania* Conserved Telomere Associated Sequences" (LCTAS) arranged in blocks of 100 bp flanqued by telomeric sequences. Different from *Trypanosoma brucei, Trypanosoma cruzi* and *Plasmodium falciparum*, LCTAS do not present genes encoding surface antigens. In contrast, they contain two conserved sequence blocks (CSB 1 and CSB 2) that show different organization and copy number in almost all species of the genus *Leishmania* studied so far. Due to the high degree of sequence conservation, it is suggested that LCTAS play an important role in chromosome segregation and that they could harbor binding sites for telomeric proteins.

The aim of this work is to study the genomic organization of the *Leishmania (L.) amazonensis* telomeric/subtelomeric sequences and identify proteins that specifically recognize them. In this report, we hybridized digested genomic DNA from *L. (L.) amazonensis* promastigotes with a G-rich telomeric sequence and with CSB 1 and CSB 2 sequences as probes. Kinetic experiments using *Bal*31 exonuclease were used to differentiate the telomeric fragments from the interstitial internal ones and to estimate the size of *L. (L.) amazonensis* telomeres. In addition, we used Pulsed Field Gel Electrophoresis (PFGE) in first and second dimensions to map and study the organization of telomeres and LCTAS in the parasite chromosome context.

The results of Southern blotting and *Bal*31 helped us to estimate that the Terminal Restriction Fragment (TRF) of *L. (L.) amazonensis* is approximately 7 Kb long and that the mean size of parasite telomeres is ~3 Kb. CSB 1 and CSB 2 appeared as blocks of 0.3-0.4 Kb, flanqued by *Hae*III restriction sites. They are present in lower copy number when compared to the telomeric sequences, although the hybridization signal with CSB 2 was stronger than with CSB 1, suggesting that *L. (L.) amazonensis* LCTAS are mainly composed by CSB 2 blocks. To confirm the above results, we hybridized *L. (L.) amazonensis* chromosomes separated by PFGE. Under our running conditions, all chromosomes of *L. (L.) amazonensis* were fractionated in a unique gel. They appeared as 25 bands, with molecular sizes ranging from 0.35 - $\geq$ 3.0 Mb, which were stained by ethidium bromide and hybridize with the telomeric sequence, confirming that they are linear chromosomes. Using second dimension PFGE, we demonstrated that *L. (L.) amazonensis* chromosomes present telomeres with different sizes, suggesting that as the same as in other eukaryotes, telomere replication in *Leishmania* is regulated at the chromosome level. We are currently analyzing telomeric/subtelomeric sequences cloned from purified chromosomes in order to study these regions in detail.

Financial support: FAPESP, WHO/TDR – UNDP Bank.

BM71 - GENOMIC ORGANIZATION AND TRANSCRIPTION OF A *TRYPANOSOMA CRUZI* REPEATED ELEMENT

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We describe here further characterization of a new *T. cruzi* repeated element of ~1.5 kb named Z25. Most of Z25 element are flanked by *Eco*RI sites, and they are composed by a ~65 bp region similar to the mucin-like pseudogene followed by a ~591 bp region that shares similarity with sequences previously described in the intergenic spacer of the genes for pyrimidine biosynthesis. The 3' end (~844 bp region) shows several repetitions of 54 bp and 55 bp arranged like beads-on-a-string. In spite of the variation in the number of these small repeats (54- and 55 bp) the whole element is highly conserved. Partial digestion of *T. cruzi* genomic DNA with *Eco*RI and hybridization with selected probes indicated that most of Z25 elements are arranged in tandem arrays which are located in six chromosomal bands. To further confirm this hypothesis we did Southern blot analysis of *T. cruzi* chromosomes separated by two dimensional pulsed field gel electrophoresis after partial digestion with *Eco*RI. There is an average of 4-6 *EcoRI* fragments per chromosomal band, indicating that Z25 are arranged in tandem arrays. Northern blot analysis showed that sequences found in Z25 share homology with 1.5 kb and 0.24 kb transcripts are expressed in epimastigotes. The 1.5-kb transcript was only found in the poly A+ RNA fraction, whereas the 0.24-kb transcript was also detected in the poly A- fraction. Nucleotide sequence identity search of the GenBank database revealed a high percentage identity of Z25 with 15 ESTs (Expressed Tag Sequences), confirming that this repeated element is transcribed. Taken together, our results indicate that Z25 is organized in tandem arrays located at specific chromosomal regions, and it is expressed as oligo(A)-terminated transcripts. Further characterization of structural and functional features of Z25 repeated elements is underway in our laboratory.

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BM72 - FISHING *LEISHMANIA (L.) AMAZONENSIS* PROTEINS USING THE TELOMERIC DNA AS A BAIT

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Telomeres are specialized nucleoprotein complexes localized at the ends of linear chromosomes. They function as a cap at the chromosome end terminus and protect them from exonucleolytic degradation and end-to-end fusions, conferring genome stability and normal cellular proliferation. Telomeric DNA is composed of tandem short repeats of G-rich sequences. The G-rich strand extends beyond the complementary C-rich strand forming a single-stranded protrusion or a 3'G-overhang, which is the substrate for telomerase replication. Proteins found at telomeres can associate to the double strand or single-strand telomeric DNA forming a high order complex at the end of the chromosome. They function as positive or negative regulators of telomere maintenance and mutation or deletions of any of these protein factors can alter telomere function leading to gross effects in cell life maintenance.

Leishmania spp. telomeres are composed by the conserved TTAGGG sequence, which is maintained by telomerase. Proteins that associate to the G-rich telomeric strand were identified in affinity purified extracts of *L. (L.) amazonensis* (Fernández et al., submitted). However, there are no descriptions of proteins that associate with the double-stranded *Leishmania* spp. telomeres. Yeast one-hybrid system was chosen to screen *L. (L.) amazonensis* telomeric proteins. To conduct the assay, two integrative vectors containing the telomeric bait cloned upstream of a reporter gene were constructed: i) the pLacZi-LaTel vector, containing *lacZ* reporter gene and ii) the pHISi-1-LaTel vector containing *HIS3* reporter gene. These two vectors were used to built three different reporter yeast strains. HisTel and LacTel containing respectively pHISi-1-LaTel and pLacZi-LaTel vectors integrated in the genome. And LacHisTel, that bears both integrative vectors. *L. (L.) amazonensis* logarithmic phase promastigotes mRNA was reverse transcribed using a mixture of random and oligo dT primers generating cDNA molecules ranging from 0.4-2.0 Kb. To construct a non-normalized library the cDNA was cloned in pGAD424 expression vector, which allows the proteins to be expressed in fusion with GAL4p activation domain. The expression of *L. (L.) amazonensis* telomeric proteins activate the reporter gene transcription providing the genetic selection of positive clones using selective medium or β-galactosidase assay.

The first attempt to identify *L. (L.) amazonensis* telomeric proteins by one-hybrid system was performed with the double reporter yeast strain LacHisTel, which resulted in the selection of 13 clones from 600 possible candidates. The clones already sequenced showed any similarity with known telomeric proteins, suggesting the existence of novel *Leishmania* telomeric proteins. To confirm the above results we are currently sequencing other clones and performing the screening of new libraries.

Supported by FAPESP and WHO/TDR – UNDP Bank.

BM73 - SPECIFICITY OF A TRANSPOSON-BASED TOOL FOR GENE IDENTIFICATION IN *LEISHMANIA*

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The completion of genome sequencing projects stimulates the definition of new strategies aimed at the systematic analysis of the information that is being accumulated. The transposon technology has proven to be a powerful tool in tagging and analyzing genes of many organisms in a systematic fashion. In many cases transposons can be modified, controlled and used in the characterization of gene expression.

The in vitro mobilization of the Mos1 transposon, isolated from the *Drosophila mauritiana* genome, led to the development of protocols tailored for the characterization of genes of the protozoan parasite *Leishmania*. In a previous report we showed that fusion proteins generated between parasite genes and reporter genes carried in transposons can be efficiently detected within the parasite cell. The detection of reporter expression was based on the use of an immunofluorescence microscopy protocol. Here we report the study of the efficiency of the element /NEO*SAT to trap *Leishmania* genes. The specificity of the trapping system was confirmed by the fact that the Neomycin Phosphotransferase (NPT) gene is expressed within the parasite only if inserted into the open reading frame of a given gene. Random insertions of the /NEO*SAT transposon into genomic libraries were characterized by *primer island* sequencing and insertions of interest were identified. Selected events bearing the reporter gene inserted in-frame into annotated genes were transfected into the parasite. Out-of-frame insertions and intergenic events were also selected and used as negative controls. Since all constructs were cloned into the shuttle vector pELHYG, transfectants were selected in hygromycin B. Transfectants bearing in-frame insertions were able to grow in the presence of G418. Those transfectants carrying intergenic or out-of-frame insertions could not be selected for the expression of NPT. This was the first indication of the specificity of the trapping tool. Immunodetection of NPT in transfectant cells confirmed that the reporter gene introduced by the transposon insertion was expressed only as part of a fusion protein.

Two other specialized transposons, carrying the b-glucuronidase (GUS) and the Green Fluorescent Protein (GFP) genes as reporters, were also constructed and tested. In the elements /GUS*SAT and /GFP*NEO the reporter genes do not contain an ATG start codon and will be expressed only if part of a fusion protein. The expression of GUS and/or GFP is screened rather than selected for. This characteristic, and the possibility of detection of reporter expression in live

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cells, is a major advantage of these elements as gene trapping tools. Work in progress is focused on defining strategies for genome-wide implementation of the established protocols.

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BM74 - SYSTEMATIC DISRUPTION OF GENES AT A *LEISHMANIA* CHROMOSOME END.

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The development of transposon technology and its ability to destroy endogenous phenotypes allow a global perspective on genome expression in a systematic manner. Such strategy is especially appealing when dealing with the protozoan parasite *Leishmania,* whose genome-sequencing project is approaching completion. However, the presence of repetitive elements at chromosomal extremities hampers the assembly and annotation of sequence while restricting the implementation of strategies aimed at the study of genes located therein.

Here we report the use of a specialized mariner transposon adequate not only as the source of reagents for precise gene disruption protocols, but also for the sequencing of repetitive elements. The extremity of the 580 Kb chromosome of *L. (L.) major* LV39 was assembled through mapping of two cosmid clones named 008B2 and 056G8, isolated from a genomic library. Southern analysis of PFGE-separated chromosomes associated these clones to chromosome 7. Southern hybridization revealed the presence of repetitive sequences common to both clones, but only clone 008B2 presented the typical telomere-associated hexameric repeats. The restriction map of these clones revealed an overlapping region of approximately 12 Kb. Clones 008B2 and 056G8 cover 60 Kb of the chromosome end.

Clone 056G8 was restricted with *Bam*HI/*Bgl*II and further subcloned into the shuttle vector pELHYG. Two of the recombinant plasmids, carrying 15 Kb and 10 Kb, were subjected random insertions of the mariner element ELNEOKO. This specialized transposon contains a selectable marker preceded by the protozoan splicing acceptor sites and a prokaryotic promoter sequence. *Primer-island* sequencing of these insertion events and *in silico* analysis of the data generated revealed the presence of two putative ABC-transporters genes. Clones carrying the element ELNEOKO inserted within a 5.2 Kb ABC transporter-like gene were identified and isolated. The plasmid DNA of a chosen clone was digested to produce a linear fragment to be used in a gene disruption protocol. The fragment was transfected into the parasite and integration events were selected in media containing G418. The homologous recombination of the selectable marker carried by the transposon, and flanked by the target sequences, was confirmed by Southern analyses of PFGE-separated chromosomes and digested genomic DNA. The clone 008B2 was used as target for transposition of the element /GFP*KAN. This transposon contains the Green Fluorescent Protein (GFP) as a reporter for translational fusion events. *Primer-island* sequencing of transposition events into 008B2 enabled not only the characterization of telomeric and sub-telomeric repetitive elements, but also the establishment of a higher resolution map of this peculiar genome structure. The strategy presented here is a valuable tool for systematic disruption and/or discovery of genes, and an important source of reagents for comparative and functional studies of *Leishmania* genome.

Supported by FAPESP and UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases.

BM75 - FUNCTIONAL STUDIES OF THE TCRHO1 GTPASE OF *TRYPANOSOMA CRUZI*: CONSTRUCTION OF MUTANTS (POSITIVE AND NEGATIVE DOMINANTS) AND RNA INTERFERENCE ASSAYS.

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Rho family proteins are members of the Ras superfamily of small GTPases. In higher eukaryotes, these proteins play critical roles in the cell mobility, phagocytosis, intracellular transport, cell adhesion and maintenance of cell morphology. Other cellular processes under Rho family control are the regulation of activity of the NADPH oxidase complex, progression of the G1 phase of cell cycle, transcription regulation and metastasis induced by different oncogenes. The TcRho1 gene identified in our previous work is the only Rho family member in *Trypanosoma cruzi.* This GTPase presents a C-terminus motif (CQLF), which is targeted to farnesylation by a *T. cruzi* farnesyltransferase. A *T. cruzi* cell line expressing a mutant TcRho1-ÄCaaX (a protein unable to be farnesylated) demonstrated poor metacyclogenesis levels in TAU-3AAG medium. To proceed with the functional characterization, we carried out site-directed mutagenesis to obtain the G15V and Q76L positive dominants (constitutive active form) and the T20D negative dominant (constitutive inactive form), which were transfected in *T. cruzi* and selected on agarose plates. Growth curve revealed a significative decrease on proliferation of the TcRho1-T20D cell line, with poor adhesion ability during metacyclogenesis process. Currently, all cell lines are under ultrastructure investigation by electron microscopy. Other functional approach to study TcRho1 function is the RNA interference. A fragment sense-loop-antisense of TcRho1 was subcloned in the pTEX and pRIBOTEX vectors to produce a dsRNA in vivo and parasites transfected with these constructions are presently under selection for posterior phenotypics studies.

This work was supported by CAPES, CNPQ and PRONEX.

BM76 - DIFFERENTIAL EXPRESSION OF GENES IN *TRYPANOSOMA CRUZI* TREATED WITH THE BENZNIDAZOLE DRUG

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The Chagas disease is one of the parasitic diseases more broadly distributed in America and it affects among 16-18 millions of people from different regions of the continent. In Colombia, it is calculated that 1.3 million people are infected and 3.6 millions are in risk of contracting the infection.

In the last years, numerous studies have been carried out about the biochemistry and physiology of the *T. cruzi*. However, the treatment of the infection taken place by the parasite is considered as one of the more unsatisfactory and the advances in the chemotherapy in order to control the Chagas disease have been very few. The treatment is based on two old and unspecific medications, nifurtimox and benznidazole. The necessity exists of looking for new chemotherapeutic targets and effective drugs against the different forms of *T. cruzi*, and with less deleterious effects than those of classic medications.

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In this work, as an approach, an analysis is shown of expression of genes when a Colombian stock of *T. cruzi* is treated with benznidazole, in comparison to the genes expressed in the stock control without treatment. Epimastigotes of *T. cruzi* were treated with the drug from 25 ug/ml to 500 ug/ml with the purpose of determining the DL_{50}. The RNA total of treated parasites and the controls were isolated, and the RNA messenger was purified with a primer poly T. The cDNAs of the control stock were cloned in the vector l ZAP and evaluated by the technique of hybridisation subtractive, with cDNA of the treated parasites and non treated ones, with the purpose of identifying genes that are expressed differentially when the parasite is exposed to the drug. The differentially expressed clones were isolated, amplified by PCR and subjected to a second screening. The doubly positive clones were sequenced and compared in the database. A great number of genes has been isolated, some of them corresponded to well-known genes, as it is the case of the histone H1, and other genes to be studied. In a future, knowing the sensibility of the stocks of Colombia to the antichagas drugs, is interesting to the identification of genes that are expressed differentially in stocks with sensitive and resistant phenotype.

Financial support: Proyecto de sostenibilidad, CODI, University of Antioquia, Medellín.

BM77 - ISOLATION OF CDNA GENES OF AMASTIGOTE SURFACE PROTEIN-2 EXPRESSED IN THE SYLVIO X10 CLONE 4 STRAIN OF *TRYPANOSOMA CRUZI*.

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Recently, independent groups studied the immunogenic properties of plasmids containing genes encoding the Amastigote Surface Protein-2 (ASP-2). Genetic immunization with *asp-2* genes generated immune responses mediated by antibodies, $CD4^+$ and $CD8^+$ T cells. Most relevant, DNA-vaccinated mice displayed remarkable protective immunity, surviving lethal infection with *T. cruzi* (Boscardin *et al*., 2003, Infect. Immun. 71:2744, Fralish & Tarleton, 2003, Vaccine 21:3070). To gain further information on the sequence polymorphism of ASP-2 genes, we isolated and characterized genes encoding members of this protein family expressed in the Sylvio X10 clone 4 strain of *T. cruzi*. RNA purified from intra-cellular amastigotes was used as template for reverse transcriptase reaction in the presence of oligo-dt primers. The cDNA was used as target for PCR in the presence of primers specific for the previously described ASP-2 gene. PCR products of ~2.1 Kb were obtained from cDNA of amastigotes and cloned into the pMOS vector. Several clones containing the ~2.1 kb inserts were analyzed by enzymatic restriction. We found that the amastigote cDNA contained at least 4 groups of genes. When compared to the previously described genes of ASP-2, their restriction patterns were completely distinct indicating a clear polymorphism. We are currently sequencing theses genes to determine their predicted amino acid sequences.

Supported by FAPESP.

BM78 - *LEISHMANIA (L.) AMAZONENSIS*: MOLECULAR AND IMMUNOLOGICAL ANALYSIS OF A CDNA LIBRARY FROM AMASTIGOTES AND MAPPING OF EXPRESSED SEQUENCE TAGS (ESTS) IN CHROMOSOMAL BANDS.

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The present work describes the construction of a cDNA expression library from *Leishmania (L.) amazonensis* amastigotes, as well as the generation and characterization of expressed sequence tags (ESTs).

The randomly analysis of 322 clones showed that all of them reacted with rabbit immune serum against *L. (L.) amazonensis* amastigotes, demonstrating the reliability of the *L. (L.) amazonensis* cDNA library. Among the isolated clones, 100 were sequenced generating new *L. (L.) amazonensis* ESTs from which 53% are not related to any other sequences in databases, whereas 47% presented significant similarities to known genes: 3.2% showed similarity to *L. (L.) amazonensis* genes, 23.4% to *L. (L.) major* chromosomal sequences, and 19% to genes from other trypanosomatids. The chromosome mapping of *L. (L.) amazonensis* resolved by pulsed field gel electrophoresis (PFGE) of 9 ESTs and 2 cysteine proteinase genes was used to study the chromosomal polymorphism between *L. (L.) amazonensis* and *L. (L.) major*. Results from these experiments indicate a possible rearrangement in chromosome 19 of *L. (L.) major*, since two clones from *L. (L.) amazonensis* which present similarity to cosmid L4766 mapped in two different chromosomes of *L. (L.) amazonensis*, one of 1250 kb (*LlaAmEST*0087) and another of 720 kb (*Llacys1* gene). On the other hand, the ESTs hybridizing in a single band may be used as a chromosome-specific marker in the determination of the molecular karyotype of the *L. (L.) amazonensis* LEM690 strain.

The ESTs characterization is useful for discovery of new genes and for the physical mapping of genomes.

Supported by CNPq and FAPESP.

BM79 - GENE SURVEY OF *EIMERIA* SPP. OF DOMESTIC FOWL USING OPEN READING FRAME ESTS (ORESTES)

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Coccidiosis of the domestic fowl is an important enteric disease caused by seven different protozoan species of the genus *Eimeria*. The most studied species, *E. tenella*, presents a genome of circa 60 million base pairs distributed in 14 chromosomes. Sequencing initiatives are being carried out, including an EST project (Washington University - USA) and a 5-fold genome coverage (Institute for Animal Health and Sanger Institute, UK). As a member of the *Eimeria* Genome Consortium (http://www.sanger.ac.uk/Projects/E_tenella/consortium.shtml), our laboratory has initiated an alternative EST sequencing project using the open reading frame expressed sequences tags (ORESTES) approach. ORESTES reads are produced by low-stringency RT-PCR of mRNAs and are biased towards the central region of the transcripts (Dias-Neto *et al.*, PNAS 97: 3491-3496, 2000). The primary aim of our study was the generation of at least 10,000 reads of each one of the three most important *Eimeria* species: *E. tenella*, *E. acervulina* and *E. maxima*. We have obtained so far a total of 7,741 high-quality reads from sporozoites and second-generation merozoites of *E. tenella* H, 10,175 reads from *E. acervulina* H sporozoites and sporulated oocysts, and 1,851 reads from *E. maxima* H sporulated oocysts. Clustering analyses resulted in 2,382, 2,233 and 549 distinct events, respectively. When the *E. tenella* reads were clustered together with 26,955 conventional 5' ESTs already deposited on NCBI, a total of 5,926 clusters were obtained. Comparing to clustering data restricted to the NCBI subset (4,571 clusters), this data suggests that our set of reads contributed with the discovery of 1,355 new putative transcripts (61% of our clusters represented novel sequences). This result is in agreement with the assumption that ORESTES-generated data is complementary to the information obtained by conventional ESTs. Preliminary annotation revealed that sporozoites and merozoites express very distinct transcript profiles. Future perspectives include

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ORESTES generation and sequencing for other developmental stages of each species, plus a comprehensive annotation aiming at identifying new potential candidates for vaccine development.

Financial Support: FAPESP, CNPq and Pró-Reitoria de Pesquisa USP.

BM80 - *TRYPANOSOMA CRUZI:* USE OF PERMEABLE CELLS AS MODEL OF STUDY THE ACTION OF DRUGS IN THE PROCESSING OF THE MESSENGER RNAS"

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Pre-mRNA maturation in Trypanosomatids occurs through a processs called trans-splicing, which involves excision of introns and union of exons in two independent transcripts; a short transcript spliced leader (SL RNA) is trans-spliced to the acceptor pre-mRNA, originating the mature mRNA. In this work we have used permeable cells from epimastigote forms of *Trypanosoma cruzi* (Y, NCS and Bolivia strains) as a model for study of drug interference in trans-splicing reaction. The cells were treated with lysolecithin and hydroxymethyl-nitrofuran, a nitrofuran-derived drug which can inhibit trypanotione reductase, an important enzyme in the metabolism of *T. cruzi*. The reciprocal drug nitrofural had its activity observed in different concentrations, showing itself a sensible reduction in the RNA processing concentration of 8mM. After newly-formed RNA extraction, they were hybridized with the SL RNA antisense sequence as a probe followed by RNAse treatment to localize SL exon and intron formation to demonstrate that trans-splicing reaction occurred.

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BM81 - IDENTIFICATION OF CANDITATE GENES WITH HEMOLITIC FUNCTION IN *TRICHOMONAS VAGINALIS*

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Trichomonas vaginalis, a flagellate parasitic protozoan, is the etiologic agent of trichomoniasis. The pathogenesis includes events such as hemolysis, cytoadhesion and secretion of proteases, causing epithelial injuries with some pathological consequences in the geniturinary tract of its host. Hemolysis is also an important source of nutrients for the protozoan. Some molecules are not synthesized *de novo* by the parasite. In particular, the microelement iron controls the metabolic activities of the parasite as well as regulation of some genes related to virulence. The hemolytic process must therefore be characterized. The objective of this study was to identify candidate genes with hemolytic function in a cDNA library of *T. vaginalis*. Messenger RNA was purified from fresh clinical isolates, grown in Diamond growth media. A cDNA library was developed from transcripts orientated for transient expression in *Escherichia coli* under control of the *lac* promoter. Additionally, functional screenings were performed in total of 5 x 10^4 clones of the cDNA library. This functional screening was based on the formation of bright halos surrounding the bacterial colonies after induction with a layer of IPTG/blood/agar. Six distinct hemolytic clones were selected based upon this screening procedure. Their sequences show that the parasite has different genes with hemolytic activity, with transcript length varying from 0.4kb to 1.5kb. Bioinformatic analysis based on homologies, domains and possible functions showed that the parasite may use different pathways to induce hemolysis in the host.

BM82 - MAPPING AND CHARACTERISATION OF THE *LEISHMANIA (L.) AMAZONENSIS* GENOMIC REGION CONTAINING THE META 1 GENE: COMPARATIVE STUDY WITH THE *L. (L.) MAJOR* GENOME

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The metacyclic upregulated meta 1 gene is conserved in both Old and New Wold *Leishmania* species. Overexpression of the meta 1 protein in *L. (L.) amazonensis* generates parasites that are more virulent *in vivo,* suggesting an important function for this protein during infection (Uliana et al, Exp. Parasitol. 92:183-191,1999). Previous efforts to characterise other transcripts derived from the region surrounding the meta 1 gene in *L. (L.) amazonensis* allowed the description of a meta 1-related, metacyclic upregulated gene named meta 2 (see Ramos and Uliana, this meeting).

Three other fragments of the same genomic region identified as complementary to cDNAs prepared from metacyclic stage parasites were characterized. The first fragment, 5 kb in length, was derived from the 5' region of the meta 2 gene and hybridized to 2 transcripts: a 1,5 kb transcript upregulated in metacyclic promastigotes and an unregulated 1 kb RNA. The analysis of the nucleotide sequence of this clone enabled the identification of two ORFs and a possible coding strand switch region. The translated sequence of one of this ORFs is similar to the "VsrD" protein of *Pseudomonas solanacearum,* a virulence related, transcription regulator protein.

The second fragment, located between meta 1 and meta 2 genes, identified a 2,2 kb transcript predominantly expressed in promastigotes and encoded a putative protein without significant similarities to proteins previously described.

The last clone, obtained from a fragment located 3' to the meta 1 gene, identified a 2 kb transcript upregulated in amastigotes. The nucleotide sequence of this fragment indicates the presence of an ORF similar to the NOD3 human protein, involved in immune reactions.

The contig composed by all these *L. amazonensis* gene fragments was aligned with a *L. (L.) major* contig (*L. (L.) major* Genome Project) revealing a high degree of similarity. Regions containing ORFs, as expected, are more conserved, with sequence similarities ranging from 87 to 92%, while intergenic/untranslated regions are more heterogeneous, with sequence similarities ranging from 58,66 to 87,02%.

Supported by FAPESP.

BM83 - CHARACTERIZATION OF THE ABC TRANSPORTER GENE *PRP1* RELATED TO PENTAMIDINE RESISTANCE IN *LEISHMANIA (L.) MAJOR*

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Pentamidine (PEN) is a second-line agent in the treatment of leishmaniasis whose mode of action and resistance is not well understood. Here we used a genetic strategy to search for genes able to mediate PEN resistance when overexpressed in *Leishmania (L.) major*. A shuttle cosmid library containing

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genomic DNA inserts was transfected into wild type promastigotes and screened for PEN-resistant transfectants. Two different cosmids identifying the same locus were found, which differed from other known *Leishmania* drug resistance genes. The PEN resistance gene was mapped by deletion and transposons mutagenesis to a protein belonging to the P-glycoprotein/MRP ABC transporter superfamily that we named PRP1 (Pentamidine Resistance Protein 1). The predicted PRP1 protein encodes 1,807 amino acids with the typical dimeric structure involving 10 transmembrane domains and two nucleotide-binding domains. PRP1 mediated PEN resistance could be reversed by verapamil and PRP1 overexpressors showed cross-resistance to trivalent antimony but not to pentavalent antimony (glucantime). Although PEN resistance was modest (1.7-3.7 fold), this may be potentially significant in clinical drug resistance given the marginal efficacy of PEN against *Leishmania*.

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BM84 - MULTILOCUS GENOTYPE DATA PROVIDE EVIDENCES THAT HYBRID *TRYPANOSOMA CRUZI* STRAINS CONSTITUTE A THIRD MAJOR LINEAGE

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Several molecular markers corroborate the existence of two major lineages for *Trypanosoma cruzi*, denominated *T. cruzi* I and *T. cruzi* II, related to zymodeme 1 and 2, respectively. However, a number of strains presenting hybrid characteristics cannot be classified into any of these two lineages. The major goal of this work was to ascertain the evolutionary relationship of these hybrid strains, by performing a multilocus analysis in 98 *T. cruzi* isolates. Two nuclear genes, one mitochondrial gene and five microsatellite repeats were chosen for this analysis. Maximum parsimony phylogenetic inferences based on microsatellite genotypes showed a good correlation with the 24Sa rRNA dimorphism, but revealed the existence of a third major cluster that included all hybrid strains studied. Likewise, single-nucleotide polymorphism of *TcMSH2* gene and RFLP analyses of the mitochondrial *COII* gene support the existence of three major lineages (haplotypes A, B and C) with all strains displaying hybrid characteristics being classified into *MSH2* haplogroups B and C or the mitochondrial clade B. The hybrid nature of these strains were confirmed by median-joining network analyses for the microsatellites loci. In the light of these results, we discuss new insights into the evolutionary origin of hybrid strains of *T. cruzi* and propose that these strains may constitute a third major lineage in the population.

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BM85 - CHARACTERIZATION OF A ZINC FINGER PROTEIN IN *TRYPANOSOMA CRUZI*

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Zinc fingers are compact protein domains composed of an a-helix and a b-sheet held together by a zinc ion. Tandem arrays of zinc fingers are commonly used to recognize nucleic acids. Among other activities, they are involved in the processes of replication, transcription, and DNA repair. The nucleocapsid protein of HIV-1 contains a zinc finger motif $CX_2CX_4HX_4C$ that contributes to multiple steps of the viral life cycle, including the proper encapsidation of HIV RNA. In trypanosomatids, only a few of the proteins that contain such fingers are studied. In *Leishmania chagasi*, a protein containing nine zinc finger motifs $CX_2CX_4HX_4C$ was identified. This protein, called HEXBP, binds to the 5'unstranlated region of the most abundant membrane glycoprotein of this protozoan and is likely to be involved in DNA replication, structure and repair. In *Trypanosoma cruzi*, a protein containing five zinc finger motifs $CX_2CX_4HX_4C$ was identified. This protein, called TcZinc1 or PDZ5, is homologous to the *Crithidia fasciculata* UMSBP protein that is probably involved in minicircle replication. Here, we report the identification and characterization of a protein in *Trypanosoma cruzi* containing eight zinc finger motifs $CX_2CX_4HX_4C$, denominated TcZinc2. Molecular cloning of the *Tczinc2* gene and heterologous expression of TcZinc2 as a fusion with an His-tag was performed in *E. coli*. The recombinant protein TcZinc2 was purified by immobilization in metal affinity chromatography Ni-NTA (Nickel-nitrilotriacetic acid) and used for antibody production in rabbits. Western blot analysis using total protein extracts of the three forms of the parasite has shown that TcZinc2 is expressed equally in all of them. Western blot experiments using cytoplasmic and nuclear/mitochondrial fractions from epimastigote forms have shown that TcZinc2 is present at the nuclear/mitochondrial fraction. Since the TcZinc2 protein does not have peptide signal for mitochondrial localization, it is probably a nuclear protein. SELEX (Systematic Evolution of Ligands by Exponential enrichment) experiment will be performed in order to determine the nucleic acid nature, as well as the consensus sequence of the target molecule to which this specific protein binds. For that purpose, molecular cloning of the *Tczinc2* gene and heterologous expression of TcZinc 2 as a fusion with GST (Glutathione-S-Transferase) were performed in *E. coli*. The recombinant protein TcZinc2 was purified by affinity chromatography to glutathione. This study will provide valuable information regarding the function of this protein in *T. cruzi*.

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BM86 - TCZFP1: A *TRYPANOSOMA CRUZI* PROTEIN THAT BINDS TO C-RICH SEQUENCES

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The control of mRNA stability plays a fundamental role in the regulation of eukaryotic gene expression. This is particularly relevant in trypanosomes where the organization of the genome into polycistronic transcription units implies that most genes must be regulated at the post transcriptional level. This control can be influenced by the basal mRNA decay machinery, regulatory factors that respond to various stimuli and sequence-specific decay components.

We have cloned a *T. cruzi* gene encoding a zinc finger protein named TcZFP1. TcZFP1 is homologous to the TbZFP1 (67.6%) which was implicated in the regulation of the morphogenesis and differentiation in *T. brucei*. These proteins share the unusual zinc finger motif (CCCH) found in a diverse range of RNA-binding proteins involved in various aspects of the control of cell homeostasis and differentiation. Other proteins bearing this motif are intimately associated with RNA stability, transport or translation and operate at different steps of gene expression regulation. Electrophoretic mobility shift assay (EMSA) showed that TcZFP1 binds specifically to some synthetic oligoribonucleotides containing C-rich sequences. This kind of sequences are present in the untranslated region of several mRNAs of trypanosomatids, raising the possibility that TcZFP1 might interact with RNAs or ribonucleoprotein complexes in the cell via the CCCH domain, regulating the mRNA stability or translation.

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BM87 - THE *META 2* GENE: GENOMIC COMPARISON IN TRYPANOSOMATIDS AND CHARACTERIZATION OF THE META 2 PROTEIN OF *LEISHMANIA (L.) AMAZONENSIS*

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We had previously identified the *meta 2* gene in the region flanking the *L. (L.) amazonensis meta 1* gene. Both are single copy genes upregulated in metacyclic promastigotes and conserved in all *Leishmania* species analyzed to date.

The region of the *L. (L.) amazonensis* genome containing the *meta 1* and *meta 2* genes was compared with orthologous *L. (L.) major*, *Trypanosoma brucei* and *T. cruzi* contigs provided by Genome Projects. Conservation of synteny and linkage between these species was observed in a segment comprising at least five genes. Interestingly, a cluster of eight meta 1 related genes was found in *T. brucei* with five highly conserved copies of the *meta 1* homologue and three copies of the *meta 2* gene. On the other hand, in *T. cruzi* three copies of *meta 1* gene were identified while the *meta 2* gene was not present.

The *meta 2* gene had been structurally characterized and an open reading frame (ORF) encoding a 444 amino acid protein was identified. The transcribed amino acid sequence contains three copies of the META domain, which is defined as a conserved domain present in secreted proteins of bacteria and in the meta 1 protein of *Leishmania*, followed by a sequence similar to calpain-like proteins. The recombinant meta 2 protein, expressed in fusion to the maltose binding protein (MBP), lacks the C-terminal end. We immunized Swiss mice with this protein in order to obtain anti meta-2 polyclonal sera. Immunofluorescence experiments suggest that the meta 2 protein is localized in vacuoles dispersed in the cytoplasm of the cell. These sera will be used in immunobloting experiments against *L. (L.) amazonensis* protein extracts prepared from log and stationary phase promastigotes and amastigotas to investigate the pattern of expression at the protein level.

Functional analysis of the meta 2 protein has been initiated. We are currently preparing constructs to be employed in obtaining meta 2 and meta 2-GFP ovexpressing mutants. These mutants will be characterized by *in vitro* and *in vivo* infection experiments.

Supported by FAPESP.

BM88 - SEQUENCING AND CHARACTERIZATION OF GENES WHICH CODE FOR ANTIGENS FROM *L. (L.) CHAGASI* COMPOSED OF MULTIPLE REPETITIVE DOMAINS.

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The visceral leishmaniasis (VL) is a potentially fatal disease caused by *Leishmania chagasi* in Brazil, responsible for about 3.500 new cases each year. It is present in almost all the Brazilian territory, but it occurs mainly in areas where the health services are scarce. Due to the difficulties in the diagnosis of VL in low income areas and the lack of non toxic treatment, many alternatives are being investigated for the development of simple, economical and non-invasive tests for the early diagnosis of VL or the production of a vaccine against this disease. The identification of new antigens from *L. (L.) chagasi* which could be used as part of such tests/vaccines is a major goal in the *Leishmania* research.

In this report we describe the sequencing and characterization of genes that were previously selected from a *L. (L.) chagasi* amastigote cDNA library in a screening with sera from dogs infected with this parasite. Our results show that of the 6 analyzed clones (LC 9, LC 12, LC 14, LC 16, LC 18 and LC 30), 5 codify for proteins composed of regions that contain multiple repetitive domains, varying in size from 14 to 74 aminoacids. The 6th clone (LC 30) lacks the small repeats, but is nevertheless composed by two identical sequences *in tandem* (over 1000 aminoacids in lenght). Clones LC 9 and LC 12 code for the same protein differing only in the number of repetitive units. The same is observed for clones LC 14 and LC 16. The individual repeats from the LC 9/LC 12, LC 14/LC 16 and LC 18 proteins are unrelated and of different sizes, suggesting that, at least in *L. (L.) chagasi*, proteins with repetitive regions are good inducers of humoral immunity irrespective of their sequences. These antigens therefore could be important components of kits for the diagnostic of visceral leishmaniasis.

This work was supported by BioManguinhos, FINEP and FIOCRUZ.

BM89 - CHARACTERIZATION OF A HIGHLY REPETITIVE ANTIGEN FROM *L. (L.) CHAGASI*

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The visceral leishmaniasis (VL) is an endemic parasitic infection which occurs in both tropical and sub-tropical countries. In Brazil it is caused by *Leishmania (L.) chagasi* and it is considered an important cause of morbidity and mortality. This disease still presents great difficulty in its diagnosis and treatment, with the current chemotherapy being highly toxic. Control of LV therefore requires the development of better diagnostic methods as well as alternatives to reduce the number of affected individuals, such as development of a vaccine which could prevent its occurrence in both humans and dogs, its natural reservoir.

In earlier work we identified by immunoscreening of a *L. (L.) chagasi* amastigote cDNA library several clones coding for proteins containing long stretches of tandem repeats. One of these proteins, named LC 14, codes for 22 copies of a 14 amino-acids (aas) long repeat followed by a unique 230 aas C-terminus. A homologue from *L. (L.) major* has also been identified containing over 100 copies of the 14 aas repeat plus a very similar C-terminus. Here we start the functional characterization of this protein in *L. chagasi* by subcloning its cDNA in a prokaryotic expression vector (pRSET), expression of the recombinant protein in *Escherichia coli* fused to a N-terminus His-tag and production of specific rabbit polyclonal antiserum. The recombinant protein transcribed and translated *in vitro* as well as the bacterially expressed protein behaves abnormally in SDS-PAGE gels, migrating with an apparent molecular weight much higher than expected. We believe this may be a consequence of the highly repetitive region. Western-blotting of total *L. (L.) chagasi* extract with the specific antibody produced multiple bands, several of which with molecular weights higher than 170kDa. A different profile is observed in *L. major* extract, with a reduced number of bands, although several bands with high molecular weights can also be observed. In both *L. (V.) braziliensis* and *Trypanosoma cruzi* the recognition by the antibody is impaired, and in the latter only a minor band is detected indicating that these antigens are not very conserved within the order Kinetoplastida and even within the *Leishmania* genus. Further work will be required to understand the reason for the multiple protein bands recognized by the antibody as well as the protein's function and intracellular localization in *L. (L.) chagasi*.

This work was supported by BioManguinhos, FINEP and FIOCRUZ.

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BM90 - CHARACTERIZATION OF THE PTERIDINE REDUCTASE 1 GENE (PTR1) OF *LEISHMANIA (VIANNIA) BRAZILIENSIS*

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A critical step in the infectious cycle of *Leishmania* is the differentiation of parasites from the non-infective stage to the highly infective metacyclic promastigote stage. The gene Pteridine reductase 1 (*PTR1*) of *Leishmania major* was shown to play an important role in differentiation process, the metacyclogenesis. PTR1 acts reducing biopterin into its active form, tetrahydrobiopterin (H_4B). A decreased level of intracellular H4B is an important factor controlling the extent of metacyclogenesis. The disruption of *L. (L.) major PTR1* gene resulted in transfectants with an increased virulence phenotype, as judged by the rate of lesion formation in mice. The increased virulence observed in these transfectants is connected with an increased number of metacyclic forms. In *L. (L.) major* the *PTR1* gene is located in the H region, a 45 Kb locus of chromosome 23. The H locus can be easily amplified and codes for genes involved in drug resistance, such as *PTR1* (methotrexate resistance), *PGPA* (antimonials resistance) and *HTBF* (terbinafine resistance). Therefore, the H_4B levels, being so important in the differentiation of infective forms of the parasite, could be affected or controlled by the amplification of *PTR1*. The aim of this work is to characterize the expression of *PTR1* in *L. (V.) braziliensis*, as well as the effect of its inactivation. The availability of *PTR1* mutants of *L. (V.) braziliensis* will permit the correlation between levels of gene expression and the number of metacyclics forms found in stationary-phase cultures.

The *L. (V.) braziliensis PTR1* (*LbPTR1*) was isolated from a partial genomic library using the *L. (L.) major* gene as a probe in Southern analysis. The disruption of the gene will be done by homologous recombination of a disruption fragment generated by in vitro transposition into the cloned *LbPTR1*. The isolated clone was subjected to transposition of two specialized transposable elements ELNEOKO and ELSATKO. Both transposons carry a shuttle selectable marker preceded by the protozoan splicing acceptor sites and a prokaryotic promoter sequence. The sequence of the *LbPTR1* is being determined by *Primer-island* sequencing of these insertion events. The availability of the gene interrupted by two different selectable markers permit the production of two different reagents for homologous recombination into the genome. Therefore, it is possible that the strategy employed will generate *LbPTR1* null mutants. Although the overexpression of PTR1 had no effect on the *L. (L.) major* virulence, *LbPTR1* was also cloned into the expression vector pXG and will be transfected into the parasite. The effect of *LbPTR1* inactivation and/or overexpression will be accessed by Northern analysis and by the determination of number of metacyclic forms in stationary-phase cultures of the parasite.

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BM91 - CLONING AND CHARACTERIZATION OF *LEISHMANIA (VIANNIA) BRAZILIENSIS* H-REGION ASSOCIATED GENES

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Gene amplification is a mechanism of cellular self-preservation observed in the protozoan *Leishmania* cell lines subjected to drug pressure. The R and H region of *Leishmania (L.) major* genome are the best studied loci that undergo amplification in response to unrelated drugs. The H region is 45 Kb present in *L. (L.) major* chromosome 23. Three genes associated to drug resistance were identified within this locus. The genes *PTR1* (Pteridine reductase-1), *PGPA* (P-glycoprotein A) and *HTBF* (H region associated terbinafine resistance gene) are involved in resistance to methotrexate, antimonials and terbinafine respectively. In spite of the importance of the phenomenon, and its relevance to the definition of therapeutic strategies, the H region has not been studied in the parasite of the *Viannia* subgenus, *L. (V.) braziliensis*.

We have used the genes mentioned above and two other loci as probes in Southern analysis in order to draw a comparison between the H locus of *L. (L.) major* and that of *L. (V.) braziliensis*. The presence of the four loci in the same chromosome of 800 Kb was confirmed by Southern analyzes of PFGE-separated chromosomes. Although all loci analyzed were present in the same chromosome, the genomic DNA Southern data revealed important differences in their restriction pattern. The mapping of the different loci in the *L. (V.) braziliensis* genome was the basis for the construction of partial genomic libraries. We have used these libraries to isolate three *L. (V.) braziliensis* H region genes. Clones bearing the genes *PTR1*, *TTRS* (Tryptophanil-tRNA synthetase) and *V-ATPase* were isolated and are being characterized. The isolated clones were subjected to the in vitro transposition of the specialized transposable element /GFP*NEO. This transposon carries a shuttle selectable marker (NEO) preceded by the protozoan splicing acceptor sites and a prokaryotic promoter sequence. /GFP*NEO also contain the reporter gene GFP (Green Fluorescent Protein), which will be expressed only if part of a fusion protein. The use of the in vitro transposition system will allow not only the determination of the sequence of these *L. (V.) braziliensis* genes, through *primer-island* sequencing, but also the characterization of their expression. The functional characterization of these genes can be furthered due to the fact that the insertion of the /GFP*NEO transposon generates the reagents for protocols of gene disruption.

We are also investigating the possible amplification of the H locus through the characterization of a terbinafine-resistant *L. (V.) braziliensis* cell line selected in 9mg/ml of terbinafine. This inhibitor of esqualene-epoxidase mediates the amplification of the H region as circular amplicons in *Leishmania* species of the *Leishmania* subgenus.

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BM92 - HOMOLOGS OF THE YEAST LONGEVITY-ASSURANCE GENE (*LAG-1*) IN *TRYPANOSOMA CRUZI*: UNRAVELING THE MECHANISMS OF CERAMIDE BIOSYNTHESIS

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Ceramide, originally identified as mere lipid moiety of membrane sphingolipids such as sphingomyelin and surface glycoconjugates, is now emerging as a vital lipid mediator that influence key aspects of cell growth, regulation, differentiation and death in eukaryotes. Ceramides are synthesized *de novo* or by recycling pathways after the *N*-acylation of dihydrosphingosine (DHS), reaction catalysed by the enzyme ceramide synthase. Recently, two highly homologous ER membrane proteins associated to longevity in *Saccharomyces cerevisae* (Lag1p and Lac1p) were shown to be essential for the acyl-CoA-dependent ceramide synthase reaction. Recently, *LAG1* homologous sequences have been identified in several eukaryotes, including human. In yeast, the *LAG1Sc* and *LAC1Sc* genes are essential for viability since in haploids, deletion of both genes result in a nonviable strain.

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In the present work, we assayed microsomal membranes from epimastigotes with [^{3}H]-DHS, and substrates such acyl-CoA derivatives, free fatty acids or an *in situ* acyl-CoA generation system to identify and characterize the ceramide synthase activities of *Trypanosoma cruzi*. The radiolabelled products were extracted, separated on TLC using $CHCl_3$: MeOH: 2N NH_3OH (40:10:1, v/v) and visualized after autoradiography. In order to obtain further molecular evidences about the existence of the ceramide synthase in the parasites, the *T. cruzi* genomic database in the web was searched for putative *LAG1* homologues using BLAST and the yeast Lag1p-motif "RKDYKELVFHHIVTLLLIWSSYVFHFTKMGLAIYITMDVSDFFLSLSKTLNY". Underlined amino acids in this sequence are conserved in all Lag1-proteins described to date.

Using the cell-free assay system described above, we have observed that the *T. cruzi* ceramide synthase was able to use only acyl-CoA derivatives as substrates, but not free fatty acids. The activity was completely blocked by Fumonisin B1 (FB1), a mycotoxin able to inhibit the acyl-CoA-dependent ceramide synthases from fungi, plants and mammals. However, contrary to previous observations with yeasts and mammals, FB1 was not toxic to the parasite. From the total Lag1p-motif related individual reads found in the *T. cruzi* whole genomic sequence database at TIGR (http://www.tigr.org), 20 sequences (between 600 and 1000 bases each) producing high-scoring segment pairs (E = 3.9e-172) and presenting variable degrees of overlap to each other were used for the construction of two mini-contigs. The first one contained a putative open reading frame (ORF) of 1212 (*LAG1TcA*) bases and the second contig contained another putative ORF of 1218 (*LAG1TcB*) bases. As observed for *LAG1*Sc and *LAC1*Sc, the predicted amino acid sequence of *LAG1TcA* is 74% identical to the *LAG1TcB* protein sequence. Therefore, in analogy to yeast, the first sequence was named *LAG1Tc* and the second *LAC1Tc*. Both sequences presented homology to *LAG1* genes from other eukaryotes. Further evidence of homology came from hydrophobicity plots and suggested that the *T. cruzi* putative *LAG1* members encode membrane proteins with a predicted 45kDa molecular mass.

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BM93 - CHARACTERIZATION OF TIF34 AND PRT1 HOMOLOGS IN *TRYPANOSOMA CRUZI*: TWO SUBUNITS OF THE EUKARYOTIC INITIATION FACTOR EIF3

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Translation is an important step in gene expression, since the genetic information encoded in mRNAs has to be, ultimately, translated into proteins. Initiation of protein synthesis is promoted by at least 10 initiation factors. The largest eukaryotic initiation factor is eIF3, a multisubunit complex that stabilizes the ternary complex eIF2-GTP-$tRNA_i^{Met}$ and promotes mRNA binding to the 40S ribosomal subunit. eIF3 also functions as a ribosome subunit anti-association factor. In yeast, eIF3 is composed by five subunits, including TIF34, TIF35 and PRT1.

Through the analysis of sequences from a normalized *T.cruzi* epimastigote cDNA library, we have identified a gene that encodes a TIF34 homolog protein (56% of similarity), which was named *TcTIF34*. *TcTIF34* is a sigle-copy gene, displaying an open reading frame of 1,0 kb and encodes a polypeptide of 37 kDa, that presents several WD repeats, which are suposed to facilitate the interactions among subunits of multiprotein complexes. It has also been shown that in yeast eIF3, TIF34 interacts directly with TIF35 and PRT1. The presence of these two other genes in the *T.cruzi* genome was investigated from the analysis of the sequences that were deposited from the *T.cruzi* genome sequencing project (www.tigr.org). We have identified only a *T.cruzi* PRT1 homolog gene 2.0 Kb when the yeast PRT1 and TIF35 were used as query sequences in the BLAST search. *TcPRT1* is a single copy gene that encodes a polypeptide of 80 kDa, which shares 39% homology with yeast PRT1.

Polyclonal antisera were raised against the recombinant TcTIF34 and TcPRT1 in order to analyse the expression pattern of theses proteins along *T.cruzi* cell cycle and differentiation. Furthermore, we are currently using a bacterial two-hybrid system to investigate whether TcTIF34 and TcPRT1 can physically interact with each other.

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BM94 - STRUCTURE OF PDZ5 CODING GENE OF *TRYPANOSOMA CRUZI*: A ZINC FINGER PROTEIN WHICH BINDS TO UNIVERSAL MINICIRCLE SEQUENCE

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PDZ5 is a protein of *Trypanosoma cruzi* that is homologous to the *Crithidia fasciculata* UMSBP (Universal Minicircle Sequence Binding Protein). We have previously characterized the PDZ5 as a five zinc-finger domain protein that is recognized by the antibody generated against the protein UMSBP from *C. fasciculata*, and also binds to UMS – the Universal Minicircle Sequence, (Coelho, E.R. International Journal for Parasitology 33: 853-858, 2003). The UMS is a dodecamer found in all minicircles of the trypanosomatids and it is involved with the minicircle DNA replication. The UMSBP binds to UMS just before replication of the minicircles and was first characterized in *C. fasciculata*. It is believed that UMSBP recruits the protein complex responsible for the replication itself.

The gene pdz5 is localized in the chromosomal band XX of *T. cruzi* genome (da Silveira, Gene 308: 53-65, 2003), downstream to the proteasome beta 5 subunit gene. Preliminary results indicate that the pdz5 gene is present as a single copy. We have mapped the trans-splicing and polyadenylation sites. Reverse transcription assays suggested that this gene is transcribed as a polycistronic unit. We have found a DNA polymorphism represented by a 72 base pair deletion in the proteasome-pdz5 intergenic region. Analysis of other genera or strains of Trypanosomes indicates that this DNA polymorphism is present in some strains and absent in others. We are investigating the role of this polymorphism in mRNA transcription.

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BM95 - GENE CHARACTERIZATION OF THE MOLECULAR CHAPERONE HSP10 OF *TRYPANOSOMA CRUZI*

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The HSP10 it's a component of a proteic multimeric apparatus composed of HSP60 and HSP10 proteins. This chaperone machine is responsible for helping cellular proteins to reach their stable tridimensional conformation and also reduces protein denaturation in stressing conditions and prevents the formation of protein aggregates. Both HSPs are inducible proteins and must be coordinately regulated, and are, therefore, good models for studying kinetoplastid gene regulation. The *Trypanosoma cruzi* HSP60 gene has been previously isolated and characterized in our laboratory. As a first step to characterize the coordinated regulation, we begun by studying the other component of the *T.cruzi* chaperone machine, HSP10.

The complete coding sequence was obtained from the EST sequencing effort of the *T. cruzi* genome project (Verdun *et al*, 1998 - Infect. Immun. 66(11):5393-

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5398). The comparison of the predicted amino acid sequence of *T. cruzi* HSP10 with other HSP10 sequences shows small conserved regions spread over the entire length of the protein. These regions matches with the hydrophobic and hydrophilic peaks of the peptidic chain. In this alignment we also detected a 5 amino acid deletion conserved only in tripanosomatids. When molecular modeling methods were performed in silico, this deletion provokes a two fold increase in the size of the orifice located on the upper surface of the HSP10 structure, upon comparison with the E.coli analog protein (Gro ES). Moreover, the tripanosomatids maintain a negatively charged circle around the orifice, putting there an aspartate since they lost the glutamate that performs this role in E.coli. This information could help to elucidate the still obscure role of this orifice on the chaperone machine function.

A phylogenetic tree built from the sequences mentioned above point to a vertical evolution of the HSP10 protein. Southern blot experiments suggest that HSP10 is present as a multicopy gene arranged in tandem, estimated to be between 5 and 10 copies based on gene equivalent experiments. The genes are located at chromosomal band XVIII, which was verified by PFGE. Comparison of the genomic pattern of different strains of *T.cruzi* revealed a restriction fragment length polymorphism (RFLP). The level of HSP10 mRNA, of about 0.5 Kb, does not increase upon heat shock at 37°C and 40°C. However, a smaller mRNA has an expression increase in higher temperatures. The complete coding sequence was cloned in an expression vector and transformed into bacteria (E. coli BL21 strain) in order to produce recombinant HSP10 as a GST fusion protein. The fusion protein generated inclusion bodies, which were solubilized using urea. The isolated recombinant protein was then tested against chagasic human serum, and the HSP10 protein shown to be immunogenic. We are currently amplifying by RT-PCR the 5´ and 3´ UTRs in order to identify RNA processing sites.

BM96 - IDENTIFICATION OF THE HEAT SHOCK ELEMENT(S) IN THE POST-TRANSCRIPTIONAL REGULATION OF HSP70 GENES OF *TRYPANOSOMA CRUZI*.

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Kinetoplastid protozoa show unusual mechanisms of RNA processing, such as trans-splicing, polycistronic transcription and RNA editing, and a predominance of regulation of gene expression at the post-transcriptional level. Post-transcriptional control of gene expression have been shown in kinetoplastids, as in other eukaryotes, to be mediated by sequence elements present in untranslated regions (UTRs) of mRNAs and/or intergenic regions.

The HSP70 genes of *Trypanosoma cruzi* are organized as 7-10 copies arranged in tandem, and the protein is synthesized at normal temperatures. Upon heat shock, both HSP70 synthesis and mRNA levels are increased in a transcription-independent manner. As a first step to identify the heat shock responsive elements, the HSP70 trans-splicing acceptor and polyadenylation sites were identified. We found a major and a minor trans-splicing acceptor site, and three distinct cleavage/polyadenylation sites. In addition,analysis of several 3'UTR sequences cloned by RT-PCR shows polymorphism of the length of a central TTA repeat region. Plasmids containing the chloranfenicol acetyltransferase (CAT) reporter gene are being constructed. In these constructs the CAT gene is flanked by segments of the HSP70 intergenic region containing either the 5' UTR or 3' UTR and their respective regulatory sequences. Rab7 UTRs containing sequences are being used as control plasmids. The reporter genes will be under the control of the 18S ribosomal RNA promoter. The promoter containing sequence is currently being validated in transient transfection essays. CAT enzymatic activity and mRNA levels resulting from transfection of the plasmid constructs will be determined.

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BM97 - CHARACTERIZATION OF THE FIRST *TRYPANOSOMA CRUZI* RAB-LIKE ENCODING GENE

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Rab GTPases are small GTP-binding proteins that are involved in several steps along the exocytic and endocytic pathways. Here we report the characterization of the 552 bp-complete coding sequence of *TcRABT*, the first Rab-Like gene of *Trypanosoma cruzi*. *TcRABT* is present in a discrete number of copies and the chromosomal location of its locus is being performed. *TcRABT* is transcribed as a single 1.2 kb mRNA in epimastigotes and its presence is being investigated in the other parasite forms. The *TcRABT* open reading frame predicts a 183 amino acid polypeptide and a 20.5 kDa protein that shares the highest values of similarity and identity with human and rat Rab23, presenting all the five GTP-binding domains. However, the cystein residues at the C terminus, which is carried by all members of Ras superfamily, essential to isoprenylation, an absolute prerequisite for the membrane association of those proteins, are not present in *TcRABT*. This finding may indicate that *TcRABT* may represent a novel member of a small GTPase family as it does not seem to be a typical Rab, neither an obvious member of another family. Polyclonal antibodies raised against a polypeptide of the *TcRABT* C-terminus were able to recognize a single 20 kDa band in DM28c and CL Brener *T. cruzi* strains as well as a single 49 kDa band corresponding to the GST fusion protein expressed in *Escherichia coli* BL21 strain. Functional and immunolocalization assays are being performed in order to better understanding *TcRABT* function.

This work was supported by CNPq and FAPERJ.

BM98 - CLONING AND CHARACTERIZATION OF TOPOISOMERASE IV GENE IN THE ENDOSYMBIONT OF *CRITHIDIA DEANEI*

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In eubacteria there are four topoisomerases which present distinct roles in DNA topology. Topo I and III are type I topoisomerases, that make single-stranded breaks in DNA, whereas the type II topoisomerases, DNA gyrase and Topo IV promote double-stranded breaks in DNA. Topo I and IV counter gyrase activity in order to maintain DNA supercoiling and play essential roles for cell viability, participating in processes as DNA replication, transcription and recombination. Regarding type II topoisomerases, gyrase introduces negative supercoils, which are required for the initiation of replication and removes positive supercoils to allow fork progression. The main role of Topo IV in replication is to unlink the catenated DNA generated during the replication or recombination, allowing chromosome segregation.

Some trypanosomatids present a obligate symbiotic bacterium in the cytoplasm, which divides in synchronicity with the host cell. How the symbiont

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co-evolves with the host protozoon is a question of great interest, since it is related to the origin of organelles as the mitochondrion and the chloroplast. In the present study, we investigate the presence of type II topoisomerases in the endosymbiont of *Crithidia deanei*. For this purpose, the DNA used in molecular assays were obtained from isolated symbionts after cell fractioning. Primers were constructed from conserved domains of several prokaryotic Topo IV in order to amplify such gene in the symbiotic bacterium. PCR amplifications produced fragments sharing high similarity with those described in bacteria from *Pseudomonas* genus. A polyclonal antiserum raised against the recombinant Topo IV was produced to detect this protein by Western blotting. A band of approximately 70 kDa was detected in extracts of *Escherichia coli* which super-expressed the protein and in *C. deanei*, but not in the aposymbiotic strain of this protozoon. Immunocytochemical approaches were performed using the same antiserum, in order to determine the localization of this protein. A spefic labeling was observed over the symbiotic bacterium, but not in other structures of *C. deanei*. Further studies are necessary to better characterize other topoisomerases and to elucidate how the expression pattern of these enzymes affect the division process of this endosymbiotic bacterium.

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BM99 - CHARACTERIZATION OF A *TRYPANOSOMA CRUZI* GENE ENCODING A PROTEIN BEARING REPETITIVE EPITOPES

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Trypanosoma cruzi, the etiological agent of Chagas disease has a complex life cycle that involves at least three distinct developmental stages: epimastigote, trypomastigote and amastigote. The differentiation of *T.cruzi* epimastigote into metacyclic trypomastigote involves the transformation of a replicative, non-infectious form into a non-replicative, infectious stage, by a process named metacyclogenesis. The identification of genes that play a role in cell differentiation of this unicellular organism is essential to determine how the parasite survives in different hosts and which molecules are involved in its infectiveness. Among the genes identified from the sequencing of ESTs from a *T.cruzi* metacyclic trypomastigote normalized cDNA library, there is one, named Tc445, that encodes a protein bearing repetitive epitopes. The Tc445 epitopes are highly conserved each other and contains 149 amino acid residues. Data base searching revealed that Tc445 protein shares a 48% of similarity with NUP-1, a protein of *Trypanosoma brucei*, which is associated with the nuclear lamina. NUP-1 is believed to be an orthologue of metazoan lamins. Since *T.cruzi* metacyclic trypomastigote is a non-replicative form, Tc445 protein might have a distinct role from the metazoan lamins which are involved in the reorganization of the nuclear envelope after the mitosis. Instead, Tc445 protein might play a role in the organization and maintenance of the highly condensed chromatin level that is observed in the trypomastigote stage. Antiserum was raised against peptides corresponding to either the sequence of Tc445 repetitive epitope or a hydrophilic non-repetitive region of the protein and it is currently being tested in imunolocalization assays.

Financial support: PRONEX, CNPq, Fiocruz

BM100 - CLONING AND CHARACTERIZATION OF THE PHOSPHOGLUCOMUTASE (PGM) GENE FROM *TRYPANOSOMA CRUZI*.

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The surface of *Trypanosoma cruzi* is covered by a dense coat of highly O-glycosylated sialoglycoproteins, which are thought to play a key role in host cell adhesion and invasion by trypomastigotes. There is evidence that the degree of surface sialylation of galactopyranose residues (Galp) is critical to parasite virulence. In order to further study the requirement of O- sialylglycans for *T. cruzi* survival and virulence, we aim to reduce the amount of Galp residues in the parasite by down regulating the activity of the enzyme phosphoglucomutase (PGM). PGM catalyses the reversible conversion of Glc-6-PO4 to Glc-1-PO4. The latter phosphorylated product is a key compound for the formation of UDP-Glc that is transformed into UDP-Galp by UDP-Galp-4-epimerase. In *T. cruzi* this is the only pathway for generation of UDP-Galp. In order to identify the *PGM* gene by PCR, we used a combination of degenerated primers based on sequence similarity with the *PGM* gene from others organisms and specific primers based on the 3' end of the *T. cruzi* PGM sequence (GenBankAI066127). The full lenght gene was isolated by inverted PCR of Dm28c genomic DNA, cloned and sequenced. The *PGM* gene of T. cruzi shows approximately 60% identity with that of Leishmania major and approximately 50% sequence identity with the *PGM*1genes from human, mouse and *A. thaliana*. Southern blot analysis indicates the presence of one *PGM* gene copy and Northern blot analysis revealed similar PGM RNA levels in epimastigotes and tissue culture trypomastigotes. The heterologous expression of the full lenght gene is underway.

BM101 - STRUCTURAL AND FUNCTIONAL ANALYSES OF *TRYPANOSOMA CRUZI MSH2*, A GENE INVOLVED IN THE MISMATCH REPAIR PATHWAY

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Recent studies have demonstrated that the species *T. cruzi* can be divided into three distinct haplogroups, named A, B and C. This division was based upon polymorphisms found in the *MSH2* of 32 *T. cruzi* strains and is supported by another study with a *T. cruzi* antigen, *TcAg48*. The *MSH2* codifies for a protein involved in the mismatch repair pathway (MMR), which is responsible for the correction of mismatched bases in DNA. It was recently verified that strains belonging to haplogroups B and C (JG and Cl Brener, respectively) present a less efficient MMR when compared to Colombiana, a haplogroup A strain, after treatment with cisplatin and hydrogen peroxide. Since each of the haplogroups is characterized by a distinct *MSH2* isoform and as this protein has a central role in MMR, we decided to investigate whether *MSH2* could account for the difference in MMR found between the haplogroups. The *MSH2* was amplified from genomic DNA of two *T. cruzi* strains, Colombiana (haplogroup A) and Cl Brener (haplogroup C) and its whole coding region was sequenced. A high percentage of the SNPs found between the *MSH2* from these strains have resulted in amino acid substitutions, some of them in regions described as important for the protein's structural or functional maintenance. Analysis *in silico* through the program SIFT and modeling by Swiss model have indicated that the differences found between the *MSH2* of Colombiana and Cl Brener do not lead to significant variations in this protein's function or structure. Aiming to verify the protein's activity i*n vitro*, we have expressed the Colombiana's MSH2 and the purified protein was used in an ATPase assay. Through this assay it was possible to verify the ATPase activity of the Colombiana MSH2 protein

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BM102 - CLONING AND CHARACTERIZATION OF *TRYPANOSOMA CRUZI* GENES ENCODING PUTATIVE NUCLEOLAR PROTEINS

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Trypanosoma cruzi, the causative agent of Chagas disease, shows several peculiarities related to the mechanisms of gene expression regulation. This parasite regulates its gene expression predominantly by a post-transcriptional process, involving either the processing of long polycistronic transcripts by trans-splicing and poly-A tail addition or mechanisms based on stage-specific changes in mRNA stability and translation. Less attention has been payed, however, to the role of the ribosomal RNA processing as a stage-specific gene expression control mechanism.

Most of the steps of ribosome biogenesis in eukaryotic cells, like transcription and processing of ribosomal RNAs (rRNAs) take place primarily in the nucleolus and are performed by small nucleolar ribonucleoproteins (snoRNPs). We have shown that a gene named *TcImp4*, encoding for a protein putatively associated with U3 small nucleolar ribonucleoprotein (U3snoRNP), is present in *T.cruzi* epimastigote forms but not in metacyclic trypomastigote forms. This observation associated to the fact that there is an extensive reorganization of the nucleolus of trypomastigotes of *T.cruzi*, leaded us to hypothesize that components of some snoRNPs, might be absent in this stage and consequently might contribute to shut down the translation of mRNAs, by blocking the synthesis of new ribosome particles. Recently, we have identified two other genes encoding for homolog nucleolar proteins Nop10p and Nop58p, which are involved in the rRNA processing in yeast. *T.cruzi* homolog proteins, named TcNop10 and TcNop58, were expressed in *E.coli* and antisera were raised against either synthetic peptides or the recombinant proteins, in order to study the expression of these proteins during *T.cruzi* metacyclogenesis process and give support to our hypothesis of differential gene regulation through the modulation of nucleolar protein synthesis.

Financial support from Pronex, CNPq, Fiocruz

BM103 - CLONING AND CHARACTERIZATION OF A *TRYPANOSOMA CRUZI* GENE ENCODING FOR A PROTEIN WITH A STRUCTURAL SIMILARITY WITH THE HEAVY CHAIN OF DYNEIN

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Trypanosoma cruzi is the protozoan parasite causative of Chagas disease, which afflicts millions of people in Central and South America. Its life cycle involves at least three distinct developmental stages: epimastigotes, trypomastigotes and amastigotes. The epimastigote forms replicate in the midgut of the insect host and develop into non-replicative metacyclic trypomastigote forms by the process of metacyclogenesis. Metacyclic trypomastigotes are released in the excreta of insects of the *Reduviidae* family (triatomine insects) during feeding and invade the cells of the mammalian host. Within the host cells, the parasite differentiates into the replicative amastigote form, which in turn differentiates into bloodstream trypomastigotes, which infect new cells. The analysis of sequences of ESTs from a *T.cruzi* cDNA library has identified a gene that was named *Tc22*. The larger *Tc22* ORF is 530 bp long and encodes a 20kDa polypeptide. Southern blot analysis indicated that *Tc22* is a single copy gene in the *T.cruzi* Dm28c genome. The search for homology using the BLAST algorithm (www.ncbi.nlm.nih.gov) showed that Tc22 shares considerable structural similarity with an extensive region of the heavy chain of dynein. In eukaryotic cells, dynein, in association with microtubules, is required for vesicular and organelles transport, as well as flagellar motility. To investigate whether Tc22 is a novel *T.cruzi* microtubule-associated protein, we have expressed this gene in *E. coli* in order to raise an antiserum against the recombinant protein, which will be used for *in situ* localization approaches by electron and imunofluoresce microscopy. Furthermore, the expression pattern of Tc22 protein will be analysed by western blot to determine if the protein is present in all stages of *T.cruzi* life cycle.

Financial support: PRONEX, PADCT/CNPq, FIOCRUZ.

BM104 - MOLECULAR CLONING AND EXPRESSION OF THE *TRYPANOSOMA CRUZI* CATHEPSIN B

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Cysteine proteases are involved in several aspects of host-parasite interactions and are the most abundant proteinases in most parasitic protozoa. Cathepsin B was recently identified in all forms of the *Trypanosoma cruzi*. In order to characterize this enzyme both molecularly and functionally, the procathepsin B gene was inserted into the pET15b expression vector to generate an N-terminal His-tagged recombinant protein. rTCCB was successfully expressed in *E. coli* and showed approximately a 36 kDa protein in SDS-PAGE under reducing conditions. Recombinant protein was then purified from the inclusion bodies fraction utilizing a column charged with nickel. rTCCB was solubilized in denaturants and was refolded. After extended incubation in acidic conditions, rTCCB showed activity on the fluorogenic substrate Phe-Arg-AMC. The antibodies against the recombinant protein were developed in mice and rabbit. The expression of an active recombinant allows a better inhibitor screening technique and the production of TCCB crystals to determine the three-dimensional structure. The complete *T. cruzi* TCCB characterization can elucidate its relevance to the metabolism of the parasite as well as the evaluation of its potential as a drug target.

This research is supported by CNPq.

BM105 - GENETIC, ENZYMATIC AND STRUCTURAL PROPERTIES OF PROLYL OLIGOPEPTIDASE FROM *TRYPANOSOMA CRUZI*

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We have demonstrated that *Trypanosoma cruzi* produces an 80 kDa enzyme (Tc80 proteinase) that is secreted and hydrolyzes purified and native collagens. Lesser activity is observed on fibronectin, but none on BSA, laminin and immunoglobin G. We have suggested that this proteinase could be involved in

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the infection process by facilitating parasite migration through extracellular matrix (ECM). Previous data have indicated that this *T. cruzi* protease is a prolyl oligopeptidase (POP Tc80). POP Tc80 selective irreversible inhibitors have blocked parasite entry into host cells, reinforcing its role in infection. Here, we report the identification of the POP Tc80 gene located on a 3.5 Mb chromosome as a single copy. Recombinant POP Tc80 expressed in *E. coli* presents biochemical and kinetic properties similar to those of the native enzyme, and is strongly enhanced by reducing agents. POP Tc80 expression was found to be higher in infective than in non-infective parasitic forms. Three-dimensional modeling based on crystalized porcine POP structure indicates that POP Tc80 is composed of the a/b-hydrolase domain containing the catalytic triad Ser548-Asp631-His667 and of the seven-bladed b-propeller non-catalytic domain.

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BM106 - MOLECULAR STUDIES OF *TRYPANOSOMA BRUCEI* ADENOSINE DEAMINASES TAD2P AND TAD3P.

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Inosine is found at position 34, the wobble (first) nucleotide of the anticodon of several tRNAs. Inosine at this position (I_{34}) is believed to play an important role in protein synthesis by allowing the base pairing with U, C or A in the third position of the codons. Biochemical studies have revealed that I is in tRNAs is a result of deamination of a genomically encoded A and suggest the involvement in this process of a tRNA-dependent deaminase. Such A34I tRNA deaminase has been cloned from yeast *Saccharomyces cerevisiae* and recently from *Trypanosoma brucei*.

In *Trypanosoma brucei* it has been identified a mitochondria specific editing process that changes the C34 residue of tryptophane tRNA to an I residue. This process results in a $tRNA^{trp}$ that can incorporate a tryptophane at the in frame stop codon AUG. The TAD2p and TAD3p genes have been cloned from *T. brucei* cells for molecular characterization.

These genes consist of 675bp (TAD2p) and 1011bp (TAD3p) sequences encoding proteins of 225 and 337kDa, respectively. Both open reading frames have been cloned into the expression vector pTrcHis2-TOPO (Invitrogen) and introduced into BL21(DE3) *Escherichia coli* cells for overexpression experiments. Several pilot expression experiments have been done in an attempt to obtain the two proteins in the soluble fraction of *E. coli* lysates. Here we report the initial results of this project aim at the structural and functional characterization of *T. brucei* TAD2p and TAD3p proteins and their involvement in the kinetoplast $tRNA^{trp}$ editing.

Financial Support: FAPESP and PRONEX.

BM107 - DEVELOPMENT OF RECOMBINANT REPLICATION DEFICIENT ADENOVIRUSES EXPRESSING *TRANS*-SIALIDASE OR AMASTIGOTE SURFACE PROTEIN-2 OF *TRYPANOSOMA CRUZI*.

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Chagas's disease is caused by the intracellular parasite *Trypanosoma cruzi*. This disease is still a major health problem for many countries in Latin America, where it affects more than 15 million of people and is the cause of thousand of deaths every year. Presently, there is no vaccine candidate able to prevent the infection by this parasite. Moreover, the effectiveness of conventional chemoterapy, specially during the chronic phase of the infection, is very poor. On the other hand, results of various experimental studies have suggested that CD8 T cells could play an important protective role against *T. cruzi* infection. Epitopes recognized by *T. cruzi* specific CD8 T lymphocytes were recently found in the *trans*-sialidase (TS) and amastigote surface protein 2 (ASP-2) of *T. cruzi*. In recent years, one of the most successful approaches for induction of potent CD8 cellular immune responses has been the use of recombinant viral vectors as vehicles to deliver antigens. Among the most studied viral vectors, replication-defective adenoviruses possess several attractive features with regard to the development of sub-unit vaccines. They generate self-limited infections, have the ability to infect antigen-presenting cells, possess strong protein expression capabilities, and can be purified to high titers.

In the present work, we describe the construction of genetically stable replication-defective recombinant adenoviruses that express either TS or ASP-2 antigens. The *in vitro* characterization of these recombinant viruses was performed following infection of 293 A cells. By immunoblot, we detected high levels of expression of both *T. cruzi* antigens. We are currently evaluating whether the recombinant adenoviruses expressing TS or ASP-2 can induce an effective immune response against *T. cruzi* in mice.

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BM108 - AUTO-REGULATED EXPRESSION OF ALPHA AND BETA TUBULIN GENES OF *TRYPANOSOMA CRUZI*

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We have been studying the molecular mechanisms responsible for the expression of alpha and beta tubulin genes in *T. cruzi* as a model to understand the control of stage-specific gene expression in trypanosomatids. In higher eukaryotes, tubulin mRNA levels are subjected to auto-regulatory mechanisms affecting mRNA stability: when the pool of free tubulin increases in the cytoplasm, polysomal tubulin mRNAs are destabilized by binding of protein factors to nascent N-terminal tubulin tetrapeptide. It is hypothesized that this binding results in recruitment of RNAses that degrade polysomal tubulin mRNAs. Using vinblastine and taxol, drugs that disrupt tubulin dynamics by opposite mechanisms, we found evidences indicating similar regulatory process operates in *T. cruzi*. Vinblastine causes significant morphological alterations in *T. cruzi* epimastigotes whereas taxol does not alter the shape of these parasites. This result parallels with the effects of these same treatments on the levels of microtubule-associated tubulin: vinblastine causes significant despolimerization of microtubule-associated tubulin whereas taxol maintains the microtubule structure unchanged. In accordance with these effects, only vinblastine treatment was found to alter the levels of alpha and beta tubulin mRNAs. Two hours after the addition of 50 mM vinblastine, treated parasites present a significant reduction in the levels of both tubulin mRNAs. Experiments using actinomycin D showed that this reduction is due to a decrease in the half-life of alpha and beta tubulin mRNAs. Western blot analyses are in agreement with the auto-regulatory model: epimastigotes, which have higher levels of tubulin mRNA, contain less amount of free tubulin subunits compared to amastigotes and trypomastigotes. To investigate the involvement of the sequences within tubulin mRNAs, particularly

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the regions encoding the N-terminal tetrapeptide which have been shown to be a target for the regulatory process in other eukaryotes, plasmids containing the luciferase reporter gene associated with these sequences have been constructed. These plasmids are presently being used in transient and stable transfections assays of epimastigotes.

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BM109 - PHOSPHOGLYCERATE KINASE GENES FROM *LEISHMANIA (L.) MAJOR* AS TOOLS FOR THE STUDY OF GENE EXPRESSION.

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All members of the order Kinetoplastida contain microbodies harboring glycolytic enzymes. Some enzymes are exclusive to the glycosome, whereas others are present in both glycosome and cytosol. *Leishmania* has two *PGK* genes. One, *PGKC,* encodes a glycosomal variant and the second gene, *PGKB*, encodes the cytosolic enzyme. We have previously localized those genes in one end of chromosome 20 of *Leishmania (L.) major* (Pedrosa *et al.* MBP 114:71, 2001). At the nucleotide level these genes are 99.5% identical. Our aim is to study some aspects of the control of expression of each PGK isoform. Fragments containing PGKB and PGKC genes were subcloned in pX63Neo and transfected in *Leishmania*. Both transfectants were submitted to high levels of drug pressure (G418) to specifically overexpress the corresponding gene in the parasite. We observed that cells bearing the exogenous *PGKB* would respond to the drug pressure and increase the episome copy number. On the other hand, the PGKC overexpressor could never reach similar number of copies of the recombinant, the estimated difference between them is about 18 times. Furthermore, a similar difference was observed in the transcript levels of *PGKC* and *B* in northern experiments, even if drug concentration is 10 times higher. To extend this analysis we are currently investigating the PGKC and PGKB protein levels. Our data supports the speculation that an element present either in the 3' or 5' UTR of *PGKC* controls the episome replication. This is also affecting the level of the *PGKC* transcript. Therefore, we decided to investigate such regions and the strategy chosen involved the construction of chimeras of both *PGK* recombinants for further transfection and phenotype analyses. *PGKC* and *PGKB* recombinants were digested and the fragments were used to build four chimeras in which 5' of one of the PGKs was ligated to the 3' of the other gene and vice versa. Transfectants were recovered from plates and are currently under analysis.

Supported by FAPESP

BM110 - MOLECULAR CHARACTERIZATION AND FUNCTIONAL COMPLEMENTATION OF *LEISHMANIA (L.) AMAZONENSIS* AP-ENDONUCLEASE (*LAMAP*) HOMOLOGOUS GENE

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Introduction: *Leishmania* spp. parasites are able to thrive inside fagolisosomes despite of the constant generation of oxygen and nitrogen reactive species, resulting in the emergence of abasic (apurinic and apyrimidinic - AP) sites, considered cytotoxic and mutagenic to prokaryotic or eukaryotic cells. It is believed that these parasites might possess efficient DNA repair mechanisms, extremely important for its intracellular survival. Objectives: Our goal was the isolation, molecular characterization and functional complementation studies in mutant *E. coli* strains of *Leishmania (L.) amazonensis* homologous genes involved in the repair of oxidative DNA lesions. Methodology: Based on the *L. major* AP-endonuclease (APEX) sequence *L. (L.) amazonensis* PCR primers were designed, cloned into TOPO TA cloning vector and subjected to automated DNA sequencing. These amplicons within the expected size (1.34 kb), were used in functional complementation assays of Exonuclease III (*xth*$^-$), Endonuclease IV (*nfo*$^-$), Endonuclease III (*nth*$^-$) *E. coli* strains challenged with various concentrations of H_2O_2 and methylmethanesulphonate (MMS). This product was cloned into *Leishmania* sp. shuttle vector (pXG-GFP$^+$) at both orientantions. The transfected parasites expressing the *L. (L.) amazonensis* homologous *apex* gene, named *Lamap*, and consequently sense and antisense mRNAs will be used in *in vitro* assays with culture and peritoneal macrophages as well as in *in vivo* assays with BALB/c mice. Results: The *L. (L.) amazonensis Lamap* gene displayed a 95% overall homology with the *L. (L.) major* one. We have observed a positive correlation for the *apex* gene and its ability to repair oxidative and alkylating lesions, conferring higher resistance to MMS and a less significant one to H_2O_2. Conclusions: We have isolated an AP-endonuclease homologous that allowed us to conclude that the activities for Endonuclease III and Exonuclease III enzymes are the most significant ones detected at this parasite, being the former more relevant when alkylating agents are involved and more effective when coupled to the second one. The Endonuclease IV activity is irrelevant when compared to the other ones. These data point towards a divergence on what concerns the activities previously shown in *L. (L.) major* and might be further exploited for the characterization of oxidative responses displayed by this protozoan parasite.

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BM111 - EIF4A HOMOLOGUES FROM *LEISHMANIA (L.) MAJOR*: QUANTIFICATION AND FUNCTIONAL PROPERTIES OF TWO DIFFERENT HOMOLOGUES

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In the kinetoplastid protozoans the presence of a modified cap, cap4, plus the spliced leader sequence on the 5'end of the mRNAs suggests the existence of differences in the way the mRNA is recruited for translation. However very little is known about the translation machinery in these pathogens. In plants, yeast and animals, initiation of protein synthesis starts with the binding of the translation initiation complex eIF4F - eIF4A, eIF4E and eIF4G – to the monomethylated cap present on the 5'end of the mRNAs. This complex allows the recognition of the mRNAs by the small ribosomal subunit and the initiation of translation. eIF4A is a very conserved protein within the eukaryotes and possibly even in prokaryotes. It has a helicase activity and is a member of the DEAD box family of RNA helicases. In translation eIF4A binds eIF4G and, with the help of another translation initiation factor – eIF4B, is responsible for melting secondary structures along the mRNA 5'UTR allowing the small ribosomal subunit to scan the mRNA and find the translation initiation codon. We have previously reported the initial characterisation of one *L. (L.) major* eIF4A sequence (LmeIF4A1). Here we describe a second eIF4A homologue from this parasite (LmeIF4A2) identified within the *L. (L.) major* partial genome sequence available online at the Sanger Center Home-PAGE. In order to characterise it functionally the LmeIF4A2 sequence was amplified the gene,

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cloned and expressed as a recombinant protein in *Escherichia coli* fused to a tag of 6 Histidines on its N-terminus. His-tagged recombinant LmeIF4A2 was then used for the production of polyclonal sera in rabbit and its expression compared with that of LmeIF4A1 through Western-Blots. So far we have not been able to detect LmeIF4A2 in *L. (L.) major* promastigotes, in contrast with LmeIF4A1 which is expressed as a very abundant protein with over 10^6 molecules per cell. Also, in pull down assays to investigate interactions between the *Leishmania* His-eIF4A homologues and a candidate eIF4G protein from the same organism, labelled with 35S, LmeIF4A2 differs from LmeIF4A1 homologue in that it doesn't seem to bind the eIF4G protein. Further experiments will be required in order to define LmeIF4A2's function in the *Leishmania*'s protein synthesis and during its complex life cycle.

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BM112 - IDENTIFICATION AND PRELIMINARY CHARACTERIZATION OF PUTATIVE HOMOLOGUES OF THE TRANSLATIONAL INITIATION FACTOR EIF4G FROM *LEISHMANIA (L.) MAJOR*.

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Our research has as its major objective the study of protein synthesis in protozoan parasites of the order Kinetoplastida, which includes major human pathogens responsible for diseases such as Chagas' disease, sleeping sickness and the leishmaniasis. With the sequencing of the *L. (L.) major* and *T. brucei* genomes and the availability of the sequences in the internet (Sanger Center and NCBI databases), we opted to investigate translation initiation and mRNA recognition in these pathogens. Our approach was the identification within these databases of homologues of translation initiation factors, through homology analysis with known vertebrate factors. We concentrated on the translation initiation complex eIF4F (eIF4E, eIF4G and eIF4A) since in other eukaryotes it binds the mRNA at its capped 5' end and recruits it for translation. eIF4G is a very large polypeptide (approx. 200 kDa), which provides a scaffold for the other eIF4F subunits, and has binding sites for other proteins such as the eIF3 initiation factor (which binds the 40S ribosomal subunit) and the poly(A) binding protein. In metazoans eIF4G is the subject of multiple regulation events required to control protein synthesis and is able to bind RNA on its own. Here we describe the preliminary characterization using bioinformatic and biochemical tools of four putative eIF4G homologues identified within the *L. (L.) major* genome database (LmeIF4G1-4). They all share the central conserved eIF4A binding domain which allowed their identification. However they lack any significant similarities outside this domain and no binding sites for the other eIF4G partners could be identified. Likewise, homology to described eIF4G proteins from other organisms is restricted to the eIF4A binding domain. The four sequences differ significantly in the size and sequence content of their N and C-terminus, but homologues for the 4 proteins can be clearly identified in *T. brucei* genome sequences indicating their conservation within the order Kinetoplastida. To begin the functional characterization of the LmeIF4G1-3 their sequences were amplified, cloned, expressed and used for antibody production. So far we have been able to confirm expression of LmeIF4G3 in *L. (L.) major* promastigotes but LmeIF4G1-2 are either expressed in very low amounts or are expressed in others stages of the *Leishmania* life cycle.

This work was supported by FIOCRUZ, CAPES and The Wellcome Trust.

BM113 - CLONING AND EXPRESSION OF RECOMBINANT *LEISHMANIA* SP. TRYPANOTHIONE REDUCTASE

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Leishmania parasites have elaborated a variety of strategies to evade the host defense mechanisms, among them a redox system involving trypanothione (T)/trypanothione reductase (TR), equivalent to the glutathione (G)/glutathione reductase (GR) system in mammals. Trypanothione is a tripeptide (glutathione) associated to spermidine, and it is maintained intracellularly as a dithiol $T(SH)_2$ through a unique enzyme, the trypanothione reductase (TR, EC1.6.4.8). Based in previous data from our group demonstrating this system in *Leishmania amazonensis* promastigotes and axenic amastigotes (Castro-Pinto et al., 2003), we decided to better characterize this protein through recombinant DNA technology. In a preliminary comparative analysis of coding sequences for TR in different trypanosomatids, a high degree of conservation was observed among the amino acid sequences from the species studied up to now, allowing the design of oligonucleotides in order to amplify the TR gene of *L. (L.) amazonensis* and *L. (V.) braziliensis*. The PCR fragments obtained were subcloned in a sequencing DNA vector and expressed in *E. coli* (pBAD/Thio - TOPO). The nucleotide sequences from both genes were determined through automatic sequencing of double stranded DNA (ABI Prism 377). *E. coli* strains over-expressing recombinant TR of *L. (L.) amazonensis* and *L. (V.) braziliensis* were obtained and, after purification, these proteins will be used to obtain polyclonal sera in mice and for functional and structural studies.

Supported by CNPq/PDTIS/PAPES/IOC-FIOCRUZ

BM114 - MOLECULAR AND BIOCHEMICAL CHARACTERIZATION OF *LEISHMANIA (L.) MAJOR* ADENYLOSUCCINATE LYASE (ADSL).

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Many species of *Leishmania* are responsible for serious visceral or skin diseases that show high incidence in tropical and subtropical regions. These Kinetoplastid protozoa parasites are auxotrophs to purine nucleotides and require those compounds from the medium or host cell. As a consequence, they have developed a specific set of enzymes involved in the purine nucleotide salvage pathway, such as adenylosuccinate lyase (ADSL). The characterization of purine salvage enzymes would contribute to our knowledge of this central biochemical pathway in the kinetoplastid protozoa. In this context, the present work aims to characterize the *adsl* gene and the recombinant ADSL enzyme from *Leishmania (L.) major* Friedlin. The *adsl* gene was already cloned into an expression vector and a purification protocol of the recombinant enzyme was established. The mature mRNA transcript, containing 2032 nucleotides, was defined by 5' and 3'RT-PCR. Restriction analysis and Pulse Field Gel Electrophoresis (PFGE) followed by Southern hybridizations showed that *adsl* is a single copy gene and is located in the chromosome 5 of this parasite. These characteristic allows to future knockout experiments. The tetrameric form of the recombinant ADSL

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enzyme was confirmed by native gel electrophoresis and Dynamic Light Scattering. ADSL has an experimental pI of 6.07 and exhibited maximum enzymatic activity at pH 8.5. The kinetic parameters were analyzed by a Lineweaver-Burk plot. K_m, V_{max} and K_{cat} values of 10.0mM, 14.3mmoles/min, and $33s^{-1}$, respectively, were obtained for adenylosuccinate substrate. A polyclonal mouse antibody raised against ADSL was produced and have been tested. In addition, X-Ray diffraction data, from a Cesium derivative crystal, were collected and are being processed.

Work supported by: FAPESP, HHMI, WHO/TDR, PRONEX and the University of São Paulo

BM115 - CLONING, EXPRESSION AND PURIFICATION OF A *LEISHMANIA (L.) MAJOR* MITOCHONDRIAL TRYPTOPHANYL-TRNA SYNTHETASE.

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The fidelity of protein synthesis is dependent on the correct charging of tRNAs with their cognate amino acids. This process is catalyzed by aminoacyl tRNA synthetases (aaRSs) specific for each particular tRNA. From the comparison of sequence homologies and crystal structures, aminoacyl-tRNA synthetases could be divided into class I and II. Tryptophanyl-tRNA synthetase (WARS) belongs to class I aaRS, which share the consensus sequences "HIGH" and "KMSKS" and the Rossman fold domain. The mitochondria *L. (L.) major* tryptophanyl-tRNA synthetase, LmWARS2, plays an important role in the organelle protein synthesis were it is required to distinguish and charge the edited and the unedited $tRNA^W$. This unique feature may render LmWARS2 a potential target for the design of novel inhibitors.

Sequence analysis of this protein revealed a putative mitochondrial signal represented by the first 24 amino acids and alignment with the sequences of others mitochondrial WARS indicated this protein has insertions along of its amino acids sequence. The mature form of this protein was amplified by the PCR and cloned into expression vectors pCR-TOPO/NT and pCR-TOPO/CT, pET-28a and pET-29a and introduced into *Escherichia coli* BL21-CodonPlus (DE3)-RIL cells. The pET-28a construct resulted in the overproduction and purification of LmWARS2 by metal chelate affinity chromatography. One construct was made by the introduction of an in-frame stop codon into vector pET29a. This LmWARS2 (LmWARS2/pET29a-DH) allowed to express the protein without a His-tag. Both constructs showed expression after induction with IPTG. The expression level of LmWARS2/pET28a was higher than LmWARS2/pET29a and LmWARS2/pET29a-DH. A purification protocol has been developed to this protein, however, the resulting LmWARS2 is unstable, precipitating shortly after purification. We have obtained mouse polyclonal antibodies against LmWARS2 for further imunolocalization and imunoprecipitation experiment.

Work supported by: FAPESP, HHMI, WHO/TDR, PRONEX and University of São Paulo

BM116 - MOLECULAR CHARACTERIZATION AND COMPARATIVE ANALYSIS OF THE MITOCHONDRIAL GENOMES OF *EIMERIA* SPP. OF DOMESTIC FOWL

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Most of the protozoan parasites of the phylum Apicomplexa, including the genus *Eimeria*, present three distinct DNA genomes: nuclear, mitochondrial and plastid genomes. The nuclear genome comprises 14 chromosomes and is currently the subject of a major genome sequencing effort (http://www.sanger.ac.uk/Projects/E_tenella/). The mitochondrial genome of Apicomplexa has been characterized in *Plasmodium* spp., corresponding to a linear molecule composed by 15-150 tandemly repeated copies of a 6 kb element. In *Theileria*, however, the genome is comprised by a 7 kb monomeric element. In both organisms three cytochrome genes were found (*cy*b, *cox*I and *cox*III), as well as some stretches of rRNA genes, but no tRNA genes were present. Dunn *et al.* (1998) have reported in *E. tenella* the occurrence of 170-220 kb molecules hybridizing to a mitochondrial probe. Since the mitochondrial genome is maternally inherited (through the macrogametocyte), it represents an interesting target for monitoring cross-infections. Moreover, this genome can also be used for phylogenetic and epidemiologic analyses. In order to characterize the mitochondrial genome of *Eimeria* spp., our group has determined the complete mitochondrial sequences of the seven *Eimeria* species that infect the domestic fowl. In addition, the mitochondrial genomes of 5 distinct strains of *E. tenella* were also sequenced, thus allowing the characterization of the intra-specific variability. All the genomes sequences showed a size of circa 6 kb and contained the cytochrome genes reported for other Apicomplexa. A multiple sequence alignment, using sequences derived from the seven *Eimeria* species, revealed a similarity of around 90% and a conservation of the synteny and gene order. When the mitochondrial genomes five *E. tenella* strains were compared, a very high conservation was observed. Two haplotypes were found, with two consecutive timines being deleted in strains MC (Brazilian) and Wisconsin (North American) when compared to strains H, TA and Wey (isolated in the UK). This high level of conservation was already reported for *Plasmodium falciparum* strains isolated from different continents (Conway *et al*. 2000), and may reflect a very recent common origin of the strains. Comparing the mitochondrial sequences of several species of *Eimeria*, *Theileria* and *Plasmodium*, we observed that the gene order and orientation are conserved among different species of any genus, but not across distinct genera. This result suggests a gene "shuffling" event during the evolution of these mitochondrial genomes. A possible explanation can be found on the replication mechanism of this genome, which is primarily based on recombination events and rolling circle activity (Preiser *et al*. 1996). Further studies will be carried out to better understand this finding.

BM117 - FUNCTIONAL CHARACTERIZATION OF TRYPSIN AND CHITINASE GENES OF *LUTZOMYIA LONGIPALPIS* THROUGH POLYCLONAL ANTIBODIES.

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While transgenic mosquitoes incapable of transmitting malaria have already been created, little is known about molecules involved in feeding and infection by *Leishmania* in *Lutzomyia longipalpis.* By using differential display techniques and EST sequencing, we have already identified many candidates for a role in these processes. Among these is a gut-specific chitinase gene with high transcription levels after 72 hours of blood feeding, probably involved in the degradation of the peritrophic matrix.. We have also identified 3 trypsin genes, expressed early (6 hours) after the bloodmeal. We are interested in carrying out functional studies of these genes, and for that we have as objective the production of polyclonal antibodies. Not only it is well know that levels of RNA not always reflect presence of active protein, due to post-transcriptional gene regulation, but also, polyclonal antibodies also permit immunolocalization studies. We are using two different approaches for the production

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of these antibodies: production of recombinant antigens in the expression vector pET28a, and use of the vector pCDNA3 as a DNA vaccine. The pET28a vector is designed with enhanced features to permit easier sub-cloning, detection, and purification of target proteins. pET28a has a promoter that directs transcription of the T7 RNA polymerase gene, which is inducible by isopropyl-b-D-thiogalactopyranoside (IPTG). Addition of IPTG to a growing culture of the lysogen induces the T7 promoter, which in turn transcribes the target DNA cloned in the plasmid. pCDNA3 is a DNA vector designed for high-level stable and transient expression in eukaryotic hosts, being effective in a wide range of mammalian cells. The pCDNA3 vector is designed to be used as a DNA vaccine. These plasmids, containing specific genes, enter the cells, where the DNA is transcribed and translated resulting in the production of specific proteins. The produced proteins are processed similarly to intracellular viral antigen resulting in the activation of the immune system and production of antibodies. PCR was used to amplify both cDNAs (chitinase and trypsin) and the fragments were cloned into pCRII. The inserts were purified and ligated into either the pET28a or pCDNA3 plasmids. The positive clones were confirmed by sequencing. The pET28a constructs were successfully induced for expression by IPTG (1mM) and the recombinant protein is presently being purified for inoculation of rabbits. The pCDNA3 constructs are presently being inoculated in rabbits. It will be interesting to evaluate the efficiency of antibody production using both techniques, since it is so much easier to work with DNA vaccines. These antibodies will be used in Western blots and immunolocalization studies of the chitinase and trypsin proteins, for temporal and quantitative studies.

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BM118 - STUDIES ON FLAG, A FLAGELLAR PROTEIN OF *LEISHMANIA*

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The pathogen causing leishmaniasis is a protozoan with basically two morphologies along its life cycle, an amastigote form in the mammalian host and a flagellated form called promastigote in the sand fly vector. A successful infection by the promastigote in the invertebrate depends on the capacity to adhere to the epithelial cells in the gut of the sand fly. Some molecules were implicated in this process. The main one is the parasite surface LPG (lipophosphoglican). Another *Leishmania* molecule, a protein named FLAG, recognized by anti-flagellar monoclonal antibodies (mAb), seems to be involved also in this phenomenon. Its location is exclusively flagellar and there is no function formally described for it. Evidences showed that the anti-FLAG mAb can inhibit the adhesion of *Leishmania* promastigotes to the epithelial cells of the sand fly midgut (Warburg *et al*.,1989, *J. Protozool*., 36:613). There is a lack of information about flagellar proteins of *leishmania*. Also, the precise mechanism by which proteins are directed to the flagellar pocket and flagellum remains unsolved. We have sequenced the FLAG gene in many species of *leishmania*, and found a high level of conservation. Data base searches also found similar sequences in trypanosomes. We intend to study the sorting signal of FLAG and in this way contribute to the formal characterization of this protein and to the current knowledge of flagellar targeting. For this purpose we are constructing fusion proteins of FLAG and GFP (green fluorescent protein). Two *leishmania* expression vectors with the GFP cassette were used to obtain FLAG linked to the C- or the N-terminal of the reporter protein. These constructs are being transfected into leishmania, that will be examined by fluorescence microscopy. Another objective is the production of policlonal antibody, a useful tool for many different assays. This will be done by the injection into rabbits of either recombinant FLAG or by DNA vaccines.

Supported by PAPESIII-Fiocruz.

BM119 - TRAFFICKING OF THE CYSTEINE PROTEINASE LPCYS1 IN *LEISHMANIA*: FUNCTION OF THE PRE-PRO DOMAIN

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Leishmaniasis is a serious disease with about 400,000 news cases per year, that nowadays is also considered an opportunistic disease in AIDS. *Leishmania* is a dimorphic parasite that has its cycle of life in two hosts, a sand fly vector, and a mammal. In the invertebrate host, it presents promastigote forms (with flagellum), and in the mammalian host the amastigote form (without external flagellum). Cysteine proteinase may be linked to many pathological processes caused by parasites. Two distinct lysosomal cysteine proteinases have been identified in *Leishmania (L.) pifanoi,* Lpcys1 and Lpcys2. Studies of the targeting of Lpcys2 showed the lack of a role for glycosylation in this process, although a role for the pro domain has been determined. Assays of immunolocalization have shown the presence of both proteinases in the lysosome, but of relatively more abundant quantities of Lpcys1 in the flagellar pocket than of Lpcys2. Lpcys1 does not have the C-terminal domain characteristic of most cysteine proteinases of trypanosomatids, that is also present in Lpcys2, and have some sequence differences in the pre-pro domain. We are interested in investigating possible differences in targeting mechanisms between Lpcys 1 and 2. Constructs that express the pre-pro domain of Lpcys1 fused with GFP (green fluorescent protein) were made in a *leishmania* expression vector. To study the targeting of this protein to the lysosome, these constructs are being transfected into *Leishmania* through electroporation. In this technique the parasite, in the presence of DNA, is submitted to an electric shock, when pores are formed in its membranes and the plasmids enter the cell. The transfected parasites are incubated in medium with a concentration of selective drug determined by EC50 (dose of drug needed to reduce the growth in 50%), and the transfected cells are examined by fluorescent microscopy.

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BM120 - LYSOSOMAL TRAFFICKING IN *LEISHMANIA*: USE OF YEAST TWO-HYBRID SYSTEM AND STUDIES OF SIGNAL CONSERVATION

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Leishmaniasis are diseases caused by protozoan haemoflagelates of the genus *Leishmania* that affect many people in various parts of the world, predominantly poor and underdeveloped countries. The lack of efficient vaccines and therapies leads to the need for the development of new targets for control measures. Molecules involved in mechanisms of cellular trafficking in *Leishmania* can be used as a potential target for disease control. Cysteine proteinases have been implicated in processes that might be important in parasite invasion, infectivity and pathogenicity, making them a good investigation target. Previous studies showed that in Lpcys2, an abundant lysosomal cysteine proteinase from *L. (L.) pifanoi*, the pro domain plays a role in targeting (Costa-Pinto et al., 2001). We are presently going deeper into these studies and searching for molecules that interact with the pro region signal utilizing the yeast two-hibrid system. The system is based on yeast co-transformation of the vectors: pBD-GAL4 Cam containing the pro domain sequence of cysteine proteinase (bait) and HybriZAP-2.1 containing an amastigote leishmania cDNA library (target). Subsequently, molecule interaction activates the b-galactosidase and Histidine gene reporter

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expression, identified by the appearance of blue colonies. Until this moment no positive colonies were identified. We are also investigating the evolution of trafficking signals by fusing the Lpcys2 prepro and pro domains to the reporter GFP (green fluorescent protein) and transfecting these constructions into yeast, *Saccharomyces cerevisiae* YRG-2. For this purpose we used two yeast specific expression vectors: pGFP-N-FUS34 and pGFP-C-FUS23. The cells transfectants were examined by fluorescence microscopy. When the signal sequence was fused to the GFP amine end, the cells showed a compartmentalized fluorescence compatible with the vacuole, the equivalent to the lysosome of *Leishmania*. This observation indicates the recognition of the *Leishmania* signal by yeast, and might explain the lack of positive results by the yeast two-hibrid system.

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BM121 - CHARACTERIZATION OF GENES ENCODING PROTEINS INVOLVED IN INTRACELLULAR TRAFFICKING IN *TRYPANOSOMA CRUZI*.

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In this study we propose to characterize two cDNAs isolated from a *Trypanosoma cruzi* library of amastigote forms screened with sera derived from of chagasic patients. Sequence analyses of *Tc*AG38 and *Tc*AG49 cDNAs reveal homologue sequences and indicated that they corresponds to a new class of *T. cruzi* gene, which encodes proteins presenting significant homology with VIP36 and ERGIC 53, mammalian proteins involved in cellular sorting or recycling. The complete sequence of the *Tc*AG38 cDNA was determined revealing an ORF of 550 amino acids and the *Tc*AG49 sequence is slightly bigger. Using GFP tranfections, we have previously showed that these proteins localized near to the flagellar pocket and co-localizes with kinatoplast and golgi region markers. Another strategy in the briefing of the localization of this protein is the analysis of the distribution of this pAG38 in cells of mammals. For this, already we are carrying through the construct of the vector presenting the codding region for pAG38 in fusion with GFP for transfecção of fibroblasts. We identified a microssatelite within the coding region which is translated into a poli-Glu region. PCR amplifications of this region reveled polymorfism among diferent strains of parasite. Southern blot analysis indicated that the antigen is encoded by a one to three copies of this gene in the genome of parasite. Northern blot analysis revealed that *Tc*AG38 is constitutively transcribed into a 2,5 kb mRNA, which is slightly more abundant in amastigote than epimastigote and trypomastigote forms. Transformed *E.coli* expressing the recombinant proteins as GST fusions were produced using the pGEX vector (Pharmacia). GST::pAG38 and GST::pAG49 were submited to purification protocol and are being used to generate specific antibodies.

APOIO: WHO, CNPq e CAPES

BM122 - STUDIES ON THE TYPE II DNA TOPOISOMERASE OF THE TRYPANOSOMATID *BLASTOCHRITHIDIA CULICIS*

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Type II topoisomerases (topoII) are enzymes that catalyze changes in the topology of the DNA molecule by introducing transient double-stranded cuts. They are involved in many cellular processes, including DNA replication, transcription, recombination and chromosome segregation. Moreover, topoII are targets for many antitumoral agents and might be also important targets in the chemotherapy of diseases caused by parasites, including Chagas disease and leishmaniasis. We have cloned and characterized the gene encoding *TOP2* from the monoxenic trypanosomatid *B. culicis* (*BcTOP2*), since this enzyme might be used as a prototype for topoII enzymes from pathogenic trypanosomatids and consequently a good model for future structural and functional studies. BcTOP2 was expressed in *E. coli* and antiserum was raised against the recombinant protein. Western blot analysis revealed a polypeptide of 138 kDa, comparable to that observed in extracts of *C. fasciculata* (CfmtTopoII). In *C. fasciculata*, monoclonal antibodies against CfmtTopoII showed that the enzyme is localized in the mitochondrium. On the other hand, in *T.cruzi*, topo II was localized in the nucleus. However, in *Leishmania (L.) donovani* and *Bodo salt*ans, topo II imunolocalizes in both compartiments. In view of this conflicting results we decided to determine the cellular compartiment in which BcTopo II is localized, in order to approach the role that this enzyme could perform either in the kDNA replication or in chromosome replication and segregation.

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BM123 - *TC*RABS FUSED TO GFP - AN ALTERNATIVE STRATEGY TO STUDY THE LOCATION OF *TC*RAB7 AND *TC*RAB5A PROTEINS IN *TRYPANOSOMA CRUZI*.

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Rab proteins are specifically found at different cellular compartments. They are small GTPases anchored in the organelle membranes cytoplasmatic face, with a role in the vesicular traffic control of the eukaryotic cells. The *rab7* and *rab*5A genes of *Trypanosoma cruzi* have already been sequenced and characterized in our laboratory. We have located *Tc*RAB7 protein in the Golgi Apparatus of epimastigote forms by immunoelectronmicroscopy using a specific polyclonal antibody and now we are performing the *Tc*RAB5 localization. A heterologous antibody, raised against a carboxi-terminal region of Rab5A protein of *Trypanosoma brucei*, is being used to localize Rab5A protein in *T. cruzi* and we have already produced recombinant protein in order to produce a specific antibody against *Tc*RAB5A.The use of the GFP (green fluorescent protein) fusion protein is another powerful strategy to localize proteins in live cells, distinguishing their functional sites from their synthesis or recycling pathways. To further investigate *Tc*RAB7 site and also in a first attempt to localize the *Tc*RAB5A, we have constructed and will express *TcRAB* genes fused in their N-terminal regions to GFP. Oligonucleotides were drawn to amplify, from the cloning vector pEGFP-C1, a DNA cassette (850 kb fragment) containing a truncated version of the coding region for GFP, without stop codon, suitable for expression of the protein fusion. Two vectors (pRIBOTEX-GFP and pTEX-GFP) will be used to insertion of *Tc*RAB7 and *Tc*RAB5 ORFs. The first vector will be originated from the pRIBOTEX plasmid whose expression is driven by a ribosomal RNA gene promoter. Another vector will be originated from the pTEX whose expression is controled by flanking regions of the GAPDH genes. The constructs will be sequenced, amplified and transfected in epimastigote forms. The cells, live or fixed, will be examined for GFP expression using the Confocal Laser Scanning microscope Zeiss LSM 310.

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BM124 - REGULATION OF POLYSOME ASSOCIATED POLYPETIDES AND HSP47 BIOSYNTHESIS BY ANTISENSE OLIGONUCLEOTIDES IN *HERPETOMONAS SAMUELPESSOAI*

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Herpetomonas samuelpessoai corresponds to a Trypanosomatidae parasite from the *Herpetomonas* genus. These parasites can be grown in culture medium *in vitro*. Hsp47, an endoplasmic reticulum resident protein, has binding properties and has been hypothesized to function as a molecular chaperone in regulating protein folding and assembly. In this study, we investigate the interacion of Hsp47 with polysome- associated proteins following antisense oligonucleotide treatment directed to Hsp47 in *Herpetomonas samuelpessoai* parasites. For these studies, we employed phosphorothioate oligodeoxynucleotides directed to the first five codons of Hsp47 that straddle the predicted translation initiation site of Hsp47. The levels of Hsp47 were assessed by immunoprecipitation and/or Western Blot analysis. Labeled nascent polypeptide chains were isolated from polysome preparations as peptidyl-tRNA complexes using ion exchange chromatography. The eletrophoretic profiles of labeled polysomes revealed that the majority of Hsp47 was associated with nascent chains of polypeptides. Treatment of *Herpetomonas samuelpessoai* with antisense Hsp47 oligonucleotides after 48 days reduced the levels of Hsp47 and caused a "knock out" of the synthesis of nascent polysome associated polypeptide chains. This study revealed that diminished levels of Hsp47 induced by antisense oligonucleotide treatment of *Herpetomonas samuelpessoai* cells also reduce the levels of polysome associated polypeptides. The results provide further evidence that Hsp47 is associated with polysomes at a very early point during translocation of polysome associated nascent polypetide chains.

This study was sponsored by FAPEMIG and PROBIC-PUC-MINAS.

BM125 - TYPE I DNA TOPOISOMERASES OF *TRYPANOSOMA CRUZI*: CELLULAR LOCALIZATION OF TOPOISOMERASE I AND CHARACTERIZATION OF TOPOISOMERASE III

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Type I topoisomerases are classified in two subfamilies, IA and IB, based on differences in amino acid sequence and reaction mechanisms. The type IA enzymes link covalently to cleaved DNA through the 5' phosphate. They are represented by reverse gyrase, bacterial topo I and topo III and the eukaryotic topo III enzymes. Type IB topoisomerases, in contrast, become attached covalently to 3'-phosphate end of the cleaved strand of the DNA. The presence of at least one type I DNA topoisomerase in all organisms indicates that these enzymes play a key role in replication and transcription processes, and recent studies have pointed to their role in recombinational repair and in chromosome segregation. Furthermore, topo I is a target for clinically anticancer drugs, such the analogous of camptotecin. Hence, we decided to clone and characterize the genes encoding these enzymes in *T. cruzi* (*TcTOP1* and *TcTOP3*) in order to get insight into its importance during the cell cycle and differentiation of the parasite and evaluate them as potential targets for chemotherapic treatment of Chagas disease. The sequencing of *TcTOP3* gene showed that it contains an ORF of 2,5 Kb, encoding for a polypeptide of 95 kDa. The deduced amino acid sequence of *TcTOP3* shares a similarity of ~ 49% with Topo IIIb of *Xenopus laevis* and *Homo sapiens*. We have also initiated the characterization of a gene encoding a second topo III, which the sequence showed to be divergent from that first one and shares significant homology with topo III of *Arabidopsis thaliana*, instead. It indicates that *T.cruzi* might have more than one topo III with different functions. Recombinant Tctopo III was expressed in *E. coli* and an antiserum was raised for further studies of cellular localization.

Regarding to Tctopo I, which the gene was previously characterized by us, immunofluoresce analysis shows that this enzyme immunolocalizes into the nucleus of epimastigote forms of *T.cruzi*, as expected. Our preliminary data also suggest that TcTopo I enzyme might have a differential expression pattern through the cell cycle of *T.cruzi* epimastigote forms.

Financial support: PRONEX, PADCT/ CNPq.

BM126 - EVALUATION OF THE ROLE OF THE MINIEXON GENE IN THE VIRULENCE OF *LEISHMANIA (V.) BRAZILIENSIS.*

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Trans-splicing is the preferred route for the generation of mature mRNA among trypanosomatids and the miniexon gene plays a central role in the splicing process of mRNAs. The splice leader is added to the 5' extremity of each mRNA supplying its 5' capped structure. In previous studies carried out in the laboratory we have shown that the over expression of the miniexon gene leads to an attenuation of the virulence of *Leishmania (L.) major* in BALB/C mice (Antoniazi et al. MBP 107: 57, 2000). To verify if the same phenomenon would be observed in different species of *Leishmania*, we have selected three distinct strains of *L. (V.) braziliensis* (2904, Ba 27b2 e Ce 3227). The EC50 for Hygromycin B (HYG) was calculated for each one of the *L. (V.) braziliensis* strains, in order to define the drug concentration for selection of transfectants. The drug concentration to be used in liquid media is 6ug/ml, 4ug/ml e 6ug/ml, for strains 2904, Ba 27b2 e Ce 3227, respectively. These strains were transfected with clone 32D05 that contains approximately 100 copies of the miniexon gene, or with the vector (cLHYG) with no insert, as a control. The transfectants of *L. (V.) braziliensis* had been selected on solid media and submitted increasing drug pressure to stimulate the overexpression of the episomal genes. Molecular characterization of the transfectants confirmed the identity of the episome and was followed by *in vivo* investigation of virulence. A suspension of 10 million promastigotes from stationary phase (Ce 3327) was injected in the hind footpad of hamsters. Evaluation of lesion development was carried out for five months by weekly measurements of the footpad with a kaliper, the contra-lateral footpad was used as a control. Each experimental group was composed of 10 animals infect with: (1) parental line, (2) cLHYG transfectant, (3 and 4) transfectant with 32D05 under low (12 ug/ml of HYG) and high selective pressure (120 ug/ml of HYG), respectively. Lesion development was observed in groups 1 and 2, but not in animals from groups 3 and 4. Present data confirms that the gene miniexon, when overexpressed, attenuates the virulence of *L. (V.) braziliensis*. We are currently evaluating another strain of the same species and also investigating the possible mechanism that leads to the observed attenuation.

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BM127 - PILOT SURVEY OF EXPRESSED SEQUENCE TAGS (ESTS) FROM THE ASEXUAL BLOOD STAGES OF *PLASMODIUM VIVAX* IN HUMAN PATIENTS

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Plasmodium vivax is the most widely distributed human malaria, responsible for 70–80 million clinical cases each year and large socio-economical burdens for countries such as Brazil where it is the most prevalent species. Unfortunately, due to the impossibility of growing this parasite in continuous *in vitro* culture, research on *P. vivax* remains largely neglected. A pilot survey of expressed sequence tags (ESTs) from the asexual blood stages of *P. vivax* was performed. To do so, 1,184 clones from a cDNA library constructed with parasites obtained from 10 different human patients in the Brazilian Amazon were sequenced. Sequences were automatedly processed to remove contaminants and low quality reads. A total of 806 sequences with an average length of 586 bp met such criteria and their clustering revealed 666 distinct events. The consensus sequence of each cluster and the unique sequences of the singlets were used in similarity searches against different databases that included *P. vivax*, *Plasmodium falciparum*, *Plasmodium yoelii*, *Plasmodium knowlesi*, Apicomplexa and the GenBank non-redundant database. An E-value of $<10^{-30}$ was used to define a significant database match. ESTs were manually assigned a gene ontology (GO) terminology. A total of 769 ESTs could be assigned a putative identity based upon sequence similarity to known proteins in GenBank. Moreover, 292 ESTs were annotated and a GO terminology was assigned to 164 of them. These are the first ESTs reported for *P. vivax* and, as such, they represent a valuable resource to assist in the annotation of the *P. vivax* genome currently being sequenced. Moreover, since the GC-content of the *P. vivax* genome is strikingly different from that of *P. falciparum*, these ESTs will help in the validation of gene predictions for *P. vivax* and to create a gene index of this malaria parasite. Most important, as these ESTs represent parasite genes expressed during the stages responsible for the pathology associated with vivax malaria, sequence comparisons with the data from the *P. vivax* genome should assist in identifying SNPs for genetic mapping and population diversity studies.

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BM128 - ROLE OF THE SPLEEN IN EXPRESSION OF VARIANT GENES FROM *PLASMODIUM CHABAUDI* IN BALB/C MICE.

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Antigenic variation has been noted in all *Plasmodium* species where it has been sought, including the simian malarias, *P. knowlesi* and *P. fragile*, the rodent malarias, *P. chabaudi* and *P. yoelii* and the human malarias, *P. vivax* and *P. falciparum*. Significantly, a key role of the spleen in the expression of variant antigens from *P. knowlesi* was initially observed in splenectomized monkeys. Thus, a schizont-infected cell agglutination test allowed demonstrating that splenectomized monkeys no longer expressed a highly variant multigene family, *pk235*, involved in antigenic variation whereas normal monkeys expressed different *pk235* genes in the course of chronic infections. Recently, a multigene family likely involved in antigenic variation in *P. chabaudi* has been identified and termed *cir* (*P. chabaudi* variant genes). *cir* genes, along with nine other multigene families whose function and subcelular localizations are unknown, are located within the subtelomeric regions of different (if not all) chromosomes. We are thus using mouse models to determine the role of the spleen in the expression of these multigene families in *P. chabaudi*. To this end, we have established chronic infections in normal and splenectomized BALB/c and CBA/J mice by using non-sterilizing treatments with chloroquine at each peak of parasitemia. This treatment allows us to study the pattern of transcription of all the subtelomeric gene families, including the *cir* genes, and to determine the extent of cytoadherence of these parasites after several passages in splenectomized animals.

Supported by CAPES, FAPESP and CNPq

BM129 - SYMPTOMATIC AND NON-SYMPTOMATIC MALARIA INFECTIONS ARE CAUSED BY THE SAME SET OF *PLASMODIUM FALCIPARUM* GENOTYPES

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In order to elucidate if genotypes of *Plasmodium falciparum* parasites causing either symptomatic or non-symptomatic malaria infections were different from each other, *Plasmodium falciparum* parasites obtained from residents of different suburbs of Porto Velho were compared by means of microsatellite analysis.

Fourty-four samples, divided in five groups were analyzed: two groups of samples from non-symptomatic infections (Candelaria, taken at different timepoints) and three groups of samples from symptomatic patients (from Candelaria, Bate-Estaca and CEPEM, all from suburbs of Porto Velho-RO, Brazil) were analyzed using eleven different microsatellite markers. For each distinct group, the diversity index, the fixation index (F_{st}-value) and the percentage of mixed (polyclonal) infections was determined. All goups presented multiple infections to a similar degree. As expected, the group of samples from CEPEM showed the highest degree of heterozigosity, since samples were from patients of several different locations. There was no significant difference in heterozygosity between samples from asymptomatic and symptomatic infections of the same location. The largest genetic differentiation was observed between Candelária (asymptomatics) and Bate Estaca (symptomatics) samples, in spite of the geographical proximity of the two locations. This study confirms that the rather similar circulating *Plasmodium falciparum* genotypes, determined by microsatellite analysis, can either both cause symptomatic or asymptomatic infetcions and that the reason for this probably lies in the differential expression of virulence associated genes.

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VE1 - CHAGAS DISEASE IN MEXICO: MORBIDITY, MORTALITY, RISK AREAS AND DISEASE BURDEN.

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Chagas disease is the most important parasitic disease in Latin America, as in Mexico, where 1.5-2% of blood donations are contaminated with anti-*Trypanosoma cruzi* antibody. Through environmental and population based stratification, we estimate that 91 million inhabitants are at risk (78% through residence), 1,768,376 individuals are infected, and mortality may oscillate between 25,500 and 63,000 individuals/yr (830 of these are under 5 yrs old). The disease incidence is estimated at 69,000 cases/yr and approximately 530,500 individuals are currently in chronic phase. More than 96% of the transmission occurs via the vector, and niche modeling with GARP estimates that 67% of the transmission occurs via one of the 6 primary *phyllosoma* complex species.

The economic loss due to incapacity is estimated at US$ 3,160,000,000/yr, while diagnostic and treatment costs currently could attain US$ 126,000,000/yr. In the absence of a vector control program, disease burden could duplicate in 25 yrs, while chronic case treatment will augment by a factor of 45 over the same period.

VE2 - MORPHOMETRIC ANALYSIS OF MEXICAN AND GUATEMALAN POPULATIONS OF *TRIATOMA DIMIDIATA*.

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Current estimates suggest that 2% of the Mexican population is seropositive for *Trypanosoma cruzi*, and the incidence of Chagas disease may supercede 69,000 cases/yr, with mortality equally 5-6% (25,000 cases) of the country´s total burden. Among the 18 vector species found in the country, *Triatoma dimidiata* plays a critical role in transmission principally in Gulf coast and all states south of the Istmus of Tehuantepec. Current molecular studies using ITS-2 rDNA and mitochondrial DNA markers, suggest that the populations found in the Yucatan peninsula may be an ancestral stock, from which other Mexican, Mesomerican, and Colombian/Venezuelan populations derived. Given profound genetic differences between yucatecan and all other populations, phenotypic differences might be expected using head and wing morphometric and symmetry discriminate analysis. 187 specimens of *T. dimidiata* collected from the states of Yucatán, Veracruz, and San Luis Potosí (SLP), were compared to a population from the Petén, Guatemala, and *T. phyllosoma* from Oaxaca as outgroup (:33 specimens per species). 12 head and 14 wing characters were measured separately for male and female samples, and measurements analyzed using multigroup principal component and discriminate analyses, sexual dimorphism and wing asymmetry. Guillaumin profiles indicated no size difference between SLP and Veracruz populations, while Peten specimens were smaller, and those from the Yucatan the smallest. None of the populations were separated completely using principal component analysis, although there was a clear tendency for separation. No shape differences were observed between SLP and Veracruz populations although they were clearly separated from both the Yucatan and Peten populations using discriminate analysis. Yucatan and Peten populations showed a clear tendency for separation, although this was not complete. Sexual dimorphism and fluctuating wing asymmetry were observed within all populations. There is a substitution of 24 to 27 nucleotides in the ITS-2, and a sequence divergence using Cyt*b* and ND4 mtDNA markers from 3.9 –14.1% between Yucatan and all other populations studied herein. While SLP and Veracruz populations appear to be identical morphometrically, they were clearly differentiated from the other two populations. This was not the case between the Peten and Yucatan populations. Genetic distance between the latter two populations was not reflected in differences in head and wing phenotype characters. Future studies with other marker systems may elucidate this disparity. From an operational viewpoint, head characters were the most informative to differentiate among all populations, suggesting their potential use for differentiating re-infestations during control programs.

VE3 - EGGSHELLS MORPHOMETRY AND MORPHOLOGY OF *TRIATOMA COSTALIMAI, TRIATOMA GUAZU* AND *TRIATOMA WILLIAMI* (HEMIPTERA, REDUVIIDAE).

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The morphometrics and morphologic researchs contribute for the specific characterization as well as for the phylogenetics studies of the Triatominae. Then was performed a morphometric and morphologic study of *T. costalimai, T. guazu* and *T. williami.* With regard to morphometry of the eggshell of *T. guazu* the averages had been, length of 2.24mm, width of 1.44mm and diameter of opercular opening of 0.59mm. *T. costalimai* presented the following average measurement: length of 2.14mm, width of 1.31mm and diameter of 0.53mm. *T. williami* got a average in the measurement of 2.04mm of length, 1.16mm of width and 0.60mm of diameter. The formats of the three species had presented ellipsoid eggshell and the lateral flattening was present in the three species also. The presence of neck and of collar it was not verified in none of the three species, it was verified the presence of a band in very narrow ring form contiguous and plain that makes the connection between operculum and the body of eggshells. The examination by means of scanning electron microscope showed that the exochorio of the body of *T. costalimai* presented exochorial cells with pentagonal and hexagonal formats, the junctions are little evident, the cells has scasse and unclear perfurations. *T. guazu* presented plain exochorial cells with no symmetrical form (pentagonal and hexagonal) the junction between the cells is well evident, the perforations are concentrate in the internal face of the exochorial cells and the number of perforations varied of 16 the 20 for cell. *T. williami* presented a predominance of hexagonal exochorial cells that was bigger than in *T. guazu* and *T. costalimai,* the junction between the cells of the exochorio is well evident, the cellular exochorio perforations is distributed in irregular way (center, edge and junction) and the number of perforations exceeded 20 for cell. About relation aeropyle and micropyle these structures had been evidenced in the three studied species. Amongst three studied species *T. guazu* presented the biggest length (2.16mm) and the biggest width (1.39mm). *T. williami* showed the average greater in the diameter of the opening (0.60mm). Morphologically, *T. costalimai, T. guazu* and *T. williami* presented lateral flattening but none of them have neck e collar. *T. costalimai* presented the exochorial cells with well distinct characteristics of the others two species (edges little defined, little or inexistence of perforations, plain surface). *T. williami* presented exochorial cells with size and number of bigger perforations than the others two studied species.

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VE4 - EVALUATION OF VETORIAL CAPACITY OF *TRIATOMA INFESTANS* AND *TRIATOMA KLUGI* HYBRIDS

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The genus *Triatoma* is wide spread in Latin American Countries, comprising sylvatic, peridomestic and domestic triatomines, which represents almost 50% of all triatomine species. Some species of the genus *Triatoma* such as *T. infestans* have great importance in the transmission of *Trypanosoma cruzi*, the causative agent of Chagas disease. Our group has recently described a new silvatic triatomine species (*Triatoma klugi*) in the State of Rio Grande do Sul (Southern Brazil). Having successfully established a colony of *T. klugi*, we have detected the ability of these insects to mate with *T. infestans* under laboratory conditions. Previous data showed that couples formed by *T. klugi* females and *T. infestans* males produce of high number of viable eggs. The aim of this work was to evaluate the vectorial capacity of *T. infestans*/*T. klugi* hybrids. For that, groups of 50 4th-5th instars of hybrids (*T. klugi* females/*T. infestans* males) were fed for 2-3 hours on anesthetized Swiss mice in the peak of blood parasitemia, infected with *T. cruzi* (Y strain) and *T. rangeli* (Choachi strain). Nymphs of *T. infestans* and *T. klugi* were infected with the same strains and used as control. Triatomines were searched for the presence of flagellates in their feces and hemolymph every 30 days. The infection rate of hybrids was of 70.6% for *T. cruzi* and no *T. rangeli* was observed. The infection rates in control *T. klugi* and *T. infestans* groups was of80.0% and 92% for *T. cruzi* and 42.3% and 30,3% for *T. rangeli*, respectively. Since *T. infestans* and *T. klugi* are sympatric at Rio Grande do Sul State, the high hybrid production and their susceptibility to T. cruzi may assume a epidemiological relevance in the context of Chagas disease.

Supported by UFSC

VE5 - *TRIATOMA INFESTANS* WILD FOCI IN MESOTHERMIC ANDEAN VALLEYS OF COCHABAMBA, BOLIVIA. IMPLICATION OF *TRYPANOSOMA CRUZI* IN NATURAL CYCLE.

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The success of *Triatoma infestans* elimination in Southern Cone countries relied on the fact that this species is almost exclusively domestic. This is not the case of Bolivia where the recent detection of *T. infestans* wild foci throughout various ecosystems calls attention. True wild populations of this species are now well documented from mesothermic Andean valleys (altitude 2,500 m), from the high-Chaco (1,350 m) and the low-Chaco (500 m). With the exception of the arboreal *T. infestans* dark morph from the low-Chaco, all other populations occur in terrestrial habitat.

A survey of *T. infestans* in the wild environment was performed in the Andean focus of Quillacollo, Cochabamba Department. Of 346 traps placed among rocks (30 traps/day), 46% were positive for *T. infestans* counting 478 insects between nymphal instars and adults. Nymphs predominated thoughout and 60% of fecal samples from the examined insects (n =202) were infected with *Trypanosoma sp*. Precipitin test demonstrated that wild *T. infestans* were mostly associated with rodents and marsupials.

Also small rodents (Caviidae and Muridae families) (n = 13) and marsupials (Didelphidae) (n = 3) were examined. Infection by *T. cruzi*, was evidenced in three rodents (23%) and one marsupial (33,33%) by microhematocrit method.

MLEE analysis of 14 genetic loci, demonstrated that all 35 *Trypanosoma cruzi* from wild

T. infestans and four isolates from wild mammals displayed only micro-heterogeneity. PCR amplification of the non-transcribed spacer of the miniexon gene showed that all isolated were in the genotype *T. cruzi* I.

In spite of both genotypes (*T. cruzi* I and *T. cruzi* II) being prevalent in Bolivia, in our study area only *T. cruzi* I is being transmitted wild *T. infestans*, rodents and marsupials.

Supported by CAPES/IRD

VE6 - POPULATION PHENOTYPIC PLASTICITY LINKED TO ECOLOGICAL ADAPTATIONS IN TRIATOMINAE

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Triatomine bugs are vectors of *Trypanosoma cruzi* – causative agent of Chagas disease. Sylvatic bug populations maintain enzootic infection cycles by sharing microhabitats with their mammalian hosts; populations adapted to synanthropic environments transmit human Chagas disease. Phenotypic plasticity has been recognised by morphometric and genetic analyses, with examples of convergence and divergence described at various taxonomic levels. Such phenotypic changes have been attributed to ecological transitions: habitat shifts (eg. sylvatic?domestic) involving subsets of genetically homogeneous populations (leading to within-species divergence); or the sharing of comparable habitats by genetically distinct populations (resulting in convergence or the retention of plesiomorphic phenotypes). When using phenotypic characters only, systematists are thus at peril of describing spurious morphospecies or overlooking cryptic taxa.

Following this rationale, we combined phenotypic characterisation (qualitative+quantitative) with mtDNA analysis to study five putative *Rhodnius ecuadoriensis* populations spanning most of the geographic/ecological range of the species (Ecuador: Andean-sylvatic [wet forest], Coastal-sylvatic [seasonally dry forest], Andean-domestic [seasonally humid forest], Andean-domestic [dry forest]; and Peru*: Andean-domestic [very dry forest]).

Qualitative phenotypic assessment revealed differences customarily associated with distinct morphospecies: all synanthropic bugs (3 populations) had comparable, typical phenotypes (small-pale bugs, short-stout heads), whereas Andean-sylvatic specimens were very large and dark with elongated-slender heads. Coastal-sylvatic bugs had intermediate (medium-pale with slender heads) phenotypes. ANOVA and traditional canonical variate analysis (CVA) of head measurements revealed size-related changes associated with microhabitat (large bugs?palms; small bugs?houses). CVA-based reallocation of specimens to their original ecological groups (sylvatic/domestic) was almost perfect (kappa>0.9), supporting the use of discriminant analysis for reinfestation surveillance. While all synanthropic phenotypes were qualitatively comparable regardless of general ecological conditions, sylvatic phenotypes consistently varied when Andean (wet) and coastal (seasonally dry) life zones were compared.

These phenotypic-ecological groups were not recognised by mtDNA analysis. A 663bp fragment of the cytochrome *b* gene was sequenced and analysed using character state- and distance-based methods. Domestic Peruvian bugs (synanthropic-like phenotypes) were separated from the closest Ecuadorian population (coastal-sylvatic) by >3.9% sequence divergence (26 point mutations), suggesting they constitute independent lineages. The maximum distance between Ecuadorian haplotypes was <2% (13 mutations). Andean-sylvatic bugs presented a single haplotype; it was shared with other Ecuadorian bugs (coastal-sylvatic) in spite of their strongly divergent phenotypes. Size-free CVA (head

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measurement-based) and geometric analysis (wing landmark-based) revealed patterns of difference/similarity compatible with mtDNA-based clades, with a distinct Peruvian cluster. Explicit size partitioning or geometric analysis of form were therefore required for consistently assessing genetic differences using metric data.

These results revealed (1)phenotypic convergence (involving size-related characters) of genetically distinct synanthropic populations (Peruvian?Ecuadorian) and (2)phenotypic divergence within genetically homogeneous clades (sylvatic?synanthropic; coastal-sylvatic?Andean-sylvatic). This remarkable phenotypic plasticity within a single triatomine species was apparently associated with ecological adaptations and microhabitat.

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VE7 - SALIVARY PAF-ACETYLHYDROLASE ACTIVITY OF *TRIATOMA INFESTANS*

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The saliva of blood-sucking insects shows several pharmacological activities that antagonize the host's hemostatic response. Amongst these activities, the inhibition of platelet aggregation plays an important role as an anti-hemostatic mechanism during blood feeding. In accordance with this feature, we wanted to see if the saliva of *Triatoma infestans* would display hydrolytic activity on the platelet-activating factor (PAF). In this study, a PAF-acetylhydrolase (PAF-AH) activity was identified in the saliva of this insect using the PAF-AH fluorogenic substrate 2-(6-(7-nitrobenz-2-oxa-1,3-diazol-4-yl) amino) hexanoyl-1-hexadecanoyl-*sn*-glycero-3-phosphocoline in a Ca^{+2}-independent manner. The purification of a protein that mediates this enzymatic activity was achieved by a two-step FPLC procedure using ion exchange and hydrophobic interaction columns. The PAF-AH activity was associated with a single 17 kDa saliva protein on SDS-PAGE under reducing conditions. By means of peptide mass fingerprinting analysis it was possible to confirm the identity of the protein as a member of the phospholipase A_2 family. This enzyme was shown to be immunogenic as it was capable to induce specific IgG antibodies in mice. Host PAF hydrolysis by this *T. infestans* enzyme could be related to the inhibition of platelet aggregation, thus helping the insect to obtain his blood meal. Also, it may reduce the inflammation process at the site of the insect bite and that could facilitate the transmission of *Trypanosoma cruzi* to mammal hosts. Furthermore, other possible functions of this activity would be lysis of blood cells and decreasing host's nociceptive response. These features suggest that enzymes with such activity would be good candidates to the development of vaccines against vector-borne diseases like Chagas' disease.

This research is sponsored by: CNPq.

VE8 - PHOSPHOLIPASE INHIBITION PROTECT THE *VENEZA ZONATA* (HEMIPTERA COREIDAE) AGAINST SEPTICEMIA CAUSED BY TRYPANOSOMATID PARASITE 563DT ISOLATED FROM *EUSCHISTUS HEROS* (HEMIPTERA PENTATOMIDAE

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The *Veneza zonata* is a Hemiptera insect of the Coreidae family, whose geographic distribution is wide, ranging from the U.S.A. to South America. These insects feed on corn, sorgo, bean, tomato, soy, guandu and various legumes and fruits. In the Londrina region, the presence of the insect in cornfields is predominant and it is considered a plague by many agriculturists. Besides its agricultural importance, *V. zonata* is frequently infected with trypanosomatids of the *Phytomonas*, *Leptomonas*, *Herpetomonas* and *Crithidia* genera. The 563DT (*Leptomonas*) strain, isolated from the digestive tract of *Euschistus heros* (Hemiptera Pentatomidae), pathogenic for *Veneza zonata*, was analyzed for phospholipases inhibtion in living cells. *Veneza zonata* specimens were collected on rural properties in Londrina, Paraná, southern Brazil. After inoculating *V. zonata* with 563DT strain, insect death was observed in approximately 24 h after infection, with intense bacterial proliferation in the hemocoel. When 563DT trypanosomatids were previously incubated with a phospholipase inhibitor called Palmitoyl-carnitina, there was a relevant reduction in insect deaths, showing that phospholipases are probably involved in the pathogenic mechanism. In a previous work, we showed an increase of survival rate of insect treated with proteases inhibitors. In this work, we observed that insects inoculated with proteases plus phospholipases inhibitors had their survival rate increased. The insects treated with serino-proteases and phospholipases inhibitors presented a survival rate of 91% from control insects inoculated only with sterile NaCl 0.85%.

Key Words: Trypanosomatids, phospholipases, *Veneza zonata*,

VE9 - ISOLATION AND CHARACTERIZATION OF DEFENSIN, AN ANTIMICROBIAL PEPTIDE OF THE CHAGAS DISEASE VECTOR, *TRIATOMA BRASILIENSIS*.

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The humoral immune response in insects includes the synthesis of several classes of proteins, e.g., lysozyme and defensin. However, not only in the haemolymph but also in the gut these antimicrobial peptides appear: cecropin is expressed in the gut of *Drosophila* and *Bombyx* and defensin in the gut of the blood-sucking insects *Stomoxys calcitrans*, *Aedes aegypti*, *Anopheles gambiae* and *Rhodnius prolixus*.[1, 2] Since triatomines ingest sterile blood, there seems to be no necessity for intestinal antibacterial compounds. However, triatomines swallow air before moulting, offering air-borne bacteria access to the intestine. In addition, the development of triatomines strongly depends on possessing endosymbiotic bacteria, which they obtain via coprophagy. These bacteria multiply after blood ingestion in the cardia and stomach. The passage of the blood from the stomach to the digesting small intestine causes considerable destruction of symbiont populations, and only about 0,01% of the total population is still present in the rectum.[3] Since this development can not be correlated to the activity of lysozyme we are investigating other antibacterial compounds, e.g., defensins.

We have isolated and characterized from the intestine of *T. brasiliensis* the cDNA encoding a defensin gene. The complete nucleotide sequence of 282 bp was amplified by PCR using degenerated oligonucleotides derived from the known amino acid sequences of defensins A, B and C from *Rhodnius prolixus*. RACE was used to amplify the 5'- and 3'-end of the defensin encoding cDNA. The overall amino acid identity between *T. brasiliensis* and *R. prolixus* defensin was ca. 75%. The deduced protein has a size of ca.10 kDa with a putative signal peptide after the amino acid residue *Ser*-19 and an activation cleavage site at *Lys*-50.

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VE10 - EVALUATION OF ITS2 POLYMORPHISM IN *TRIATOMA* SPECIES BY PCR-RFLP

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The internally transcribed spacer 2 (ITS2) of the ribosomal DNA (rDNA) of four *Triatoma* species; *Triatoma arthurneivai*, *T. sordida, T. infestans* and *T. tibiamaculata* was evaluated by restriction fragment lenght polymorphism (RFLP).

T. infestans is considered to be the main vector of Chagas disease in the South America countries, showing a closely association with the man. *T. sordida* is also recognized as an important domestic transmitter. Although *T. arthurneivai* and *T. tibiamaculata* remain in sylvatic ecotypes as secondary vectors, they are competent *T. cruzi* hosts being able to invade houses and to attack man.

T. tibiamaculata previously considered member of the *T. infestans* complex, has its status questioned in accordance with recent mitochondrial DNA (mtDNA) study, showing a tendency to cluster in the vicinity of the *Panstrongylus* clade. The same study includes *T. arthurneivai* within *T. sordida* complex.

In contrast to *T. infestans* and *T. sordida*, *T. arthurneivai* and *T. tibiamaculata* do not have its ITS2 studied.

ITS2 has been shown to be a excellent marker for sistematic and phylogenetic inferences: it accumulated a very substantial degree of structural diversity during evolution, possess great number of copies per cell and display specie homogeneity.

The DNA was extracted from six macerated legs of each live specimen using a protocol of an phenol-cloroform extraction procedure. DNA quantification and purity analysis was performed by spectrophotometry.

ITS2 amplification was carried out by Heminested-PCR. The first reaction was performed in higher hybridization stringency using the primers reported by Bachellerie and Qu: forward 5' GTGAACCTGCGGAAGGATCA and reverse 5' ATCCTGGTTAGTTTCTTTTCCT. The second series was carried out with primers: forward 5' GTCGATGAAGAACGCAG and reverse5' ATCCTGGTT AGTTTCTTTTCCT. For both reactions the amplification parameters and conditions were standardized.

The products obtained of approximately 400 bp were tested against a set of restriction enzimes: AccI, EcoRV, HaeIII, HhaI, HinfI, RsaI, XbaI. The digestion profile were resolving in polyacrylamide gel (10%) visualized by silver staining.

HinfI restriction patterns was the same as waited for *T. arthurneivai* and *T. sordida.* Moreover, *T. tibiamaculata* showed identical profile (4 bands). Although *T. infestans* showed distinct restriction sites (only 3 bands). After restriction with HaeIII and HhaI *T. arthurneivai* and *T. infestans* coincided in its profile (3 and 2 bands respectively) leading a not expected result and displaing a possible ITS2 relationship between these species. *T. infestans* ITS2 still presents restriction sites for RsaI and HhaI, which is not seen in other studied species. Other tested enzimes had not cut the sequence.

These preliminary findings show that ITS2 PCR-RFLP method could be a new and useful tool for sistematic and phylogenetic studies being relatively less laborious and expensive than the sequencing.

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VE11 - LYSOZYMES OF *TRIATOMA INFESTANS*

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Lysozymes act in many animals as part of the non-specific immune response against bacteria. In some animals, which ingest large amounts of bacteria, lysozymes have been adapted to digestive purposes and are produced in the intestine and salivary glands, e.g.*Drosophila melanogaster* or *Anopheles gambiae*. Since triatomines ingest sterile blood, there seems to be no necessity for intestinal lysozymes or other antibacterial compounds. However, like all insects, triatomines swallow air before molting, offering air-borne bacteria access to the intestine. Triatomines also possess symbionts, which they obtain from the faeces of other bugs, i.e. via coprophagy. The symbionts strongly multiply after blood ingestion, mainly in the two anterior midgut regions cardia and stomach. The passage to the digesting and resorptive small intestine causes a considerable breakdown of the symbiont population, and only about 0.01% of the total population is present in the rectum.[1] This development can not be correlated to the lysozyme activity which increases parallel to the number of symbionts in the stomach and is much lower in the small intestine.[2]

Using fifth instars of *Triatoma infestans*, the pH-optimum of antibacterial activity was determined by the lysis of *Micrococcus luteus* cell walls in substrate plate tests. Using haemolymph and homogenates of fatbody, cardia, stomach, three parts of the small intestine, rectum and salivary glands, the activity of all samples showed an optimum at an acidic pH, while only the big, transparent salivary gland D1 and the little yellow salivary gland D2 possessed a second optimum at a basic pH.

After using degenerate oligodeoxyribonucleotide primers and obtaining a 174 bp fragment by PCR-amplification, a cDNA gut library of *T. infestans* was screened with this fragment. A clone containing the 3´-end was isolated. The 5´-end was amplified via RACE. Sequencing of the complete lysozyme cDNA revealed a deduced 417 amino acid sequence with high identity (40-50%) with other chicken-type lysozymes. The expression pattern of the lysozyme gene in the digestive tract of the bugs at different molting and feeding stages showed that this gene was upregulated directly after the molt and after feeding.[3] Investigating the expression of lysozymes in all parts used for activity tests via PCR amplification with specific lysozyme-primers, we obtained DNA-fragments by using cDNA of cardia, stomach, the final part of the small intestine and the salivary glands D2 and D3. Sequencing indicated that different lysozymes are expressed in the gut and salivary glands.

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VE12 - USE OF MOLECULAR MARKERS TO STUDY GENETIC VARIABILITY IN THREE POPULATIONS OF *TRIATOMA INFESTANS.*

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Sweden. 3.PROIMI. San Miguel de Tucuman, UNT. Argentina

The Triatominae is a subfamily of the Reduviidae that is composed of 14 genera and 118 known species. All of them are obligated bloodsuckers, regardless of age and sex. More than half of them were proven to have the ability to carry *Trypanosoma cruzi*, the flagellate that causes Chagas disease. The most important vectors of Chagas disease are *Triatoma infestans*, *Triatoma brasiliensis*, *Triatoma dimidiata*, *Triatoma sordida*, *Rhodnius prolixus* and *Panstrongylus megistus* (Schofield 1994). They are distributed from eastern and southern Brasil and from the southern half of Bolivia, down to the Argentinian province of Chubut. They are also present in Paraguay and in the largest part of Uruguay. At the east of the Andes, they can be found in northern Chile and Southern Peru. The species are almost exclusively domestic and peridomestic. Sylvatic colonies are only reported from Bolivia.

In the past decade there has been a remarkable increase in the use of genetic markers to characterize genetic diversity in different species. Some of these genetic markers have a different molecular basis, but all of them are focused to understand the organization of genetics structure of natural and cultivated populations. In additions, these markers have been used to determine the genetics similarity among and within populations avoiding environmental influence.

In this work we show the results of a genetic diversity study on intra an inter-population of *Triatoma infestans*, collected from three different cities in the Argentinean provinces of Cordoba, Catamarca and Mendoza. To obtain fingerprints of each populations a total of 38 RAPD primers belonging to the OPA, OPI and OPB series were assayed. Bands were recorded in the binary form i.e (1)=presence and (0)=absence, and assembled in a data matrix table. The UPGMA algorithm was used for hierarchical cluster analysis. Pairwise comparisons were calculated using Simple Matching (SM) coefficient. In addition a dendrogram was built using NTSYS-pc package (Rohlf 1990).

VE13 - FEEDING BEHAVIOUR OF *TRIATOMA BRASILIENSIS*: INTRAVITAL MICROSCOPY ON HAIRLESS MICE

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The feeding behaviour of *T. brasiliensis* on mice was analysed through intravital microscopy. Second (n=19) and third instars nymphs (n=10) were fed on the ear of hairless mice previously anaesthetised. The mouse ear was gently extended over a transparent platform and the animal was then placed under an optical microscope. Microscopy images were recorded by a charge-coupled device video camera and transferred to a video system for off-line analysis. The mean number of bites was 2.6±0.3 bites per insect. The analysis of the images showed that the probing time depends on the proximity between the bite and vessel. Concerning the type of vessels used for feed, 74% of the nymphs fed on venules, 12% on arterioles and 15% on vessels that could not be identified. It was not observed vasodilatation in any of the assays. However, in 43% of the cases it was observed vasoconstriction in the vessel where the mouthparts were inserted. In 62% of the assays where vasoconstriction occurred, this phenomenon was also observed in a vessel near to that in which the insect was feeding. Haemorrhage was observed in 48% of the assays, mainly during the probing time or after the withdrawal of the mouthparts. Interruptions during the bloodmeal were observed in 25% of the experiments. Usually, when interrupted, the triatomine searched for another vessel. The experiments performed through intravital microscopy add information to the study of triatomine feeding behaviour allowing a broad vision of the phenomena that occur during the bloodmeal in mammal hosts.

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VE14 - SYNANTHROPIC TENDENCY OF TRIATOMINAE VECTORS OF TRYPANOSOMATIDAE IN NORTH WESTERN PERU: VECTOR CAPACITY OF *RHODNIUS ECUADORIENSIS, TRIATOMA CARRIONI, PANSTRONGYLUS CHINAI AND PANSTRONGYLUS RUFOTUBERCULATUS*

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The development of strategies for the adequate control of the vector transmission of Chagas Disease depends upon the availability of updated data on the species of triatomines present in each region, their geographical distribution, natural infection by *Trypanossoma cruzi*, eco-biological characteristics and, synanthropic behavioral tendencies. This paper summarizes and updates, critically, information available in published reports and obtained by our field and laboratory studies over the last three years in the North-Western region of Peru. Resulting from these observations is the realization that three triatomine species exhibit a strong synanthropic behavior and vector capacity, being present into domestic and peridomestic environments: *Rhodnius ecuadoriensis*, *Panstrongylus herreri* (synonymus of *Panstrongylus lignarius*) and *Triatoma carrioni*. The first is the only *Trypanosoma rangeli* corroborated vector, but with sporadic natural infection by *T. cruzi* a situation that apparently has not changed in the last two decades. *P. herreri*, with populations in active geographical expansion, continues to be the most effective vector for *T. cruzi* and of human Chagas Disease in the North-Western region. Currently, *Triatoma carrioni* spreads itself out very quickly in the domiciles of the Sullana and Ayabaca Provinces of Piura Department. The existing range of dispersion toward the south of the country and natural infection by *T. cruzi* are unknown. The three species should be given continual attention by Peruvian public health authorities. The possibility of a favorable vector control strategy would be guaranteed if all of them did not have silvatic populations within the ecosystems in which they are distributed. *Panstrongylus chinai* and *Panstrongylus rufotuberculatus* are bugs with an increasing potential in their role as vectors according to their demonstrated synanthropic tendency, wide distribution, broad ecological valence and, trophy eclecticism. Remains to be proved the real epidemiological role of *Panstrongylus geniculatus*. We do not have explanation yet for the apparent absence of *Triatoma dimidiata* from the previously reported geographic distribution in Peru. There is a pressing need to carry out studies on the genus *Rhodnius* species,to evaluate their present Trypanosomatidae vector capacity in the Peruvian North-Eastern Amazon.

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VE15 - THE SERIOUS PROBLEM OF BOLIVIA: CHAGAS DISEASES AND THE BUGS: *TRIATOMA INFESTANS*

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Chagas disease is one of the most serious public health problems in Bolivia, in terms of its magnitude and its social impact. The regions at risk represent 60% of the Bolivian territory and cover more than a half of the municipalities(André Medici,et.al2001). Recall that in 1998, the population at risk was estimated to be more than 4 million people, which would require to

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spray about 700 thousand households, near 40% of the national total. The population, the number of households and localities in areas at risk of Chagas.Up to three and a half million Bolivians are at risk or already infected with *T. cruzi*, the protozoan parasite that causes Chagas disease. Because treatment options are limited and no vaccine exists, vector extermination and elimination of vector habitats in and around houses are the most effective control measures (Arata et al. 1994). The prevalence of Chagas disease in Bolivia is highest in rural areas, where poverty, lack of education, and poor housing favor infestation by the Triatomid bugs (vinchucas) that carry *T. cruzi*. Baseline surveys (1991) revealed *T. cruzi* sero-prevalence rates in humans ranging from 40 percent to 80 percent in these areas, with 38 percent to 78 percent of the homes infested with the vector, *Triatoma infestans* (Klug, 1834). Over 30 percent of the insect vectors captured in and around the houses were infected with *T. cruzi* (Arata et al. 1994). In Los Tiempos Journal in april 13th in 2003. In Bolivia 1 of 2 boliviens can have chagas'disease and 60% of the country have these disease and 6 of the nine departments. The ideas it was wrong because in the first time, chagas'disease it is not only a problem in rural areas in Bolivie. The chagas'disease is so much important in the great cities too, as Cochabamba, In this city the risk with this infection is so much hight as the rural areas, is increivel that there is in new houses and hight buildings in the center of the city. Although the direct vector infection represents around 82% of all cases(André Medici et. al.2001)

The intense rural-urban and inter-departamental migration in Bolivia show for us the magnitude and its social impact in our country.

Our results show for us that the porcent of infestation in the different homes in the different regions at risk in cochabamba Bolivie were the next: Sacabamba(0,2%9), Anzaldo (2,2%), Tarata (1,0%), Arbieto (2,0%), Aiquile (6,0%), Pasorapa(10,8%), Omerque (14,3%), Mizque (5,4%), Vila Vila (2,9%), Capinota (7,4%), Santibáñez (10,9%), Sicaya (12,7%), Tapacarí (7,5%), Punata (14,3%), San Benito (10,0%), Cliza (3,7%), Tolata (4,3%), Toco (3,2%). The principal results were: Endemics areas (60%), Province with bugs (83), Municipalities with bugs (168), Comunities with bugs (10.321), homes with bugs (700.000), population in risk (4.800.000) seroprevalence(40%), chagásic Cardiopathy (15 a 28%), principal vector(*T.infestans*), Congénital cases (9%).

VE16 - NATURAL PARASITISM OF TRIATOMINAE EGGS AND CONSERVATION IN LABORATORY CONDITIONS

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Telenomines are parasitoids of the eggs of other insects in the orders Lepidoptera, Hemiptera, Homoptera, Diptera, and Neuroptera. Some 800 species are presently recognized, but this probably represents at most half of the true number. In our insectary of Inmunoparasitology and chagas'disease laboratory, Biology Department in San Simón Univeristy we maintain a population of the microhymenoptera *Telenomus sp.* endoparasitoid of the eggs of several species of Triatominae (Hemiptera). This insect (*Telenomus sp*.) was inside the eggs of the *Triatoma infestans*, the principal Bolivian vector. Among the 780 eggs examined was found that *T. infestans* egss, were parasitised by *Telenomus sp,* and the temperature that we maintained the telenomus it is in 25 centigrades and 40% atmosphere In 1990 Fernandes et. al. observed parasitoid/egg average was10% in *T. infestans* the present report has special importance considering the real possibility of *Telenomus sp* infestation due to the access of infested triatomine eggs from field captures inducing great damage. These eggs are carefully examined, isolated to study the biology, ecology, and taxonomic are reviewed. Suggestions for greater use of this parasitoid and research needed for improving its biologic control capabilities in the field. Also, the future successful use of *Telenomus sp* to control populations of *Triatoma infestans* and other bugs of the triatominae family. For this our proposal is to do first a strong study inside the laboratory and after we want to go in the little towns in our city Cochabamba and show the effectivite capacity of the telenomus in the biologic control, for this our interested is try to work with some groups interested to study this Hymenoptero. Our city Cochabamba is considered one of the cities in Bolivia, with more infestation,the population at risk consists of the population that lives from 300 to 3.500 meters above sea level, which corresponds to the population in the departments of Tarija, Chuquisaca, Cochabamba, Santa Cruz and part of that of Potosí and La Paz (IDB, 1998). In 1992, the estimate of the population at risk was 3.5 million persons; Although the direct vector infection *T.infestans* represents around 82% of all cases, the intense rural-urban and inter-departamental migration in Bolivia leads to the additional transmission through blood transfusion (15% of the cases). An evaluation carried out in 1994 for Carrasco et. al. showed that the seroprevalence of Chagas in blood banks reaches very high magnitudes in the Departments of Santa Cruz (51%), Tarija (41%), Sucre (39%), Cochabamba (28%), Potosí (24%) and smaller proportions in La Paz (5%) and Oruro (6%).

VE17 - DETERMINATION OF LIPID STORAGE VARIATION IN THE FAT BODY OF *RHODNIUS PROLIXUS* DURING THE DAYS AFTER A BLOOD MEAL

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In the days after a blood meal, *Rhodnius prolixus* addresses large amounts of lipids to the developing ovary where a great number of eggs are produced in relatively short time. The fat body is an organ, which concerns functions of both liver and adipose tissues from vertebrate organisms. When tissue needs are suppressed, during digestion, exceeded lipids are transported to fat body to be stored as triacylglycerol, the main lipid form stored by insects.

The objectives of this work were to determine the variation of lipid storage in fat body during the days following a meal and to analyze the effect of decapitation on this process. Fat bodies from females, males or decapitated males in different days after a blood meal were dissected, lipids were extracted and the amounts of triacylglycerol were determined by TLC. Our results showed that females in the 2º day after blood meal presented the triacylglycerol level of 58 mg / fat body, which increased to around 250 mg / fat body in the 4º day. Triacylglycerol amount was stable until the 13º day, when levels of lipids regularly decreased, reaching about 30 mg of triacylglycerol / fat body in the 20º day. Male storage showed a different profile, increasing gradually until the 10º day, when triacylglycerol was around 460 mg / fat body. After that, levels decreased reaching 200 mg of triacylglycerol / fat body in the 20º day. We can conclude that males store more lipids then females during all digestion cycle. This is probably because males have a metabolic demand smaller than females (responsible for egg production) and also have a slower digestion.

In order to investigate a possible involvement of factors released by head in the accumulation of lipids by the fat body, a few hours after feeding, insects were decapitated and in different days after blood meal the amount of triacylglycerol in the fat body was measured. Results showed that, even without head, insects were able to incorporate and store lipids, achieving the maximal value of 430 mg of triacylglycerol / fat body on the 13º day. Considering these results, we can exclude the possibility of a hormonal factor from head to be involved in the storage of lipids by fat body.

VE18 - CHARACTERIZATION OF LIPASE ACTIVITY FROM THE FAT BODY OF *RHODNIUS PROLIXUS*

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In insects, the fat body is the main site of storage of lipids and that are accumulated in the form of triacylglycerol (TG). Lipids are transported by lipophorin, a major hemolymphatic lipoprotein, to the different tissues and the interaction of lipophorin with fat body, as well as, midgut and oocytes is mediated by specific binding sites at cell surface. After a blood meal, *Rhodnius prolixus* lipophorin takes up lipids from the midgut and transports them to the fat body. This transfer of lipids is greater in the fourth day after blood meal, as well as, the binding of lipophorin to this organ. During digestion of blood the fat body accumulates lipids and this reserve is maintained at the same level until the thirteenth day, when the amount of lipids decreases. These results suggest that a TG-lipase is probably involved in the control of lipid mobilization in the fat body of *R. prolixus*.

In order to study TG-lipase activity from the fat body of *Rhodnius prolixus*, females ten days after blood meal were dissected and the fat bodies homogeneized. The homogenates were centrifuged at 20,000 x g for 30 min at 4°C and infranatants were used as enzyme source. The infranatants were incubated with radiolabelled triacylglycerol (^{3}H-triolein) in the presence of Triton X-100 and unlabelled triolein, and the amounts of released fatty acids were determined for lipase activity characterization. The optimal concentration of Triton X-100 for determination of lipase activity was 26 mM. The time course of lipase activity was linear for at least 120 min of incubation, suggesting that the enzyme was stable under incubation conditions used. NaF and ATP, known inhibitors of TG-lipase activity, were capable to inhibit the lipase in 30% and 54%, respectively. The study of triacylglycerol hydrolysis is important for the understanding of the process of lipid storage and mobilization in the fat body of *Rhodnius prolixus*.

Supported by CNPq and Faperj

VE19 - BIOLOGICAL PARAMETERS DURING THE OVIPOSITION PERIOD OF *PANSTRONGYLUS MEGISTUS* (BURMEISTER, 1835) (HEMIPTERA-REDUVIIDAE) FEMALES AND EGGS MORPHOMETRY.

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Panstrongylus megistus is a Triatominae that has a significant importance in the transmission of Chagas' disease, with *Triatoma infestans, T. dimidiata, T brasiliensis, T. pseudomaculata, T. sordida* and *Rhodnius prolixus* are the main vectors of *Trypanosoma cruzi* in Americas according to Silveira (1985). *P. megistus* is a specie that remains importance in epidemiology because it persist in residual woods as verified by Barata et al (2000) in Araraquara town. However the biological parameters to the oviposition period of *P. megistus* females are unknown. To try to elucidate that, a project was elaborated that evaluated individually five females of *P. megistus*, which had been mated after the 5th instar ecdise. In this summary are presented the partial results referring to two females. For these females had been evaluated: time of oviposition, egg number ranks for female, as well as the variability of eggs size. The length, width and opercular opening diameter of eggshells were mensured by stereomicroscope Leica MZ APO and QWin image analysis system. The statistical analysis was effected using the INSTAT program. The female 1 values in millimeters are: average length 1,909 with standard deviation of 0,12 maximum and minimum values respectively 2,003 and 1,757; average width 1,387 standard deviation of 0,13 and maximum width of 1,459 and minimum of 1,230; average diameter of 0,650 standard deviation of 0,05 with maximum of 0,704 and minimum of 0,596. For female 2 average length 1,882 standard deviation of 0,09 with maximum of 1,936 and minimum of 1,819; average width of 1,341 standard deviation of 0,12 with maximum of 1,457 and minimum of 1,275; average diameter of 0,642 standard deviation of 0,03 with maximum of 0,660 and minimum of 0,621. The two females had different period of oviposition, one for 11 and the other one 12 times in a period of 57 and 61 days. The maximum and the minimum numbers of eggs for the two females occurred in different dates, during the oviposition were perceived great variability in eggshell mensuration. The diameter average values of the female 2 eggshell opening presented a bigger average than female 1, while the length and the width of the eggs of female 1 presented bigger average than the female 2. The female 2 oviposition was bigger than the female 1 and the female 2 presented born nymphs tax of 61,19% while the female 1 was 10,81%.

VE21 - EFFECTS OF THE LIGNAN ISOLATED FROM *PODOPHYLLUM PELTATUM* ON THE DYNAMIC DEVELOPMENT OF *TRYPANOSOMA CRUZI* IN *RHODNIUS PROLIXUS.*

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Trypanosoma cruzi, the causative agent of Chagas disease is transmitted to mammals by hemipteran insects, the triatomines. This flagellate develops as epimastigote in the gut with differentiates to infective form, metacyclic trypomastigotes in the rectum on the invertebrate host. Herein, we described experiments demonstrating different developmental forms of the *T.* cruzi (Dm28c strain) when the insect vector is orally treated with a lignan (Pp) isolated from *Podophyllum peltatum.* The infection of 5th instar larvae of *R. prolixus* were performed by the insects feeding on blood meal containing parasites as controls and parasites plus lignan (Pp) as experimental group.

The observed results were: (i) *in vivo* experiments with *T. cruzi*, comparing the controls with the group treated with 10 mg Pp/ml, showed that in the crop and intestine, no difference in the *T. cruzi* development was observed at days 5, 10, 15 and 20 after infection; (ii) a high significantly accumulation of epimastigotes forms of *T. cruzi* was detected at day 10, 15 and day 20 after feeding, 92%, 84% and 95% respectively in the rectum of treated insects; (iii) in contrast, *in vivo* experiments control group with *T. cruzi* demonstrated that decreased the population of the epimastigotes forms of the parasites, 88%, 10% and 67% respectively, in the rectum of *R. prolixus*; (iv) low numbers of metacyclic trypomastigotes were observed in the rectum treated with the lignan 1 – 3% at 5 to 20 days post infection (p.i.). The control group presented 2 – 21% of the metacyclic trypomastigotes forms of *T. cruzi*; (v) division stages of the parasites were not observed in the rectum of the treated insect. These results suggest that lignan (Pp) of the *Podophyllum peltatum* perhaps affect the metacyclogenesis of *T. cruzi* in this bloodsucking vector insects.

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VE22 - CHARACTERIZATION OF AN OVARY TREHALASE ACTIVITY IN *RHODNIUS PROLIXUS*.

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The eggs of insects accumulate various nutrients during oogenesis to be used as nutrient sources during embryogenesis. Large amounts of macromolecules are synthesized and stored in the oocytes. Proteins and lipids are mainly produced by extra ovarian organs and sequestered by developing ovary. Carbohydrate are taken up by the oocytes and are stored as glycogen. Hemolymph trehalose appears to be the major source for glycogen synthesis, since this disaccharide is the predominant sugar in the hemolymph of most insects. In *Rhodnius prolixus*, we showed that glycogen was accumulated during oocyte growth and utilized during embryogenesis. The major increase in glycogen content occurred when oocytes grew from 1.0 to 1.5 mm in length and, in fertilized eggs, this content decreased after oviposition until 15th day. A trehalase activity was identified in the ovaries of vitellogenic females and in this work we describe the kinetic characterization of this activity. At 7th day after blood meal the ovaries were dissected, washed, homogenized and trehalase activity was determined. This activity was linear with time and protein concentration. Trehalase activity showed a Michaelis-Menten profile and the apparent K_m was estimated to be 1.5 mM. Activity was maximal at pH 4.5 - 5.5 and metal ions had no significant effect on it. Thus, it is possible that this activity is involved in the uptake and hydrolysis of hemolymphatic trehalose by the oocytes to provide glucose for glycogen synthesis, to be used by embryo during its development.

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VE23 - LIPID DIGESTION IN *RHODNIUS PROLIXUS* MIDGUT: CHARACTERIZATION OF A TRIACYLGLYCEROL LIPASE ACTIVITY

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One of the major functions of the midgut is to digest dietary lipids, and absorb and process the digestion products for export into the hemolymph. Triacylglycerol is a major lipid component of the diet and the main form for fatty acid storage. The digestive process has been characterized to suggest two models of lipolysis in the midgut lumen, the complete hydrolysis of triacylglycerol to fatty acids and glycerol and the formation of fatty acids and monoacylglycerol. Triacylglycerol lipases are enzymes that preferentially hydrolyze ester links of triacylglycerols and act only on the water-lipid interface. Insect midgut triacylglycerol lipases have been studied in few insects and only in crude preparations. The data suggests that these enzymes preferentially release fatty acid from the 1- and 3- positions, and show a preference for unsaturated fatty acid, and are activated by calcium ions, thus resembling the action of mammalian pancreatic lipases.

In order to study the triacylglycerol lipase activity from the midgut of *Rhodnius prolixus*, two days after blood meal midguts were dissected from adult females and luminal contents was removed. After this, midgut tissue and luminal content were homogenized and incubated with radiolabelled triacylglycerol (^{3}H-triolein) in the presence of Triton X-100 and unlabelled triolein, and the amounts of released fatty acid were determined for the lipase activity characterization. The optimal concentration of Triton X-100 for determination of lipase activity was 0.26 mM and the ratio of molar concentration of triolein to Triton X-100 was calculated to be about 1:130 mM. The time course of lipase activity exhibited linearity for at least 120 min of incubation, suggesting that the enzyme is stable under incubation conditions used. The amount of free fatty acids released was proportional to the amount of homogenate added, thereby indicating that the release of fatty acids was due to lipase activies. The study of triacylglicerol hydrolysis is important for the understanding of the process of lipid digestion in the midgut of *Rhodnius prolixus*.

Supported by CNPq

VE24 - VE-FURTHER INVESTIGATIONS OF LIPID TRANSFER FROM MALES TO FEMALES DURING MATING IN *RHODNIUS PROLIXUS*.

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Lipophorin is the major lipoprotein in insect hemolymph. It is known to transport lipids of many classes between tissues involved in lipid absorption, storage and utilization. In *Rhodnius prolixus* lipophorin (Lp) transfers lipids to the testis and can be reloaded in fat body and midgut. In this work we are studying the transfer of these lipids from males to females during mating.

Virgin males were injected with Lp labeled in neutral lipid moiety with ^{3}H (^{3}H-Lp) and put together with virgin females. After five days, testis and female spermatheca were dissected and radioactivity estimated. It was demonstrated that ^{3}H-lipids were taken up by male testis and transferred to female spermatheca during mating. When Lp labeled in phospholipid moiety with ^{32}Pi (^{32}P-Lp) was injected, no transfer to females was observed. The ^{3}H-lipids transferred to spermatheca during mating were analyzed by a thin-layer chromatography. Diacylglycerol, triacyglycerol, and free fatty acids were the major lipids found.

To confirm those results, Lp fluorescently labeled in phospholipid moiety with Texas Red phosphatidilethanolamine (TRPE-Lp) and in neutral lipid moiety with Bodipy palmitic acid (Bodipy-FA-Lp) were injected into virgin males. After mating, testis and female spermatheca were dissected and the fluorescence was analyzed by a epifluorescence microscopy (Nikkon Eclipse TE 300). Both fluorescent lipids were visualized in testis but only green fluorescence was found in female spermatheca.

To examine whether the entire Lp particle or only lipids were transferred during mating, Lp was labeled in apolipoprotein moiety with ^{125}I (^{125}I-Lp). Males were injected with ^{125}I-Lp and put together with females. Five days later, no radioactivity was found in female spermatheca.

Supported by CNPq, FAPERJ, PADCT, Pronex.

VE25 - HEME DEGRADATION IN *R. PROLIXUS*: CHARACTERIZATION OF A NEW PATHWAY

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Rhodnius prolixus, a Chagas disease vector, presents protective mechanisms against heme-induced oxidative damages generated by the digestion of blood hemoglobin. One of them is the degradation of heme in tissues such as heart and midgut. Heme degradation is well known in mammals where it produces the isomer a of biliverdin IX (BV), carbon monoxide (CO) and free iron. This reaction is catalyzed by heme oxygenase and it‘s highly specific, producing only the isomer a of BV. BV isomers other than the a are widespread among several organisms but their mechanisms of heme degradation are still unknown. We demonstrated that the product of heme degradation in *R. prolixus* is a BV g bound to two cysteine residues. This new BV compound suggests a novel heme degradation pathway. In this work we began the characterization of this new pathway by the identification of its intermediates. Ten days after feeding, females were injected with heme and Sn-protoporphyrin IX. Hearts were dissected after 48 hours, homogenized and analyzed by reverse-phase HPLC. In this conditions, two peaks eluted after *R. prolixus* BV are intensified, suggesting that they correspond to intermediates of this pathway. Curiously, they present high similarity with heme absorption spectrum, suggesting that both have heme in

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their structure. Purified intermediates were analyzed in an Electronspray Mass Spectrometry for mass determination (ES-MS) or collision-induced fragmentation for structure identification (ESMS/MS). The intermediates have 972 and 794Da. Fragmentation of the ion species corresponding to the intermediates were performed and a specie of same molecular mass than heme was obtained in both cases. The mass difference between the intermediates and heme suggests the addition of two carboxymethylcysteines to the heme molecule before its degradation into BV. Thereafter, these carboxymethylcysteines would be properly processed to cysteines.

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VE26 - PRELIMINARY MOLECULAR CHARACTERIZATION OF RETINOIC X RECEPTOR FROM *RHODNIUS PROLIXUS*

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Retinoids are a class of compounds derived from b-carotene, which play various roles in biological systems. These compounds have been shown to play a central role in different processes such as cellular proliferation and differentiation as well as their involvement *in vivo* as natural morphogens specifying the antero-posterior axis in vertebrate development. Retinoic acid (RA) is one of the retinoids with morphogenetic activity and this is mediated through interaction with specific nuclear receptors named retinoic acid receptor (RAR) and retinoid x receptor (RXR). Both receptors, in turn, regulate the expression of various genes such as the homeobox (*Hox*), which are involved in patterning specification during development. Little is known about the roles and the enzymes involved in retinoids metabolism in invertebrates. The RXR analog *ultraspiracle* (USP) has been already cloned and characterized in some insects such as *Drosophila*, *Bombyx* and *Aëdes*. Based on the fact that the vertebrate blood is a good source of retinoids and that hematophagous organisms usually ingest large amounts of it to reach their nutritional requirements our group is interested to investigate the effects of retinoid supplementation and the characterization of enzymes of retinoids metabolism in blood-feeding arthropods. The main objective of the present work is to investigate the presence of a putative retinoid x receptor (RXR) in the blood sucking insect *Rhodnius prolixus*. Our first task was to assess whether *R. prolixus* possesses a sequence similar to RXR(USP) genes by southern hybridization of genomic DNA obtained from fat bodies and using the DNA-binding domain (DBD) of the RXR1 of *Schistosoma mansoni* as a probe. Under low stringency conditions a unique band was observed suggesting that *R. prolixus* has a sequence homologous to DBD of RXR. Next, total RNA from the fat bodies of blood-fed adult females of *R. prolixus* were extracted and utilized for cDNA synthesis. PCR reactions were performed by using three different combinations of primers designed to amplify amplicons of 80, 170 and 227bp inside the DBD region of RXR. Three products of 80, 170 and 227bp were amplified and subsequently cloned in a TOPO TA system, indicating that possibly these amplicons correspond to the DBD of *R. prolixus* RXR. Attempts to obtain the sequence of these products are currently underway in our laboratory. In conclusion, the preliminary results presented here suggest that *R. prolixus* contains a putative RXR (USP) which may be involved in important biological roles in this blood-feeding insect.

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VE27 - EFFECTS OF RETINOIDS ON THE OOGENESIS, EMBRYOGENESIS AND MOULT OF *RHODNIUS PROLIXUS*

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Retinoids comprise a class of compounds derived from b-carotene, which the most known is retinal. It has been demonstrated that retinoids are implicated in different biological processes such as cellular proliferation and differentiation as well as their involvement *in vivo* as natural morphogens specifying the antero-posterior axis in vertebrate development. Little is known about the metabolism of retinoids in invertebrates, especially in arthropods. Since the vertebrate blood is a good source of retinoids and that hematophagous organisms, usually ingest large amounts of it to reach their nutritional requirements, the main objective of the present work is to investigate the metabolism of retinoids and their possible role in oogenesis and embryogenesis of the blood feeding arthropods *Rhodnius prolixus*. Blood-fed adult females of *R. prolixus* were injected with 60 pmols of all-trans retinoic acid (at-RA), 60 pmoles 9-cis retinoic acid (9cis-RA) or 5,8 nmoles all trans retinol (at-ROH) in the hemocoel. We observed that at-RA accelerates egg laying, while 9cis-RA delayed egg laying. at-ROH injection did not altered egg laying. The viability of these eggs was decreased in at-RA injected insects while at-ROH did not show any effect. The time of hatching was delayed by both at-RA and 9cis-RA, whilst at-ROH did not modify the time of laying eggs compared to control. When nymphs of 5th. instar were injected with at-RA or 9cis-RA, we observed remarkable changes in the external morphology of these insects such as losses of antennae and legs segments as well as changes in leg morphology. Taken together, these results indicate that retinoids may be exerting some effects in oogenesis, embryogenesis and moulting of blood-feeding insects.

Financial support: TWAS, CNPq, Pronex, HHMI, Faperj, FUJB.

VE28 - POPULATION GENETICS OF *RHODNIUS BRETHESI* IN THE BRAZILIAN AMAZON

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In the Brazilian Amazon, Chagas disease has traditionally been considered as a disease of wild animals which circulates solely within the sylvatic foci. However, because of deforestation and human colonization of natural ecotopes, Chagas disease is now seen as an emerging disease in this region. Several cases have been recently reported in the Amazon and 10 of the 18 triatomine species that there occur have been shown to be infected with *T. cruzi*. Of particular interest is the transmission cycle that happens in certain areas of the Rio Negro. In these areas, piassava gatherers will leave their homes to go up tributaries of the Rio Negro after certain locations where there is an abundance of the palm tree *Leopoldinia piacaba*. As these locations are usually far from their homes, they may spend months there collecting piassava fibers. During such periods, at night, these workers experience the "attack" of hungry adult *Rhodnius brethesi* that come flying from the piassava palms to bloodfeed.

We are in the process of investigating two issues regarding the triatomine vector species involved in this transmission cycle, *R. brethesi*, by means of mitochondrial DNA sequence analysis: we want to (1) determine the levels of genetic diversity of natural populations (i.e. wild triatomine populations that have never before been exposed to insecticides); and (2) compare different populations in order to determine the degree of genetic structuring and infer levels of gene flow among them.

We have used live bait traps to collected over 200 insects from several piassava

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palms from six sites along two tributaries of the Rio Negro (Aracá and Padauirí rivers). Geographic distance between individual palm trees sampled ranges from five meters to 200 km. So far 30 insects have been sequenced for a 650 bp region of the mitochondrial cytochrome *b* gene (cyt *b*). Our preliminary findings suggest that, surprisingly, the levels of genetic diversity for these natural *R. brethesi* populations, are extremely low. Among the insects analyzed, 29 share a single haplotype, and one individual has a second haplotype that differs by a single silent substitution. This seems to indicate that these populations have gone through a very recent bottleneck that has drastically reduced the levels of gene variation. We believe that the analysis of a greater number of insects will give us a better understanding of the genetic structure of these *R. brethesi* populations in this area.

Financial support: CNPq

VE29 - EFFECT OF PLATELET-ACTIVATING FACTOR (PAF) IN THE OVIPOSITION AND ECLOSION OF *RHODNIUS PROLIXUS*

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Blood-sucking arthropods, especially insects, have been widely used in biological, biochemical, molecular, pharmacological and immunological studies. The main medical interest of these studies is due to the fact that these arthropods may be vectors of virus, bacteria, protozoan and worms that cause emergent and reemerging diseases, like Lyme disease, malaria, leishmaniasis, Chagas' disease and others. The strategy for controlling these diseases is based upon understanding some molecular aspects of vectors and parasites. Platelet-activating factor (PAF) is a phospholipid involved in diverse biological and pathophysiological processes, like cell differentiation, inflammation and allergy. PAF is produced by mammals, other vertebrates, invertebrates, fungi and protozoan. Previous data from our group showed that PAF induces cell differentiation of *Trypanosoma cruzi* and *Herpetomonas muscarum muscarum*. In the present study we observed the effect of PAF in the oviposition and eclosion of *Rhodnius prolixus*. The insects were artificially fed with rabbit blood in the absence or in the presence of the following modulators: 10^{-6} M PAF, 10^{-6} M WEB2086 (a PAF antagonist) or both 10^{-6} M PAF plus 10^{-6} M WEB2086. There was not significant difference in the number of eggs or in the percentage of eclosion among the four groups of insects. However, a significant number of the eggs from the treated insects led to defective nymphs: 5.6%, 5.3% and 7.7% eggs from PAF-, WEB- and PAF plus WEB-treated insects respectively led to defective first instar nymphs, as compared to the eggs from the control insects, which were all perfect.

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VE30 - ECDYSTEROID IN DIGESTIVE TRACT OF *RHODNIUS PROLIXUS*

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Investigations on the effects of decapitation and azadirachtin treatment on the maturation and organization of the perimicrovillatr membranes (PMM) have been developed in our laboratory. In these studies, we demonstrated that ecdysone is an important hormone to establish *Trypanosoma cruzi* infections and reorganize the *R. prolixus* midgut structures. Herein, we described the results obtained by ecdysteroids radioimmuneassay (RIA) measurements in the stomach (anterior midgut or crop), intestine (small intestine or posterior midgut) and rectum (hindgut) at different days after feeding, treatment with azadirachtin and decapitation of *R prolixus* larvae. The 3 gut compartments were removed from 6 insects, separately homogenized and ecdysteroids extracted with methanol. RIA was performed using ecdysone antiserum, which binds ecdysone and 20-hydroxyecdysone, according to Chang and O' Connor (1979). RIA unit is defined as pg equivalent to ecdysone since this hormone was used as standard.

The main results obtained were: (i) at anterior midgut of control insects ecdysteroids levels began to increase 24 h after feeding and revealed the presence of peak on day 10 after feeding (10 ng/crop); (ii) at the posterior midgut the ecdysteroid peak occurred on day 11 after feeding (12 ng / intestine); in the rectum the peak was of 3 ng/ rectum at day 11 after feeding; (iv) decapitation and azadirachtin treatment drastically reduced the levels of ecdysteroids in the 3 gut compartments during the entire experiment.

Some dates of our lab have shown that *T. cruzi* attachment in the gut is important for both differentiation and multiplication (Garcia and Azambuja, 1991). Gonzalez et al (1999) demonstrated that azadirachtin treatment and decapitation drastically decreases the rate of shedding of the PMM. We now are developing gut cellular cultures to investigate the biosynthesis of ecdysteroids in vitro. The significance of these results will be discussed in relation to the success of the establishment of *T. cruzi* infection in its vector, *R. prolixus*.

This work was supported by grants from Conselho Nacional de Desenvolvimento Científico e Tecnológico, Fundação Oswaldo Cruz (Papes) and PADCT. PA; ESG, CBM and MSG are CNPq, and DF is Faperj/ Fiocruz research fellow.

VE31 - VE31 - DOSAGE OF HEMEPROTEINS IN THE SALIVARY GLANDS OF RHODINIINI SPECIES

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The saliva of the hematophagous insects presents a series of biological activities that interferes not only in the haemostatic mechanism (platelet aggregation, blood clotting and vasoconstriction) as in the inflammatory reaction and the immune response of the host. The nithrophorins are the most abundant salivary proteins in the Rhodniini tribe. These proteins present a heme group, which binds reversibly to the NO. The effects of the nitrophorins are vasodilation, due to the liberation of the NO in the skin of the host, anticlotting and antihistaminic activity. The microdosage of hemeproteins was standardized from the metahemoglobin cyanide methodology based on a clinic test for hemoglobin dosage (Doles reagents ®). The following species were used in the present study: *Rhodnius prolixus*, *R. neglectus*, *R. nasutus*, *R. robustus* and *Psammolestes tertius*. The samples were constituted by the content of four salivary glands diluted in 25mL of water. In the hemeprotein dosage 20 mL of the samples were combined with 200 mL of the Color Reagent (Potassium phosphate 1 mM; Potassium ferricyanide 0.6 mM; Potassium cyanide 0.77 mM and Triton X-100 0.82 mM). After three minutes, 200mL of this solution was transferred to a microplate and read at 550 nm. The standard used for the dosage was equine myoglobin and the results obtained with saliva were expressed as myoglobin equivalents. The results obtained in mg of myoglobin equivalent were: *R. prolixus* (10.96 ± 1,10); *R. neglectus* (14.79 ± 1.07); *R. nasutus* (19.73 ± 4.75); *R. robustus* (36.24 ± 4.34); *P. tertius* (8.54 ± 1.02). In order to corroborate the results further tests will be performed using graphite furnace atomic absorption spectrophotometry.

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VE34 - *TRYPANOSOMA RANGELI* UPTAKES THE MAIN LIPOPROTEIN FROM *RHODNIUS PROLIXUS*

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Trypanosoma rangeli is a hemoflagellate that employs a wide variety of mammalian hosts and hematophagous insects in its life cycle, including *Rhodnius prolixus*. *T. rangeli*, when infects invertebrate host, penetrates the gut epithelium reaches the hemolymph where they can obtain resources for their metabolism. In insects, lipophorin (Lp) is the major hemolymphatic lipoprotein, which carries and distributes lipids. We have previously demonstrated the Lp uptake by *T.rangeli*. In this work we observed the fate of this lipoprotein in *T. rangeli* epimastigotes. In adition investigate the process of Lp endocytosis in *T. rangeli*.

T. rangeli was incubated with [Texas Red Phosphatidylethanolamine]-Lp for 1h. Parasites were chased in a medium free of fluorescent Lp for different times the fluorescence was analyzed by microscopy. Lp was localized in, anterior region of the cell, i.e. close to the flagellar pocket, and in vesicles at the posterior region. Suggestive that fluorescence observed in parasites was derived from Lp lipids. *T. rangeli* group was incubated with H^3-palmitic acid for 24 hours. After lipid extraction the lipids were analyzed by thin-layer chromatography, followed by plate exposition with phosphoimager screens. We observed that the parasites incorporated radioactivity. The free fatty acids were utilized for *de novo* lipids synthesis. Cholesterol-ester, TG, DG and phospholipids were the major lipids found. The presence of specific Lp receptor in the parasites was determined. *T. rangeli* was incubated at 28ºC or 4ºC, with I^{125}-Lp. Endocytosis of I^{125}-Lp, by *T. rangeli* at 28ºC was higher than at 4ºC, and an excess of BSA did not affect the process. Unlabeled Lp was able to abolish the I^{125}-Lp binding in a concentration dependent manner. These results suggest that *T. rangeli* is able to receive lipids from Lp and Lp uptake is mediate by a specific receptor.

Suported by CNPq

VE35 - SOLUBLE FACTORS WITH ANTIMICROBIAL ACTIVITIES ASSOCIATED WITH THE EGGSHELL OF *RHODNIUS PROLIXUS.*

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The great evolutive success of the insects can be attributed in part to its capacity of reproducing in a relatively short period time. The egg that is laid in nature has to be protected against mechanical damage and also against microorganisms. The first barrier is physical, and when this barrier is exceeded, diverse chemical factors are set to ensure the elimination of the pathogen. However, the mechanisms of antimicrobial protection associated with eggs are not well known. It was previously described the presence of antimicrobials peptides associated with the exochorion of insects, secreted by the female accessory gland of *Ceratitis capitata* (Marchini and cols., 1997), by the male accessory gland of *Drosophila melanogaster* (Lung and cols., 2001) and by salivar glands of *Pseudacanthoterme spiniger* (Lamberty and cols., 2001). Previous studies, carried out in our laboratory identified an water insoluble protein of 45 kDa, constituent of chorion with antifungal activity. Here, we present the dose dependent inhibition of 45 kDa protein against *Aspergillus niger* AD 102. Besides that we describe here the presence of a water soluble proteins (possibly peptides) associated with the egg, with antifungal activity, of *R. prolixus*. The soluble proteins were extrated by washing the eggs in water or PBS and concentrated. These proteins, extrated from *R. prolixus* eggs were assayed in a microplate with 96 wells containg *A. niger* AD 102 in a Potato-Dextrose medium or incubated on slides into Petri dishes. The results clearly shows the presence of factors capable of inhibiting the growth of fungi. The concentration of 1,25 mg/ml of *R. prolixus* extract inhibited 60% of normal growth in 48 h of incubation. The factors are thermolable.

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VE36 - INTERACTION OF *PHYTOMONAS SERPENS* WITH THE SALIVARY GLANDS OF THE INSECT VECTOR *ONCOPELTUS FASCIATUS*

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Some members of the family Trypanosomatidae present great medical, veterinary or economical importance. *Phytomonas* spp are parasites of plants and invertebrates. Some of these species are the causative agents of plant diseases in plantations of economical importance, such as coconut, coffee, oil palm and several fruit. In 1957 Gibbs found flagellated parasite in tomatoes (*Solanum lycopersicum*), which was denominated *Leptomonas serpens* and later *Phytomonas serpens*. However, little is known about the pathogenicity of *P. serpens* for either plants or insects. Dipteran and hemipteran insects are involved in the transmission of trypanosomatid parasites of plants. The phytophagous hemipteran insect *Oncopeltus fasciatus* is the natural host of *Phytomonas elmasiani*. The colonization of the salivary glands of the vector is a major event in the life cycle of *Phytomonas* spp. In the present work, the salivary glands of *O. fasciatus* were extracted from the bodies and the dissected parts were washed with PBS pH 7.2 previously to the interaction assay. The parasites were harvested and washed with PBS pH 7.2. These protozoans were allowed to interact with the dissected salivary glands. In this work parasites of the species *P. serpens* adhered tightly to cells of the dissected salivary glands of *O. fasciatus*. Taking this result into account, we decided to study the molecular aspects of this interaction. Total protein extract of *O. fasciatus* was separated by SDS-PAGE and transferred onto a nitrocellulose sheet, which was exposed to sulfo-NHS-biotin-labeled *P. serpens*. These labeled parasites were able to bind to a protein of 130 kDa, present in the protein extract of the *O. fasciatus* salivary glands. Further experiments are required aiming the purification and identification of this protein.

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VE37 - PHYLOGENY AND POLYMORPHISM OF THE *KERTESZIA* SUBGENUS ASSESSED BY THE SECOND INTERNAL TRANSCRIBED SPACER (ITS2) OF RDNA.

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Kerteszia is a small subgenus that includes neotropical Anopheles species. Some of these species, such as *An. bellator*, *An. cruzii* and *An. homunculus*, can be sympatric and vectors of human and monkey malaria in Southeastern and Southern Brazil. Another species, *An. laneanus*, has been suspected to be a malaria vector in Bolivia. The identification of *An. cruzii* and *An. homunculus* specimens, based on morphological characteristics, is troublesome. In order to evaluate molecular tools to assess the polymorphisms and phylogeny of the *Kerteszia* malaria vectors we cloned and sequenced their ITS2 and compared them with data obtained from other anophelines.

Adult females (*An. cruzii*, *An. bellator*, *An. homunculus*) and larvae (*An. laneanus*) were collected in the State of São Paulo and identified. DNA extraction, PCR amplification, DNA cloning and sequencing, were performed as previously described (Malafronte et al., 1999). Sequence data were aligned using the CLUSTAL W (1.60) and the phylogenetic trees were constructed by MEGA (Molecular Evolutionary Genetics Analysis ver. 1.01, Kumar et al. 1993).

Analysis of the sequences showed ITS2 regions of those anophelines with lengths varying from 332 to 354 nucleotides and their GC contents varied from 53 to 62,5%. Comparing to *An. cruzii*, the major vectors of Atlantic Forest, the alignment of the ITS2 sequences showed divergences such as 0,5% *An. cruzii*/ *An. laneanus*, 1,5% *An. cruzii*/*An. bellator* and 9,5% *An. cruzii*/*An. homunculus*. Comparisons of the ITS2 sequences also showed differences between the sibling species *An. cruzii* and *An. homunculus*, which can be used as tools for the identification, at the molecular level, of these species.

VE38 - MORPHOLOGICAL ANALYSES OF *ANOPHELES (NYSSORHYNCHUS) AQUASALIS* INFECTED WITH *PLASMODIUM (PLASMODIUM) VIVAX*.

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The human malaria has been considered a serious problem of health in the world. The interaction studies involving New World vectors of the human *Plasmodium* are still very limited. Structural and ultrastructural analysis was accomplished with the objective of knowing the normal aspect and the migration of the *P. vivax* in the *A. aquasalis* midgut. Histological techniques, confocal laser, transmission and scanning electron microscopy were done in order to study the organization of the cellular types, the cell ultrastructure, the kinetics of the modifications of the midgut when blood fed and the establishment of the infection for *P. vivax* following the cycle in the mosquito vector *A. aquasalis*. The alimentary tract of mosquitoes is generally divided into three regions: foregut, midgut and hindgut. The foregut and the hindgut have ectodermal embryological origin, distinctly from the midgut, which is originated from the endoderm. The midgut itself can be divided into two topographic regions according to their location: thoracic and abdominal midguts. The histology showed the midgut composed by a columnar single epithelium of microvillar cells supported by a basal membrane. We observed in both areas of the midgut, a cellular heterogeneity as for the afinity for the staining revealed mainly by the presence of acid components. The external and internal aspects of the midgut presented a regular surface. Secretory vesicles were found frequently in the cells of the thoracic region; the abdominal region, besides revealed cells with vesicles, also showed frequently organelles related with the protein's synthesis. The cytochemical aspects of the apical and basal surface demonstrated the presence of anionic components, as well as, of the carbohydrates. The *P. vivax* infection was determined by the presence of oocysts, which were considered young when they showed sporoblastoid center, organization of sporozoites and irregular wall; mature oocyst presented flat surface and disorganized sporozoites inside. We visualized the escape of the sporozoites from the oocyst, the liberation to the hemocel and the invasion of the secretory cells in the salivary gland of the *A. aquasalis*, which were not synchronically after the infection. Throughout the confocal laser microscopy, we observed ookinete into the epithelium and preferentially in the intercellular space, without to demonstrate alterations in the cytoskeleton, with little modification in the microvilli. The ookinete migrate to the basal portion of the epithelium, where the oocysts modify themselves. This work is showing the details of morphological observations of the interaction using human *Plasmodium* in a New World vector.

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VE40 - BIOCHEMICAL CHARACTERISATION AND PARTIAL PURIFICATION OF A INTESTINAL α-GLUCOSIDASE AFTER SUGAR FEEDING OF THE BRAZILIAN MALARIAN VECTOR MOSQUITO, *ANOPHELES AQUASALIS*.

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The main source of sugar in nature is the nectar, which is composed by the most abundant dissacharide, sucrose. Mosquitoes, in general, feed constantly on sugar, once the digestion of this substance is very important due to its hole on supplying the energy required for female ovarian maturation and wings beating, which affect directly the vectorial capacity of these insects. The hydrolysis of the bounds between a carbohydrate and anyother molecule is carryed out by the glycosidases, and within this group are the a-glucosidases, which may cleave the bounds Glc a-1,2; a-1,3; a-1,4e a-1,6. These enzymes have been already described in midgut and salivary glands of flebotomines, however, in mosquitoes, a-glucosidases activities have been only detected in salivary glands. Molecular analysis have shown two genes that encodes to this enzyme in the midgut of the mosquito *Anopheles stephensi*, but activity assays have not still been done. In the current study, we have identified at least one a-glucosidase after female feeding with 10% sucrose. Others glycosidases have shown not to be present when only sucrose is given to the mosquitoes. Our data have shown that a-glucosidases are present in the female midgut even before feeding of these mosquitoes with sucrose, and it is slightly activated after that. Biochemical characterization has been performed, and the enzyme is present in the midgut, in both soluble and insoluble extracts. The major portion of the activity has been found associated with the microvilli cells, in according to the exohydrolases properties. The optimal pH is around 5.5, and the enzyme is sensitive to the presence of Tris, even though in low concentrations (2 mM). The a-glucosidase activity is also affected by the incubation of the enzyme at 45 ºC. The enzyme has been partialy purified by molecular exclusion (Superdex 200 HR 10/30) and hidrophobic interaction (Resource Phe) cromatographies. In parallel, the polypeptide sequences of a-glucosidases from some insects have been aligned, and degenerated oligonucleotides are going to be constructed, based on the conserved sequence regions, for the gene characterization.

Supported by: FAPESP

VE41 - HEME DEGRADATION IN THE MOSQUITO *AEDES AEGYPTI*

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In hematophagous animals, blood digestion results in the release of peptides, amino acids and the hemoglobin prostetic group heme; a molecule with a high oxidative potential. The independent evolution toward a common set of problems, related with the oxidative stress, has led to some quite independent solution or strategies to deal with heme, in these animals. In some organisms, many of these mechanisms have been described. In *Rhodnius prolixus,* for example, heme is degraded in the midgut epithelium and heart, producing a green pigment which is a modified biliverdin, different from the one produced by the enzyme heme oxygenase present in mammals, plants and bacterias. In *Aedes aegypti*, the vector of dengue and yellow fever viruses, it is known that heme other anti-oxidative defenses were described, as the presence of anti-oxidative enzymes and the peritrophic matrix that has a role in haem binding. In this work, we intend to study the heme degradation mechanism in the mosquito *A. aegypti.* and in a mutant of *Anopheles quadrimaculatus larvae*.

During blood digestion, a large amount of a green pigment is produced and secreted to the intestinal lumen of this mosquito. We have been trying to investigate the chemical nature of this molecule by a liquid chromatography analysis in reverse phase with a C18 column in HPLC, with an acetonitrile gradient (5% to 80%) in a trifluoroacetic acid 0,05% solution. We've compared this pigment with biliverdin and, specially, with the one produced by *R. prolixus*.

It has been shown that *R. prolixus* biliverdin has a higher hidrophobicity comparing with gama-biliverdin. Preliminary analysis showed that *A. aegypti* pigment seems to be more hydrophobic than the *R. prolixus* biliverdin. Additionally, the spectrum of *A. aegypti* pigment is very similar to gama-biliverdin spectrum.

Considering the different chemical nature of these pigments, that reflects divergent haem degradation vias, one can conclude that the insects developed different strategies against blood-feeding challenge.

VE42 - *AEDES AEGYPTI* HEMOLYMPH PROTEINS AND ANTI-DENGUE GENES.

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We are developing transgenic mosquitoes that are resistant to dengue virus to test the hypothesis that genetically-engineered vectors can be used to block transmission of the disease. The focus of our research project is the study of interactions between the dengue virus and its mosquito vector. Conventional approaches to disease control such as drugs, insecticides, or vaccines have been thus far ineffective. Our research aims to understand the molecular mechanisms of virus development and interference with the mosquito host. This research will facilitate the development of new control measures through genetic manipulation of mosquito vectorial capacity.

Recombinant monoclonal antibodies (scFv-Mab; *s*ingle *c*hain *F*ragment IgG *v*ariable region *M*onoclonal *anti*body) are powerful weapons for blocking vector-borne diseases. We have already successfully developed and expressed the variable portion of a Mab (N2-scFv) that prevents *Plasmodium gallinaceum* sporozoites invasion of mosquito salivary glands (de Lara Capurro et. al., 2000). We are currently developing five recombinant scFv-Mab´s that recognize all four dengue virus serotypes. These scFv-Mab´s will be used to test if they can be expressed in transgenic mosquitoes to block virus transmission. The corresponding heavy- and light-chain variable regions encoding the anti-dengue 1A10-2 Mab, 1B7 Mab, 2H2 Mab, 9A Mab and 3H5 Mab were engineered to produce single-chain antibody constructs, 1A-scFv, 1B-scFv, 2H2-scFv, 9A-scFv and 3H5-scFv. We are at the final steps to test the ability of these recombinant antibodies to block transmission in a transient expression system. Moreover, they can provide a valuable tool in the search for the mosquito receptor(s) for dengue viruses.

As a second goal from our research we are studying the expression of hemolymph proteins in dengue infected mosquitoes. To start this approach fat bodies libraries were obtained from females sugar fed and 24 h after blood feed. We start a EST catalog of these libraries. The partial data show that from 200 clones analysed. We have 106 contigs and from these contigs we have at the least 65 new putative proteins.

Supported by FAPESP

VE43 - HYDROGEN PEROXIDE DETOXIFICATION BY CATALASE IN THE MIDGUT OF *AEDES AEGYPTI* AND ITS MODULATION BY HEME

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Aedes aegypti females ingest large amounts of vertebrate blood in a single meal. The hydrolysis of hemoglobin in the midgut lumem releases huge amounts of heme, its prosthetic group. Since free heme can generate reactive oxygen species, which are able to oxidize lipids, nucleic acids and proteins, this blood-sucking mosquito is subjected to an oxidative chalange imposed by blood feeding. To counteract this heme toxicity *Aedes aegypti* possesses antioxidant mechanisms that make possible its survivor, among these is catalase, an antioxidant enzyme that detoxifies hydrogen peroxide into water and oxygen. The importance of this reaction is to avoid hydrogen peroxide interaction with heme or iron leading to hydroxyl radical formation, the most toxic oxygen radical.

Once blood is a source of oxidative stress we fed *Aedes aegypti* with blood, plasma or plasma plus hemoglobin. Different hours after the meal (0, 12, 24, 36 and 44 hours) the peritrophic matrix and the midgut epithelium were dissected, homogenized and catalase activity was measured according to Aebi (Aebi et al., 1984). In the epithelium, this activity showed the same profile for the three kinds of meal, reaching its maximum among 24 – 36 hours after the meal, but was greater in mosquitoes subjected to blood and plasma plus hemoglobin than in mosquitoes subjected to plasma feeding. In the peritrophic matrix we observed a small catalase activity during all the digestive process in mosquitoes fed with plasma or plasma plus hemoglobin. However, in mosquitoes fed with blood this activity was higher 12 and 24 hours after meal.

We inhibited catalase activity from mosquitoes fed with rabbit blood *in vitro* and *in vivo* using different concentrations of 3-amino-1,2,4-triazole, a specific catalase inhibitor. The *in vitro* inhibition profile of the enzyme present in peritrophic matrix was different from the enzyme present in midgut epithelium, indicating that they are different. Probably, the enzyme from the peritrophic matrix is provided by the rabbit blood since its *in vitro* inhibition profile is quite similar to the rabbit blood inhibition profile.

To perform the *in vivo* inhibition assays we fed mosquitoes with blood plus different concentrations of 3-amino-1,2,4-triazole. The *in vivo* inhibition profile from the midgut epithelium was similar to the observed in the *in vitro* experiments. Nevertheless, catalase present in the peritrophic matrix was not inhibited. Since inhibition of catalase by 3-amino-1,2,4-triazole is dependent on hydrogen peroxide, we expect that hydrogen peroxide produced in the epithelial cells doesn't diffuse to the peritrophic matrix, but this hypotesis needs to be tested.

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VE44 - *AEDES* HEMOLYMPH PROTEINS EXPRESSION DURING *PLASMODIUM GALLINACEUM* OOCYST FORMATION.

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Universidade de São Paulo – Instituto Ciências Biomédicas II – Depto. de Parasitologia.

The quantity of vitellogenin (VG), ferritin (FE), lipophorin (LP), lipophorin receptor (LPR), vitellogenin receptor (VGR), transferrin (TF) and carboxypeptidase (CBX) transcripts was measured in *Aedes aegypti* following infection with *Plasmodium gallinaceum*. *Aedes* females were infected with *Plasmodium* by feeding upon infected chicken. After 6 days the females laid its eggs and a second blood meal in a non-infected chicken was taked by the infected *Aedes* females. The total RNA in infected females showed a lower level, when compared to control non-infected females, 24 hours after second blood meal (2^{nd} BM) in carcass and at 48 h (2^{nd} BM) in ovaries.

A lower level of VG mRNA also occurred 24 h (2^{nd} BM). However, by 48 h (2^{nd} BM), higher levels of VG transcripts were detected in fat bodies. The levels of VGR and LPR transcripts showed significant reductions in ovaries. Nevertheless, by 24 h (2^{nd} BM), CBX transcripts were accumulated in fat bodies.

The levels of LP, TF and FE showed no differences between infected and non-infected females. These results suggest that vitellogenesis is affected by *Plasmodium* infection on *Aedes aegypti*.

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VE45 - *AEDES AEGYPTI* MIDGUT IS AFFECTED BY *BLASTOCRITHIDIA CULICIS* COLONIZATION

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The mosquito *Aedes aegypti* is an important vector of human diseases such as dengue, yellow fever and filariasis. The interaction of monoxenic trypanosomatids with hematophagous insects acquired more importance after descriptions of opportunistic infection of currently non-pathogenic trypanosomatids in humans with immunodeficiency and also in immunocompetent patients. The mosquito midgut is the first tissue that pathogens interact and is formed by a single layer of columnar epithelial cells with two distinct morphologies: densely microvillated cells, that are predominant, and less microvillated cells with electron-lucent cytoplasm. Ultrastructural studies of the midgut invasion by *Plasmodium gallinaceum* showed that ookinetes interact with epithelial cells and induces morphological changes as cell swollen, surface blebs and vesicles development. Previous studies have proposed that *Plasmodium* ookinetes invade a specific cell type which do not stain with basophilic dye and is less osmiophilic. Differential interference contrast microscopy showed that ookinetes invade a midgut cell at the lateral apical surface and might lyse it. The mechanism that results in death of invaded midgut cell, necrosis or apoptosis, is not yet clear.

In this work we analyzed by scanning and transmission electron microscopy the colonization of *Aedes aegypti* midgut by *Blastocrithidia culicis*, an endosymbiont-bearing trypanosomatid, which is monoxenic and presents typical features such as a modified cytoskeleton and different surface properties. In this work we extend previous observations of our group showing that *B.culicis* was the endosymbiont-bearing trypanosomatid species which better interacted with explanted midguts of *A. aegypti*. We demonstrated that *B. culicis* was able to survive and multiply in *A. aegypti* guts for >30 days after in vitro feeding. Ultrastructural analysis by scanning electron microscopy evidenced that *B. culicis* interacts with microvillated columnar cells initially via flagellum, then protozoa are observed inserted in the microvilli with part of the cell body. The microvillated columnar cells revealed bare bodies protruding and being released to the gut lumen. Although these cells present a normal cytoplasm electrondensity by transmission electron microscopy, the released bare bodies showed an electron-lucent aspect. The disturbance in the aspect of gut epithelia seems to be associated to the protozoa presence, since it was not observed in uninfected midguts. Protozoa did not penetrate epithelial gut cells and haemocoele invasion was not observed, although destruction of columnar cells may suggest this possibility.

Taken together, results provided by this work are interesting for comparative studies involving pathogens-mosquito models, since *B. culicis*, a non-pathogenic trypanosomatid, promoted an epithelial response in *A. aegypti* midgut similar to that observed for *Plasmodium*.

This work was supported by: CNPq, FAPERJ, FUJB AND PRONEX.

VE46 - XANTHURENIC ACID (XA): A MAJOR MOLECULE IN THE GUT PHYSIOLOGY OF *AEDES AEGYPT*

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Xanthurenic acid (MW 205.2 g/mol) is a tryptophan derivative that was recently (1998) characterized as being the exflagellation factor for the malaria parasite. XA shows a preeminent coordination chemistry (it binds Ca^{2+}, Fe^{2+} and heme). In addition, XA can protect soluble proteins against OH* damage. XA is present in larvae and in saliva of mosquitoes but until now nothing was proposed about what is its function in the mosquito physiology.

In this work, measuring by HPLC, the XA content (identified by it's absorption and mass spectra) of the gut during de course of blood digestion, we are showing that XA is present in the gut in a concentration about ten milimolar. The time course of XA concentration during digestion shows a peak in 24 hours (coinciding with the peak of free heme). Furthermore, we observed that XA can protect azolectin (a soybean phosphatidylcholine-rich fraction) from heme-catalyzed oxidation.

Taken together, our results suggest that XA is an important molecule in the gut physiology of *Aëdes*. It is present in a high concentration in the digestive scenario and interacts with all of the other principal characters: heme, calcium, iron and free radicals. The time course of XA concentration, with a peak at 24 hours after blood meal, indicates that XA is synthesized by the intestinal epithelium, but not derived from the ingested saliva.

VE47 - CHARACTERIZATION OF BINDING OF HEME TO THE *AEDES AEGYPTI* INTESTINAL MUCIN – 1 (AEIMUC1)

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The gastrointestinal tract of adult female mosquitoes possesses an extra cellular structure, the peritrophic matrix (PM), which surrounds the ingested blood and separates it from the epithelial cells of the midgut. We have shown previously that a large amount of heme (a pro-oxidant molecule) is generated during blood digestion, and that this is bound by the PM and subsequently excreted with the feces (Páscoa, V. et al., 2002). This suggests that PM may act as an antioxidant defense and a heme detoxification mechanism.

Little is known about the proteins present in *Aedes aegypti* PM. The first protein described, called *Aedes aegypti* Intestinal Mucin-1 (AEIMuc-1), is a mucin-like protein induced by metal feeding in both larvae and adult, and by blood feeding in adults (Rayms–Keller, A. et al., 2000). Here we show that this protein (herein called B1) is capable of binding large amounts of heme and is a potential candidate to be involved in the role of PM in the adaptation of this insect to blood feeding.

This protein is composed of 3 chitin-binding domains and one mucin-like domain. Deletion constructs were made in order to identify which domains are important for binding heme and assayed using calorimetric and spectrophotometric titration. All constructs tested were capable of binding heme, but with different stoichiometries. The whole protein was capable of binding up to 12 heme molecules per polypeptide chain, 9 of which had absorption spectra similar to the so-called regulatory heme-binding domains, attributed to cysteine and proline motifs. Cysteine-proline motifs are also present in the chitin binding domains of B1 were they form intramolecular disulphide bounds and allow interaction with chitin. Calorimetric assays also indicated that there is more than one type of heme binding site (apparently 3) with different affinities, and indicated that there is a different kind of event happening after saturation of binding sites, suggesting the formation of an aggregate. Together with the fact that B1 was unable to prevent heme toxicity this data suggests that this protein may be acting as a heme aggregation nucleation centre, but this hypothesis needs to be tested.

Circular dichroism spectra were performed in order to investigate whether bound heme changes the secondary structure of this protein. Results indicated that both the whole protein and the constructs have a secondary structure mainly composed of random coil and that bound heme didn't lead to conformational changes.

Supported by: HMMI, Gorgas Memorial Institute, Faperj, Pronex, CAPES, PADCT and CNPq.

VE48 - APPLICATION OF A NEW SURVEILLANCE METHOD OF *AEDES AEGYPTI* IN BOTUCATU, SÃO PAULO.

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The World Health Organization (WHO) keeps searching for effective means on dengue control. The main problem relates to control of the major vector, *Aedes aegypt*i. Currently in Brazil, the control program applied by Fundação Nacional de Saúde/ Ministério da Saúde (FNS/ MS) in *Aedes albopictus* and *Aedes aegypti* populations are larval surveys. There are many problems that compromise the reliability of this technique. An interesting alternative is the using of a new control strategy named PCI (Premise Condition Index). This model relates the property condition, like house condition, yard condition, and degree of shade; to the occurrence of *Aedes* spp. oviposition. The PCI validation is achieved by association for the three property variables (house, yard and shade) calculated in scores from 3 to 9. The lowest scores point to properties in good conditions and unfavorable breeding environment. In opposite, the highest scores prove high risk properties to *Aedes* spp. infestation. The present study is based on the application of PCI in properties located in the urban area of Botucatu to confirm the effectiveness of this new tool. With the support of SUCEN (Superintendência de Controle de Endemias) and Secretaria de Saúde de Botucatu, ovitraps have been set in 105 spots all over the city and their properties qualified. Results showed that 64,7% of properties with scores 8 and 9 were positive to *Aedes albopictus* while only 19% of properties with scores 3 and 4 were positive. This preliminary analysis has demonstrated the accuracy of PCI method, since the major occurrence of *Aedes* spp. has been observed in properties with highest scores. The analysis for *Aedes aegypti* has been not significant due its low incidence during the survey. New surveys will be done in order to expand the results.

VE49 - PRESENCE OF CHITINASE ACTIVITIES IN THE GUT OF *AEDES AEGYPTI* (DIPTERA:CULICIDAE) LARVAE FOR DIGESTION OF CHITIN-RICH STRUCTURES.

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Mosquito larvae are believed to be capable of digesting chitin, an insoluble polysaccharide of N-acetylglucosamine, for their nutritional benefit. Studies based on physiological and biochemical assays were conducted in order to detect the presence of chitinase activities in the guts of the detritus-feeding *Aedes aegypti* larvae. Larvae placed for 24 h in suspensions of chitin azure were able to digest the ingested chitin. Semi-denaturing PAGE technique using glycol chitin and two fluorogenic substrate analogues showed the presence of three distinct chitinase activities: an endochitinase that catalyzed the hydrolysis of chitin; an endochitinase that cleaved the short substrate [4MU$(GlcNAc)_3$], and an exochitinase that hydrolyzed the chitobioside [4MU$(GlcNAc)_2$]. The endochitinase had an extremely broad pH-activity against glycol chitin and chitin azure, ranging from pH 4.0 to 10.0. When the substrate [4MU$(GlcNAc)_3$] was used, two activities were observed at pH ranging from 4.0 to 6.0 and 8.0 to 10.0. Chitinase activity against [4MU$(GlcNAc)_3$] was detected throughout the gut, displaying the highest specific activity in the hindgut. The pH values of the gut content were determined with the color shift indicators after larvae feeding. A correlation was observed between pH measured in guts of feeding larvae (pH 10-6.0) and pH of activity of the gut chitinases. Considering the obtained data, it is possible to postulate that gut chitinases may be involved in the digestion of the ingested chitin-containing structures, and in partial degradation of the chitinous peritrophic matrix (or membrane) in the hindgut.

Supported by: FENORTE – CNPq – WHO/TDR

VE50 - *BOOPHILUS* YOLK CATHEPSIN (BYC), AN ASPARTIC PROTEASE FROM *B. MICROPLUS* EGGS THAT LACKS THE SECOND ASP-RESIDUE FROM ITS CATALYTIC SITE

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The hard tick *Boophilus microplus* is a bovine ectoparasite responsible for great economical losses in tropical and subtropical areas. In Brazil, the annual losses due to this tick may reach 268 million dollars. In order to solve these problems, new effective control methods must be developed.

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Two aspartic proteases were previously characterized in our laboratory from *B. microplus* eggs, named BYC and THAP (Logullo, 1998, Sorgine 2000). *Boophilus* yolk cathepsin (BYC) was tested as a potential component of a protective vaccine against this tick, being able of inducing an immune response in cattle (da Silva Vaz, 1998).

Recently BYC was cloned by RT-PCR and the analyses of its amino-acid sequence demonstrated great similarity with other aspartic proteases. In spite of this similarity, BYC's sequence shows many important differences in the putative active site of the enzyme. The most important one is the lack of the second Asp residue, highly conserved in this class of protease. Although the classical mechanism for aspartic proteases catalysis requires both Asp residues for the nucleophilic attack to the peptidic bond, BYC was shown to be active against haemoglobin (Hb), tick vitellin (VT) and synthetic substrates. Since BYC was proteollyticaly active (although lacking an important part of it's active site) we believe that BYC might have a catalytic mechanism different from the other aspartic proteases.

Sequences predictions of BYC secondary and tertiary structure were made using Swiss Prot database. BYC is a Beta-sheet protein as confirmed by Circular Dichroism analysis. The sequence alignments of BYC and Renin showed a high conserved secondary structure identity, making possible to create a molecular model of BYC's tertiary structure and analyse its active region closely, so as to determine the residues missing in the active site.

As vitellin proteolyses is being show to be controlled by fosforilation in *B. microplus* eggs, we decide to investigate if BYC had fosforilated amino-acid residues by Western blot. The assays were positive for tyrosine residues but not for serine residues. We are now testing the effects of defosforilation on enzyme activity.

Supported by: HHMI, Gorgas Memorial Institute, Faperj, Pronex, CAPES, PADCT and CNPq

VE51 - PARTICIPATION OF *RHIPICEPHALUS SANGUINEUS* (ACARI: IXODIDAE) AND *CTENOCEPHALIDES FELIS FELIS* (SIPHONAPTERA: PULICIDAE) IN THE EPIDEMIOLOGY OF CANINE VISCERAL LEISHMANIASIS

Maria Teresa Zanatta Coutinho, Lilian Lacerda Bueno, Annelise Sterzik, Ricardo Toshio Fujiwara, Evaldo Nascimento, Edilene Matias do Amaral, Débora Cristina Carline Alvarez, Odair Genaro "*in memoriam*" e Pedro Marcos Linardi.

The vectorial competence of the tick *Rhipicephalus sanguineus* and the flea *Ctenocephalides felis felis* is discussed in relation to the epidemiology of canine visceral leishmaniasis, taking into account its strict association with dogs and the low indices of natural infection presented by its known vector, the phlebotomine sand fly *Lutzomyia longipalpis*.

To determine the viability of the parasites in *R. sanguineus* and *C. felis felis*, 72 hamsters were inoculated orally and peritoneally with macerates of ticks and fleas removed from 18 dogs symptomatic for visceral leishmaniasis (nine for ticks and nine for fleas). Six months later, the hamsters were sacrified and necropsied. Slide smears of spleen and liver, as well as PCR of these viscerae and IFAT (Indirect Fluorescent Antibody Test) from serum of hamsters were used as test to determine the parasite infection. Twenty hamsters inoculated by macerates of ticks gave positives results for *L. (L.) chagasi*. Sixteen hamsters that have been inoculated by macerates of fleas showed positive results. Eleven (68,7%) of them have been infected peritoneally and five (31,2%) orally. Tick macerates could infect 20 hamsters, being 14(70,0%) peritoneally and six (30,0%) orally.

Based on these findings, we suggest that the vectorial capacity of *R. sanguineus* and *C. felis felis* for *L. (L.) chagasi* should be evaluated further, opening new perspectives in the epidemiology of ZVL.

Apoio Financeiro: CAPES, CNPq.

VE52 - LUMINAL PH, PROTEOLYTIC ACTIVITY AND MACROSCOPIC ANATOMY OF THE DIGESTIVE TUBE OF *LUTZOMYIA LONGIPALPIS*' LARVAE (DIPTERA, PSYCHODIDAE).

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Phlebotomine sand flies are important in public health as vectors of *Leishmania* ssp. Despite their parasitological importance, the biological and molecular knowledge about immature forms is very restrict.

The objective of this work was to describe the macroscopic anatomy of the digestive tube of *Lutzomyia longipalpis* larvae as well as to measure the pH and characterize the proteolytic activity in their gut lumen. *Lutzomyia longipalpis* larvae have a bulk midgut flanked by a short and narrow foregut, and a hindgut with a conspicuous conical rectum.

pH measurements in the gut lumen were performed by using three different vital indicator dies mixed with larval meal. It was observed a pH gradient inside the midgut varying from >9 in the initial portion of this region to 6.5 just before the pylorus. A high pH like that observed in the initial portion is a common feature in detritivorous insects. Proteolytic activity was assayed by using the unspecific substrate azocasein and the synthetic substrates BApNA and N-CBZ-L-Phenylalanine-p-Nitroaniline which are specific to trypsin and chymotrypsin-like enzymes, respectively. Preliminary results, obtained with midgut extracts and azocasein as substrate, showed a remarkable proteolytic activity at higher pH values with one peak around pH 10.5. At least two peaks of trypsin activity were observed when BApNA was the substrate. The first peak had maximal activity at pH 8.5 and the other at pH 10. Just one peak at a high alkaline pH (pH 10 - 11) was observed when chymotrypsin was assayed.

More detailed studies concerning the proteolytic activity in the *Lutzomyia longipalpis*' larvae will be carried out.

Supported by: CNPq and FAPEMIG

VE53 - IDENTIFICATION AND CHARACTERIZATION OF GENES POTENTIALLY INVOLVED IN THE IMMUNE RESPONSE OF *LUTZOMYIA LONGIPALPIS*.

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Lutzomyia longipalpis is the main vector of visceral leishmaniasis in Brazil. While parasite-insect vector interaction has been well studied in malaria, with the development of transgenic mosquitoes incapable of transmitting the parasite, little is know about the interaction leishmania-sand fly. We are studying molecules potentially involved in feeding and infection by leishmania in *Lutzomyia longipalpis*. We have done EST sequencing and differential display studies using RNAs extracted at different times after blood-feeding and infection with leishmania. Through DDRT-PCR we have identified some genes potentially involved in insect immune response: MAP-kinase, TGF-β, Cactus. It is well known that insects use a variety of strategies to fight pathogens, from physical barriers to defense peptides, that were shown to be produced by sand flies in response to bacterial infection. In *Drosophila*, specific recognition appears to be achieved by membrane receptors of the Toll family, equivalent to IL-1 receptor in vertebrates. Cactus is active in this response cascade, and a similar mechanisms could exist in sand flies. MAP-kinase, which is also involved in innate immune response, is very conserved among organisms as far as mammals, plants and arthropods. The gene found in *L.*

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longipalpis showed similarity to the *Drosophila* gene. TGF-b may be involved in immune response, and is also very conserved. In *Anopheles stephensi*, TGF-b may have a function in the immune response against *Plasmodium* infection. The *L. longipalpis* TGF-b gene was identified through DDRT-PCR from RNA of sand flies fed on infected blood, indicating a potential immune response role for this molecule in this vector as well. We have previously partially sequenced *L. longipalpis* Map-kinase, TGF-b and Cactus genes. We are presently sequencing the whole gene by different techniques: probing cDNA libraries with the gene fragments in search for full sequences, 5'RACE and by the amplification of the 5' end of the gene by PCR amplification of a cDNA library, using a reverse homologous primer and a forward primer situated on the cloning vector. Fragments have been obtained and are presently being sequenced.

Supported by PAPESIII-Fiocruz, PDTIS, CNPq.

VE54 - CHARACTERIZATION OF TRYPSIN GENES IN *LUTZOMIA LONGIPALPIS*

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According to WHO, leishmaniasis is among the main infectious disease and is widely distributed around the Americas. In Brazil, the number of cases is increasing gradually over the last 20 years. *Leishmania* is transmitted to mammal hosts from wild reservoirs by Phlebotomine sand flies. *Lutzomyia longipalpis* is the main vector of *Leishmania (L.) chagasi,* that causes visceral disease in Brazil. We are studying molecular aspects of blood feeding and interaction between *Leishmania* parasites and their insect vector host. Knowledge of the vector biology and physiology can be an important tool to intervene in this interaction mechanisms. We intend to characterize specific molecules that might have a significant role in feeding and in this interaction and use such molecules as potential targets for the development of new strategies in the fight against the spread of leishmaniasis. There is already lots of information from studies with the malaria vector *Anopheles gambiae.* Various mosquito genes which may participate in feeding and in parasite interaction have been identified and characterized. Much of the attention has been placed on midgut specific genes and promoter elements. Midgut-specific proteins like trypsin, chymotrypsin, chitinase and others are being considered potential targets for the development of transgenic insect populations unable to harbor a parasite or transmit it to the vertebrate host. Although most of the data available comes from mosquitoes, this same approach can be applied to other insects such as phlebotomine sand flies. We isolated, from an expression library from blood-fed midgut, trypsin codifying cDNAs. These genes are regulated by blood ingestion and are probably involved in blood digestion. Partial sequencing of these clones showed that there are at least two different genes, with similarity to other insect trypsins. We are presently completing the sequencing by either 5'RACE or by PCR from the expression library, using internal reverse primers and a forward primer situated on the plasmid. We are also interested in characterizing a genomic clone, for the identification of introns and regulatory sequences. For that a genomic library in EMBL3 is being probed with the trypsin gene fragments. Positive clones have been obtained. These clones are being characterized by mapping, hybridization and sequencing.

Supported by PAPESIII-Fiocruz, PDTIS, Faperj, CNPq.

VE55 - AMERICAN TEGUMENTAR LEISHMANIASIS IN THE PONTAL OF PARANAPANEMA RIVER, SP, BRAZIL, AND THE RELATIONSHIP WITH MST (LANDLESS MOVEMENT FOR LAND).

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Introduction: American tegumentar leishmaniasis (ATL) is a zoonosis and transmission depends on many factors due to ecological changes or by invasion of man to natural niches where vectors and reservoirs are present. Seasonal variations and different susceptibility of the population also happens.

Objective: To present a survey of the American tegumentar leishmaniasis (ATL) in Pontal of the Paranapanema River, SP.

Methods: Retrospective and prospective study of cases of leishmaniasis in the Pontal of the Paranapanema River was carried out. The clinical forms were obtained from data of the Center of Epidemiological Vigilance (CEV), of the Health State Secretary of São Paulo, from May 1995 to December 2001. The studied variables were submitted to statistical analysis.

Results: The total of registered cases were 89. Most notified cases were from the district of Teodoro Sampaio (29,2%). The predominant clinical form was cutaneous lesion (78,65%). The most frequent lesion was in exposed areas of the body, mostly in the down limbs (36,36%). The higher occurrence was in male (67,42%). Individuals with age up to 54 years corresponded to 75% of the cases. The most notified cases were from rural settlements or MST (Landless Movement for Land) camps existing in the Pontal of Paranapanema. The higher number of notifications was in the winter and spring.

Conclusion: The data show the transmission in Pontal of Paranapanema occurs when man gets into the natural habitat of the zoonosis. Based in the epidemiological data, ATL occurs predominantly in the male, with age from 30 to 60 years, in the classical cutaneous form, where most with rural occupation or habitation.

VE56 - CAPTURED *LUTZOMYIA INTERMEDIA* SAND FLIES ARE SUCCESSFUL INFECTED WITH *LEISHMANIA BRAZILIENSIS* USING AN EXPERIMENTAL MODEL.

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Lutzomyia intermedia has been demonstrated as major vector of the ACL due to *Leishmania (V.) braziliensis* in Southeast Brazil. The aim of this study is to understand interactions between *Leishmania (V.) braziliensis* and its vector *Lutzomyia intermedia*, both from an endemic area of American Cutaneous Leishmaniasis (ACL). About two-thousand *L. intermedia* sand flies were captured and subjected to infection with *L. (V.) braziliensis* amastigotes. The flies were allowed to feed throughout a chick skin membrane in an artificial feeding device containing heparinized mouse blood seeded with parasites for 3h. After that, flies were maintained in sugar diet. Following infection, the gut of each sand fly was dissected in differents time points until to complete the digestion (1st to 10th day). The guts were examined to observe the presence, location, morphology and density of the parasites. Three-hundred and eighty-eight sand flies were examined and we observed an infection load of 86.5% at the first day and 42.9% at the last day after the blood meal. Interestingly, we demonstrated that procyclic promastigotes represented 100% of the population at the 1st and 2nd days, and metacyclic forms accounted for 40% at day 5 following infection. Haptomonads, nectomonads and paramastigotes promastigotes were present at different levels during the study.

This work is basis for subsequent studies which are being developed in our laboratory for better understanding interactions between *L. (V.) braziliensis-L. intermedia*, including comparison with others vectors present in the same endemic area, such as *Lutzomyia whitmani* and *Leishmania (L.) amazonensis.*

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VE57 - SPATIAL ORGANIZATION AND STRUCTURAL MODIFICATION OF MIDGUT MUSCLE NETWORK RELATED WITH BLOOD MEAL IN *LUTZOMYIA LONGIPALPIS* AND *PHLEBOTOMUS DUBOSCQI* SANDFLIES, VECTORS OF LEISHMANIASIS.

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Sand flies are vectors of leishmaniasis in vertebrates including man. Sand flies are able to feed on plant nectars and vertebrate blood, but hematophagy is an essential and exclusive behavior of females, which needs the blood meal to develop its eggs. The alimentary tract of sandflies is divided into three regions: foregut, midgut and hindgut. The midgut itself can be divided into two topographic regions according to their location: thoracic and abdominal midguts. The blood meal is stored and digested in the midgut. Muscle fibers are responsible for extensive changes in the midgut volume during the blood ingestion and digestion. Therefore, the knowledge of the structure of the muscle fibers present in the midguts of vectors of human diseases is important in order to correlate it with the organ functions. We describe and compare the spatial organization and the modifications of the midgut muscle fibers related with blood meal process. The midguts were morphological analyzed immediately after blood meal ingestion until its complete digestion, following the periods of 0 to 72 hours for *Lu. longipalpis* and 0 to 96 hours for *P. dubosqi*, since they finished their digestive processes at distinct times. The muscle components are placed over the entire midgut region as circular and longitudinal fibers forming a well-arranged muscle network. The muscle fibers are striated due to alignment of the Actin filaments clearly demonstrated by Phalloidin labeling and scanning electron microscopy. The thoracic midguts of the two sand flies do not distend after blood meal and do not change the arrangement of their muscle networks. On the opposite, their abdominal midguts suffer several modifications in the muscle network organization, which appeared to be completely related to the blood meal journey into the midgut. It is also remarkable to observe that after the digestion of the blood meal was finished, the muscle fibers of the *P. duboscqi* midgut returned to a better-organized muscle network than the *L. longipalpis* midgut fibers. This fact could be due to the differences in the arrangements of the muscle network of the two sand fly midguts, or even because of the distinct biochemical compositions of the midgut muscles of the two sand flies species, which were not detected by ours observations. In conclusion, the actin labeling with fluorescent Phaloidin and SEM allowed us to visualize in details, for the first time, the muscle organization of the midguts of two sand fly species, important vectors of leishmaniasis. This muscle network presented structural modifications related with the ingestion, storage and digestion of the blood meals in the midguts. This knowledge is important for a better understanding of the organ physiology and disease transmission by these insects.

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VE58 - STUDY OF THE OVARIAN DEVELOPMENT AND EGG EXOCHORION OF *CULEX QUINQUEFASCIATUS* USING SCANNING ELECTRON MICROSCOPY

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Culex quinquefasciatus is the mosquito vector of *Wuchereria bancrofti*, parasite that causes human filariasis in Brazil. It is considered a tropical–cosmopolitan with a high level of anthrophylia. The feeding adaptation allows it to feed during nighttime, which coincides with the peripherical circulation of the parasite optimizing vector competence. In order for the hematophagus female to lay healthy viable eggs, the ingestion of blood from a warm-blooded vertebrate is needed to complete the protein requirement for the egg's development.

The female reproductive system consists of spermtheca, accessory glands and ovaries, which are composed of ovarioles that will develop into eggs. This study focuses on different stages of the ovarian maturation and development, and the hatched eggshell using the scanning electron microscope (SEM). The mosquitoes were blood fed on quails (*Coturnix* sp.). Ovaries of five females were dissected at different times after blood meals (from 0-72h). Samples were processed and observed in the SEM. The measurements were made using microscope software. The size and the morphology of ovaries from 0 to 6h were similar (357µm X 140µm). These ovaries have dense and compact membranes, which appear to be composed of overlapping filaments of different sizes. At 6h, these membranes were ruptured and it was possible to observe the ovarioles measuring 15um X 20um. At 12h, a similar ruptured membrane allowed us to see intact and ruptured ovaries measuring 523µm X 180µm and 493µm X 203µm, respectively. The ovaries of 24h, 36h and 48h with ovarian membrane measured 560µm X 262µm and without the membrane measured 764µm X 430µm. The ovarioles from these times, which were seen under the ruptured membrane, measured 72um X 71um. At 72, the ovaries (1,580µm X 720 µm) and ovarioles (439µm X 101 µm) increased in size, and the ovarian membrane was very thin. The ovarioles were almost completely formed and lost another membrane that exposes their adherent surface of the egg. In the hatched eggshells, it was possible to see the exochorion, which is the outer layer of the egg. The egg's exochorion was composed of tubercles arranged in orthogonal arrays. The operculum from where the larvae will escape was also seen. In conclusion, it appears that in the first hours, the ovaries do not show any significant changes but around 36h there is a rapid growth, probably due to the end of the digestion process. The ovarian membrane is responsible for containing the ovarioles in formation. In this study was possible to observe several ultrastructural events related with the mosquito egg´s development.

VE59 - MOLECULAR ANALYSIS OF GLUTATHIONE PEROXIDASE GENES: COMMON CHARACTERISTICS SHARED BY PLANTS, ARTHROPODS AND YEAST

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Glutathione peroxidase (GPX) is one of the most important enzymes involved in cell redox regulation. GPx is usually described as a selenoenzyme. But, until now, all glutathione peroxidases homologous described in arthropods and yeast do not have the amino acid selenocysteine and plants have just one example of GPX-Se[1]. We have recently cloned a GPX gene homologous in the cattle tick *Boophilus microplus* (Bmgpx) which shares about 58% and 54% amino acid sequence identity with plant (*Momordica charantia*) and mammalian (*Mus musculus*) PHGPXs, respectively. As other arthropods GPX homologous, Bmgpx do not code for a selenoenzyme, since it does not have the opal codon UGA. Molecular analysis of GPX genes from arthropods, yeast and plants revealed that they have two common features: they don't code for selenoenzymes and they are similar to phospholipid hydroperoxide glutathione peroxidase (PHGPX).