B-1 – ADHESION SPECIFICITY TO ENDOTHELIAL CELL RECEPTORS OF *PLASMODIUM FALCIPARUM* ISOLATES FROM RONDONIA (WESTERN AMAZON REGION, BRAZIL)

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Parasite molecules expressed at the surface of infected red blood cell's membrane mediate endothelial cell adhesion and sequestration of mature forms of *Plasmodium falciparum* and were identified as belonging to the EMP1group of "variant" antigen proteins encoded by the multigenic family of var genes (review by Borst, Cell 82:1-4, 1995). It has been proposed that different forms of severe malaria, in particular cerebral malaria, depends on the receptor affinity of individual var antigens to specific endothelial cells receptors expressed at different tissues. To examine this hypothesis it is necessary to correlate adhesion specificity of parasites isolates with clinical forms of the infection they produce and to specific var gene expressed by the corresponding parasite population.

In the present study, falciparum malaria patients of the Porto Velho area (Rondonia State, Brazil) were examined to define clinical and biological parameters of severity of infection, according to WHO criteria . Purified red blood cells from the patients containing ring forms were incubated in RPMI-albumax medium at 10% hematocrit in candle jar for 20-24 hours. Trophozoites and schizonts were concentrated and re suspended in RPMI-1640 to perform adhesion assays. For these assays were used CHO transfected cells expressing CD36, ICAM-1, V-CAM, E-selectine receptors (gift of A. Scherf, Pasteur Institute) and native CHO cells expressing Chondroitin sulfate A (CSA) residues at the cell surface. Among twenty one isolates examined, three types of adhesion profiles were recognized: 1) three isolates showed specific adhesion to just one of the receptors; 2) five isolates showed specific binding to two receptors; 3) the remaining isolates showed adhesion to various receptors examined at different levels of efficiency. Using panning techniques (A.Scherf, EMBO J 17:5418-26), some pluri-specific adherent isolates were shown to consist of heterogeneous population . To identify the var antigen molecules expressed by the studied parasite population, RNA was extracted from the parasites and used in TR PCR amplification procedure to amplify the conserved segment of the DBL1 segment of the corresponding var gene allowing its' sequencing and identification.

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B-2 – BIOCHEMICAL ANALYSIS OF MEGASOME BIOGENESIS IN *LEISHMANIA AMAZONENSIS*

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Stage-regulated cysteine proteinases are abundant in Trypanosome and *Leishmania*, usually present in lysosomal compartments, playing an essential role in pathogenesis, host-parasite interactions and parasite survival. In amastigote forms of *Leishmania* species belonging to *mexicana* species, cysteine proteinases are found in large lysosomes called *megasomes* that are absent in the promastigote stage. In order to investigate megasome development during promastigote differentiation into amastigote stage in an axenic culture of *L. amazonensis*, cysteine proteinase activity was analyzed by gelatin SDS-PAGE and the appearance of a stage specific cysteine proteinase was demonstrated by immunobloting assay.

Infective promastigotes, grown in Warren's medium supplemented with 10% fetal calf serum, were transformed by gradual temperature shift from 25º C to 32º C and samples were collected daily during 6 days. After electrophoretic separation of total lysates under nonreducting conditions in SDS-PAGE containing copolymerized gelatin, gels were incubated in the presence or absence of a specific cysteine proteinase inhibitor (E-64) and then were stained with Coomassie blue. In the same way, for immunoblotting assay, total lysates were separated by electrophoresis in SDS-PAGE, but in reducing conditions and then transferred to nitrocellulose membrane. Cysteine proteinase was detected using an anti-cysteine proteinase polyclonal antibody that was captured with goat anti-rabbit IgG alkaline phosphatase conjugate and revealed with substrate (BCIP/NBT).

Preliminary results obtained by gelatin SDS-PAGE analysis revealed the appearance of a 40-kDa cysteine proteinase from 3rd day on. Higher proteolytic activity was observed in lysates of established axenic amastigote culture. Immunoblotting assay showed a gradual increase of the amastigote specific cysteine proteinase since 4th day, culminating in a high level of this stage specific enzyme in axenic amastigote. These results are in accordance with morphometric analysis, performed in parallel that showed the arising of membrane-bound organelles on day 3.

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B-3 – ISOLATION AND PARTIAL CHARACTERIZATION OF *TRYPANOSOMA RANGELI* SEQUENCES RELATED TO *TRYPANOSOMA CRUZI* GP85/SIALIDASE SUPERFAMILY

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Trypanosoma cruzi trypomastigotes express surface glycoproteins (gp85, Tc-85, gp90 and gp82) which are involved in cell invasion and induction of protective immune response in mammalian hosts. These glycoproteins belong to a superfamily of surface molecules whose members are characterized by the presence of two partially conserved amino acid motifs: subterminal repeat Fn3 (VTVxNVxLYNR) and Asp box (SxDxGxTW). Previous results of our laboratory suggested that sequences related to the gp82, gp85 and gp90 genes can be found in the non-pathogenic protozoon *Trypanosoma rangeli*. Here we report the isolation and partial characterization of *Trypanosoma rangeli* sequences related to *Trypanosoma cruzi* gp85/sialidase superfamily.

A *T. rangeli* genomic library was constructed in pUC18 using DNA fragments (size = ~4.0 kb) obtained by mechanical shear, and probed with *T. cruzi* surface glycoprotein genes. Screening of 576 recombinant clones with probes derived from gp82 and gp85 genes yielded 169 positive clones (29%): 132 clones hybridized with both probes; 21 clones reacted only with gp82 probe; and 16 clones hybridized only with the gp85 probe. This result indicates that sequences related to the gp82 and gp85 genes are present in multiple copies in *T. rangeli* genome.

T. rangeli sequences homologous to the *T. cruzi* surface glycoprotein genes were also isolated from genomic libraries were constructed in plasmid pUC18 using *T. rangeli* DNA fragments obtained by digestion with *Sau*3AI or *Eco*RI restriction enzymes. Several recombinant clones were isolated from this library using the gp82 gene as a probe. Nucleotide sequence analysis revealed the presence in these clones of several blocks of 20-60 nucleotides that share 85%-95% of identity with gp82 and gp85 (TSA-1, SA85-1) genes. The translated peptides from the open reading frames (ORFs) have 35%-50% of identity with the C-terminal domain of the gp85 surface glycoproteins including the motif VTVTNVFLYNR. These results suggest that there are many clusters of the gp82/gp85 gene family spread throughout the *T. rangeli* as it has been reported for *T. cruzi* (Chiurillo et al. 1999, *Mol Biochem Parasitol* 100:173). A comparative analysis of the *T. cruzi* gp82/gp90 and *T. rangeli* related sequences may aid in the determination of the features of gp82 and gp90 proteins that contribute to its function in host cell interactions.

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B-4 – INVOLVEMENT OF PHOSPHATIDYLINOSITOL 3-KINASE (PI3K) IN *TRYPANOSOMA CRUZI* INVASION OF HOST CELLS

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The invasion process of *Trypanosoma cruzi* in non phagocytic cells has been the subject of extensive studies. Several signal transduction events have been described in this process as a consequence of the interaction of parasite molecules and host cells. Recent evidence have shown that phosphoinositide kinases are involved in many cellular functions, such as regulation of the cell cytoskeleton, mitogenesis, cell survival and vesicular trafficking. In particular, host cell PI3K has been associated with the invasion processes of different bacteria and viruses. Therefore, it could be possible that this enzyme could also be involved in the process of parasite and host cell interaction. In the present work we present evidence that both parasite and host cell PI3K are implicated in the infection of non phagocytic cells by trypomastigotes of *T.cruzi*.

In order to analyze if the host cell enzyme was required for succesful parasite invasion, two specific PI3K inhibitors were used: Wortmannin and LY294002. Both compounds showed a marked dose dependent inhibition of the invasion when the non phagocytic cell lines Vero, NIH 3T3 and L_6E_9 were preincubated for 1 h with these inhibitors before infection. Maximal inhibition of invasion (75-95%) was obtained with 50 μM of LY294002 and 100 nM of Wortmannin.

Based on the results that PI3K inhibitors negatively affected *T.cruzi* entry, the next step was to assess if activation of this enzyme in the host cells could be a mechanism triggered by the contact of these cells with trypomastigote membranes. Using this model, Vero cells were stimulated at different times with trypomastigote membranes and PI3K activation was evaluated by immunoprecipitation of cell lysates with an anti-PI3K antibody and assesment of the *in vitro* lipid phosphotransferase activity. Contact of Vero cells for 15min with trypomastigote membranes induced a 10-fold increase in enzyme activity compared with unstimulated controls. The activation was dependent on the parasite stage since treatment of cells with epimastigote (non infective stage) membranes did not induce any effect on PI3K activity. Treatment of cells for 1h with LY 294002 50 μM before the addition of parasitic membranes abolished the stimulatory effect of the trypomastigote fraction.

On the other hand, the role of *T.cruzi's* PI3K was also studied using the same inhibitors. When trypomastigotes were incubated for 1h prior to infection with Wortmannin 1-500 nM or LY294002 25-100 μM, the results showed an dose-dependent inhibitory effect on the infection percentages of Vero cells. As *T.cruzi* PI3K has not been described up to day, determination of the enzyme activity of trypomastigotes was performed and a basal phosphatidylinositol 3'kinase activity could be measured in this stage. In conclusion, the pathway involving PI3K seems to be another example of the signalling events that are subverted in the host cell by intracellular pathogens for their own benefit.

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B-5 – STRUCTURE OF *T.CRUZI* GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE IN COMPLEX WITH THE NATURAL PRODUCT INHIBITOR CHALEPIN

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Aiming at the design of specific inhibitors of the enzyme GAPDH from the parasite *Trypanosoma cruzi*, causative agent of Chagas' disease, the crystallization and structure determination of this enzyme was undertaken. We report here the structure of the complex of *T.cruzi* GAPDH with the natural product inhibitor chalepin, a coumarin derivative. Crystals were obtained in microgravity at 22º C (VDA2) by vapor diffusion, with protein solution at 8 mg/ml, 5 mM of chalepin, 0.1 M sodium cacodilate at pH 7.1, 0.1 M calcium acetate and 16% PEG 8000. The crystals have grown to a size of 0.2x0.3x0.5 mm after the 9 days, and belong to the space group $P2_1$ with unit cell dimensions a=82.2 Å, b=85.1 Å, c=105.4 Å, β=96.4º and one tetramer per asymmetric unit. Diffraction intensities were collected at 100K to 1.95 Å resolution and 78% completeness with an R_{merge} of 10.7%. The structure was determined by molecular replacement with a search model of *T. cruzi* glycosomal holo-GAPDH cryo-crystal structure refined in our laboratory at 2.15 Å. Initial refinement aimed at the possible identification of the binding of the inhibitor chalepin was conducted without NCS symmetry using REFMAC, with a model containing 359 aminoacids per monomer and 848 water molecules in the asymmetric unit.. The structure has no NAD bound to the enzyme, therefore adopting an open conformation analogous to the apo enzyme structure observed for other species. The catalytic domain remains essentially unchanged whereas the NAD binding domain shows a global rotation of 2.8°, followed by internal changes that lead to an opened NAD binding site, with modifications at the hydrophobic pocket adjacent to the adenine moeity originally observed in the holo-form. Extensive electron density was observed at the active site and could be interpreted as the inhibitor. Active site Cys166 shows a conformational change to accommodate the inhibitor. Final refinement in underway.

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B-6 – *TRYPANOSOMA CRUZI* INVASION OF ENDOTHELIAL CELLS IS POTENTIATED BY KININ-INDUCED STIMULATION OF G-PROTEIN COUPLED BRADYKININ RECEPTORS

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Endothelial cell contraction and smooth muscle relaxation are examples of biological responses which G-protein coupled (GPC) bradykinin receptors (BKR) transduce when signaled by vasoactive kinin peptides. In previous studies, we have hypothesized that kinins generated by trypomastigotes may induce endothelial cell contraction, thus enabling parasite migration across blood capillaries *(Svensjo et al., Microv. Res. 54:93,1997; Del Nery et al. J. Biol. Chem. 272:25713,1997*). We also advanced the proposition that cruzipain-isoenzymes may be involved in this process because (1) the substrate specificity of cruzipain 2 (*Lima et al.. MBP 67:33-37,1994; Reis et al., this volume)* was similar to that of tissue kallikrein (2) vasoactive kallidin was generated by incubating cruzipain with kininogen (3) Cruzipain converted plasma pre-kallikrein (PPK) into plasma kallikrein (PK), thus indirectlypromoting release of bradykinin from HK (4) potent increases in the capillary permeability were induced upon topical application of active cruzipain on the hamster cheek pouch microcirculation (Svensjo et al., 1997). Given the evidence that BK stimulation of the prototypic B_2-BKR subtype induce cytosolic $[Ca^{+2}]$i transients, we predicted that TCTs could exploit this system to render BKR^+-targets more susceptible to invasion. Here we sought to test this hypothesis by performing invasion assays using TCTs (Dm28), HUVEC and CHO-cells transfected with the rat B_2-BKR gene (B_2-BKR.CHO). As predicted, the latter exhibited heightened susceptibility to invasion (6Xfold) as compared to CHO.MOCK, but the potentiation only occurred when captopril (CPT), a synthetic inhibitor of a kinin-degrading peptidase (ACE or kininase II), was added to the cultures. Infection indexes on B_2-BKR.CHO+CPT returned to baseline levels when the cultures were treated with the B_2-BKR antagonist HOE-140 (100 nM), o with excess of BK. Addition of a mAb directed against the BK moiety of HK/LK has significantly reduced parasite invasion of B_2-BKR-target cells, indicating that parasite-induced kinin-release depends on the accessibility of the HK/LK substrate at the host cell surface. Importantly, the maneuvers that have positively or negatively modulated host cell invasion were fully reproduced with primary cultures of human endothelial cells (HUVEC) as well as with smooth muscle cells. However, the invasion of skeletal muscle lines, Vero cells or fibroblasts does not seem to require signaling by B_2-BKR. As predicted, TCTs induced rapid and potent $[Ca^{+2}]$i transients in Fura 2-treated B_2-BKR.CHO+CPT, whilst CHO-MOCK+CPT yielded low responses; the $[Ca^{+2}]$i elevations observed in B_2-BKR.CHO+CPT were markedly reduced by HOE-140, added prior to the addition of TCTs. The B_2-BKR signaling route triggered by TCT also led to the activation of PI3K, as measured by PKB phosphorylation. Interestingly, purified cruzipain (but not E64-cruzipain) stimulated striking $[Ca^{+2}]$i transients in B_2-BKR.CHO+CPT; this response was abolished by HOE-140 and did not yield elevations in CHO.MOCK+CPT. Notably, synthetic inhibitors for PK profoundly inhibited TCT invasion. Collectively, our data suggest that TCTs may accelerate kinin production because it interferes with the homeostatic mechanisms controlling the formation and degradation of these short-lived peptides. We suggest that the parasites may focus cruzipain-isoenzymes onto sites in which HK-PPK complexes are transiently assembled, at the surface of endothelial cells. Secretion in areas formed by opposed membranes may protect the parasite proteinases from inactivation by fluid phase host proteinase inhibitors. To our knowledge, this is the first report in which a biochemically defined Ca^{+2} signaling peptidergic agonist and a cognate GPC surface receptor are directly implicated in mechanisms of host cell invasion by *T. cruzi*.

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B-7 – EXPRESSION OF CRUZIPAIN AND OF A CATHEPSIN B-LIKE TARGET (TC-CB) IS INVERTED IN A *TRYPANOSOMA CRUZI* CELL LINE DISPLAYING RESISTANCE TO SYNTHETIC INHIBITORS OF CYSTEINE-PROTEINASES

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The therapeutic potential of synthetic inhibitors to the major cysteine-proteinase from *Trypanosoma cruzi* (cruzain or cruzipain) was recently demonstrated in animal models of Chagas' disease. A possible limitation of this strategy would be selective pressure allowing for emergence of parasite populations that develop resistance to cysteine-protease inhibitors (CPI). Here, we describe the properties of a phenotypically stable *T. cruzi* cell line (R-Dm28) that displays increased resistance to Z- (SBz) Cys-Phe-CHN_2, an irreversible CPI which preferentially inactivate cathepsin L-like enzymes. Isolated from axenic cultures of the parental cell line (IC_{50} 1.5 μM), R-Dm28 EPI exhibited 13-fold (IC_{50} 20 μM) higher resistance to this CPI. The resistance phenotype seems selective, given that no cross-resistance was observed by testing them with unrelated trypanocidal drugs, such as benzonidazole and nifurtimox. Western blotting (with mAb), affinity labelling (with biotin-LVG-CHN_2) and FACS analysis of R-Dm28 epimastigotes (log phase) revealed the cruzipain target was expressed at lower levels, as compared to the parental cell line. Interestingly, this deficit was paralleled by increased expression of an unrelated Mr 33,000 cysteine-protease whose activity was somewhat refractory to inhibition by the CPI. N-terminal sequencing of the affinity-purified biotin-LVG-proteinase complex allowed its identification as Tc-cb, a previously characterized cathepsin B-like enzyme (Garcia et al. MBP 1998;91:263-72). Ultrastructural studies revealed that Tc-cb accumulates in reservosomes, and that these organelles are grossly enlarged and highly electron-dense in R-Dm28. In summary, the decreased availability of the cruzipain target may have reduced the threshold required for CPI induced parasite death. On the other hand, the increased expression/accumulation of Tc-cb, a less sensitive target for Z-(SBz) Cys-Phe-CHN_2, may compensate for the deficit in metabolic functions which cruzipain would otherwise undertake.

Support from PRONEX II, FAPERJ, CNPq, FUJB.

B-8 – GENETIC DIVERSITY IN *TRYPANOSOMA CRUZI* ISOLATES FROM HUMANS, VECTORS AND RESERVOIR HOSTS IN THE DISTRICT OF BARCELOS, AMAZONAS STATE: EPIDEMIOLOGICAL IMPLICATIONS

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In rural areas of the district of Barcelos , northern part of the State of Amazonas, Brazil, cases of Chagas disease are sporadic and the human infection can be ascribed to either accidental contact with infected sylvatic vectors or to human migrations. However, it was recently reported (Coura et al.. 1994 *Rev. Soc Bras. Med. Trop. 27*: 251-253; Coura et al. 1996 *Trans.R.Soc.Trop. Med.Hyg. 90*: 278-279) the "attacks" by *Rhodnius brethesi* on "piaçava" (*Leopoldinia piassaba*) gatherers in a rural locality on a tributary river of the Rio Negro, district of Barcelos. In the present study, the genetic diversity, of 22 *Trypanosoma* isolates from *R. brethesi* (16), *Didelphis marsupialis* (2) and humans (4), proceeding from Barcelos and different "piaçava" areas in that district, was demonstrated by multilocus enzyme electrophoresis (MLEE) using a system of 11 enzymatic loci, restriction fragment length polymorphisms of kDNA (RFLP) and molecular hybridization analyses. Aiming to contribute to the epidemiology and to the understanding of the dynamics of the disease' transmission in such areas the pheno and genotypic typing of the circulating strains was carried out adding further information to previous studies (Coura et al. 1996 *loc. cit.*; Fernandes et al. 1998 *Am.J.Trop.Med.Hyg. 58*: 807-811). The heterogeneity of the *T. cruzi* isolates was remarkable at both nuclear and mitochondrial DNA levels. MLEE and phenetic analyses have allowed the discrimination of the isolates into two distinct phenetic groups separated by a coeficient of similarity lesser than 0.25. Among the circulating *T. cruzi* populations 6 distinct isoenzymatic patterns were distinguished. Although 62.5% of the isolates from *R. brethesi* had been clustered within the group of sylvatic zymodemes (Z1), three phenotypic patterns , one identical to the Z1 prototype and two Z1 variants could be included in the same cluster. The corresponding Z1 variant zymodeme of *T. cruzi* was found infecting opossuns and triatomines in a same area. In addition, such Z1 variant zymodeme seems to be a wide spreading genotype as it was found in vectors and reservoir hosts in Barcelos and in "piaçava" areas. *T. cruzi* populations recovered from human together with three isolates from *R. brethesi* were clustered in a second group. In this group, zymodemes detected in humans and vectors were distinct suggesting, at least by this parameter, that such *T. cruzi* population found in *R. brethesi* was not responsible for human infections. Likewise, no association was found neither with Z1 nor with Z2 zymodemes. One out of the four human isolates studied was typed as an isoenzymatic variant of the San Agostin reference strain of *T. rangeli* and clustered in a separated group. Results were confirmed by hybridization with specific probes. This finding corroborates previous data on PCR amplification of mini-exon gene (Coura et al 1996 *loc. cit*). Genotypic heterogeneity was detected in human isolates by RFLP analyses. The patterns obtained have shown more polymorphisms in *T cruzi* than in *T. rangeli* populations. Interestingly, parasites retrieved from a vector displayed the same RFLP pattern when compared to the human *T. rangeli* isolate. Subsequent hybridizations with a subgenus *Schizotrypanun* specific oligonucleotide (Pacheco et al. 1996 *Parasite 3*: 207-209) also revealed no sequence homology. The data reported in this study certified the high heterogeneity of *T. cruzi* populations that circulate in sylvatic environments. Further investigation will be necessary to correlate genetic diversity with clinical and biological peculiarities.

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B-9 – ENZYME MARKERS OF CARDIAC LESION OF *TRYPANOSOMA CRUZI*-INFECTED MICE SUBMITTED TO CHEMOTHERAPY WITH BENZNIDAZOLE

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Cardiac involvement is a prominent feature of both the acute and chronic stages of the Chagas'disease. Creatine kinase (CK) is a marker of muscle lesion oftenly used in clinics, and its isoenzyme CK-MB, is a choice molecular marker in ischemic heart lesion. We monitored CK and CK-MB changes during the experimental Chagas' disease in outbred (Swiss) and inbred (C57bl/6 – B6) mice infected or not with *T. cruzi*. Mice infected with 10^4 trypomastigotes (Y strain of *T. cruzi*) developed high parasitemias, presenting a peak at 8^{th} day post infection (dpi). Animals infected with 10^2 parasites presented a lower peak of parasitemia at 11^{th} dpi. Cumulative mortality was 100% and 37,5% respectively for Swiss mice infected with 10^2 and 10^4, 90% and 50% for B6. Infected Swiss mice submitted to treatment with Bz (100 mg/Kg for 9 days or 0.25 mg/ml for 50 days) had no parasitemia and 100 % of survival. We adapted the assay of CK and CK-MB activities to small volumes (5µl) and measured them before and during infection. The levels of enzyme activity in non-infected animals did not change significantly over 32 weeks, with a cut off level of 0,025 ΔO.D./min for CK and 0,032 ΔO.D./min for CK-MB. About 40% of the Swiss mice infected with 10^4 trypomastigotes presented high levels of both enzymes, and the high CK-MB levels was positively correlated with the intensity of the inflammatory process, measured by the number of inflammatory infiltrates present on heart tissue sections. Results were similar when B6 mice were infected. Using a lower inoculum of 10^2 parasites, about 60% of Swiss mice presented high CK-MB levels in the chronic phase. Bz treatment of mice infected with 10^4 parasites prevented the appearance of acute phase lesions until the 3^{rd} week post-infection, but at the 4^{th} week 40% of the infected and treated mice presented high CK-MB levels. The treatment with Bz in the drinking water of mice infected with 10^2 parasites reverted the increase in CK-MB during the acute phase, but high enzyme level was still observed during the chronic phase. This work confirmed that CK and CK-MB enzyme activity in plasma increase in an important percentage of infected animals, that these levels were positively correlated with the degree of inflammation. Animals treated with Bz in different schemes still have myocardial lesions monitored by CK e CKMB plasma levels, during late acute phase and in the chronic phase. CK-MB is then a reliable humoral marker of experimental acute and chronic myocarditis described up to now.

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B-10 – PARTIAL PURIFICATION AND CHARACTERIZATION OF A PHOSPHOLIPASE A_1 OF *TRYPANOSOMA CRUZI*

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In recent years, enzymes degrading phosphatidylcholine (PC) have been shown to play significant roles in signaling processes in eukaryotic cells. Thus, phospholipase D in combination with phosphatidic acid phosphatase is important in the generation of diacylglycerol signals for activation of C-kinase pathways. PC-phospholipases A_2 have also been implicated in the activation of this kinase. Recent studies suggest that enzymes attacking positions sn-1 in phospholipids may also serve signalling roles. While a phosphatidylinositol signaling pathway has been demonstrated in *T. cruzi*, the enzymes degrading PC have not been examined in this cell. PC-phospholipase activities have been the subject of a number of studies in African trypanosomes. The levels of Plase A_1 varied widely, with very high activity in the pathogenic *T. brucei* and relatively low in the non-pathogenic *T. lewisi* species.

In view of their possible roles in various aspects of *T. cruzi* biology, we have undertaken the present study to characterize PC hydrolyzing enzymes in this parasite.

Upon incubation of 1-palmitoyl-2-[1-^{14}C]oleoyl-PC with homogenates of *T. cruzi* epimastigote, the only radiolabeled product was lysoPC, indicating that phospholipase A_1 (Plase A_1) activity in this cell. The activity has acidic pH optimum and is sedimentable in homogenates prepared under isotonic conditions. Sedimentability is lost by addition of 0.1 % Triton X-100 or freezing and thawing, in a parallel fashion with the behavior of a- mannosidase, a lysosome marker. Thus Plase A_1 appears to be a lysosomal enzyme in *T.cruzi*. Near homogeneous preparations were obtained using DEAE-Trisacryl and Concanavalin A-Sepharose with a 240-fold increase in specific activity and a 32 % recovery. Polyacrylamide gel electrophoresis analysis showed a major band of 38 kDa. Accordingly, the activity eluted from Sephadex G-75 as a single peak of 40 kDa. *T. cruzi* Plase A_1 was unaffected by the addition of 1-5 mM Na_2 EDTA or 1-5 mM $CaCl_2$ and was activated by 1 % Triton X-100. Plase A_1 was also the only PC-degrading activity detected in homogenates of *T. cruzi* cultured amastigotes, metacyclic trypomastigotes and culture trypomastigote forms. In all of these stages, Plase A_1 showed markedly higher specific activity than in the epimastigote form. Incubation of killed *T. cruzi* epimastigotes for 12 hours resulted in an almost complete deacylation of endogenous phospholipids with accumulation of free fatty acids and lysocompounds. Thus, as referred by different authors, Plase A phospholipid breakdown can generate potentially toxic compounds, which could contribute to the inflammation processes observed in host tissues around degenerating amastigotes. Therefore Plase A_1 activity may contribute to the pathogenesis of Chagas' disease.

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B-11 – KINETICS OF PROTEIN DEGRADATION IN RESERVOSOMES OF *TRYPANOSOMA CRUZI*.

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Reservosomes are the site of accumulation of proteins and lipids endocytosed by epimastigote forms of *T. cruzi*. Proteins internalized by fluid phase and receptor mediated endocytosis through the cytostome are accumulated in reservosomes that were characterized as late endosomes. Previous studies indicated that pre-loaded reservosomes with a 15 nm BSA-gold complex during 4 hours can be loaded again by a second pulse of 10 nm BSA-gold. Gold particles present in reservosomes were flocculated consistent with proteolytic degradation. The present study analyses the kinetics of BSA degradation from 15 minutes to 4 hours of incubation. Some samples were submitted to incubation with BSA-gold complex during 4 hours followed by incubation in conjugate-free medium for 20-72 hour chase. Samples were fixed in 4% formaldehyde, 2.5 % glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.2, post-fixed in 1 % osmium tetroxyde in 0.1 M sodium cacodylate buffer, pH 7.2, containing 0.8 % potassium ferricyanide and 5 mM calcium chloride, dehydrated in acetone and embedded in Epon. Small aggregates were observed after 30 minutes of incubation and the number of gold particles per aggregate increases until 4 hours of incubation in the present of the BSA-gold complex when few large aggregates or even only one aggregate were observed inside the same reservosome section. Epimastigotes submitted to a 4-h pulse and a 20-h chase showed an uncommon distribution of reservosomes observed in the anterior portion of the parasite body close to the Golgi complex and the flagellar pocket. The presence of the aggregates inside the flagellar pocket suggesting a process of exocytosis of the gold particles was not observed even after 48 or 72 h of chase. Endoplasmic reticulum profiles were observed in close contact with the loaded reservosomes. Pre- loaded samples submitted to a 4-h pulse and 20 h chase were submitted to the cytochemical detection of acid phosphatase activity. Co-localization of gold aggregates with electron dense precipitates from the cytochemical reaction was not seen even when the loaded reservosomes were observed in the anterior end of the parasite.

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B-12 – *TRYPANOSOMA CRUZI* MEMBRANES PERMEABILITY TOWARDS PEROXYNITRITE

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Macrophages play an important role against *T. cruzi* infection, via superoxide, nitric oxide and presumably peroxynitrite formation. Indirect evidence of peroxynitrite transmembrane diffusion in *T. cruzi* has been reported that lead to inactivation of key metabolic pathways of the parasite. Herein we study the ability of peroxynitrite to diffuse through *T. cruzi* membranes.

Peroxynitrite dependent-oxidation of low molecular weight sulfhydryls was used as an intracellular marker for the diffusion of peroxynitrite through the parasitic membrane. Intact and freeze-disrupted epimastigote cells were exposed either to peroxynitrite or a peroxynitrite generating system (external flux of nitric oxide plus menadione-induced superoxide production). Total glutathione ([GSH] + ½[GSSG]) concentration was determined by the glutathione reductase assay in the presence of DTNB (Sies & Akerboom, Methods in Enzymol.105: 445-451). Increasing concentrations of peroxynitrite (0-250 µM) exponentially decreased the total glutathione concentration of intact as well as lysed epimastigote cells. The concentration of peroxynitrite required to decrease the initial glutathione concentration by 50 % was 3.5 µM for lysed cells and 22 µM for intact cells. In most eukaryotic cells glutathione constitutes a key scavenger of peroxynitrite, due to its elevated intracellular concentration and its high reaction rate constant (1350 $M^{-1}.s^{-1}$, pH 7.4 and 37ºC (Koppenol, et al, 1992, *Chem. Res. Toxicol.* 5: 834-841)). Trypanosomatids contain most of their GSH conjugated as bis-glutathionyl spermidine (trypanothione - T (SH_2)) and glutathionyl spermidine (GSH-SP). The effect of peroxynitrite on the low molecular weight thiols of *T. cruzi* was investigated. Sulfhydryls were derivatized with the fluorescent thiol reagent monobromobimane and then separated by reverse-phase high-performance liquid chromatography (HPLC). Cell lysates exposed to 50 µM peroxynitrite showed a 60% GSH, 85% GSH-SP , and 97% T $(SH)_2$ oxidation. While the same concentration of peroxynitrite added on intact cells only decreased the sulfhydryl content in less than 40 %. The possibility of mixed disulfide formation was explored in the presence of reductants; DTT, cyanide, and $NaBH_4$ were used, after and before protein precipitation. Protein mixed disulfides appeared as the main product of sulfhydryl oxidation.

Protein tyrosine nitration was also used as a marker for peroxynitrite arrival to the intracellular space of the parasite. Western blot analysis using 3-nitrotyrosine polyclonal antibodies was performed to determine membrane-bound and soluble protein nitration. The main nitrated band (~50 kDa) of the whole cell preparation exposed to peroxynitrite seemed to correspond to tubulin, one of the most abundant proteins in the cytosolic fraction of the parasite. Protein nitration was more extense and intense when lysed cells were exposed.

Our results indicate that eventhough the T. cruzi membrane respresents a hindrance for peroxynitrite; the oxidant is able to reach intracellular parasite targets.

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B-13 – 58-KDA PROTEIN A MACROMOLECULE ASSOCIATE WITH THE GOLGI COMPLEX IN MAMMALIAN CELLS COULD BE USED AS MARKER TO *TRYPANOSOMA CRUZI*

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The 58-kDa Protein is a macromolecule reported to be localized at the citoplasmic side of the Golgi complex and the available data suggest that in mammalian cells it provides an anchorage site for microtubules on the outer surface of the Golgi. In pathogenic protozoa, particularly in *Trypanosoma cruzi*, there are few works on the biochemical characterization and the physiology of Golgi complex. In this work we report that the 58-kDa protein can be used as a marker to characterize the Golgi complex of epimastigote forms of *T. cruzi*. Firstly we have obtained a highly enriched Golgi complex fraction which was characterized using biochemical and microscopic approaches. Immunoblotting assays showed that the antibody that recognizes the 58-kDa protein labeled bands all fractions obtained during the purification of the organelle and that the higher reactivity was observed in the purified Golgi fraction. Immunocytochemistry analysis using the monoclonal antibody anti-58 K protein, associated to high-resolution scanning electron microscopy, allowed to see gold particles, which detect the 58-kDa protein, at surface of the Golgi complex elements. Furthermore, immunofluorescence studies using the same antibody showed an intense labeling on the anterior region of the parasites.

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B-14 – CHARACTERIZATION OF THE SUBSTRATE SPECIFICITY OF CYSTEINE PROTEASE ISOFORMS FROM *T. CRUZI*: A COMPARATIVE STUDY BETWEEN CRUZIPAIN 2 AND CRUZAIN

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Studies on protozoan parasites have focused attention on enzymes that play key roles in parasite survival. In *Trypanosoma cruzi*, structure-functional studies on cysteine proteases were thus far limited to the parasite's major isoform, the lysossomal protease designated as cruzipain, cruzain or GP57/51. This enzyme is encoded by a large family of polymorphic genes that can be distinguished from the mammalian homologues by the presence of an unusual C-terminal extension. Studies have demonstrated that inhibition of cysteine proteases prevents growth and differentiation of the parasite *in vitro* (Meirelles, N. M. *et al*, 1992, Mol. Biochem. Parasitol. 52:175-184; Harth, G., *et al*, 1993, Mol. Biochem. Parasitol. 58:17-24) and *in vivo* (Engel, J. C., *et al*, 1998, J. Exp. Med. 188:725-734), validating these enzymes as potential targets for anti-trypanocidal drugs.

Years ago, we have identified an isoform of cruzipain, cruzipain 2, that shares 86% of sequence identity with cruzipain (Lima, *et al*., 1994, Mol. Biochem. Parasitol. 67:333-338). Despite the high degree of homology between cruzipain 2 and cruzipain, the clustering of non-conservative substitutions in the catalytic domain suggested that they might be enzymes with different substrate specificity and/or kinetic parameters. Recombinant cruzipain 2, expressed in *S. cerevisiae*, shows different pH stability, substrate specificity and sensitivity to natural and synthetic inhibitors of cysteine proteases, as compared to recombinant cruzain.

In this work, we set out to investigate the substrate specificity of this new isoform using a series of intramolecularlly quenched fluorogenic substrates with variations in the P_1, P_2 and P'_1 positions. Substrate hydrolysis was followed by measuring the increase in fluorescence with time at 37°C. Determination of K_m, K_{cat} and K_{cat}/K_m, indicated that cruzipain 2 isoform differs substantially from cruzain in regard to the P_2 position. While cruzain accepts equally large hydrophobic and charged residues in P_2, cruzipain 2 has more strict requirements in this position. Considerable differences between the isoforms were also observed in analyzing the P'_1 position. Cruzain does not discriminate between large or small hydrophobic residues in P'_1, while cruzipain 2 does not accept well bulky hydrophobic residues. Additional kinetic studies are required to define the fine specificity of these isoforms, thereby instructing the design of isoform-specific inhibitors. Such reagents are required to assess more rigorously the physiological roles of these individual cruzipain isoforms.

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B-15 – STUDIES OF CALCIUM IONS DURING THE INTERACTION OF *TRYPANOSOMA CRUZI*-CARDIOMYOCYTES

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Signaling events involving intracellular free calcium were investigated during the invasion process of *Trypanosoma cruzi* with mouse cardiomyocytes. Using Fura 2-AM, we found that the parasite was able to induce Ca^{2+} signal in the host cell, which started after 500s of interaction and remained continuous. This signal was independent of the excitation-contraction signal and seems to be associated with the adhesion of parasite to the host cell surface. The mean value of the cytosolic calcium signal frequency in the normal cardiomyocytes after 72hs of cultivation was 0.3 cycles per seconds. The role of the intracellular Ca^{2+} levels during on *T. cruzi* invasion was evaluated using 2,5 and 5mM EGTA in addition to 0,5mM calcium ionophore A23187 and we found dose dependent inhibition levels of 41,7% and 72%, respectively. When we used only 2,5mM and 5mM EGTA it also ocurred a dose dependent invasion inhibition of 15,2% and 35,1%, respectively. Intracellular free Ca^{2+} movements in cultured cadiomyocytes were observed by scanning confocal microscopy with the Ca^{2+} - sensitive fluorescence probe fluo 3-AM. Images of the intracellular free Ca^{2+} movements were obtained at 0.4 – 2.0 seconds intervals. One type of cytoplasmic Ca^{2+} movement observed was a simultaneous increase in $[Ca^{2+}]_i$ in the nucleus and cytoplasm termed "spike" and another type was a local increase in $[Ca^{2+}]_i$ propagating in the cytoplasm, termed "wave". These movements are triggered by depolarization-induced Ca^{2+} influx across the sarcolemma (spike) or a propagating local increase in Ca^{2+} due to Ca^{2+} release from the sarcoplasmic reticulum ("sparks" → "waves"). In infected cardiomyocytes, the parasite altered the passage of both simultaneous and local Ca^{2+} increases that propagated in the cytoplasm and this signal passed through the parasites. These results indicate that *T. cruzi* interferes with calcium homeostasis of the cardiomyocytes.

We also studied the distribution of the Ca^{2+} binding sites at cardiomyocyte sarcolemma using the trivalent catium lanthanum. This tracer displaces the surface Ca^{2+} that is bound to the sarcolemma bilayer phospholipid involved in the muscle cells contractility. Both normal and *T. cruzi* infected cells from 24 to 96hs displayed lanthanum precipitate distributed at the sarcolemma surface. Our results indicate that the parasites did not affect the calcium sites at the sarcolemma bilayer during the whole kinetic studied. Also, the presence of lanthanum nitrate at all times of infection points to the presence of molecules rich in anionic sites responsible for the extracellular calcium pools probably involved in the maintainance of contractility observed until later than 120h of parasite interaction. Early times of infection will be analysed in order to see if these Ca^2 sites displaced by the tracer are affected during parasite invasion.

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B-16 – INTERACTION OF CHAGASIC ANTIBODIES WITH THE ALLOSTERIC SITE OF ATRIAL MUSCARINIC ACETYLCHOLINE RECEPTORS

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Previous studies have shown that antibodies from chagasic patients induce desensitization of Human M_2 muscarinic acetylcholine receptors (M_2AChR) expressed in CHO cells (J Biol Chem 272:12989-93 1997) and suppress cardiac electrogenesis when assayed by the functional Langendorff setup (Circulation 96 2031-37 1997). In the present study we demonstrate the interaction of CrCh-IgG with a putative allosteric binding site in the second extracellular loop of M_2AChR by displacement radioligand binding techniques in the presence or absence of Mg^{2+}. Porcine right atrium membranes with M_2AChR were obtained by density gradient (13 and 28% sucrose) centrifugation. Isothermal binding curves revealed a Bmax = 275 fmol/mg of total protein and a K_D = 130 pM for [^{3}H]-N-methyl scopolamine ([^{3}H]-NMS). CrCh-IgG were purified from chagasic patient's (classified as group II or III by the Los Andes criteria for the Chagas' disease) sera using a DEAE ion-exchange chromatography. The binding assays were performed using concentrations of the orthosteric ligand, [^{3}H]-NMS against increasing concentrations of CrCh-IgG ($1x10^{-7}$ to $1x10^{-6}$ M). These experiments were done over a range of 0.1 to 1 nM [^{3}H]-NMS. When the competition assays were performed in the presence of 1 mM Mg^{2+}, [^{3}H]-NMS binding was inhibited but there was no shift in the binding curves using different orthosteric ligand concentrations. However, Ki values for CrCh-IgG varied from $5.032x10^{-7}$ ±$2.65x10^{-7}$M to $2.364x10^{-7}$ ±$7.14x10^{-8}$M for the lowest and highest concentrations of [^{3}H]-NMS. By contrast, when Mg^{2+} is absent, a shift to the right is observed in a mass action law manner and the Ki values remain the same ($8.990x10^{-7}$ and $2.82x10^{-6}$ M). In order to study [^{3}H]-NMS dissociation kinetics, assays were prepared at adequate time intervals over a period of at least 180min. The dissociation of [^{3}H]-NMS from M_2 receptors induced by the allosteric ligand Gallamine was antagonized by CrCh-IgG in a competitive fashion. Koff values were 0.0035 min^{-1} for Gallamine and 0.0068 min^{-1} for Gallamine in the presence of CrCh-IgG. From our results we conclude that CrCh-IgG binds to a site distinct from the orthosteric ligand binding site and the bound antibody modulates ligand binding at the latter site.

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B-17 – CHARACTERIZATION OF A RAB11 HOMOLOGUE IN *TRYPANOSOMA CRUZI*

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Vesicle trafficking between organelles occurs through fusion of donor and specific acceptor membranes. This process is highly regulated and ensures proper direction in sorting and packaging of a number of molecules in eukaryotic cells. Monomeric GTPases of the Rab family play a pivotal role in the control of membrane fusion and vesicle traffic. In this work we characterize a *Trypanosoma cruzi* Rab 11 homologue (TcRab11) which shares at amino acid level 40% similarity with human rab11, *Arabdopsis thaliana* rab11 and yeast rab11 homologue genes. Western blot analysis, using a polyclonal rabbit antiserum raised against a synthetic peptide derived from the COOH-terminus of predicted TcRab11 protein, reacted to a 25kDa protein. In immunofluorescence assays, TcRab 11 showed to be expressed in epimastigote and amastigote forms but not in trypomastigotes. Interestingly, TcRab11 product seems to be located at the reservosome complex, a site of active endocytosis and vesicle fusion, present only in epimastigote stage. Therefore, TcRab11 may represent the first molecular marker of this peculiar organelle.

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B-18 – PHOSPHOLIPASES FROM *TRYPANOSOMA CRUZI* ACTIVE ON PHOSPHATIDYLINOSITOL AND INOSITOLPHOSPHOCERAMIDE

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The lipid moiety in the glycosylphosphatidylinositol anchors of glycoproteins of *T. cruzi* consists of an alkylacylglycerol (AAG), a *lyso*-alkylglycerol or a ceramide. We showed previously that the inositolphosphoceramides are the major components in the precursor inositolphospholipids of epimastigote (Bertello *et al*., 1995, *Biochem. J*., 310, 255-261) and trypomastigote forms (Uhrig *et al*., 1996, *Biochim. Biophys. Acta*, 1300, 233-239). None of them contain lignoceric acid, a constituent of the lipopeptidophosphoglycan (LPPG), a major component of epimastigote membranes with an anchor-like structure. Also, the lipid in the anchor of the mucins of *T. cruzi* changes from AAG to ceramide when epimastigotes differentiate to metacyclic trypomastigote forms (Acosta Serrano, *et al*., 1995, *J. Biol. Chem*., 270, 27244-27253). These results strongly suggest that the precursor lipid is remodelled either before or after transfer to proteins. Using [^{3}H] labeled subfractions of inositolphosphoceramides (IPCs) and phosphatidylinositol (PI) as substrates and a cell free system we now demonstrate the association of several enzyme activities with the trypanosomal membranes. The particulate material was prepared by hypotonic lysis of epimastigotes and incubated with labeled IPC or PI, containing AAG and diacylglycerol (DAG) species, for different times. The lipids, extracted with butanol after the incubation, were analysed by TLC and fluorography.

Two activities of phospholipase C have been detected, one releasing ceramide from IPC and the other AAG, alkylglycerol or DAG from PI. The phospholipase C activity was inhibited by *p*-chloromercuriphenylsulfonic acid as reported for other PI-PLCs. Also, it was shown that the amount of AAG was increased by the presence of Ca^{2+}, although the enzyme was also effective in the absence of added Ca^{2+}. In the case of the IPC hydrolyzing activity, although inhibition by EDTA was observed the activity was slightly increased by Ca^{2+}. Both PLC activities were not affected by *o*-phenantroline.

The main degradation observed by incubation of radioactive PI with membranes was the release of fatty acid in a time dependent reaction, showing endogenous PLA activity which generated three *lyso*-PIs. For their identification they were separately eluted from the plate and aliquots were treated with PI-PLC, PLA_2 or saponified, leading to the conclusion that both, PLA_1 and PLA_2 membrane activities were working on the PIs containing DAG. Probably the *lyso*-lipid containing alkylglycerol is generated by the same PLA_2. At a slower rate fatty acid was released by a lipase directly from DAG. An acyltransferase acting on the *lyso*-lipids and introducing cold endogenous fatty acid would account for a larger amount of free fatty acid compared with the radioactivity found in the *lyso*-lipids. This hypothesis was confirmed by incubating [^{3}H]-palmitic acid with the membranes, showing incorporation of radioactivity into the IPLs. Also, fatty acid is released when incubating radioactive IPC with the membranes.

In conclusion, the phospholipase activities demonstrated in the membranes of epimastigotes may be important for the generation of second messengers. More experiments are necessary for defining their role in remodelling reactions leading to mature GIPLs or to the anchor of glycoproteins of *T. cruzi*.

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B-19 – FURTHER PURIFICATION AND LOCALIZATION OF *O*-α-*N*-ACETILGLUCOSAMINYL TRANSFERASE OF *TRYPANOSOMA CRUZI* Y-STRAIN

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Previously, we have characterized in microsomal membrane preparations from epimastigote and trypomastigote forms an UDP-*N*-acetylglucosamine:polypeptide-*O*-α-*N*-acetyl glucosaminyl-transferase (Previato *et al.*,1998. *JBC* 273: 14982-14988), which catalyzes the attachment of *N*-acetylglucosamine units to threonine residues in the first step of the biosynthesis of the surface sialoglycoproteins of *T. cruzi*. Here, we present further studies on partial purification of this glycosyltransferase. Among several detergents tested at different concentrations, for enzyme solubilization in microsomal membrane, the higher activity was observed with CHAPS 0.5%. The CHAPS-soluble enzymatic activity was applied onto a Sephacril S-500 column equilibrated with Tris-HCl buffer pH 7.4, containing DTT and 0.5% CHAPS. An increment of three times of the transferase specific activity was observed in the void volume of Sephacril S-500 column. In addition, 85% of the total protein were eliminated during this purification step. In another approach, a highly enriched Golgi complex fraction was obtained using differential and discontinuous sucrose gradient centrifugation after cell rupture (Morgado Días *et al.*, 1998. Memórias do Instituto Oswaldo Cruz, 93 BI-133). In the Golgi-rich fraction, was observed an increase of four times of the enzymatic specific activity. Other purification steps are under way, starting from enriched Golgi fraction to attempt the complete *T. cruzi* UDP-GlcNAc transferase purification in order to realize molecular and kinetic studies of this unique *T. cruzi* enzyme.

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B-20 – CHEMICAL SYNTHESIS OF LONG-CHAIN FATTY ALCOHOLS, 1-*O*-ALKYL AND 1-*O*-ALCYL-DIHYDROXYACETONE-3-PHOSPHATE AND THEIR ANALOGUES FOR STUDIES OF THE ETHER-LIPID BIOSYNTHESIS IN *TRYPANOSOMA CRUZI*

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The mechanisms of ether-lipid biosynthesis are associated with organelles called microbodies that include peroxisomes in animal cells and glycosomes in trypanosomatids. The initial steps comprise the dihydroxyacetonephosphate (DHAP) pathway which are catalysed by the enzymes DHAP acyl-transferase, alkyl-DHAP synthase and acyl/alkyl-DHAP reductase. All these enzymes were described to be associated with glycosomes in protozoan parasites from the trypanosomatid group including *Trypanosoma brucei* and *Leishmania donovani* and *L. mexicana*. Alkyl-DHAP synthase, one of the key enzymes in the process, is quite easy to assay but the substrates are not comercially available. Understand how these ether-lipids are synthesized and transported inside the cell may bring important advances in studies of Chagas' disease (caused by *T. cruzi*), and other human diseases including rhizomelic chondrodysplasia punctata, cerebrohepatorenal syndrome (Zellweger) and cancer.

The objective of the present work is to chemically synthesize long-chain fatty alcohols, 1-*O*-alkyl and 1-*O*-alcyl-dihydroxyacetone-3-phosphate and their analogues for studies of the ether-lipid biosynthesis in *T. cruzi*.

In order to synthesize the long-chain fatty alcohol, it was possible to obtain the intermediate $C_{16}H_{33}OCH_2CO_2H$ which will be transformed into the target products. In the analogues and possible inhibitors series, it was possible to prepare several diazo methyl derivatives following the methodology described by Hajra et al. (Chem. Phys. Lipids, 1983, 33: 179). In addition, an allyl naphtyl ether derivative was obtained by the reaction of allyl bromide and alpha-naphtol following the reaction described by Takahashi et al. (J. Am. Chem Soc., 1990, 112: 5876). The reaction presented an yield of 51,3% and the product was characterised by Infrared Spectroscopy (IR) and ^{1}H Nuclear Magnetic Resonance (^{1}H-NMR). This allyl naphtyl ether product was further oxidized with $KBrO_3$ and the products are currently under IR and ^{1}H-NMR analyses.

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B-21 – EFFECTS OF FUNGAL SPHINGOLIPID BIOSYNTHETIC PATHWAY INHIBITORS OVER *TRYPANOSOMA CRUZI* IN AXENIC CULTURE

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The plasma membrane of *T. cruzi* insect stage forms is covered by glycosylphosphatidylinositol (GPI) anchored mucin-like glycoproteins and glycoinositolphospholipids. The lipid moieties of these GPI anchored components may be formed by ether lipids or sphingolipids. Despite the extensive compositional analyses done so far, little is known about the mechanisms of ether-lipid and sphingolipid biosynthesis in *T. cruzi*.

The initial steps of sphingolipid biosynthesis in fungi and animal cells are conserved. However, the description of selective inhibitors suggested functional differences between the enzymes present in the two groups. Therefore, those enzymes became excelent targets for the action of new and potent anti-fungal chemotherapeutic agents. In addition, fungi do not form sphingomyelin like mammalian cells but concentrate in the production of inositolphosphoceramide (IPC) containing lipids. Because of the similarity in the production of several components containing IPCs, the sphingolipid biosynthetic pathway may also be explored as a putative chemotherapeutic target against *T. cruzi*.

In the present work, we have used 3 inhibitors that act at different steps of fungal sphingolipid biosynthesis in order to study their effects over epimastigote and metacyclic forms of Y strain of *T. cruzi*. Opposite to *Cryptococcus neoformans*, australifungin and rustmicin, inhibitors of dihydrosphingosine *N*-acyl transferase and inositolphosphoceramide synthase respectively, did not cause any effect in *T. cruzi* viability as judged by the Alamar Blue staining method. However, optical microscopy observations indicated morphological changes in the trypanosomes incubated with (or above) 5-7 ug/ml of australifungin. Equivalent or higher concentrations of rustmicin did not show any effect. Concentrations higher than 200 ug/ml of beta-chloro-alanine, an inhibitor of serine-palmitoyl transferase, produced intense parasite lysis with lost of viability after 48-72 h of incubation.

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B-22 – EVIDENCE FOR A SIALOSYL CATION TRANSITION-STATE IN THE SIALIDASE ACTION OF *TRANS*-SIALIDASE FROM *TRYPANOSOMA CRUZI*

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The protozoan parasite *T. cruzi*, takes advantage of a *trans*-sialidase (TS) enzyme to scavenge sialic acid from exogenous sialoglycoconjugates and transfer it to mucin-like molecules on parasite surface. The TS-mediated surface sialylation is thought to play an important role in host-parasite interactions. *In vitro* studies have shown that *T. cruzi* TS preferentially catalyzes the transfer of sialic acid residues from Siaα2-3Galβ1-*x*-containing donors and attaches them in α2-3linkage to terminal β-galactopyranosyl-containing acceptors. In absence of a suitable acceptor TS irreversibly transfers sialic acid to a water molecule, thus functioning as a sialidase similar to viral, mammalian and bacterial sialidases. Sequencing of *T. cruzi* TS genes shows that although TS differs from those sialidases in acceptor specificity it is a member of the sialidase superfamily. The catalytic site region in the *N*-terminal domain of *T. cruzi* TS contains all amino acid residues which are known to interact with sialic acid in substrate binding pocket of all other sialidases studied, it also contains the FRIP motif and five conserved Asp box sequences, which are found at similar positions in bacterial sialidases. In a recent work, we demonstrated by ^{1}H NMR that TS from *T. cruzi* hydrolyzes the sialyl glycosydic linkage with retention of configuration at the anomeric center of sialic acid as found for all sialidase studied (Todeschini *et al.*, 1999. Glycobiology, In Press). Here, we show that methanol can acts as nucleophile in the TS catalyzed hydrolysis of 4-methyl-umbelliferyl-N-acetylneuraminic acid. Our date indicate that the amount of free sialic acid production decreases with increasing methanol concentration, due to simultaneous formation of α-Neu5Ac2Me with no effect on the release of 4-methyl-umbelliferone. In addition, prolonged incubation of α2-3sialyllactose with an excess of TS produced a trace of 2-deoxy-2,3-didehydro-N-acetylneuraminic acid (Neu5Ac2en) which was identified by NMR spectroscopy and by gas liquid chromatography/mass spectrometry. The latter compound is a transition-state analogue of the oxicarbonium ion intermediate proposed to be formed during sialoside hydrolysis by viral sialidases. All together these results suggest that TS from *T. cruzi* catalyzes the hydrolysis reaction involving a oxicarbonium ion as key transition-state. During this reaction, planarization of the sialic acid around the ring oxygen, C1, C2 and C3 in the active center of TS induces the release of the ketoside bound to the glycosidic oxygen at C2. This release generates a sialosyl cation intermediate, which is rapidly attacked by nucleophiles such as water or methanol. A proton elimination at C3 during the formation of the oxicarbonium ion intermediate leads to the formation of Neu5Ac2en. These results suggest that transition-state analogues may provide a strategy for the rational design of trans-sialidase inhibitors which could be highly effective agents for the chemotherapy of acute phase *T. cruzi* infections.

Financial support: PADCT, PRONEX, FUJB, HHMI, CNPq, CEPG/UFRJ.

B-23 – TANDEM NANO-ELECTROSPRAY ANALYSES OF *O*-LINKED-*N*-ACETYL GLUCOSAMINE-CONTAINING OLIGOSACCHARIDES FROM SIALIC ACID ACCEPTOR GLYCOPROTEINS OF TRYPOMASTIGOTE FORMS OF *TRYPANOSOMA CRUZI* (COLOMBIANA STRAIN)

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Highly *O*-glycosylated proteins on the cell surface of *T. cruzi* function as sialic acid acceptors in the *trans*-sialidase reaction, and have been strongly implicated in the molecular mechanism of attachment to and invasion of mammalian cells. Most of the structural information on the oligosaccharides of these glycoproteins has been gained from the study of axenically-cultured non-infective epimastigotes (Previato *et al*., Biochem. J. 301: 151-159, 1994, Previato *et al*., J. Biol. Chem. 270: 7241-7250, 1995, Jones *et al.*, Abtracts of Eurocarb17 (Utrecht), B36, 1997), rather than from infective trypomastigote forms (Almeida *et al.*, Biochem. J. 301: 793-802, 1994), because of the difficulty in obtaining glycans from the latter in sufficient quantity for structural study by currently available methods.

ESI-MS (electrospray ionization-mass spectrometry) is a powerful tool for the characterization of oligosaccharides. The recent development of hybrid quadrupole / time of flight (Q-Tof) spectrometers, equipped with nano-flow sources now enables ESI analysis of underivatized oligosaccharides at picomolar sensitivity. In this study, tandem mass spectrometry with collision induced dissociation (CID) was used to determine the composition of oligosaccharide-alditols liberated by reductive β-elimination of sialic acid acceptor glycoproteins purified from trypomastigote forms of (Colombiana strain). Trypomastigotes were obtained from MK_2 cells infected with tissue culture-derived trypomastigotes, maintained at 37ºC in modified Dubelco medium containing 1% fetal calf serum. After initial fractionation, the oligosaccharide-alditols were further purified by HPLC on a porous graphite column (Hypercarb) and were analysed by ESI-MS with a Micromass Q-Tof instrument. It was found that the sialic acid acceptor glycoprotein of tissue culture-derived trypomastigotes of *T. cruzi* Colombiana strain contains at least five *O*-linked-*N*-acetyl glucosamine-linked oligosaccharide chains with the following compositions; Hex-HexNAc-ol, $(Hex)_2$-HexNAc-ol, $(Hex)_3$-HexNAc-ol, $(Hex)_4$-HexNAc-ol, and $(Hex)_5$-HexNAc-ol. Studies are currently in progress to determine the complete monosaccharide, linkage and branching structures of these oligosaccharides.

Supported by: HHMI, CNPq, FINEP, PADCT, CEPG/UFRJ.

B-24 – MOLECULAR CHARACTERIZATION OF GLYCOINOSITOLPHOSPHOLIPID (GIPL) OF *TRYPANOSOMA CRUZI* - COLOMBIANA STRAIN

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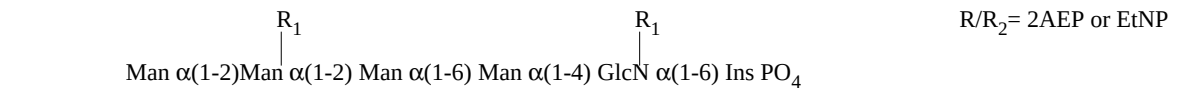
GIPLs are surface glycolipids synthesized in abundance by *T. cruzi* epimastigote forms. There are structural diversities in GIPLs from different species of trypanosomatids, which could be related to the host specificity. GIPLs from many *T. cruzi* strains have been characterized. Although they share the same Man 4-GlcN-Ins-PO_4 core sequence, they can be classified into two series on the basis of the substituent on the third Man distal to Ins. Series 1 has a phosphorus containing group (EtNP or AEP) and Series 2 has a β-D-Gal*f* unit.

To extend this analysis, GIPLs from *T. cruzi* Colombiana strain, a drug resistant strain, were extracted with phenol-water and purified by solubilization in chloroform/methanol/water (10:10:3). The results from chemical determination, nuclear magnetic resonance spectroscopy anf mass-spectrometry showed that the structure of Colombiana-derived GIPL is identical to the one expressed by *T. cruzi* Tulahuen strain which synthesizes a GIPL lacking Gal*f* residues:



Supported by: CNPq, FINEP, Pronex, CEPG/UFRJ

B-25 – A MOLECULAR MODELING COMPARATIVE STUDY BETWEEN AMINO GUANYL AMIDES AND GUANYL HYDRAZONES LIKE TRYPANOCIDE AGENTS USING EMPIRICAL METHOD

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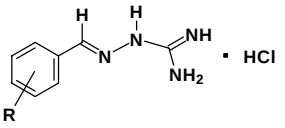
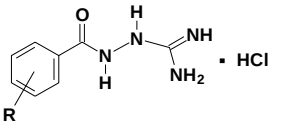
The monocationic guanyl hydrazones (1) have been studied as trypanocides substances, showing *in vitro* and *in vivo* activity against *Trypanosoma cruzi*. Another class of monocationic substances containing a guanidine group, the aminoguanyl amides (2), which may present a shorter half life and lower toxicity, were designed in our group. The presence of an amide moiety in the aminoguanyl amides suggests taht these drugs may be more metabolizable than guanyl hydrazones. A comparative study of both types of compounds was carried out using molecular mechanics as well as some *in vitro* activity results. In this comparison a CVFF and AMBER were utilized like forcefields, and a docking methodology was used too study the intermolecular energy of the interaction drug-DNA. A previously minimized B-DNA dodecamer was used a model for B-DNA in the docking studies with the chosen aminoguanyl amides and guanyl hydrazones. The conformational analysis was carried out for 5 aminoguanyl amides and the equivalent 5 guanyl hydrazones using Torsion Drive methodology, with the angles changing for about 18º. For the bioassays, trypomastigote forms of the parasite were obtained from mices inoculated intraperitoneally with 10^5 cells of Y strain *T. cruzi*. , and the values of IC_{50} were obtained by linear and polynomial regression analysis.

The results show that the degree of planarity of the molecule is related with lower docking energies, with the aminoguanyl amides being more planar than the guanyl hydrazones. This greater stability of the aminoguanyl amide-DNA complex is followed by lower values of IC_{50} indicating that the drug-DNA interaction may play a role in the mechanism of action of these compounds. For example, the docking energy for the aminoguanyl amide of benzaldehyde is 4 kcal/mol lower than the enregy for the respective guanyl hydrazone (205 μM) and is also more active (ID_{50} 175 μM).



(1)



(2)

Financial suport: PADCT/CNPq, FAPERJ, CAPES.

B-26 – RELATIVE BINDING AFFINITIES OF ENZYME INHIBITORS: MOLECULAR MODELING OF *T. CRUZI* CYSTEINE PROTEASES INHIBITION

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We use molecular modeling methods to investigate interactions of inhibitors with the catalytic site of Cruzain and Cruzipain2, important cysteine proteases in vital cycle of protozoan parasite *T. cruzi*, the etiologic agent of Chagas' disease. We present a new approach to estimate relative interaction affinities of enzyme inhibition, making use of the crystal structure of the complexes as well as modeled structures.

We submit these complexes to an energy minimization produce and molecular dynamics simulations From theses structures we predict the relative interaction affinity between different protease-inhibitor complexes. We have tested this protocol using the E-64 compound, a potent irreversible inhibitor of many cysteine proteases. The experimental results show that E-64 is 30 fold more potent inhibitor for cruzain than for cruzipain2.(Lima,A.P. unpublished data).

The methodology used to identify important aminoacids of enzymes of interaction inhibitors - catalytic site, was based on the alignment of primary sequences of papain, cruzain and cruzipain2 (ALING program). The cruzipain2 was modeled using homology methods based on the crystal structure of cruzain. A automatic modelling program have been used - SWISS-MODEL program.. The E-64 -cruzain and E-64 - cruzipain2 were modeled based on the crystal structure of the actinidin - E-64 (WATHIF program).

The geometry optimization of E-64 - Cruzain and E-64 - cruzipain2 were performed and the potential energy for each complex was determined using molecular mechanics (steepest descent / conjugate gradient algorithms) and dynamics simulations (THOR program). M.D. simulations were performed during 1ns at 300K using ε=1. The energy of E-64 - cruzain and E-64 - cruzipain2 complexes was compared to the enzymes without inhibitor, in order to obtain the difference between the energies variations to form the complexes. All numerical calculations were carried out on ORIGIN 2000-SILICON GRAPHICS computer.

The potential energies of these structures were -1125,39 Kcal/mol for cruzain, -1036,29 Kcal/mol for cruzipain2, -1205,16 Kcal/mol for cruzain-E-64 and -1162.53 Kcal/mol for cruzipain2-E-64. Comparative calculations showed that the binding of E-64 molecule is much more energetically stable with cruzain than cruzipain2. The energy-minimized structures of E-64- cruzain and E-64-cruzipain2 complex showed the formation of five hydrogen bonds, for E-64 - cruzipain2 and six hydrogen bonds for cruzain – E-64 structures in the interactions of the catalytic site. The movement of Glu 205 in the cruzipain2 - E-64 complex suggested that Glu 205 might adopt inhibitor-directed conformation while in the cruzain – E-64 the lateral chain of Glu 205 is directed to the S2 pocket. These results suggest that the presence of hydrogen bonds and the movement of Glu 205 could have a play an important role in the affinity of binding of E-64.

Financial Support: CAPES, PRONEX.

B-27 – STRUCTURAL STUDIES OF PEPTIDES INVOLVED IN AUTOIMMUNITY RESPONSE IN CHAGAS' DISEASE BY CIRCULAR DICHROISM AND NUCLEAR MAGNETIC RESONANCE

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Previous studies suggest that antibodies (Ab) directed against the ribosomal P protein of *Trypanosoma cruzi* cross-react with the human P protein and the human heart muscarinic acetylcholine receptors (mAChR) in patiens with chronic Chagas heart disease (cChHD). The target of this cross-reaction was mapped as a region possessing a short sequence of negatively charged residues present in the C-terminal end of the parasite protein, known as R13 (EEEDDDMGFGLFD). This sequence is also present in same region of the human P protein (peptide H13: EESDDDMGFGLFD) and in the second extracellular loop of the mAChR (VRTVEDGECYIQFFSNAAVTFGTAI). These peptides of the P protein also have homology with the C-terminal region of P protein of the *Leishmania braziliensis* (peptide A13: EEADDDMGFGLFD). However A13 has no cross-reaction with anti-P Ab and R13.

Probably this effect is due to structural similarities between R13, H13 and the loop of the mAChR. We analyze the structural properties of these peptides[1] by Circular Dichroism (CD) and Nuclear Magnetic Resonance (NMR). The CD spectras have shown the influence of different solvents on secondary structure induction and similarities in the behavior of the R13 and H13 peptides. The preliminary results obtained from NMR 2D experiments indicated a residual structure for the R13 peptide.

[1]Kindly provided by Prof. Antônio Carlos Campos de Carvalho (IBCCF, UFRJ, Rio de Janeiro)
Financial Support: CNPq, PRONEX.

B-28 – EFFECT OF PLATELET-ACTIVATING FACTOR ON ECTO-PHOSPHATASE AND ON ECTO-CASEIN KINASE ACTIVITIES OF *LEISHMANIA TROPICA*

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The leishmaniasis, which in its worst form is fatal, affects 12 million people, threatening 350 million people worldwide. These diseases are caused by different species of the genus *Leishmania*, transmitted by the bite of an insect vector, the phlebotomine sandfly. During their life cycles these trypanosomatids face several different environmental conditions, as they infect both invertebrate vectors and vertebrates. In the vertebrate hosts, these protozoa need to escape the host mechanisms of defense. Several molecular mechanisms have been described in the last few years, which ensure the parasite infection despite the host efforts. Protein phosphorylation-dephosphorylation is one of the most powerful mechanisms able to control cell activities. A series of studies have demonstrated the role protein phosphorylation may play in the coordination of parasite defenses. Ecto-phosphatase, ecto-nucleotide diphosphohydrolase and ecto-kinase activities have been described in trypanosomatids such as *Trypanosoma*, *Herpetomonas* and *Leishmania*. Platelet-activating factor (PAF) is a phospholipid which belongs to the family of autacoids and is a critical mediator in diverse biological and pathophysiological processes, including inflammation, allergy and cellular differentiation. PAF is synthesized and released from a variety of stimulated cells including macrophages. In recent reports, we have demonstrated that 10^{-6} M PAF triggers the process of cell differentiation of *H. muscarum muscarum* and of *Trypanosoma cruzi*, as well influence the ecto-phosphatase activity of these protozoa. In this work we have investigated the effect of PAF on the ecto-phosphatase and on the ecto-casein kinase activities of *Leishmania tropica*. The parasites were grown in BHI medium supplemented with 10% of fetal calf serum, at 28°C, for five days. Living parasites were assayed for the enzyme activities in the absence or in the presence of 10^{-6} M PAF. The assays were also performed using living parasites pre-treated with this mediator. PAF promoted an inhibition (approximately 32%) of the ecto-phosphatase activity and of the secreted one (inhibition of approximately 30%). An antagonist of PAF, WEB 2086, was able to revert the PAF effect on the ecto-phosphatase activity. The pre-treatment of the cells with 10^{-6} M PAF, promoted a two-fold increase in the casein kinase activity of the intact cells and of the secreted enzyme. We have already shown in a previous report that PAF promotes an increase in the number of macrophages infected with *L. tropica*. These results could be explained, at least in part, by alterations in the signal transduction of the parasites, potentially induced by PAF.

Supported by: CNPq, FAPERJ, FINEP and PRONEX.

B-29 – EFFECT OF PLATELET-ACTIVATING FACTOR ON THE INTERACTION OF *LEISHMANIA AMAZONENSIS* WITH PERITONEAL MOUSE MACROPHAGES: INFLUENCE OF MODULATORS OF PROTEIN KINASE C

[1]Rosa, M.S.S; [1]Lopes, A.H.C.S; [2]Pereira, A.F; [1, 2]Vieira, R.B. and [1, 3]Dutra, P.M.L.
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Parasites of the genus *Leishmania* are the causative agents of leishmaniasis, which can be fatal, affecting 12 million people worldwide. These parasites are transmitted to their mammalian hosts by the bite of an insect vector, the phlebotomine sandfly. During their life cycles these trypanosomatids face several different environmental conditions, as they infect both invertebrate vectors and vertebrates. In the vertebrate hosts, these protozoa need to escape the host mechanisms of defense. Platelet-activating factor (PAF) is a potent lipid autacoid which exerts a wide range of biological activities, including cellular differentiation, inflammation and allergy. PAF is synthesized and released from a variety of stimulated cells, including macrophages. All PAF effects described to date occur through specific receptor on the cell surface and signal transduction pathways, that induce activation of protein kinase C. In recent reports, we have demonstrated that 10^{-6} M PAF triggers the process of cell differentiation of *Herpetomonas muscarum muscarum* and of *Trypanosoma cruzi*, as well as modulates the ecto-phosphatase activity of these protozoan. Here we analyzed the effects of 10^{-6}M and 10^{-9}M PAF, in the presence or in the absence of modulators of protein kinase C (20 ng / ml TPA or 50 ng / ml sphingosine) in the interaction of *Leishmania amazonensis* promastigotes with tioglicolate-elicited peritoneal mouse macrophages *in vitro*. These assays were also performed in the presence or in the absence of an immunogenic *L. amazonensis* cytoplasmic fraction. This fraction was prepared by a combination of conventional cell fractionation procedures. In order to obtain this fraction, *L. amazonensis* promastigotes were grown in BHI medium containing 10% fetal calf serum. The number of infected macrophages, as well as the number of parasites within each macrophage was evaluated after 24 hours of infection. Our results indicate that the percentage of infected macrophages was higher when the parasites were treated with PAF prior to the infection. Also, in this case, the number of parasites in the infected macrophages was higher than when the macrophages were infected with non-treated parasites. On the other hand, when the macrophages were pretreated with PAF, a severe inhibition of the infection was observed. We could also observe an inhibition of this infection when both the parasites and the macrophages were pretreated with PAF. This inhibitory effect in the interaction between *L. amazonensis* and mouse macrophages was even more significant when either the macrophages or the parasites were pretreated with the *L. amazonensis* cytoplasmic fraction. We here demonstrate that sphingosine and TPA influence the interaction between *L. amazonensis* and mouse macrophages, both in the presence and in the absence of PAF.

Supported by: CNPq, FAPERJ, FINEP and PRONEX.

B-30 – EFFECTS OF HEAT SHOCK ON ECTO-ENZYME ACTIVITIES IN *LEISHMANIA AMAZONENSIS*

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Protozoa of the genus *Leishmania* parasites the tissue macrophages of their mammalian hosts, where they survive and profile inside the phagosome. This intracellular proliferation is the key for their survival inside the mammalian host.*Leishmania* parasites encounter heat stress as a regular feature of their digenic life cycle during the transmission of the promastigote stage from poikilothermic sandfly vectors into mammalian. It has been described that elevated temperature encountered within a mammalian host serve as a key trigger for the development from the promastigote into the intracellular stage, the amastigote (Zilberstein, D. & Shapira, M., 1994 *Annu. rev. Microbiol.* 48, 449-470). In some *Leishmania* species, elevated temperature alone can induce stage development (Bates, P. A., 1993 *Parasitol. Today* 9, 143-146). A temperature upshift similar to the one encountered during host invasion can indeed induce heat shock protein synthesis (Hsp), and it has been proposed that such elevated Hsp synthesis may protect the parasite against the adverse effects of higher temperature (Hunter, K. W. et al. 1984 *Biochem. Biophys. Res. Commun.* 125, 755-760). Moreover, the major (Hsps) such as Hsp 70 and Hsp83 (Hsp90) are highly in *Leishmania* promastigotes under all culture conditions and thus not likely to play amastigote stage-specific roles during infection (Brandau, S. et al. 1995 *Biochem. J.* 310, 225-232). We have previously characterized a Mg-dependent ecto-ATPase in *L. tropica* (Meyer-Fernandes et al. 1997 *Arch. Biochem. Biophys* 341: 40-46). The physiological role of this enzymes is still unknow but it is related to the Heat Shock 70 (Smith, T.M. & Kirley, T.L. 1999 Biochemistry, 38. 321-328). In this study we investigated the differences on ecto-enzymes activities of promastigote forms of *Leishmania amazonensis* under conditions of increasing temperature (22^{o}C to 28^{o}C) during the cell growth. We observed that increasing temperature on growth, stimulated ecto-ATPase activity at about 5.0-fold and ecto-3'nucleotidase activity 1.5-fold, but no effects could be observed on the ecto-5'-nucleotidase or ecto-phosphatase activity. In order to determine if the ecto-ATPase activities at 22^{o}C and 28^{o}C are deriving of the same enzyme, we used suramin, a competititve antagonist of P_2 purinoceptors and inhibitor of some ecto-ATPases, as well as, the non cell permeable agent DIDS (4'-4'-diisothiocyanostylbenzene-2-2'disulfonic acid). These two regents inhibited similarity both activities. Showing Ki values to suramin (210 μM to cells growth at 22^{o}C and 230 μM to cells growth at 28^{o}C), and Ki values to DIDS (90 μM to cells growth at 22^{o}C, and 106 μM to cells growth at 28^{o}C). We suggest here that the ecto-ATPase present in *Leishmania amazonensis* could be related to the Heat Shock proteins present in this parasite.

Financial Support: FAPERJ, CNPq, FINEP and PRONEX.

B-31 – BIOCHEMICAL CHARACTERIZATION OF THE PARASITOPHOROUS VACUOLE CONTAINING LEISHMANIA AMAZONENSIS PROMASTIGOTE AND AMASTIGOTE FORMS

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Leishmania amazonensis, the aetiological agent of cutaneous leishmanioses, presents two developmental stages: promastigote, the flagellated form that is inoculated by the sand fly bite inside the mammalian dermis and the amastigote, the intracellular form that resides in the macrophage inside a membrane bound compartment, the parasitophorous vacuole (PV). Thus, depending on the Leishmania form different surface molecules may "select" different receptor sets at the macrophage surface which will probably alter the PV membrane composition at the beginning of the phagocytic process. In order to understand the composition of the PV membrane surrounding Leishmania amazonensis promastigote and amastigote forms were allowed to interact for short periods of time with biotin labelled bone marrow derived macrophages. To verify the efficiency of biotin labeling, bone marrow derived macrophages were cultivated onto round cover slips for seven days in RPMI medium containing fetal calf serum (FCS) 10%, glutamine 2 mM and L929 conditioned medium > 10%, labeled with biotin -NHS for 20 minutes at 4C, washed and incubated with PBS/CM containing bovine serum albumin for 20 minutes at 40C, to block free biotin, and allowed to interact with promastigotes or amastigotes for 15 and 60 minutes, > fixed with paraformaldehyde, permeabilized in metanol at - 20C, blocked overnight with PHEM buffer pH 7.3 containing 1% BSA and ammonium chloride, mounted and observed by confocal laser scanning microscope. To isolate the parasitophorous vacuole from bone marrow derived macrophages were cultivated labeled with biotin- NHS and allowed to interact with parasites for 60 minutes. Afterwards the cells were scrapped in lysis buffer (Hepes 20 mM, sucrose 0.25 M containing protease inhibitors) and lysed through passage by two coupled needles 23 gauge (20x),incubated with Dnase, > centrifuged at 211g for 10 minutes. The supernatant (homogenate) was poured onto the sucrose gradient and centrifuged at 845g for 30 minutes. The fraction corresponding to PV was collected and centrifuged at 5000g for 30 minutes. The PV were ressuspended in Hepes buffer containing protease inhibitors, frozen at - 70C and submitted to SDS-PAGE and Western Blotting. Confocal microscopy showed that the whole macrophage surface was stained. No significant labeling of the PV was observed after 60 minutes of interaction. However, some structures located near the PV were labeled. SDS-PAGE of the whole homogenate displayed a large number of proteins corresponding to macrophage and Leishmania proteins. After Western blotting several high molecular weight proteins, that represent macrophage surface proteins, were revealed. The isolated PV displayed a small number of proteins and after blotting only three major proteins could be clearly observed. One protein of 75 kDa was observed in the PV containg both promastigote and amastigote forms. In the PV containing infective promastigote two other proteins of high molecular wheight, around 220 and 204 kDa were clearly observed.These bands wele lightly labeled in preparations cotaining amastigotes and were not observed in vacuioles containing non infective promastigote forms.

Supported by Pronex, CNPq, FINEP and FAPERJ.

B-32 – INHIBITION OF PROTEASOME ACTIVITY IMPAIRS INTRACELLULAR DEVELOPMENT OF *LEISHMANIA AMAZONENSIS* AMASTIGOTES

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Proteasomes are multicatalitic and multisubunit endopeptidase complexes widely distributed in eukaryotic cells. These enzymes are central proteases in cytosol and nucleus and are involved in removal of abnormal, misfolded or incorrectly assembled proteins, in the processing or degradation of transcriptional regulators in stress response, in degradation of cyclins in cell cycle control, in destruction of transcription factors or metabolic enzyme in cell differentiation and metabolic response and in the processing of protein antigens. Little is known, however, about the function and regulatory mechanisms of proteasomes in parasitic protozoan. We have previously purified a 20S form of proteasome from *Leishmania amazonensis* and shown that lactacystin, a specific proteasome inhibitor, halts promastigotes proliferation *in vitro.* In the present work, we show that the intracellular development of the parasite is also impaired by pretreating promastigotes with lactacystin. Promastigotes from early stationary phase were treated for four hours with different concentrations of lactacystin, washed and used to infect mouse peritoneal macrophages. The cultures of infected macrophages were kept on chambers over glass slides at 34ºC, and cells were dyed with May-Grünwald Giensa at different intervals. Treatment with lactacystin caused no effect on morphology, mobility or viability of promastigotes. Infectivity of treated parasites was the same as of untreated cells. However, on the fourth day of culture, the number of intracellular parasites previously treated with lactacystin was significantly lower than of the control. This effect was not observed if the promastigotes were treated with 50 μM E-64, a thiol-protease inhibitor, or 2 mM PMSF, a serino-protease inhibitor. It remains to be determined whether the cause of diminished number of intracellular amastigotes is a result of an increased rate of parasite death, a arrest on cell division, or a combination of the two processes.

Financial support: CNPq, CAPES and FAPEMIG

B-33 – *LEISHMANIA AMAZONENSIS* AND *LEISHMANIA GUYANENSIS* PROMASTIGOTES ESCAPE FROM LYSIS BY LEISHPORIN BY INTERFERING WITH PORE FORMATION

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We have recently described in *L. amazonensis* and *L. guyanensis* extracts a cytolytic activity. In *L. amazonenis*, this activity is associated with a membrane pore-forming protein, named leishporin, that lyses erythrocytes and nucleated cells (Noronha et al., 1996, *Infect. Immun*. 64 (10): 3975-3982; Horta, 1997, *Trends in Microbiol*. 5 (9): 363-366). We have previously reported that leishporin is secreted by *L. amazonensis* promastigotes without lysing the parasites, suggesting that promastigotes are resistant to their own cytolysin. Indeed, we have shown that *L. amazonensis* promastigotes incubated with their own cytolytic extract are more resistant to lysis than erythrocytes and nucleated mammalian cells. This resistance increases during its growth *in vitro*, reaching a peak at late logarithmic phase. In the present work, we show that *L. guyanensis* promastigotes are also resistant to lysis by *L. amazonensis* leishporin. The resistance of *L. guyanensis* to lysis by leishporin was investigated by incubating viable promastigotes with a cytolytic promastigotes extract (p-ext) at 37ºC for 30 min at pH 5.5, the optimal conditions for the cytolysin full activity. After staining with Erytrosin B, dead and alive parasites were counted. We have observed, however, that unlike *L. amazonensis*, *L. guyanensis* resistance is stable during their growth *in vitro*. To determine whether parasite surface proteins are involved in resistance of the promastigotes to lysis by leishporin, viable promastigotes of *L. amazonensis* or *L. guyanensis* were incubated with proteinase K, trypsin and chymotrypsin in different concentrations (6.25, 12.5, 25 and 50 (g/ml) for 2 hours at 34ºC). Parasites were then washed, incubated with p-ext for 30 minutes at 37ºC and assayed for viability. We have found that proteinase K and trypsin caused a dose-dependent reduction in *L. amazonensis* and *L. guyanensis* promastigotes resistance to lysis by leishporin. Chymotrypsin, on the other hand, had no effect on parasites' sensitivity to lysis. These results indicate that surface parasite proteins are involved in resistance of promastigotes to lysis by leishporin. Previous results from our laboratory have determined that pore formation by leishporin involves at least two distinct stages: 1) the binding to the cell membrane and 2) the pore formation itself, that probably involves the insertion and oligomerization of subunits with subsequent cell lysis. To investigate in which step the surface proteins are involved, promastigotes from both species were treated with proteinase K and incubated with p-ext for 15 min on ice. After incubation, the parasites were assayed for viability, the samples were centrifuged at 3000 rpm and the hemolytic activity of the supernatant was determined. We have observed that the supernatants of p-ext obtained after incubation with the parasites treated or not with the protease were hemolitically inactive, suggesting that leishporin binds as efficiently to treated parasites as to untreated parasites. However, after a second incubation of the same parasites at 37ºC and pH 5.5, while the untreated parasites remain intact and viable, the treated parasites are more sensitive to lysis by leishporin. These data indicate that promastigote surface proteins renders promastigotes of both species resistant to lysis by leishporin without interfere with the binding of the protein to their surface.

Financial support: CNPq, CAPES and FAPEMIG *L. amazonensis* and *L. guyanensis*

B-34 – LEISHPORIN BECOMES ABLE TO BIND TO MEMBRANE LIPIDS AFTER DISSOCIATION FROM AN OLIGOPEPTIDE INHIBITOR

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We have described in *Leishmania amazonensis* extracts a pore-forming protein, named leishporin, that lyses erytrhocytes and nucleated cells (Noronha *et al*., 1996, *Infect. Immun*. 64 (10): 3975-3982; Horta, 1997, *Trends in Microbiol*. 5 (9): 363-366). The pore formation involves at least two distinct steps: 1) the binding of the cytolysin to the target cell membrane and 2) the lysis itself that probably involves the insertion and/or oligomerization of subunits. In the present work, we show that the treatment of human red blood cells (HuRBC) with trypsin, pronase or proteinase K does not affect their susceptibility to lysis mediated by leishporin, indicating that surface proteins are not important for the binding of the cytolysin to the target cells. We also show that incubation of promastigotes extracts with glicose, manose, lactose, fructose or galactose does not interfere with the ability of the extract to lyse HuRBC, suggesting that carbohydrates either are not involved in the binding of leishporin to the target membrane. Furthemore, we demonstrate that leishporin can bind directly to lipids on the target cell membrane, corroborating the previous results. This conclusion was drawn from an experiment showing that multilamelar lipossomes made of 20 mM cholesterol and 25 mM dipalmithoilphosphatidylcholine incubated with cytolytic promastigote extracts completely remove the hemolytic activity of those extracts. Interestingly, the hemolytically inactive supernatant obtained after removing the lipossomes can be activated by incubation with proteinase K or with guanidine-chloride, previously shown to destroy or dissociate, respectively, an oligopeptide inhibitor non-covalently bound to leishporin. The newly generated hemolytic activity can again be removed by lipossomes. These results indicate that, while the inactive form of leishporin is unable to bind to membrane lipids, its active form, released by the dissociation of the inhibitor molecule, is able to bind to these lipids, probably by exposing hydrophobic sites on the molecule. The solubilized lipossome-bound proteins can be shown as two bands of M*r* of 45-66 kDa after silver staining of the gel after SDS-PAGE.

Financial support: FAPEMIG, CNPq, CAPES, PRONEX, PADCT

B-35 – *LEISHMANIA CHAGASI*: CULTURE OF AXENIC AMASTIGOTES AT 37ºC

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Parasite from the genus *Leishmania* causes a variety of disease states in humans and other mammals in tropical and subtropical areas, which include cutaneous, mucocutaneous, and visceral leishmaniasis. The parasite undergoes a digenetic life cycle between a nonmotile intracellular amastigote stage, parasiting the mammalian phagocytic cells and a flagellated, motile promastigote stage in the midgut of its sandfly vector. Promastigote can be cultured under different *in vitro* laboratory conditions and have been the subject of numerous biological and biochemical studies. In contrast, the study of the amastigote stage has been hindered by methodological difficulties in obtaining adequate amounts of live amastigotes free from host cell debris contamination. Although amastigotes are obligatory intracellular stage present in the host macrophage, some investigators have been succeeded in the culture of *Leishmania spp* axenic amastigotes. The objective of our work is to obtain *Leishmania chagasi* (strain PP-75) amastigotes to use for several experiments in our laboratory. We modified the methodology used by Doyle et. al (1991. Exp. Parasitol. 73: 326-334) for obtaining *L. donovani* amastigote. The promastigotes were maintained with weekly passage in M-199 medium added with 10% Fetal Bovine Serum, 2.5% FBS and 2,5% human urine at 26ºC. The transformation procedure was initiated with promastigotes the stationary phase culture when most of the forms are metacyclics (1-3 x 10^6) and put in flasks contain 5 ml of pure FBS or FBS plus Hemin at 37ºC and 5% CO_2. No differences were observed between the growth rate of axenic amastigotes and promastigotes. At 48 h of culture all the parasites were already transformed into amastigotes. The cell transformation was confirmed by scanning electron microscope examination. The viability to transform back into promastigotes was checked by put them back at 26ºC and through the infection of *Lutzomyia longipalpis*, a natural vector of *L. chagasi.* With this method we obtained large amounts of *in vitro* amastigotes to do interaction and biochemical experiments.

Financial support: FIOCRUZ, WHO, CNPq and PRONEX.

B-36 – INTRACELLULAR PROTEIN DEGRADATION DURING *IN VITRO LEISHMANIA AMAZONENSES* DIFFERENTIATION

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

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B-37 – *LEISHMANIA AMAZONENSIS* SECRETED COMPOUNDS: BIOCHEMICAL AND IMMUNOLOGICAL CHARACTERIZATION PARTIAL OF THREE PROTEINS

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Extracelular compounds have been isolated from several cells and many of them demonstrated to be important biochemical tools to successfully complete their life cycles [Glycobiology 2: 509-521(1992)]. Although many compounds has been suggested to be secreted by Trypanosomatides, only a few ones has been purified and characterized such as the LPPG and an acid phosphatase. Using salt precipitation followed by two chromatographyc steps we were able to purify three abundant proteic compounds with 72, 59 and 57 kDa from *L. amazonensis* BHI supernatant medium. No significant change of molecular weight or additional bands were observed by SDS-PAGE and gel filtration HPLC analysis, after exclusion of DTT indicating that they are not assembled by thiol groups. A structural relationship between the 57 and the 59 kDa protein was observed by N-terminal amino acid analysis and immunoblot. In addition, using the detergent Triton X-114, it was demonstrated that all of them partitioning the detergent phase. These results suggest that they are amphyphylic proteins. However, it remains to be determined if these molecules are GPI-anchored proteins and if they are developmentally regulated. On the other hand, only the 72 kDa protein was recognized in *L. amazonensis* extracts by rabbit antisera raised against the three independent proteins. Nevertheless additional immunochemicals and molecular studies are necessary in order to clarify the antigenic relationship between these proteins and cellular *L. amazonensis* compound.. Preliminary immunization studies showed that lymphonodes of Balb/c and C57/BL10 mice present an increasing of CD8 (23%±5,3) than CD4 (3.1%±0.7) cells expression. The IL-4 was increased 10 times in Balb/c culture cells when compared to C57/BL10 cultures.

Supported: FIOCRUZ, CNPq

B-38 – PHOSPHORYLATIVE ACTIVITY ASSOCIATED TO A 3',5'-CYCLIC ADENOSINE MONOPHOSPHATE -DEPENDENT PROTEIN KINASE IN *LEISHMANIA AMAZONENSIS* PROMASTIGOTES

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Cyclic 3', 5'-adenosine monophosphate (cAMP) is one of the most important signalling molecule to cell growth and differentiation in several systems including protozoa parasites such as *Trypanosoma cruzi* and *Leishmania* sp. (Rangel-Aldão *et al.*, 1987; Castro *et al.*,1987; Oliveira *et al.*, 1984, 1993) . The most significative event during *Leishmania* developmental cycle is the differentiation of procyclic into metacyclic promastigotes, which is associated to the appearance of infectivity. As we have previously demonstrated that *Leishmania amazonensis* metacyclogenesis is associated to an increase of a protein kinase C activity (Aguiar-Alves *et al.*, 1996), we are continuing our studies on the cell signalling pathways and most specifically on the activity of phosphorylative enzymes. In this work our target was the adenylyl cyclase pathway in infective/non-infective forms of *L.amazonensis.* Promastigotes (MHOM/BR/77/LTB 0016 strain) were grown at 26^0C in Schneider's medium supplemented with 10% of fetal calf serum. Soluble and enriched membrane fractions of parasites were purified through a DEAE-cellulose column and the collected fractions used to evaluate the phophorylative activiy associated to cAMP. The enzyme assay was realized in presence of 3-isobutyl-1-methylxantine (IBMX) as an enzyme activator, histone as substrate, [^{32}P] g-ATP (50-200 cpm/pmol) as phosphate source and with/without cAMP. Even thought that histone is not the best substrate, since can be used by other kinases, the assay conditions would allow us to say that the phosphorylative activity observed was associated to a cAMP-dependent kinase (PKA). Moreover, a high significative difference in the enzyme activity was detected when the infective and non-infective promastigotes of *L.amazonensis* were compared, being higher in the infective one. Considering that the PKA's activity could be associated to the infectivity of the parasite, further experiments are in development using this molecule as a target for amidine derivatives compounds.

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B-39 – PROTEIN KINASE G IDENTIFICATION IN *LEISHMANIA AMAZONENSIS* PROMASTIGOTES

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The signal transduction pathway involving the production of nitric oxide (NO), stimulate soluble guanylyl cyclase (GC) and increase cyclic 3',5'-guanosine monophosphate (cGMP).

The production of cGMP results in the cGMP-dependent protein kinase (PKG) activation, which catalyze the transfer of gamma-phosphate of ATP to serine/threonine protein residues in several eukaryote systems including unicellular organisms. The discovery of signal transduction pathway has revealed how external stimuli elicit biochemical responses from target cell. Protein kinases are the most abundant members of these signaling pathway, since phosphorylative reactions are essential for every cell systems. Although there are some data concerning NO/cGMP transduction pathway in trypanosomatids (Paveto et al., 1995), cGMP-dependent protein kinase remain to be identified. In order to study the PKG activation pathway in promastigotes of *Leishmania amazonensis* (LTB0016 strain), the following experiments were carried out: a) promastigotes were grown at 26° C in Schneider's medium for four days; b) parasites were harvest and lysed in a cavitation pump under N_2 atmosphere and the soluble and enriched membrane fractions were obtained; c) soluble fraction was applied to a Mono Q column (HR5/5) and the elution was performed using a linear gradient of NaCl (0-700 mM); d) eluted fractions were used to assay PKG activity, by measuring [^{32}Pi] incorporation from gamma ATP into a substrate (Histone), in presence/absence of a cAMP-dependent protein kinase inhibitor. Preliminary results suggest that the fractions obtained at approximately 30 and 40% of NaCl, are associated to PKG activity. Further experiments are being developed in order to evaluate the role of NO/cGMP and its association with the protein kinase G activity in *L. amazonensis* promastigotes.

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B-40 – EVOLUTION OF HISTONES IN PARASITIC PROTOZOA

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Histones, the proteins involved in the organization and function of chromatin in most eukaryotes, were supposed to be very conserved proteins. However, during the past years it was found that these proteins present a high degree of divergency in several lower eukaryotes. In Trypanosomatids, it was found that histones H3 and H4, which are at the center of nucleosomal organization, showed more than 30% of divergency, while histone H1 corresponded to only one of the three amino acid domains present in higher eukaryotes. These features of Trypanosomatids may explain, at least in part, the absence of chromatin condensation to chromosomes during cell division in these parasites.

Evolution of histones was usually considered as peculiar, with several proposals which are difficult to reconcile with experimental data. In this presentation, we propose that histones followed the same evolutionary route of many other proteins. Considering that exons code for protein domains and functions, and that at the origin of eukaryotes the histones, as other proteins, were formed by "units" (mechano theory), it would be expected that these units or domains eventually were to be found in present organisms exhibiting primitive features. Furthermore, these units could work independently.

Our results on the structure of *Trypanosoma* histone genes and proteins fit this proposal. However *Giardia*, which in supposed to be more primitive than *Trypanosoma*, presents histones that seem to be similar to those of higher eukaryotes. This points to a new problem in the organization of the chromatin in parasitic protozoa, that is, the origin of their histones. Some of them may posses histones with arcaic traits and some others may obtain these chromosomal proteins by horizontal transfer.

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B-41 – PURIFICATION OF THE PUTATIVE INOSITOL DEACYLASE OF GPI BIOSYNTHESIS FROM *TRYPANOSOMA BRUCEI*

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GPI biosynthetic intermediates exist as PI-PLC sensitive and/or PI-PLC resistant species due to the absence or presence of a fatty acid in ester linkage to 2-OH of the *myo*-inositol ring [1,2]. In the bloodstream forms of the parasitic protozoan *Trypanosoma brucei*, most of the GPI intermediates exist in both inositol acylated and inositol deacylated forms [3,4]. These forms are held in a dynamic equilibrium by the action of a phenylmethylsulphonyl fluoride (PMSF)-sensitive inositol acyl-transferase [5] and a diisopropylfluorophosphate (DFP)-sensitive inositol deacylase [6]. The parasite inositol acyltransferase has different acceptor and donor substrate and inhibitor specificities to the comparable mammalian activity. It is not clear whether the putative inositol deacylase activity in mammalian cells is similar or different to the *T.brucei* activity [reviewed in 6,7].

The *T.brucei* inositol deacylase is inhibited by DFP but not by any other serine esterase/amidase inhibitor tested so far. We exploited this observation by treating washed trypanosome membranes with a cocktail of serine esterase/amidase inhibitors followed by [^{3}H]DFP. A single 50 kDa glycoprotein was labelled by this method. We subsequently purified this labelled glycoprotein to homogeneity by lectin affinity chromatography, ion-exchange chromatography and hydroxyapatite chromatography. In-gel tryptic digestion of reduced and alkylated material yielded peptides that were fractionated by capillary micro-bore HPLC and subjected to Edman sequencing. Tryptic peptide sequences were used to design degenerate oligonucleotide primers for gene cloning.

1. W. L. Roberts, J. J. Myher, A. Kuksis, M. G. Low & T. L. Rosenberry (1988) *J. Biol. Chem.*, 263, 18766-18775.
2. A. Treumann, Lifely, M.R., Schneider, P. & Ferguson, M.A.J. (1995) *J. Biol. Chem.*, 270, 6088-6099.
3. W. J. Masterson, T. L. Doering, G. W. Hart & P. T. Englund (1989) *Cell*, 56, 793-800.
4. A. Menon, R. Schwarz, S. Mayor & G. A. M. Cross (1990) *J. Biol. Chem.*, 265, 9033-9042.
5. M. L. S. Guther, W. J. Masterson & M. A. J. Ferguson (1994) *J. Biol. Chem.*, 269, 18694-18701.
6. M. L. S. Güther & M. A. J. Ferguson (1995) *EMBO J.*, 14, 3080-3093.
7. M. L. S. Güther, A. Treumann & M. A. J. Ferguson (1996) *Mol. Biochem. Parasitol.* 77, 137-145.

B-42 – EXPRESSION, PURIFICATION AND CHARACTERIZATION OF GLUCOSE-6-PHOSPHATE ISOMERASE FROM *TRYPANOSOMA BRUCEI*

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Millions of people are affected by tropical disease, being 50 millions at risk of developing sleeping sickness in African countries. The only drugs available have to be administrated in high dosage showing pronounced side effects. Aiming the development of more efficient drugs and specific against the very important enzyme glucos-6-phosphate isomerase (PGI) from *Trypanosoma brucei*, which is an enzyme belonging to glycolysis, gluconeogenesis and pentose phosphate pathways. Recombinant PGI were overexpressed in *E. coli*. The plasmids containing the *T. brucei* PGI fragments were kindly provided by Dr. Paul Michels from Research Unit for Tropical Diseases, Catholic University of Louvain, Bruxelas, Belgium.

The full lenght fragment was amplified by PCR technique and introduced into the pQE30 vector (Qiagen) containing an N-terminal His-Tag and a factor Xa cleavage site. The enzyme was overexpressed in *E. coli* M15 by inducing it with 50 μM of IPTG. The enzyme was purified by affinity Nickel column showing a single band in SDS-PAGE eletrophoresis. The *T. brucei* PGI containing the His-Tag N-terminal and the factor Xa cleavage site extensions shows K_m value very close to the native enzyme. Crystallization and spectroscopic experiment is being carried out.

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B-43 – EFFECT OF PLATELET-ACTIVATING FACTOR ON ECTO-PHOSPHATASE ACTIVITIES OF TRYPANOSOMATIDS PARASITES OF PLANTS

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The flagellated trypanosomatids of the genus *Phytomonas* and some *Herpetomonas spp* are parasites of plants. This parasitism can occur without any apparent pathogenicity but it can also cause diseases of economic significance in plantations of coconut, oil palm, cassava and coffee. Parasite membranes are known to be involved in many cellular events, including transport of nutrients and ions and protection of the parasites against host immune responses. Membrane-bound acid phosphatases are supposed to be involved with nutrition, protection and with cell differentiation and have been characterized in some genus of the family Trypanosomatidae, such as *Trypanosoma*, *Leishmania*, *Phytomonas* and *Herpetomonas*. Most *Leishmania* species are capable of secreting acid phosphatases, which are supposed to be involved with the interaction of the parasites with their host cells. Platelet-activating factor (PAF) is a potent lipid mediator, which exerts a wide range of biological activities. We have recently shown that PAF triggers the process of cell differentiation of *Trypanosoma cruzi* and of *Herpetomonas muscarum muscarum*, as well as promotes a great inhibition of the ecto-phosphatase activity of *H. m. muscarum* (Dutra, P.M.L., Rodrigues, C.O., Jesus, J.B., Lopes, A.H.C.S., Souto-Padrón, T. & Meyer-Fernandes, J.R. 1998. Biochem. and Biophys. Res. Commun. 253: 164-169). Here we have investigated the effect of PAF on the ecto-phosphatase activity of *Phytomonas serpens*, *Herpetomonas mcgheei* and *Herpetomonas* sp.The parasites were grown in a complex culture medium, supplemented with 10% fetal calf serum, at 28°C, for three days. Intact cells were incubated for one hour at room temperature in a reaction mixture containing 30 mM Tris-HCl / 75 mM sucrose buffer pH 6.8, 1 mg protein (intact cells). We analyzed 7 systems: (1) control, (2) 10^{-9} M PAF, (3) 10^{-9} M WEB 2086 (PAF antagonist), (4) 10^{-9} M WEB 2086 plus 10^{-9} M PAF, (5) 10^{-6} M PAF, (6) 10^{-6} M WEB 2086 and (7) 10^{-6} M WEB 2086 plus 10^{-6} M PAF. The reactions were started with 10 mM *p*-NPP as substrate and stopped by the addition of 2 ml 1N NaOH. For determining the concentration of the released *p*-nitrophenol (*p*-NP), the tubes were centrifuged at 1,500 x *g* for 20 minutes and the supernatant was measured spectrophotometrically at 425 nm, using a *p*-NP curve as standard. The viability of the parasites was not affected by the conditions used in this work. PAF had no effect on *P. serpens* ecto-phosphatase activity, but 10^{-6} M PAF was able to inhibit the phosphatase activity of *H. mcgheei* (inhibition of 28%) and *Herpetomonas* sp. (inhibition of 25%) and WEB 2086 abrogated these effects. 10^{-9} M PAF had no effect on *H. mcgheei* ecto-phosphatase activity, but increased (44%) the ecto-phosphatase activity of *Herpetomonas* sp. However, 10^{-9} M WEB 2086 was not able to revert this effect promoted by PAF.

Supported by: CNPq, FAPERJ, FINEP and PRONEX

B-44 – PROTEOLYTIC ACTIVITIES OF *CRITHIDIA DESOUZAI*: A TRYPANOSOMATID WITH AN ENDOSYMBIONT IN THE CYTOPLASM

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In this study, we report the characterization of cell-associated and extracelular proteinases of *Crithidia desouzai*, a trypanosomatid which harbors an endosymbiont. Cells were grown during 48 h at 28º C. After centrifugation, 10^8 cells were lysed by the addition of SDS-PAGE sample buffer (Laemmli, 1970, *Nature*, 227:680-685), and the culture supernatant equivalent to 10^9 cells was filtered in Millipore (0.22µm) and concentrated against polyethyleneglycol before the addition of sample buffer. The proteolytic activity was detected by the rapid detection method of SDS-PAGE gels containing co-polymerized gelatin as substrate (Heussen & Dowdle, 1980, *Anal. Biochem.*, 102:196-202). After electrophoresis, gels were incubated with phosphate buffer (pH 5.5) supplemented with 200mM DTT or glycine buffer (pH10) for 24 h at 37º C in the presence or absence of the proteinase inhibitors. Cell-associated proteinases were also extracted and partitioned with Triton X-114 solution (Bordier, 1981, *J. Biol. Chem.*, 256(4):1604-7).

This analysis revealed the presence of a 50 kDa band in the detergent-phase of cell extracts and in the culture supernatant of *C. desouzai*. This enzyme is an acidic metalloproteinase based on its inhibition by 1,10-phenanthroline. The detection of a protease with the same enzymatic class, molecular mass and optimum pH in both extracts suggests that this molecule may be a membrane protein which can be released in the supernatant by an endogenous phospholipase C (Schneider *et al*, 1993, *Mol. Biochem. Parasitol.*, 58:277-82). However, further studies must be done in order to ascertain this hypothesis. Besides the above proteinases, a cysteine proteinase migrating at 80 kDa was detected in culture supernatant. The optimal conditions for its activity were: pH 5.5 in the presence of DTT, which was found to be essential for detection of activity.

The influence of a symbiont in *C. desouzai* proteinase expression was not available. However, it has been proposed that the endosymbiont supplies the host with several amino acids (McGhee & Cosgrove, 1980, *Microbiol. Rev.*, 44(1):140-73). The simpler cellular pattern of *C. desouzai* in comparison to *C. fasciculata*, *C. guilhermei* and *C. lucilae* (Branquinha *et al*, 1996, *J. Euk. Microbiol.*, 43(2):131-5) led us to speculate that this reduction on proteinases expression could be related to the presence of the endosymbiont, since the anabolic activities of the endosymbiont will supply the host with several essential nutrients.

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B-45 – EXPRESSION OF PROTEINASES IN *BLASTOCRITHIDIA CULICIS*

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Blastocrithidia culicis is a flagellated protozoan belonging to the Trypanosomatidae family which harbors a bacterium-like endosymbiont in its cytoplasm. The endosymbiont supplies the host cell with essential growth factors and are capable of inducing morphological and biochemical changes in the host cell. The present study report the detection of proteinase activities in *B. culicis* when grown in Roitman´s chemically defined medium for 72h at 26ºC. Proteinases were assayed and characterized by electrophoresis on 10% SDS-PAGE with 0.1% gelatin as substrate incorporated into the gel. After electrophoresis, the gels were washed in 1% Triton X-100 for 1h, then were incubated for 40h at 37ºC in 50mM sodium phosphate buffer, pH 5.5, supplemented with 2mM dithiotreitol or in 50mM glycine-NaOH, pH 10. Cell-associated proteolytic activities were detected showing different expressions in distinct pH values tested. In acidic pH (5.5) four distinct proteinases were observed with apparent molecular weight of 140, 100, 70 and 50kDa. However, in basic values of pH (10), only the 140 and 100kDa components were detected. The 100kDa protease was in fact distributed in a broad band, ranging from 125 to 85kDa, and showed the most pronounced activity. The 140 and 100kDa components were inhibited by 10mM 1,10-phenanthroline, a zinc-metalloproteinase inhibitor. Whereas, the 70 and 50kDa bands were inhibited by 10μM E-64, a specific cysteine proteinase inhibitor. The present results contributed to a better understanding of the production of proteolytic enzymes by insect trypanosomatids.

Financial Support: CNPq, FINEP, PRONEX.

B-46 – CHARACTERIZATION OF A MG-DEPENDENT ECTO-NUCLEOTIDE DIPHOSPHOHYDROLASE ACTIVITY IN *HERPETOMONAS MUSCARUM MUSCARUM*

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Extracellular ATP has profound effects on cellular functions, like plasma membrane depolarization, Ca^{2+} influx, and cell death. The cells showed plasma membrane enzymes whose active sites face the external medium rather than cytoplasm, that are able to promoted ATP hydrolysis and are named ecto-ATPases. The protozoan *Leishmania tropica*, *Entamoeba histolytica* and *Tretrahymena termophila* possess ecto-ATPase activities membrane bound and in soluble form. In a previous report we demonstrated that the trypanosomatids *Herpetomonas muscarum muscarum*, a non-pathogenic parasite of housefly, display a Mg-dependent ecto-ATPase activity. The optimum pH was 7.5 for the ecto-ATPase activity while the ecto-phosphatase activity, also displayed by this parasite, showed a maximum activity at pH 6.5. ADP inhibited this Mg-dependent ecto-ATPase activity.

The experiments were made using intact parasites (strain ATCC 30260), after three days of culture, at 28° C. The flagellates were harvested and washed three times in TS buffer (100 mM Tris-HCl, 250 mM sucrose, pH 7.5). The viability of the trypanosomatids was evaluated using Trypan Blue staining method. Intact cells hydrolyzed ATP, at 28° C, in the presence of 5 mM $MgCl_2$ and of the phosphatases inhibitors ammonium molybdate (100μM) and sodium fluoride (5 mM). This ecto-ATPase activity was linear when evaluated as a function of time and cells density. This activity was insensitive to bafilomicine and oligomicine (both type V ATPases inhibitors), levamisole (alkaline phosphatase inhibitor), *p*NPP (substrate of phosphatases) and PAF, a lipidic mediator that inhibit the acidic ecto-phosphatase activity of this parasite. The extracellular impermeant reagent, DIDS, showed to be an inhibitor of this activity. Suramine, an antagonist of purinergic receptors, also showed a strong inhibition of the ecto-ATPase activity of this trypanosomatid. These data suggest that the *Herpetomonas muscarum muscarum* has a Mg-dependent ecto-diphosphohydrolase activity, distinct to acidic ecto-phosphatase activity developed by this parasite. This ecto-ATPase activity can probably be involved at the signal transduction, mediated by ATP, that modulate crucial events of parasite life.

Financial Support: CNPq, FAPERJ, FINEP and PRONEX.

B-47 – TRYPANOSOMATID ENDOSYMBIONTS: BIOCHEMICAL CHARACTERIZATION OF THE ENVELOPE

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The study of intracellular symbiosis is relevant to the understanding of many biological problems including the origin of organelles and the survival of parasites within host cells. *Crithidia deanei* is a trypanosomatid which harbours an endosymbiotic bacterium which is enclosed by two unit membranes and inhabits the host protozoan cytoplasm. The origin of the outer membrane of this prokaryote is controversial. Some authors consider that this membrane was acquired from the host in an early stage of cell entry (Chang, 1974), while others propose that it derives from the prokaryote itself (Gutteridge and Macadam, 1971, Motta *et al*, 1991). Previous studies using the freeze-fracture technique support this latter proposition (Motta *et al*, 1991, Soares *et al*., 1988). Furthermore, an electron microscopy analysis of protozoa incubated in the presence of Polymixin B, an antibiotic which exerts lethal action only in Gram-negative bacteria by acting in the outer membrane surface, displayed critical morphological changes in endosymbionts (Motta *et al*, 1998). Here, symbionts free from major host components were obtained by subcellular fractionation using a discontinuous sucrose gradient. Transmission electron microscopy analysis revealed that the endosymbiont-enriched fraction is mainly composed by well preserved endosymbionts, with minor residual contamination of mitochondrial fragments. Upon eletrophoresis (10% SDS-PAGE), TX-114 extraction of the symbiont fraction yielded 7 bands in the hydrophobic phase. Two of them, the 35 and the 37 kDa membrane proteins, were selectively identified in this phase and may likely correspond to well-characterized porins, major proteins which compose the outer membrane of Gram-negative bacteria. N-terminal sequence and immunoblotting analysis will be sought to confirm this prediction.

Chang, 1974. J. Protozool. 21: 699-707
Gutteridge and Macadam, 1971. J. Protozool. 18:637-640
Motta *et al*. 1991. Micr. Electr. Biol. Cel. 15:131-144
Motta *et al*. 1998. Mem. Inst. Oswaldo Cruz, Vol. 93, Suppl II pág 114
Soares and De Souza. 1988. J. Protozool. 35:370-374

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B-48 – PLATELET-ACTIVATING FACTOR (PAF) MODULATES A PROTEIN KINASE ACTIVITY IN TRYPANOSOMATIDS

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Trypanosomatids face several different environmental conditions during their life cycles, as they parasitize invertebrates, vertebrates and /or plants. Protein phosphorylation-dephosphorylation is one of the most powerful mechanisms able to control cell activities, including parasite defenses. Several enzymes located on the surface of trypanosomatids play important roles both in the parasite survival and in its ability to invade host cells. Platelet-activating factor (PAF) is a potent phospholipid mediator that produces a wide range of biological responses including inflammation, allergy and cellular differentiation, through activation of a specific receptor on cell surface. Binding of PAF to these receptors generates a cascade of signal transduction events leading to the activation of protein kinases and culminating in the modulation of several biological processes. PAF has been found to be produced by a variety of living organisms, although its physiological role in lower eukaryotes is still unknown. Recently we have demonstrated that PAF triggers the process of cell differentiation of *Trypanosoma cruzi* (1) and of *H. m. muscarum* (2), and modulates the ecto-phosphatase activity of *H. m. muscarum* (3). In the present work we describe the effect of PAF on a casein kinase activity of *Trypanosoma cruzi* and of *Herpetomonas muscarum muscarum*. In *T. cruzi* the pretreatment of the living parasites with several concentrations of PAF, ranging from 10^{-12} to 10^{-6} M, stimulated by ten to twenty fold a protein kinase activity, using exogenous dephosphorylated casein as substrate. In *H. m. muscarum* similar results were observed, both when the parasites were pretreated with PAF for one hour and when they were incubated for two days in the presence of this lipid mediator. This effect seems to be dependent upon a signal transduction network, as PAF presented no effect when added to a purified casein kinase, in the same type of phosphorylation assay.

(1) Rodrigues, C.O., Dutra, P.M.L., Souto-Padrón, T., Cordeiro, R.S.B. & Lopes, A.H.C.S. 1996. Biochem. and Biophys. Res. Commun. 233: 735-740. (2) Lopes, A.H.C.S., Dutra, P.M.L., Rodrigues, C.O., Angluster, J. & Cordeiro, R.S.B. 1997. J. Eukaryot. Microbiol. 44 (4): 321-325. (3) Dutra, P.M.L., Rodrigues, C.O., Jesus, J.B., Lopes, A.H.C.S., Souto-Padrón, T. & Meyer-Fernandes, J.R. 1998. Biochem. and Biophys. Res. Commun. 253: 164-169.

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B-49 – CHARACTERIZATION OF SECRETED ACID PHOSPHATASE ACTIVITY FROM *TRICHOMONAS VAGINALIS*

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The parasitic protozoan *Trichomonas vaginalis* is the causative agent of trichomoniasis, a common infection human 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). Phosphatases (ortophosphoric-monoester-phosphohydrolases) can remove phosphate from a number of important cellular compounds including ATP. Acid phosphatase activity has been reported in both the sediment and homogenates of *Trichomonas vaginalis* and *Tritrichomonas foetus*. Phosphatase activities have also been showed in some members of the family Trypanosomatidae, such as *Leishmania*, *Trypanosoma* and *Herpetomonas*. *T. vaginalis* and *T. foetus* were found to release large amounts of enzymes, including acid phosphatase into the medium, but there have been no other reports on the characterization of extracellular phosphatase activity in the *T. vaginalis*.

In this work we have extended our study to a full characterization of a phosphatase activity in the live *T. vaginalis* (JT strain). The parasites were grown in TYM medium for 24 h at 36 °C and incubated in the reaction medium containing NaCl (116 mM)/ KCl (5.4 mM)/ glucose (5.5 mM)/ Hepes (50 mM) and p-NPP (5mM). To investigate the secreted phosphatase activity, the intact cells were pre-incubated for 1 hour in the reaction mixture (whithout *p*-NPP), centrifuged and the supernatant resultant (cell-free) assayed for the phosphatase activity. Cellular viability was accessed, before and after incubations, by motility and Trypan blue method. All the cells were motile as detected by phase contrast microscopy.The time course of total extracelular phosphatase activity (using live parasites) was linear during the first hour a rate of 135 nmol Pi/h/10^7 cells (1 mg protein). It was also observed that this activity was directly proportional to increasing the cell density. The phosphatase activity detected in the supernatant was completly inhibited by sodium orthovanadate (200 μM), ammonium molybdate (200 μM) and sodium fluoride (200 μM), three classical inhibitors of acid phosphatase. Sodium tartrate (1mM) and levamizole (1mM), inhibited respectively 32 % and 10 % of the phosphatase activity. In the pH range from 6.4 to 8.0 the phosphatase activity reached a maximum at pH 6.4, decreasing concomitantly with the increase of pH. The dependence on *p*-NPP concentration for this extracellular phosphatase was observed. This activity shown a hyperbolic dependence of substrate concentration, where the apparent K_m found was 0.2 mM *p*-NPP and V_{max} 8.92 nmol Pi/min/mg of protein.

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B-50– IRON MODULATES THE EXPRESSION OF SURFACE PROTEINS IN *TRITRICHOMONAS FOETUS*

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Iron is an essential nutrient for most of the investigated prokaryotes and eukaryotes, playing an important role in some parasitic diseases. Surface receptors for iron proteins as well as surface iron transporters are the molecules specifically involved in the cell uptake of iron from the extracellular environment. Either receptors for iron proteins as iron transporters have been identified in bacteria, fungi and protozoa.

Pathogenic trichomonads such as *Trichomonas vaginalis* and *Tritrichomonas foetus* are iron-dependent protozoa since they are unable to survive in iron-depleted environments. In the case of *Trichomonas vaginalis* iron has crucial roles in regulating expression of surface adhesins and proteases as well as cytoadherence.

The here reported results deals with the effects of iron in the growth of *Tritrichomonas foetus* and expression of surface proteins in parasites obtained from cultivation in medium supplemented or not with different amounts of iron. Growth of the microorganisms was almost totally inhibited when the iron chelator 2,2-dipyridyl (300μM) was added to the culture medium. This effect could be reverted by the addition of 100μM ferrous sulfate to the medium.

Parasites cultured in TYM supplemented with different iron amounts (maximum at 400μM) had their surfaces submitted to biotinilation. The labeled surface components of these parasites were analyzed by SDS-PAGE, and subsequently blotted onto nitrocellulose. As iron amounts were increased in the culture medium, the parasites exhibited enriched electrophoretic profiles. High molecular weight proteins seems to be overexpressed in parasites cultivated in presence of 400μM ferrous sulfate. In addition, by using monoclonal antibodies anti-iron transporter from *Saccharomyces cerevisiae* we were able to detect a strong reaction in electrophoretic profiles which contained surface proteins from parasites.

Our data strongly support the role of iron in *T. foetus* as a metabolic regulator, as point out the presence of an iron transporter in *T. foetus* antigenically related to that observed in yeast.

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B-51 – A COMPARATIVE ANALYSIS BETWEEN TRICHOMONDS FROM CELLULAR AND ACELLULAR CULTIVATION

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Tritrichomonas foetus and *Trichomonas vaginalis* are parasitic protozoa which cause respectively sexually transmitted diseases in cattle and in human. Adhesion of the protozoa to epithelial cells is a critical step in the pathogenesis of both parasites, and is not surprising that the mechanisms of cytoadherence and the signal tranduction of this event across the membrane have been studied extensively[1].

Some animal models have been proposed to study *Trichomonas vaginalis* infection, and in such models mouse has been mostly used[2]. In order to understand the signal transduction mechanisms performed by the parasites as soon as they contact the host epithelial cells we have designed experiments focusing each one of the following: (a) surface molecules, (b) secondary transducers as well as (c) gene expression.

In the present study we compare the electrophoretic profile of microorganisms from K strain of *T. foetus* which were cultured in acellular medium before and after passages into mice (inoculated intraperitoneally). To address this question the parasites was submitted to a polyacrylamide gel electrophoresis (SDS-PAGE) with parasite whole cell lysates and polyclonal antibodies to c-Src was employed in immunoblotting assays.

Interestingly, several differences were observed in SDS-PAGE assays and both models presented positive reaction in imunoblotting carried out with c- Src polyclonal antibody.

To further investigate how far are those differences, adhesions assays were performed with laminin-1, a known inducer of signaling in *T. vaginalis.* The last experiments resulted in differences either in morphology as in cytoadhesion.

[1]Alderete, J. F.,Lehker, M. W. and Arroyo, R., *Parasitology today.* 11(2): 70. 1995
[2]Mutwiri, G. K. and Corbeil, L.B., *J. Parasitology* 84(2): 321. 1998

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B-52 – PROTEINASE ACTIVITIES IN TOTAL EXTRACTS AND IN MEDIUM CONDITIONED BY *ACANTHAMOEBA POLYPHAGA* TROPHOZOITES

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Acanthamoeba species can cause granulomatous encephalitis, and keratitis in man. The mechanisms that underlie tissue damage and invasion by the amoebae are poorly understood, but involvement of as yet uncharacterized proteinases has been suggested. Here we employed gelatin-containing gels and azocasein assays to examine proteinase activities in cell lysates and in medium conditioned by *A. polyphaga* trophozoites. Azocasein hydrolysis by cell lysates was mostly detected at acidic pHs, with an optimum between 4.0 to 5.0. The hydrolytic activity at pH 5.0 was stimulated by 2 mM DTT and strongly inhibited by antipain, leupeptin, and chymostatin, as well as by the irreversible and specific cysteine proteinase inhibitors E-64, Z-Phe-AlaCHN$_2$ and Z-Tyr-AlaCHN$_2$. These results indicated that the activity detected in trophozoite lysates was predominantly associated to the cysteinyl class of proteinases.

To monitor proteinase activities secreted in to the culture medium, log phase trophozoites were ressuspended at 2 x 10^6/ml of fresh complete medium and incubated at 28ºC for different time periods. Integrity of trophozoites during the incubation period was assessed microscopically and also biochemically by determining the activity of lactate dehydrogenase in the supernatants. The pH dependence of azocaseinolytic activity of supernatants differed from that of trophozoite lysates, since substrate hydrolysis occurred within a broad pH range (4.0 to 10.6), and at appreciably higher rates at pH 6.0 and above. The activity detected at near neutral/alkaline pH was almost fully inhibited by PMSF, indicating secretion of serine proteinases. The latter resolved at 47, 60, 75, 100 and >110 kDa in overlay, gelatin gels. Although a similar banding profile was observed in gels of trophozoite lysates, the intracellular serine proteinases were shown to be activated during electrophoresis and to split the substrate during migration in SDS gels. Overall, these results are compatible with a secretory pathway through which newly synthesized or inactivated serine proteinases are transported to the trophozoites' membrane, the enzymes being activated during/after release in the culture medium.

Blockage of serine proteinases with PMSF prior to electrophoresis in SDS gelatin gels permitted the detection of 43, 59, 70, and 100-130 kDa acidic cysteine proteinases in cell lysates, and of three (43, 70 and 130 kDa) apparently equivalent enzymes in culture supernatants. No band associated with a metalloproteinase activity could be depicted in substrate gels, although the discrete inhibition of supernatants' azocaseinolytic activity by 1,10-phenanthroline suggested secretion of some metalloproteinase.

Financial Support: FAPESP, and CNPq

B-53 – COMPARATIVE STUDY OF ECTO-ATP-DIPHOSPHOHYDROLASE ACTIVITIES OF DIFFERENT SPECIES OF *ENTAMOEBA* AND THE EFFECT OF VARIOUS SUGARS

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Entamoeba histolytica is a human pathogen with potential to destroy many tissues of its host. Surface membrane interactions between *Entamoeba histolytica* and its host are of critical importance and an understanding of the biochemical basis for the parasite adherence and cytolytic activities are crucial for vaccine development (Radvin, J. Infection Diseases 4, 1185-1207 (1989). Plasma membrane of cells contains enzymes whose active sites face the external medium rather than cytoplasm. The activities of these enzymes, referred as ecto-enzymes, can be measured using intact cells and can be modulated by divalent cations such as Mg^{2+} and Ca^{2+}. Ecto-ATPases, Ecto-ATP-diphosphohydrolases or E-type ATPases are glycoproteins that hydrolyze extracellular nucleotide tri- and/or diphosphates. We have recently characterized an ecto-ATPase activity in *Entamoeba histolytica* that is able to hydrolyze extracellular ATP. Here we shown the comparative study of hydrolytic activity of three species of amoebae. Cellular viability was assessed, before and after incubations, by exclusion methods using 0,125% eosin solution. Invasive *E. histolytica* is able to hydrolyze ATP more effectively (78 nmolPi/ h/ 10^5 cells) that noninvasive *E. histolytica* (18 nmolPi/ h/ 10^5 cells) and free-living *E. moshkovskii* (11 nmolPi/ h/ 10^5 cells). The activities of the three species was stimulated by $MgCl_2$ 5 mM, moreover the invasive *E. histolytica* activity (258 nmolPi/ h/ 10^5 cells) was more stimulated by 5 mM $MgCl_2$ than noninvasive *E. histolytica* (32 nmolPi/ h/ 10^5 cells) and *E. moshkovskii* (23 nmolPi/ h/ 10^5 cells) activities. The carbohydrates D-galactose and D-Lactose that inhibit the adhesion of *Entamoeba* to host cells had a stimulatory effect on the Mg^{2+}-dependent ecto-ATPase of invasive *E. histolytica* (68% and 82% respectively). However D-Glucose and D-threalose that did not change the adherence of *Entamoeba* to host cells did not have effect on this ecto-ATPase.

Financial Support: FAPERJ, CNPq, FINEP and PRONEX

B-54 – BADHESION SPECIFICITY TO ENDOTHELIAL CELL RECEPTORS OF *PLASMODIUM FALCIPARUM* ISOLATES FROM RONDONIA (WESTERN AMAZON REGION, BRAZIL)

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Parasite molecules expressed at the surface of infected red blood cell's membrane mediate endothelial cell adhesion and sequestration of mature forms of *Plasmodium falciparum* and were identified as belonging to the EMP1group of "variant" antigen proteins encoded by the multigenic family of var genes (review by Borst, Cell 82:1-4, 1995). It has been proposed that different forms of severe malaria, in particular cerebral malaria, depends on the receptor affinity of individual var antigens to specific endothelial cells receptors expressed at different tissues. To examine this hypothesis it is necessary to correlate adhesion specificity of parasites isolates with clinical forms of the infection they produce and to specific var gene expressed by the corresponding parasite population.

In the present study, falciparum malaria patients of the Porto Velho area (Rondonia State, Brazil) were examined to define clinical and biological parameters of severity of infection, according to WHO criteria . Purified red blood cells from the patients containing ring forms were incubated in RPMI-albumax medium at 10% hematocrit in candle jar for 20-24 hours. Trophozoites and schizonts were concentrated and re suspended in RPMI-1640 to perform adhesion assays. For these assays were used CHO transfected cells expressing CD36, ICAM-1, V-CAM, E-selectine receptors (gift of A. Scherf, Pasteur Institute) and native CHO cells expressing Chondroitin sulfate A (CSA) residues at the cell surface. Among twenty one isolates examined, three types of adhesion profiles were recognized: 1) three isolates showed specific adhesion to just one of the receptors; 2) five isolates showed specific binding to two receptors; 3) the remaining isolates showed adhesion to various receptors examined at different levels of efficiency. Using panning techniques (A.Scherf, EMBO J 17:5418-26), some pluri-specific adherent isolates were shown to consist of heterogeneous population . To identify the var antigen molecules expressed by the studied parasite population, RNA was extracted from the parasites and used in TR PCR amplification procedure to amplify the conserved segment of the DBL1 segment of the corresponding var gene allowing its' sequencing and identification.

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B-55 – ISOPRENYLATION OF PROTEINS IN *PLASMODIUM FALCIPARUM*

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In the present paper, we report that post-translational modification of cellular proteins by isoprenoid lipids (prenylation) occurs in the human malaria parasite *Plasmodium falciparum*. Upon incubation of intraerythrocytic parasites with [^{3}H]farnesyl-pyrophosphate several labeled proteins of 14 kDa, 21-24 kDa and 50 kDa were detected, while incubation with [^{3}H]geranylgeranyl-pyrophosphate resulted in the exclusive labeling of 14 kDa and 21-24 kDa proteins. Attached isoprenoid moieties were identified by thin layer chromatography analysis. Parasites incubated with mevastatin, an inhibitor of 3-hydroxy-3-methylglutaryl-CoA A reductase, and labeled with [^{3}H]farnesyl-pyrophosphate showed a specific increase in the incorporation of radioactivity into the labeled proteins. In addition, anti-human p21^{ras} and p21^{rap}1 antibodies immunoprecipitated farnesylated and geranylgeranylated proteins, respectively. Treatment of parasites with limonene and perillic acid, both inhibitors of mammalian and yeast protein farnesyl transferase and protein geranylgeranyl transferase, suppressed the incorporation of radiolabelled isoprenoids. Limonene and perillic acid arrest parasites in the ring stage, suggesting a new target for malaria drug development.