POSTERS

Biologia Celular - Cell Biology

BC01 - ACTIVITY OF NOVEL METAL CHELATORS AGAINST INTRACELULLAR TOXOPLASMA GONDII

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Tachyzoites, the infective form of Toxoplasma gondii, infect all eukaryotic cells multiplying within parasitophorous vacuole PV. We used Thiosemicarbazones and its derivates against intracellular T.gondii. Vero cells were grown in Linbro tissue plates that contained strile coverslip and maintained at 37 C. The cultures were infected with tachyzoites for 24h and incubated for 12, 24 or 48h with 1.5mM of benzaldehyde thiosemicarbazone and its 4-thiazolidinone derivate. After this were fixed with Bouin solution, stained with Giemsa and observed at Zeiss AXIOPLAN microscope using x40 objective. For ultrastructural analysis the cells were fixed solution containing 1 p/c glutaraldehyde, 4p/c paraformaldehyde, 5mM CaCl2 and 5p/c sucrose, pH 7.2. The cells were pos-fixed with 1 p/c OsO4 in 0.1M cacodylate buffer, dehydrated in acetone and embedded in Epon. Ultrathin sections were examined using Zeiss 900 Electron microscope.After incubation with the drugs, infected cells decreased and intravacuolar parasites were disorganized. When the treated cultures for 12 or 24h were incubated for 24h in medium without the drugs, the infection increased and the intravacuolar parasites returned its cell multiplication. Ultrastructural observations of treated cells for 12h showed intracelluar tachyzoites with intense vacuolization. After 24h, the parasites were destructive. In the host cells, no morphological or ultrastructural alterations were observed.

BC02 - MECHANISM OF DESTRUCTION OF INTRACELLULAR TOXOPLASMA GONDII IN PRESENCE OF THIOSEMICARBAZONE

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Tachyzoites of Toxoplasma gondii invade the host and establish within parasitophorous vacuole PV that is not recognized by host cells lysosomes, avoiding acidification. Thiosemicarbazone derivatives are of considerable interest because of their biological activity, such as antiprotozoal, antibacterial and antiturmor. We demonstrated that some derivates of thiosemicarbazone arrest the multiplication of intracellular parasites,leading its destruction. In present study we investigated cellular mechanisms involved in this destruction using one thiosemicarbazone derivate: 4 thiazolidinone. Vero cells were cultived in Linbro tissue plates with sterile coverslip and maintained at 37C. The cultures were infected with tachyzoites for 24h. After this 4 thiazolidinone - 1,5mM - was added for 12, 24 or 48 hours. The PV with parasites was analyzed with acridine orange (acid tropic marker) and lysotracker red (selective for lysosomes) to observed PV acidification in presence of the drug, using confocal microscopy. Ultrastructural cytochemistry for localization of the acid phosphatase lysosomal enzyme was used. The cells were incubated in a solution containing CeCl3 and Beta glycerol phosphate in 0,05M tris-maleate buffer, pH5.0 and pos-fixed in solution containing 1 percent OsO4 in 0,1M cacodylate buffer. Then it was dehydrated in acetone and embedded in Epon. Ultra thin sections were examined using Zeiss 900 Electron Microscope. After treatment for 24h, the parasites were disrupted morphologically. PV with destructive tachyzoites was fluorescence when stained with acridine orange or lyso tracker red. Cytochemical analyse after 24h of treatment showed reaction product at the intravacuolar tachyzoites. These results suggest that this mechanism was used to elimination of intracellular tachyzoites.

BC03 - EVALUATION OF LEISHMANICIDAL ACTIVITY OF Crotalus durissus RATTLESNAKE VENON.

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Introduction: *Crotalus durissus* ssp venom has been characterized a highly neurotoxic and its composition and mode action is different of the *Bothrops* venom. Growth inhibition of the *Leishmania* ssp and *T. cruzi* by snake venom has been reported only for bothropic venom. Objetive: In the present work we evaluated the ability of the *Crotalus durissus* snake venom on the *Leishmania* growth rate and comparing to *Bothrops moojeni* venom. Material and Methods: Venons and *Leishmania*: *C. d. cascavella* and *C. d. collilineatus* were gift from the Butantan Institute (SP-Brazil). *L. (L.) amazonensis* was grown at room temperature in RPMI 1640 medium, supplemented with 10% of fetal calf serum and antibiotics. Leishmanicidal effect: The venom was diluted to the desired concentration with PBS solution immediately before starting the experiments. The measurement of the leishmania was done by direct counting of the immobilized parasite. Biochemical analysis of venom: The venom was analyzed by molecular exclusion HPLC and Tricine PAGE-SDS electrophoresis. Results: In the presence of 1mg/ml of solution, crotalic venom was less effective than to *B. moojeni* venom. We also observed some discrepancy between both crotalic venom where *C. d. cascavella* showed high leishmanicidal effect than to *C. d. collilineatus*. Biochemical

analysis of the venom showed that crotoxin as main fraction found in both *C. d.* venoms. In case of *C. d. cascavella* and *C. d. collilineatus*, crotoxin accounting for 70% of dried venom and 47% for *C. d. cascavella*. *B. moojeni* showed higher inhibition of the leishmania growth rate than to *Crotalus* venom. Conclusion: The biochemical differences of the crotalic venom probably was involved in the leishmanicidal effect. The differences observed for crotalic and bothropic venom involve other factors that will necessary to investigate.

BC04 - Activity of a Brazilian propolis extract against Trypanosoma cruzi

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Propolis possesses a variety of biological activities and, during the last decades an increasing number of studies about the chemical composition, biological activity and therapeutic uses of propolis have been published (De Castro, Ann Rev Biol Sci 3: 49, 2001). The composition of this resin is variable and complex due to the different botanic sources where the bees collected vegetal exudates (Bankova et al. Apidologie 31: 3, 2000). In a study with a Brazilian sample, the chemical composition of its ethanol extract (Et-Bra) was determined, showing this extract activity against epimastigotes and trypomastigotes of *T. cruzi*, and several fungi and bacteria species of medical importance (Salomão et al. Lett Appl Microbiol 38: 87, 2004). In the present work we analysed the effect of Et-Bra on the infection of peritoneal macrophages and the ultrastructure of the parasite. Et-Bra inhibited both the percent of infection as well as the number of parasites/infected cell with an IC50/3 d of, respectively, 17.1 +/- 0.5 and 18.2 +/- 2.3 μg/ml indicating also an effect on the proliferation of intracellular forms. Damage of the host cell was observed only at concentrations above 60 μg/ml Treatment with Et-Bra (50 to 300 μg/ml) of epimastigotes for 24 h at 28°C led to mitochondrial damage with swelling of the organelle and scarcity of matrix and crystae, increase in the volume of the kinetoplast, cytoplasmatic vacuolization, increase in the number of reservossomes and extraction of lipids. Morphological alterations observed by transmission electron microscopy were corroborated by scanning microscopy analysis. These data encourage us to continue our study with propolis, investigating cellular targets in the parasite and toxicity to animals.

BC05 - EFFECTS OF BETA-LAPACHONE DERIVATIVES ON CHROMATIN CONDENSATION AND ON THE ENDOCYTIC PATHWAY OF *Trypanosoma cruzi*

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Plants containing naphthoquinones are employed in folk medicine for the treatment of several, and these compounds present a variety of biological activities, most of them associated with oxidative processes. For example, the effect of beta-lapachone is associated to free radicals generation. Among about 60 semi-synthetic derivatives of naphthoquinones isolated from *Tabebuia* (ipes), three naphthoimidazoles, prepared from beta-lapachone, with aromatic moieties linked to the imidazole ring (N1, N2 and N3) were the most active against trypomastigotes of *T. cruzi*(Neves-Pinto et al. Arzneim-Forsch 50: 1120, 2000; De Moura et al. J Braz Chem Soc 12: 325, 2001; De Moura et al., Eur J Med Chem 39: 639, 2004). Ultrastrutural studies of epimastigotes and trypomastigotes treated with each of these derivatives demonstrated alterations in chromatin pattern and eletrophoresis of epimastigotes total DNA showed no fragmentation of nucleic acids. These data shows that naphthoimidazoles led to chromatin condensation, possibly compromising parasite DNA transcription to mRNA. Treated trypomastigotes and epimastigotes showed also damage in mitochondrion and in the latter forms, also in reservosomes. These alterations were confirmed by, respectively, decrease of rhodamine 123 and acridine orange labeling. Incubation of treated epimastigotes with transferrin-gold for short periods led to localization of the marker similar to that found in control parasites. In relation to the endocytic pathway, the naphthoimidazoles did not affect its initial steps (formation of early compartments) but interfere with the organization and function of reservosomes,late compartments of this pathway, also involved in energy storage of the parasite. Our data taken together suggest that naphthoimidazoles compromise DNA function, energy storage and endocytosis of *T. cruzi*. Low toxicity to mammalian cells and the absence of reservosomes in mammals encourage us to perform in vivo experiments with these beta-lapachone derivatives. Low toxicity to mammalian cells and the absence of this organelle in mammals encourage us to perform in vivo experiments with these beta-lapachone derivatives.

BC06 - Correlation of the trypanocidal activity with the chemical composition of propolis from different regions in Brazil

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Propolis is a produce by honeybees from plants exsudates, and has become a subject of increasing scientific and economic attention. Our laboratory is involved for years on the

investigation of the trypanocidal and pharmacological activities of propolis, inserted in a multidisciplinary study, which aims to perform a systematic analysis of Brazilian samples. In temperate zones the main vegetal sources of propolis are Poplar species, consisting flavonoids the major class of bioactive compounds (Bankova et al. Apidologie 31: 3, 2000). In Brazil, the sources are mainly *Araucaria* spp, *Baccharis* spp and *Eucalyptus* spp, and several new bioactive compounds have been isolated from propolis ((De Castro. Ann Rev Biol Sci 3: 49, 2001). We have previously determined the activity against trypomastigotes of *T. cruzi* of 26 ethanol extracts from propolis collected in different regions in Brazil and chemically characterized by high temperature high resolution gas chromatography (n=15; series A) or by high performance liquid chromatography (n=11; series B). In this work we performed a statistical analysis searching for a correlation of trypanocidal activity with chemical components in each extract. In series A we analysed the classes of terpenic acids (TA), aromatic acids (AA), caffeoylquinic acids (CA) and flavonoids (Fl). By the method of principal components, a positive correlation was found between the trypanocidal activity and TA-288 (higher amounts of this compound present in more active extracts) and also with AA-120, and a negative one with TA-177. No correlation was found with the classes CA and Fl. For series B, there was a positive correlation between the activity against the parasite and two derivatives of hydrocynammic acid - DCBEN, DHCA4 - and a negative one for the compounds L2, not yet identified, and I (2-[1-hydroxymethyl]vinyl-6-acetyl-5-hidroxycumarane).

BC07 - LEISHMANICIDAL EFFECT OF Jacaranda puberula ON PROMASTIGOTES AND AMASTIGOTES OF Leishmania (Leishmania) amazonensis.

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Antiprotozoal activities of Colombian plants including Jacaranda caucana (Bignoniaceae) as well as the use of Jacaranda species for the treatment of leishmaniasis in an endemic area of South-western Colombia was been described. In order to study the effect of Jacaranda puberula in cutaneous leishmaniasis, leishmanicidal assays on the promastigote forms of L.(L.)amazonensis were performed in vitro. Promastigotes in logarithimic growth phase were dispatched in 24 flat botton well plate at the concentration of 105/ml. Each well contained increasing concentration of the plant extract, from 1 up to 6mg/ml. The number of viable parasites was evaluated at 24, 48, 72 and 96 hours. The evaluation of leishmanicidal activity on intracellular amastigotes of L.(L.)amazonensis was performed in BALB/c peritoneal macrophage cultures exposed to stationary phase growth promastigotes at a ratio of 5 parasites/cell for 24 hours with 5Leishmanicidal activity on promastigotes of L.(L.)amazonensis were detected when 4 and 6mg/ml of J.puberula extract were used. The concentrations of 1, 2 and 3mg/ml leaded to inhibition of the promastigotes growth when compared to the controls. There were significant differences between the groups according to the concentration of extract. Concerning to the effect of J.puberula on macrophages infected, the macrophage infection index and the number of viable parasites were decreased proportionally to the concentration of plant extract used, as well as aspects of parasite degeneration were present. The results suggest that J.puberula has leishmanicidal effect on promastigotes and amastigotes of L.(L.)amazonensis. Experiments were necessary to evaluate the citotoxicity of the extract on macrophage and its effect on the evolution of the infection in vivo.

BC08 - Growth inhibition of *Trypanosoma cruzi* epimastigotes by L-leucine methyl ester

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Amastigotes of *Leishmania* species from the *mexicana* complex present large lysosome-like organelles known as megasomes, which contain large amounts of cysteine proteinase. Previous studies have shown that amastigotes can be killed by amino acid esters such as L-leucine methyl ester (Leu-OMe) by a mechanism that involves hydrolysis of this compound by cysteine proteinases, leading to amino acid accumulation in the parasites followed by osmotic lysis. Megasomes are the most likely targets of Leu-OMe. Reservosomes are large acidic membrane-bound organelles found at the posterior end of *T. cruzi* epimastigotes, containing cruzipain (a cysteine proteinase) and ingested proteins. Due to the biochemical similarity between these organelles, the present work was designed to verify the action of Leu-OMe on *T. cruzi* epimastigotes. Five-day-old culture epimastigotes (Y strain) were cultivated for 4 days in LIT medium containing different concentrations (0.5 to 8 mM) of Leu-OMe. Effect of this compound on parasite growth was evaluated by daily counting with a Neubauer chamber. Cell viability was assessed by light microscopy, using the Trypan blue dye. To analyze the effect of Leu-OMe on the cell morphology, treated parasites were processed for conventional transmission electron microscopy. Our data showed that treatment with Leu-OMe resulted in a dosis-dependent growth inhibition. Treatment of the parasites with 4-8 mM Leu-OMe led to 100% cell death soon after the first 24 hours of cultivation. Treatment with 0.5-2 mM Leu-OMe was less effective, resulting in lower growth inhibition rates. The data obtained allowed us to estimate the ED_50 as about 1 mM. Loss of cell viability was confirmed by the dye exclusion test. Further studies by transmission electron microscopy are underway in order to analyze morphological alterations induced by the drug. This work has been supported by CNPq and FIOCRUZ.

BC09 - EFFECT OF SERINE PROTEASE INHIBITORS FROM SOYBEAN ON *TRYPANOSOMA CRUZI* GROWTH

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The flagellate protozoan *Trypanosoma cruzi* is the etiologic agent of Chagas disease, which affects 16-18 million people. Considering the toxic effects of the currently available chemotherapic drugs, development of new drugs is needed. Trypanosomatid proteinases have been characterized by several authors. In the last decade serine proteinases have acquired considerable importance, as they are involved in the host/parasite relationship and in the penetration of trypomastigotes into mammalian cells. In this study we have analyzed the potential use of the serine proteinase inhibitors SBTI (Soybean Trypsin Inhibitor) and BBI (Bowman-Birk Inhibitor) on *T. cruzi*, Y strain. Five-day-old culture epimastigotes were grown at 28^{o}C in LIT medium supplemented with 10% fetal calf serum (FCS) and different drug concentrations and their growth was evaluated for up to 96 hours, by daily counting with a Neubauer chamber. Bloodstream trypomastigotes were isolated form infected mice and maintained for 24 hours at 37^{o}C in modified Eagle medium supplemented with 10% FCS and different drug concentrations. Trypomastigote survival and viability was then assessed by counting with a Neubauer chamber and by observation of exclusion of the vital dye Trypan Blue, respectively. Treatment of culture-derived epimastigotes with both drugs showed that parasite growth was not affected by concentrations up to 10 mg/ml. Treatment of trypomastigotes with SBTI and BBI also did not affect parasite viability, except for 10 mg/ml BBI, which killed about 20% of the parasites. Ultrastructural studies by transmission electron microscopy of treated epimastigotes and trypomastigotes are underway in order to evaluate possible alterations in parasite morphology induced by these drugs. Furthermore, the enzymatic activity of serine proteinases in being assayed in parasites treated with both inhibitors. Thus, the results so far obtained have demonstrated that these serine proteinase inhibitors are not efficient to eradicate *Trypanosoma cruzi*. This work has been supported by CNPq and FIOCRUZ

BC10 - EFFICACY OF ATOVAQUONE AND SULFADIAZINE IN THE TREATMENT OF MICE INFECTED WITH *TOXOPLASMA GONDII* STRAINS ISOLATED IN BRAZIL

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The efficacy of atovaquone and sulfadiazine was examined alone or in combination for the treatment of mice infected with six brazilian *Toxoplasma gondii* strains previously genotyped using the PCR-RFLP assays of the SAG2 gene, in addition to RH strain. Female Swiss mice were infected intraperitoneally with 100 tachyzoites from each strain of *T. gondii*. Groups of 10 mice were treated with 6.25, 12.5, 25 and 50mg/Kg/day of atovaquone or 40, 80, 160 and 320mg/Kg/day of sulfadiazine. In a second experiment, mice were treated with the association of previously determined doses of each drug. Treatment started 48 hours post-infection, and lasted 10 days. Animals were followed up for 30 days post-infection. In order to assess the effectiveness of therapeutic schemes the animals survival rates from different treatment groups were analyzed. The presence of brain cysts, ELISA and subinoculation of brain suspensions were performed as additional criterion of effectiveness.The susceptibility of *T. gondii* to atovaquone and to sulfadiazine was different according to the parasite strain. It was observed strains that are susceptible to atovaquone, and strains that are resistant to it. Type I strains were more susceptible to the activity of sulfadiazine and more resistant to atovaquone. Yet type III strains were susceptible to atovaquone and to sulfadiazine. Association of atovaquone and sulfadiazine presented a synergic effect in the treatment of mice infected with two type I strains and with two type III strains. It was not possible to establish a correlation between the occurrence of the synergic effect of the association of atovaquone and of sulfadiazine and the genetic type of T. gondii strain, as this event has already been verified for both type I and type III strains.

BC11 - IN VITRO EFECTS OF THIAZOLIDINONE DERIVATES ON DEVELOPMENT OF INTRCELLULAR **Toxoplasma gondii**

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The intracellular parasite Toxoplasma gondii multiplies inside parasitophorous vacuole PV in all eukaryotic cells. In presented study we used thiosemicarbazone TS derivates: a thiazolidinone RP. TS have several pharmacological activies include antiprotozooa activities. However, this activity was dependent of molecular structure. Thus, some thiazolidinone derivates were used against infection of T. gondii where intracellular development, morphology and ultrastructural features were observed. Vero cells were cultivated in Linbro tissue plates that contained a sterile coverslip and maintained at 37 C overnight. The cells were infected and incubated for 24 hours. After that time 1,5mM of five derivates thiazolidinone RP 16, RP 24, RP 27, RP 29 and RP 33 were added to cultures for 24 hours. Later on, the untreated and treated cultures were washed with PBS, fixed with Bouin's solution and stained with Giemsa. The cells were observed with a Zeiss AXIOPLAN photomicroscope. For ultrastructural analyzis, the cells were fixed in solution containing 1 percent glutaraldehyde, 4 percent paraformaldehyde, 5mM CaCl2 in cacodilate buffer containing 5 percent sucrose, pH 7.2 and

postfixed in solution containing 1 percent OsO4 in 0,1M cacodilate buffer, at room temperature. The cells were rinsed with cacodylate buffer, dehydrated in acetone and embedded in Epon. Ultrathin sections were examined used Zeiss 900 Electron Microscope. After incubation of all derivates thiazolidinones, the percentage infected cells and number intracellular tachyzoites decreased. Besides, the intravacuolar parasites were altered morphologically. However, the host cells were not morphology altered. Ultrastructural analyzis showed untreated cells with normal morphology tachyzoites. After treatment with all compounds, intravacuolar tachyzoites were altered with vacuolization on cytoplasmic. We also observed that PV membrane was distended and inside some PV there was membranous stucture. However, structural aspects of most cells were not altered. These results showed that thiazolidinones derivates decreased infection of T. gondii in vitro.

BC12 - Azidothymidine (AZT) and Resveratrol act synergistically to inhibit Leishmania survival in macrophages.

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The available compounds for the treatment of Leishmania infection are toxic, expensive and require long term use. These characteristics, together with the emergence of parasite resistance to these compounds, stimulate the research for new antileishmanial agents. An increasing number of patients coinfected with Leishmania and Human Immunodeficiency Virus type-1 (HIV-1) has been reported, showing that HIV-1 adversely affects leishmanial infection and vice-versa. In the present study, we tested the antileishmanial activity of the nucleoside analog AZT, a potent antiretroviral agent, and of Resveratrol, a phytoalexin found in grapes, which has been reported to increase the anti-HIV-1 activity of AZT in vitro. Thus, monocyte-derived human macrophages and murine peritoneal macrophages were infected with Leishmania amazonensis for 24 hours and treated with different concentrations of AZT and Resveratrol, alone or in combination. Our results show that AZT at 25mM inhibits 39% of amastigote survival. Resveratrol demonstrated a dose-dependent capacity to inhibit amastigote survival, with 50 and 100mM inhibiting 20% and 45%, respectively. The association of both compounds resulted in a substantially higher antileishmanial activity: 5mM of AZT alone inhibited 30% of amastigote survival, while 62% inhibition was observed when this AZT dosage was combined with Resveratrol 100mM. The influence of the compounds on nitric oxide production by macrophages was analyzed by the Griess reaction, and we found that the antileishmanial activity of AZT and Resveratrol does not occur through stimulation of nitric oxide production. Ultrastructural analysis is in progress to determine possible alterations that those compounds could cause in the Leishmania.

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BC13 - EFFECTS OF ANTI-GOLGI DRUGS ON INVASION AND SURVIVE OF TOXOPLASMA GONDII

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Toxoplasma gondii is an obligate intracellular parasite and infects all eukaryotic cells. In extracellular medium a group of secretory organelles of parasite eliminate its products and invaded host cell. At the present study, we observed parasite invasion in presence of drugs against Golgi Complex and secretory vesicle's flux - Monensin and Brefeldin A. Vero cells were placed on Limbro tissue plates contained a round sterile coverslip and incubated at 37C. Tachyzoite was obtained of Swiss mice after 48 hours of infection. Then the parasites were treated for 30 minutes with Brefeldin A (40uM) or Monensin (10uM). After this the treated parasites were incubated for 2 or 24 hours in the cultures containing 199 medium without drugs. The cultures were fixed with Bouin's solution, stained with Giemsa and observed in a Zeiss AXIOPLAN photomicroscope using objective X40. The percentage of infected cells and the number of intracellular parasites was determinate. The infection was reduced when treated extracellular parasites with both drugs were incubated in the cultures for 2 or 24 hours. This decreased was more significant after treatment with Monensin. We observed that after 24 hours of incubation, the treated parasites did not proliferate into intracellular cytoplasm. Besides, the intravacuolar parasites were altered morphologically.

BC14 - The effects of oryzalin and jasplakinolide on the cytoskeleton of /emphGiardia lamblia

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Giardia lamblia is a parasitic protozoan that colonizes the upper small intestine of vertebrates, and contaminates thousands of people all over the world. This eukaryotic cell possesses a remarkable cytoskeleton in which microtubular structures are predominant. The most proeminent constituents of the cytoskeleton include an adhesive disc, four pairs of flagella, median body and funis, all of them composed of several proteins, especially tubulin and giardin, a fibrous protein found only in cells of the genus *Giardia*. In the last few years, the study of *Giardia* cytoskeleton has been increased and the relation between structure and function of cytoskeletal components is being elucidated. Several drugs that are known to interfere with cytoskeleton dynamics have been used in the study of the biology of parasitic protozoan, including *Toxoplasma gondii* and *Trypanosoma brucei*. These include drugs that act on microtubules and microfilaments. In the present study, we analyze the effects of oryzalin, a dinitroaniline herbicide, known to act depolymerizing microtubules and jasplakinolide, a peptide isolated from marine sponges that act

as a potent inducer of actin polymerization, in the cytoskeleton of *G. lamblia*. Using scanning and transmission electron microscopy, fluorescence and video microscopy, we intend to understand the functioning of *Giardia* internal structures, such as the median body which role is not yet completely known and also to know if actin plays any role in this cell. As already seen in other protists, oryzalin caused flagellar shortening, interfered in cellular division, diminished adhesion in higher concentrations and caused several damages to the cell plasma membrane. Jasplakinolide did not interfered with cell movements neither with cell ultrastructure in the concentrations used. Nevertheless, cells labeled with anti-actin antibody after jasplakolide treatment showed a concentration of labeling surrounding the adhesive disk. This work is supported by: CNPq, FAPERJ, CAPES and CNPq/PIBIC.

BC15 - ACTION OF THE CAMPTOTHECIN ON INTRACELLULAR Toxoplasma gondii

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The currently chemotherapy against toxoplasmosis - Pyrimetamin and sulfadiazine - involve undesirables side effects and it is not effective to eliminate intracellular parasite. Early studies demonstrated that combination of antibiotics with anti-toxoplasma drugs increased an effecient of toxoplasmosis control. In the recent study,we analyzed the efect of the synthetic antibiotic camptothecin (CPT) - inhibited nuclear and mitochondrial DNA topoisomerase I - on intracellular tachyzoites of T. gondii. Vero cells were cultivated in Limbro tissue plates (3-5x10/5 well) and maintained at 37C overnight were infected with tachyzoites of T. gondii for 24 hours. After that time, CPT (5uM, 10uM, 25uM,50uM and 100uM) was added to cultures for 12 and 24 hours. Culture were fixed with Bouin's solution, stained with Giemsa and observed at Zeiss AXIOPLAN microscope; or fixed in solution containing 1percent glutaraldehyde, 4percent paraformaldehyde, 5mM CaCl2 and 5percent sucrose. The cultures postfixed for 1h in 2percent OSO4, rinsed with CaCo, dehydrated in acetone and embedded in Epon. Ultrathin sections were examined using Zeiss 900 Electron Microscope. Infected host cells incubated with camptothecin for 12 and 24 hours decreased the number of infected host cells and number intracellular tachyzoites. Intravacuolar parasites were disrupted in presence of 5uM, 10uM, 25uM and 50uM of CPT. However, 100uM of CPT inhibited most intracellular parasites development but induced host cells morphological alterations. Ultrastructural analysis showed disorganization on parasite cytoplasm membraane. These results suggest that the action of substances on mitochondrial function, as camptothecin, inhibts the survival of tachyzoites in the host cells.

BC17 - Squalene synthase as a potential target against the growth of promastigote forms of *Leishmania amazonensis.*

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Parasites of *Leishmania* genus are responsible for different forms of Leishmaniasis, which are present in many countries and affect million of people around the world. The chemotherapy actually employed to the treatment of leishmaniasis is unsatisfactory, presenting frequent toxic effects and drug resistance. *Leishmania* requires for the growth and viability an amount of endogenous sterols such as ergosterol and episterol and they are not able to synthesize cholesterol, the main sterol present in mammalian hosts. Squalene synthase (SQS) is an important enzyme that catalyzes the first committed step in the sterol biosynthesis, that was characterized as an important enzyme in the ergosterol metabolism of Trypanosomatids (Urbina *et al.*, *Mol. Biochem. Parasitol.*, 125:22-33, 2002). I this work we decided to investigate the effect of different SQS inhibitors against promastigote forms of *Leihsmania amazonensis*. Ten compounds were tested to determine an inhibition growth, where five drugs were more effective with IC50 minor than 1.0 μM. The best compound was WSP 896 presenting an IC50 of 0.278 μM for 96h. Ultrastructural analysis of the parasites revealed several alterations in the morphology of promastigote forms treated with two compounds, WSP 892 and 896. The main ultrastructural change was observed in the plasma membrane that presented the formation of elaborated structures with an intense shedding. We also observed alterations in the mitochondrion-kinetoplast complex as an intense mitochondrial swelling, rupture of its membrane and an abnormal compaction of the kinetoplast. Other alterations include the appearance of multivesicular bodies, myelin-like figures and presence of parasites with two or more nuclei and kinetoplasts. We conclude that squalene synthase of *L. amazonensis* is an important enzyme to development new chemotherapy agents against Leishmaniasis. **Financial Support**: CAPES, CNPq, PRONEX, FAPERJ and European Commission.

BC19 - *IN VITRO* TRYPANOCIDAL ACTIVITY OF GALLIC ACID DERIVATIVES

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Chagas disease is one of the major health problems in Latin American Countries. There is no specific treatment for chronic infection and the drugs currently used in the acute phase are harmful with several side effects. In this study, 25 derivatives of gallic acid were tested for activity against

trypomastigotes and amastigotes of *Trypanosoma cruzi* (Y strain). The assays were performed in triplicate in 96 wells micro plates by incubating 180μl of infected mice blood ($1x10^6$ trypomastigotes/ml) with 20μl of different concentrations (1000, 250, 50 and 10 μM) of each compound at 4C for 48 hours. As controls, parasites were incubated with 1 % DMSO or crystal violet (100 μM). Trypanocidal activity was evaluated by counting the number of live parasites after incubation and tests revealing negative results on microscopic evaluation were inoculated into groups of 4 healthy Swiss mice to confirm the absence of parasites. Fresh blood examination of these animals was individually performed every two days up to 30 days of inoculation when hemoculture and serology was carried out for all negative animals. In addition, hemolytic activity of each compound was assessed through the cyanometahemoglobin method, revealing to be negative. Active compounds were also assayed against intracellular amastigotes obtained by infection of Vero cells monolayers cultured on 12mm glass slides. After a 24 hours infection period, monolayers were treated with 50, 20 and 10 μM of each compound for 72 hours at 37C. As controls infected cells were cultivated in the absence of drugs or treated with 50 μM benznidazol. After Giemsa staining, the cell infection rate was determined by counting 300 randomly chosen cells. Four out of 25 compounds were active against blood trypomastigotes: decyl gallate (IC_{50}= 40,75μM); undecyl gallate (IC_{50}= 23,39μM); dodecyl gallate (IC_{50}= 74,40μM); tetradecyl gallate (IC_{50}= 53,12μM), revealing also a strong trypanocidal activity against intracellular amastigotes. Except for infected blood treated with 250μM undecyl gallate, which showed no mice infection and negative hemoculture, all other compounds were capable to block infection only in concentrations over 1000μM. These results show that derivatives of gallic acid may be promissing molecules for Chagas disease chemotherapy.

BC20 - The anti-parasitic activity of furamidine analogues against Trypanosoma cruzi and Leishmania (L) amazonensis

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Parasitic protozoa are unicellular pathogens with a complex life cycle displaying distinct morphological stages during their development in both vertebrate and invertebrate hosts. Among the protozoa, members of the Trypanosomatidae family include a large number of species that cause human diseases such as sleeping sickness, Chagas' disease and Leishmaniasis. In this respect, diphenylfuran diamidines represent an important promising class of DNA-targeted antiparasitic agents. The best-known member of the series is the DB75 commonly referred to as furamidine and related unfused aromatic diamidines that have proven useful for the treatment of parasitic infections. Since recent literature clearly points to the need of finding more efficient and less toxic chemotherapeutic approaches for both Chagas' disease and Leishmaniasis, our present aim was investigated the in vitro effects of DB75 and its phenyl-substituted analog DB569 on both intracellular amastigotes as well as trypomastigotes of Trypanosoma cruzi (Y and Dm28c stocks) and upon promastigotes of Leishmania (L) amazonensis. Our results showed that both compounds have anti-parasitic effect against T. cruzi and Leishmania parasites. However, the DB569 was significantly more potent than DB75 at reducing the proliferation of the intracellular forms of T. cruzi besides exhibiting higher trypanocidal dose-dependent effects against trypomastigote forms of T. cruzi (from both stocks) and promastigotes of L. amazonensis. Fluorescence microscopy experiments indicated that both diamidines were mostly localized within the nuclei and kinetoplast of the parasites. Electron microscopy studies showed that the treatment of the parasites with DB75 and DB569 induces important alterations of the parasite nucleus and kinetoplast, at sites where their DNA target is localized. Altogether, the data show that furamidine displays anti-parasitic activities against T. cruzi and L. (L) amazonensis, and the phenyl substitution of its amidine termini significantly enhances the anti-parasitic activity. The furamidine analogue DB569 appears as a promising candidate for further evaluations in vivo.

BC21 - EFFICACY OF ALBACONAZOLE NANOCAPSULES IN MURINE *T. cruzi* INFECTION

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In the present work we evaluated the efficacy of nanocapsules (NC) containing albaconazole (ABZ), a new triazole derivative that possess anti-*Trypanosoma cruzi* action *in vitro* and *in vivo*. Nanocapsules were used in order to prolong ABZ plasmatic half-life in mice ($t_{1/2}$=30min) and reduce its toxic effects. Mice were infected intraperitoneally with 10^4 blood trypomastigotes of *T. cruzi* Y strain, and randomly divided in groups, in which three different treatment protocols were used. To establish a administration route, mice were treated with albaconazol, free or encapsulated, at the same dose, by different routes (oral, subcutaneous or intramuscle). On the second protocol mice were treated with different doses of ABZ-NC (20, 40, 80 and 120mg/Kg/day), by the subcutaneous route (SC). The third protocol was based on subcutaneous treatment with different doses of the free drug (20, 80 and 120mg/Kg/day). Treatment started 4 days after inoculation, during 20 consecutive days. Cure was evaluated by fresh blood exam and hemocultures. The SC was the best route, supressing the parasitemia more ef-

ficiently. ABZ-NC and free-ABZ were able to increase survival and supress parasitemia of infected animals, by the subcutaneous route. Treatment with ABZ-NC at higher doses (120mg/Kg/day) reduced the acute toxicity of ABZ, compared to the same dose of free-ABZ. The best results were achived with 120mg/Kg/day for ABZ-NC and 80mg/Kg/day for the free drug. Among the animals treated with ABZ-NC at doses of 80 and 120mg/Kg/day, 10% and 30% showed negative hemocultures, respectively, and 10% of those treated with free-ABZ presented negative hemocultures. Although serological cure was not yet confirmed, the results obtained in this work were positive in the therapeutical point of view, because ABZ short plasmatic half-life in mice seemed to be prolonged, propably because biodistribution of the free drug was modified by depot formation in subcutaneous tissue and by nanoencapsulation.

BC22 - SCREENING PLANT EXTRACT AGAINST *Leishmania (Leishmania) amazonenis* AMASTIGOTE

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This work describes the screening, *in vitro*, for leishmanicide activity of extracts obtained from plants collected in differents ecosystems ("Campo Rupestre"formation, Atlantic Forest, Savana-like, Riparian Forest) of Minas Gerais State - Brazil. The extracts were tested against axenic culture of amastigotes forms of *Leishmania (L.) amazonensis*. The tests were done in microcultive plates of 96 wells, with $1x10^8$ amastigotes of *L. amazonensis*/mL, in triplicate. The extracts were add into the cultures at final concentration of 20 μg/mL. Amphoterice B (final concentration of 0.2 μg/mL) was used as control drug. The cultures were incubated for 72 h at 32^0C. Growth parasites was measured by colorimetric method, with of thiazolyl blue tetrazolium bromide (MTT). Extracts that caused at least 80 % of parasites death were considered active. In order to monitor the quality interassays, *Levey-Jennings* graphics for negative and for positive controls were constructed. Two standart above or below the average were assumed of assays validation. From the three thousand extracts tested, 213 (7 %) presented leishmanicide activity highest then 80 %. This corresponds to 36 botanic families (29 %) from the 125 represented in our plant extract library. The family Asteraceae was the most active, presenting the biggest number of active species.
Financial support: FAPEMIG, PDTIS-FIOCRUZ

BC23 - Influence of *Trypanosoma cruzi* manipulation in the resistance pattern to benznidazole.

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The natural induction of resistance to benznidazole (Bz) in *T. cruzi* populations during the long-term chronic phase in dogs was demonstrated recently by Veloso *et al.*, (Mem Inst Oswaldo Cruz, 96:1005, 2001). In this study the Be-78C isolated from dogs with chronic infection (Bz-susceptible strain) was submitted to several manipulations in order to evaluating the stability of the Bz-resistance phenotype. Swiss outbreed mice were infected by intraperitoneal route with the Be-78C isolated from dog after seven years of infection with Berenice-78 *T. cruzi* strain. After isolation the *T. cruzi* population were maintained through sucessive cycles of Bz-treatment. Treated animals were submitted to immunosuppressive terapy with ciclophosphamide and after parasitemia reativation the trypomastigotes were inoculated in new mice that were treated again. To follow the Be-78C was maintained during the period of one year through sucessive blood passages in not-treated mice and in LIT medium. The Bz-treatment were repeated in order to observe the stability of resistance. The results showed that after one only passage in mice it was observed 80% of resistance and a Bz-resistant population was detected after 5 successive long-term treatment cycles with benznidazole (100 mg/kg/day). The increase of resistance was also showed by the spontaneous parasitemia reativation post-treatment starting from the 8th treatment cycle and by the absence of parasitemia clearence during Bz-treatment in 13th cycle. On the other hand, new changes in Bz-resistance were found during the maintenance of the parasite without drugs pressure. The Bz-treated animals maintained through sucessive blood passages in mice and in LIT medium, showed 100% and 20% of resistance, respectively. Theses results corroborate the hypothesis that the manipulation of the *T. cruzi* may influence the susceptibility/resitance phenotype of benznidazol in this *T. cruzi* population studied.
Financial support: UFOP, FAPEMIG, CNPq.

BC24 - CELLULAR COMPOSITION AND CYTOKINE EXPRESSION IN HUMAN LEISHMANIASIS LESIONS: COMPARATIVE ANALYSIS BETWEEN CUTANEOUS PATIENTS BEFORE AND AFTER TREATMENT WITH GM-CSF ASSOCIATED WITH ANTIMONIAL

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Previous studies have shown that treatment of human cuta-

neous leishmaniasis with antimonial+GM-CSF (AmGm) is more effective than antimonial alone. Our hypothesis is that the additional use of GM-CSF alters the profile of cells that compose the inflammatory infiltrate of cutaneous lesions, favoring a more efficient local response, leading to lesion cure. In this work, we determined the frequency of T-cells and monocytes in lesions from cutaneous patients before and after treatment with AmGm. Moreover, we evaluated the expression of IFN-gamma and IL-10, identifying their cellular sources and the expression of iNOS. Lesions from cutaneous patients before and after treatment with AmGm were stained with monoclonal antibodies differentially labeled and analysis of confocal microscopy was performed. Our results showed that the intensity of the inflammatory infiltrate was slightly lower after AmGm treatment, as compared to the infiltrate before treatment (p=0,07). While the numbers of CD4+ and CD14+ cells did not alter after treatment, we observed a statistically significant decrease in the number of CD8+ cells after AmGm treatment. Moreover, the frequencies of IFN-gamma+ and iNOS+ cells were significantly lower after AmGm treatment. Interestingly, the contribution of CD8+ cells for IFN-gamma expression was higher after AmGm treatment than before, while CD4 contribution remained the same. The number of IL-10 expressing cells did not change upon AmGm treatment. These results suggest that the cure observed in AmGm treated patients is related to the control of the inflammatory response, as shown by the decrease in the inflammatory infiltrate and expression of IFN-gamma, an important inflammatory cytokine. Furthermore, the decrease in CD8+ T cells and iNOS after AmGm treatment is consistent with the lower tissue destruction observed in AmGm treated patients. In conclusion, our results suggest an important role for GM-CSF-associated therapy in the induction of immunomodulatory mechanisms involved in lesion cure.

BC25 - Pharmacokinetics of antimony in the bone marrow of dogs with visceral leishmaniasis, following multiple dose-treatment with intravenous liposome-encapsulated meglumine antimoniate

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The achievement of complete cure in dogs with visceral leishmaniasis (VL) is currently a great challenge, since dogs are the main reservoir for the transmission of VL to humans and respond poorly to conventional treatment with pentavalent antimonials. With the aim of improving the treatment efficacy, we recently developed a novel formulation for meglumine antimoniate (LMA), based on the encapsulation of this drug in freeze-dried empty liposomes. Furthermore, we have reported the distribution of antimony in healthy dogs, following a single intravenous bolus injection of LMA. The latter study suggested that the critical organ for the treatment with LMA could be the bone marrow, as it showed very low antimony levels. The aim of the present study was to evaluate the impact of a multiple dose-regimen with LMA on the concentration of antimony in the bone marrow of dogs with VL, as well as the kinetic of antimony elimination after treatment. Ten dogs naturally infected with *Leishmania chagasi* (weighing 5-12 kg, from centro de controle de zoonoses, Prefeitura de Belo Horizonte)) received, by intravenous route, 4 doses of LMA (each corresponding to 6.8 mg Sb/kg body weight) with 4-days time intervals. Before each administration and different times after treatment, bone marrow was analyzed for antimony level using electrothermal atomic absorption spectrometry. The results show a significant increase of antimony concentration from 0.76 μg/kg of wet organ (4 days after the first dose) to 2.07 μg/kg (4 days after the fourth dose). A half-life for antimony elimination from the bone marrow of about 4 days was also determined. In conclusion, the present study shows that a multiple dose-treatment with LMA is effective for improving antimony levels in the bone marrow of dogs with VL. This work was supported by CNPq and FAPEMIG.

BC26 - Inhibitory effect of *Leishmania (L.) amazonensis* on the secretion of nitrite in human Schwann cell line

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Parasites of the genus *Leishmania* are transmitted by sand flies to mammalian hosts, in which they invade and multiply preferentially within macrophages of the skin, mucosas and viscera. Since the skin is richly innervated and extracellular amastigotes of *Leishmania major* have been described in nerve fragments from skin lesions of both patients and experimentally infected mice, we decided to investigate whether Schwann cells a non-professional phagocyte could also harbor parasites, which are agents of the cutaneous disease. We evaluated the infection of a human Schwann cell line ST88-14 by promastigotes of *L. amazonensis*. After a 24 h-interaction of the parasites with ST88-14 at 34°C, the cultures were washed to remove non-internalized forms and incubated at 37°C. To monitor the course of infection, the infection was quantified

at different times up to 72 h. Immediately after the interaction step as well as 6 h later, the percentage of infected glia was 15%, which decreased to 9% and 5% after 24 h and 48 h, respectively. During this time the number of parasites per infected cell tended to remain unaltered and equal to one. After 72 h, infected cells were not found. To investigate the potential effect of nitric oxide production by ST88-14 on parasite survival, nitrite was quantified by the Griess method in the supernatants of the cultures. The levels of nitrite were always significantly smaller in cultures with 6 to 72 h of infection than those of corresponding non-infected cells. Our results show that Schwann cells are able to generate nitric oxide in the absence of stimulating agents and that infection by *L. amazonensis* reduces the formation of this metabolite. However, reduction of nitric oxide was not sufficient to allow the maintenance of the infection. These preliminary results encourage us to investigate the pathway involved in the clearance of the parasite in this model.

BC27 - CHRONIC HEART FIBROSIS IS ATTENUATED IN B1 KNOCKOUT MICE INFECTED BY Trypanosoma cruzi

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Inflammation and tissue injury is usually accompanied by upregulation of the B1 kinin receptor (B1R), via the NF-kB activation pathway. Recent studies suggest that B1R stimulation by their natural agonists, [des-Arg]-kinins, drive leukocyte transmigration through the endothelium due to upregulated adhesion molecule expression. Of further interest, there is evidence that the B1R pathway contributes to angiogenesis and tissue fibrosis in various chronic pathologies. In a previous study, we showed that B2 and B1 kinin receptors are sequentially involved in the early phase of inflammatory responses induced by T. cruzi trypomastigotes (FASEB J. 17(1): 73-75., 2003). Here we asked if T. cruzi-driven stimulation of B1R may contribute to chronic carditis in mice To verify 1. if T. cruzi trypomastigotes induce B1R on human endothelial cells (HUVECs) by activating Toll-like receptors through the activity tGPI-mucins, a potent trypomastigote-specific pro-inflammatory molecule (J. Immun, 167: 416-423; 2001). 2. if chronic heart fibrosis is differentially affected in infected WT vs C57Bl/6.J129 B1R-/- mice. Confocal microscopy reveals time-dependent B1R upregulation by tGPI-mucin and T. cruzi stimulates activated HUVECS through the B1R signaling pathway. Immunohistochemistry (Picrosirius) showed reduced collagen deposition in the myocardium of B1KO infected mice (4 months pi). The TLR2-B1R signaling axis may contribute to myocardial fibrosis. T. cruzi may upregulate B1R through the TLR2-inducing activity of tGPI-mucins. Chronic fibrosis may be aggravated due to sustained activation of B1R by [des-Arg]-kinins generated through the combined activities of cruzipain and host carboxypeptidase M/N (kinase I). Support: CNPq, FAPERJ, WHO-TDR, Volkswagen Foundation

BC28 - BIOCHEMICAL EVIDENCE OF ORNITHINE DECARBOXILASE (ODC) ACTIVITY IN *Trypanosoma rangeli* AND ITS ROLE ON PARASITE DIFFERENTIATION *IN VITRO.*

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Trypanosoma rangeli is a protozoan parasite that infects triatomine bugs and several mammalian species, including man, in Central and South American countries. Having developed a method for *T. rangeli* metacyclogenesis *in vitro*, we have identified that glutamine is a major amino acid involved on parasite differentiation. Ornithine Decarboxilase (ODC) is an enzyme that regulates the intracellular levels of polyamines in eukaryotes, and the presence of this enzyme has been demonstrated in *Crithidia fasciculata*, *Leishmania* spp. and *T. brucei*, but is absent in *T. cruzi*. In the present study, the presence of the ODC activity in *T. rangeli* was evaluated, as well as the role of polyamines in parasite differentiation. LIT derived epimastigotes (Choachi strain) were washed twice in PBS and cultivated in DMEM medium pH 8.0 supplemented with 5 % of FBS at 28C for 6 days in the presence of 10mM of α-difluoromethyl ornithine (DFMO) or with DFMO and different concentrations of polyamines (putrescine, spermidine and spermine), using as control parasites incubated in DMEM only. Parasite viability and differentiation was checked daily by fresh examinations and Giemsa stained smears. Epimastigotes incubated in the presence of 10 mM DFMO presented a low differentiation rate (15 %) on the 6th day of culture whereas control parasites showed a differentiation rate of 75 %. Incubation of epimastigotes in DMEM containing 10 mM DFMO and 1.6 μM putrescine revealed a differentiation rate between 65 % and 70 % after 6 days. However, no differentiation and 100 % mortality rate was observed for epimastigotes incubated with spermidine or spermine. Our results show, for the first time, the presence and the activity of ODC in *T. rangeli* epimastigotes, suggesting that polyamines metabolism may be essential for parasite differentiation *in vitro*. Further molecular characterization of *T. rangeli* ODC is under investigation.

BC29 - Effects of Inhibitors of Kinases on the Growth and Ultrastructure of Epimastigotes of *Trypanosoma cruzi*

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Little is known about the function of PKs in parasites and the occurrence of changes in protein phosphorylation during parasite life cycles suggests that these enzymes play important roles in parasite virulence, differentiation and cell division. An increasing number of PKs of parasitic protozoa are being evaluated as drug targets. Some inhibitors of PKs have been shown to display antiproliferative effects on the protozoa. We report here the effects of Staurosporin (STA) (inhibitor of serin/threonin kinase), Genistein (GEN) (inhibitor of tyrosine kinase), and Wortmannin (WOR) (inhibitor of phosphatidylinositol 3 (PI3) kinase) on the growth and ultrastructure of epimastigotes of *Trypanosoma cruzi*. The IC50 were: STA: 6.43 uM; GEN: 6.54 uM; and WOR: 0.056 uM. All three drugs inhibited the growth of the parasites at all concentrations. STA induced a stronger inhibition at 24h treatment (76.5% with 5.0 uM). During the rest of the cycle the inhibition was approximately 30-40% at all concentrations. GEN treated parasites presented an inhibition of approximately 60-70% during all the cycle. However, WOR at the concentrations of 5 and 10 nM did not induce growth inhibition within 24h. During most of the treatment, WOR treated parasites had a growth inhibition of approximately 30-40%. The parasites treated with the three drugs presented several morphological changes. Several parasites presented abnormal body shape and abnormal chromatin condensation of the nucleus. Many cells presented myelin-like figures and autophagosomes. Some cells did not complete cell division, since they presented duplicated cell bodies but did not complete the separation. The membrane of the flagellum was also strongly altered. The mitochondrion was swollen in several parasites treated with STA and GEN. Some parasites treated with GEN were observed shedding sacks of membranes via flagellar pocket. This work was supported by the European Commission, PRONEX, FINEP, CNPq and FAPERJ.

BC30 - Changes in H2 haplotypes alter the differential tissue distribution of *Trypanosoma cruzi* in double infected congenic mice.

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Chagas' disease, caused by the protozoan *Trypanosoma cruzi*, has a variable clinical presentation, ranging from asymptomatic to severe chronic cardiac and/or gastrointestinal disease. The reason for that is not completely understood, but both parasite and host genetic traits are certainly involved. We had demonstrated that the host background is one of the determinants of the pattern of parasite distribution in tissues after artificial infection with Col1.7G2 clone and JG monoclonal strain in four lineages of mice: three inbred (BALB/c, DBA-2, and C57BlK/6) and one outbreed (Swiss). In BALB/c and DBA-2 mice we found a prevalence of JG in hearts and Col1.7G2 in recta, while in C57BlK/6 mice there was a clear prevalence of Col1.7G2 in all tissues and JG, when it appeared, it was in recta of the animals. It is noteworthy that BALB/c and DBA-2 have the same MHC haplotype ($H2^d$), while C57BL/6 presents $H2^b$. In order to investigate a possible role of H2 haplotypes for the tissue tropism we infected mice C57BlK/sJ (JacksonMice laboratories) that have the same genetic background of C57BlK/6 but have the $H2^d$ as BALB/c and DBA-2. These mice were infected with mixture of Col1.7G2 and JG tripomastigotes, after six months these animals were sacrificed and the presence of parasite was investigated in hearts and recta. In C57BL/KsJ we found a predominance of JG strain in all tissues, with Col1.7G2 clone, just in the rectum, when seen at all. These observations are opposite of what we observed in C57BLK/6 and suggest that the MHC locus might be involved in the tissue distribution pattern of *T. cruzi* strains. Further experiments using cell cultures and others congenic strains will help in the elucidation of this hypothesis.

BC31 - IN SITU EVALUATION OF IMMUNOLOGICAL EVENTS ASSOCIATED WITH THE DEVELOPMENT OF EXPERIMENTAL CUTANEOUS LESIONS PRODUCED BY L. (L.) amazonensis IN THE MONKEY Cebus apella (Primates: Cebidae)

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Clinical and histopathological aspects of the evolution of the infection caused by different species of Leishmania in C.apella confirm its susceptibility to Leishmania infection and support previous indications that this primate may be useful as experimental model for studies of cutaneous leishmaniasis. In order to characterize the immunopathological mechanisms involved in the evolution of the histopathological changes, skin biopsies collected from C.apella inoculated intradermally with 3.000.000 promastigotes of L.(L.)amazonensis were studied at 2, 30, 60, 90, 120, 150 and 180 days post-infection (PI). The skin sections were stainned by HE and immunohistochemistry performed using mouse anti-Leishmania, mouse anti-human B cell, rabbit anti-human lysozyme antibodies. The histopathological changes were characterized by focal and mild inflammatory infiltrate in the dermis by polimorphonuclear and mononuclear cells with absence of parasites at 2 days PI. The inflammatory process increased with the time of the infection and

parasitized macrophages and plasma cells was detected since 30th day. At the 60th and 90th day of infection, granuloma with giant cells and focal area of necrosis were also present. Since the 150th day of infection collagen tissue and few inflammatory cells characterized the lesion. The immunohistochemistry confirmed parasites in the lesion since 30th day till 120th day of infection. Activation of macrophages characterized by positive reaction for lysozyme was mild at the 2nd day PI, moderate at the 30th day, intense at the 60th day. Decreased in the number of positive cells were observed since 90th day and it was characterized by moderate reaction at 90th day and mild since 120th day when the parasite burden was decreased and the scar tissue appeared. Few B cells were observed since 60th day of infection. Our preliminary results suggest that the immunopathological mechanisms could be related with the sequencial events of the histopathological changes.

BC32 - EVALUATION OF THE CELLULAR IMMUNE RESPONSE IN THE DERMIS OF C57BL/6 MICE INFECTED WITH L.(L.)amazonensis AND Lu.longipalpis SALIVA.

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We have shown that saliva of *Lu.longipalpis* modifies the *L.(L.)amazonensis*-host interaction *in vivo* and *in vitro*. The exacerbation of the infection was higher in the presence of saliva from colonized than of field captured vectors (Entomol. Vect. 9 (supl.1): 83-84, 2002). In order to better characterize the cells involved in the dermis inflammatory reaction developed after *Leishmania* infection in the presence of saliva, C57BL/6 mice were inoculated intradermally into the ear dermis with 1.000.000 promastigotes of *L.(L.)amazonensis* in the presence or absence of half pair of salivary gland of field captured *Lu.longipalpis*. Cells from ear dermis were collected by sedimentation in culture medium at 24 hours and 7 days post-infection; and the cells suspension processed for flow cytometry analysis using mouse monoclonal antibodies for CD14, CD3ϵ, CD4 (L3T4), CD8a (Ly-2) and PanNK (DX5) antigens. Preliminary results show a lower number of polymorphonuclear cells; no difference on CD14 expression; and lower number of CD3ϵ, CD4, CD8a positive cells and PanNK antibodies in the cells suspension recovered from the ear dermis of mice infected in the presence of saliva when compared to mice infected in the absence of saliva at 24 hours and 7 days post infection. These data suggest that mice infected in the presence of saliva develop an inflammatory reaction in the early phase of infection that could facilitate parasite survival and the establishment of the infection. Current experiments have been designed to better characterize the ear dermis inflammatory cells phenotype as well as cytokines profile in mice infected with *Leishmania* plus saliva from field captured and colonized vectors.

BC33 - Localization of $P2X_7$ receptor in double positive (CD4/CD8) thymocytes

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During the acute phase of *Trypanosoma cruzi* infection, there is a dramatic atrophy of the thymus, which is mainly characterized by CD4/CD8 double positive (DP) cells loss. Our group showed recently that the $P2X_7$ receptor may be involved in this process. This molecule is a member of the purinergic receptors family, whose agonists are extracellular nucleotides, such as ATP (ATP_e). This receptor can open non selective pores on plasma membranes leading to cell death in a number of cell types, such as macrophages and dendritic cells. We observed that DP cells from *T. cruzi*infected mice do express the receptor on cell surface and are highly susceptible to ATP_e. On the other hand, DP thymocytes from control mice are refractory to ATP_e. Therefore, herein we decided to investigate if this receptor is not translated or expressed in a non functional configuration. We used C57Bl/6 mice intraperitonealy infected with Y strain of Trypanosoma cruzi and sacrificed the animals after 10 days of infection. We obtained total thymocytes by mechanical dissociation and purified the DP populations (90-97%) using flow cytometer cell sorting or magnetic beads. By Western blotting, we observed the expression of $P2X_7$ in total thymocytes from control mice, accounting that DP cells correspond to about 80% of thymocytes. Using purified DP cells in confocal fluorescence microscopy, we observed a specific labeling apparently on the cell surface or in the vicinity on both control and infected mice. Indeed, preliminary results using electron microscopy suggest that the receptor is sequestered in cytoplasmic vesicles adjacent to the plasma membrane. These results indicate that the receptor is expressed in normal DP thymocytes and the infection renders its functional activation through still unknown factors.
Supported by CNPq and Fiocruz.

BC34 - Intracellular and extracellular *Trypanosoma cruzi* amastigotes of isolates from chronically and acutely infected Chagas disease patients display a polymorphic pattern of carbohydrate epitopes recognized by monoclonal antibodies

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Polymorphism of surface molecules seems to be a common feature of *T. cruzi* forms and has been described in epimastigotes, tissue culture trypomastigotes, metacyclic try-

pomastigotes and amastigotes of some isolates. Here, we describe for the first time a polymorphic expression of carbohydrate epitopes in intracellular (IA) and extracellular (EA) *T. cruzi* amastigotes of isolates from chronically (569*and*588) and acutely (573*and*587) infected Chagas disease patients by using a panel of monoclonal antibodies ($mAbs$). Vero cells infected with IA of different isolates were examined by confocal fluorescence microscopy. The fluorescence distribution was very polymorphic among isolates and not all IA were stained. For 569 and 588 isolates, mAbs 1D9, 2B7 and 3B9 preferentially labeled the parasite surface while intracellular compartments were also labeled in 573 and 587 isolates. MAbs 4B9 and 2C2 did not react or reacted weakly with IA from 587 and 569 isolates respectively. By fluorescence-activated cell sorter, the epitope identified by mAb 1D9 was less represented in 569 and 588 parasites when compared to 573 isolate. However, the mean fluorescence, which measures the relative expression of the epitope in positive parasites, was higher to that measured in 573. The epitope recognized by mAb 3B9 was abundantly expressed in all isolates studied with a high number of positive parasites. The number of mAb 2C2 positive parasites was low in all isolates and even smaller in the chagasic patients isolates. On the other hand, positive parasites of 569, 588 and 573 isolates expressed intermediate amounts of this epitope, whereas 587 presented it poorly. Immunoblotting analysis of EA using mAbs 1D9 and 2B7 revealed a polymorphic pattern of antigen expression. For mAb 2B7 several bands present in some isolates were not detected in others, except a faint detection in 573 isolate. We are currently verifying the role of this carbohydrate epitope polymorphic expression in parasite infectivity as *T. cruzi* amastigotes are covered with a significant proportion of sugar residues. Financial support: FAPESP, CNPq, and CAPES.

BC35 - Biological characterization of isolates of *Acanthamoeba polyphaga*: the possible definition of a typical pathogenic phenotype *in vitro*.

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Acanthamoeba spp. is a group of free-living amoeboid microorganisms, which are dispersed in a variety of environments. They have been isolated from soil, dust, freshwater, seawater, sewage, contact lenses, lens cases, among others. Occasionally, these amebas could be infectious agents in humans, it causing severe diseases as granulomatous amebic encephalitis, a fatal nervous system disease and amebic keratitis, a sight-threatening disease of the eyes. Furthermore, disseminated infections are also observed. Despite of its clinical relevance, the knowledge concerning the elucidation of the molecular basis of pathogenesis is not well known. Some molecular events as cytoadhesion and proteolytic activity seem to be related to the cytopathogenicity of *Acanthamoeba*. Here, in this work, we performed a biological characterization of two isolates of *Acanthamoeba polyphaga*: the first, a freshwater, environmental isolate, and second a pathogenic clinical isolate, searching to achieve some biological variation that could be related to pathogenicity. We were able to determine diverse differences between the two *Acanthamoeba* isolates. Morphologically, clear differences are detected comparing the microorganisms. The environmental isolate possess a larger number of vacuoles and spiny cytoplasmatic projections (termed acanthapodia) than the clinical isolate. However, the pathogenic isolate presents highest growth ratio. Cytotoxicity against epithelial and fibroblast cells also strongly varies between the isolates here studied. It is important to notice that the clinical isolate appears to present a metastatic-cell like phenotype, showing typical behavior of a invasive cell, with high motility, strong proteolytic activity and low adhesion levels on plastic substratum. On the other hand, the environmental isolate possesses low motility, weak proteolytic activity and high adhesion levels on plastic. Taken together, these results show that pathogenic isolates of *A. polyphaga* possess a collection of phenotypic markers that result in a pathogenic protozoon. **Financial Support**: CNPq, FAPERJ, FUJB-UFRJ and MCT-PRONEX.

BC36 - CHARACTERIZATION OF *ACANTHAMOEBA POLYPHAGA* INTERACTION WITH HUMAN OSTEOBLASTIC CELLS *IN VITRO*.

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Free-living species of the genus *Acanthamoeba* are amoeboid protozoa dispersed in diverse environments. Sporadically, in certain conditions, these microorganisms could cause serious diseases in humans, as granulomatous amebic encephalitis, a fatal brain infection, and amebic keratitis that is a sight-threatening disease of the cornea. Moreover, *Acanthamoeba* spp. may be the ethiological agent of disseminated infections in diverse parts of the human body. Recent reports in the literature showed that *Acanthamoeba* is capable to cause bone infection in immunosupressed patients. Therefore, the biological interplay between *Acanthamoeba* and bone cells could be the basis for the estabilishment of such infection. Here in this work, we started to characterize the process of interaction involving *Acanthamoeba polyphaga* and human osteoblastic cells. We were able to observe that *A. polyphaga* microorganisms readily adhere to the osteoblast cell surface, exerting a cytotoxical effect. Monolayer destruction seems to be pathogen-density dependent. However, high densities of *Acanthamoeba* trophozoites were not capable to destroy osteoblasts in the interaction period of 4 hours. This result indicates that cytotoxicity in *A. polyphaga* may be saturable, in relation to the number of amebas present in the interaction medium. Taken together, the results presented in this work show that *A. polyphaga* trophozoites recognize and destroy osteoblastic cells *in vitro*. Certainly, this type of interaction is a very important event for the better biological understanding of bone infection by *Acanthamoeba*. **Finan-**

cial Support: CNPq, FAPERJ, FUJB-UFRJ and MCT-PRONEX

BC37 - Heterogeneity of *Trypanosoma cruzi* II Isolates Derived of *Leontopithecus rosalia* from Poço das Antas Biological Reserve, Rio de Janeiro, Brazil.

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Previous studies on the infection of *Trypanosoma cruzi* in the Poço das Antas Biological Reserve population of wild free ranging *Leontopithecus rosalia* (golden lion tamarin) have shown the presence of genotype *T. cruzi* II, associated in Brazil with human disease. Aiming to understand if the social groups of the tamarins were or not involved in different transmission cycles, we performed the isozymatic typing of 20 isolates of *T. cruzi* derived from eight distinct social groups of golden lion tamarin. The data were analyzed by numerical methods and a dendrogram was produced using the Jaccard coefficient and the UPGMA as the clustering algorithm. We also compared the profile of two *T. cruzi* isolates derived from two tamarins recaptured after an interval of respectively one and four years. Parasitological, biochemical and molecular characterization demonstrated that golden lion tamarins maintain stable infections by *T. cruzi* II. Moreover, infection percentages varied among the tamarins groups of the distinct home ranges. The same isozymatic profile was observed in the isolate of the animal recaptured after four years, moreover, five loci of the isolate derived from the animal recaptured after an one year interval displayed distinct isozymatic profiles. Two robust clusters (A and B) with ten zymodemes was observed in 20 examined tamarins, independent of the social structure that each individual occupies in the group or independent of the geographical distribution of each social group. Overall these results suggest that: i) golden lion tamarins of Poço das Antas Biological Reserve participate of the same sylvatic transmission cycle and may maintain the same zymodeme for a long time, ii) no correlation between zymodeme, geographical distribution, sexual behavior or age and social group of the animal was observed and iii) microenvironmental peculiarities even in a same biome modulate the sylvatic transmission cycle of *T. cruzi* in tamarins.

BC38 - Influence of *Trypanosoma cruzi* clonal diversity on recrudescence of infection in immunosuppressed mice.

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Acute reactivation of chronic Chagas disease has occurred in cases of immunosuppression. Interestinguingly, the reactivation of Chagas disease does not occur in all patients exposed to immunosuppressive agents. Since the course of experimental infection is influenced by parasite population and host immune response, in this study we report the correlation between the *T. cruzi* phylogenetic divergence and the benznidazole-terapy (Bz-terapy) in reactivation of chronic Chagas disease in cyclophosphamide immunosuppressed mice (Cy-I). For this, four *T. cruzi* stocks from the genotypes 19 and 20 (Gambá cl1 and Cuíca cl1), 39 (Bug 2148 cl1) and 32 (IVV cl4) were inoculated in groups of 60 Swiss mice as follows: group 1 and 2 received Cy-I 60 (end of the acute phase) and 120 (chronic phase) days after inoculation (d.a.i), respectively; groups 3 and 4 received Bz-terapy 10 (acute phase) and 120 (chronic phase) d.a.i. and Cy-I 30 days post-treatment (d.p.t). The controls (groups 5 and 6) were infected but not-immunosuppressed. The results showed variability in the parasitemia reactivation after Cy-immunosuppression among mice infected with different genetic groups. The Cy-immunosuppression 60 d.a.i induced parasitemia recrudescence in 100%, 80%, 25% and 0% of the mice infected with the Cuíca cl1, Gambá cl1, IVV cl4 and Bug 2148 cl1, respectively. Among Bz-treated animals in acute phase, the Cy-I post-treatment induced recrudescence in 100%, 80%, 0% and 0% of the mice inoculated with the same stocks. In animals Cy-I during the chronic phase, the reativation was observed in 50%, 40%, 25% and 0% in Cuíca cl1, Gambá cl1, IVV cl4 and Bug 2148 cl1, respectively. On the other hand, after Bz-treatment the reativation in these same stocks were observed in 10%, 60%, 0% and 0%. Data show correlation between phylogenetic distance among *T. cruzi* clonal genotypes and reactivation of *T. cruzi* infection in Cy-treated mice. Financial support: UFOP, PROBIC/FAPEMIG/UFOP

BC39 - Intracellular traffic of amastigote forms of *Trypanosoma cruzi* from the two major phylogenetic lineages: role of the intracellular bacterium *Coxiella burnetii*

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Our aim in this work is to study the fate of *Trypanosoma cruzi* amastigote forms after invading Vero cells or Vero cells persistently colonized with the obligate intracellular bacteria *Coxiella burnetii*. This bacterium resides in acidified vacuoles with secondary lysosomal characteristics and our results show it to be a modulator of the intracellular traffic of these forms of *T. cruzi*. We examined the invasion step of amastigote forms of four different parasite strains belonging to the two major groups: *T. cruzi* I (G and Tulahuen), and *T. cruzi* II (CL and Y). Our findings revealed that persistent *C. burnetii* infection per se increased invasion of both G and CL strains, and when we raised the vacuolar or cytoplasmic pH with Bafilomycin A1 or Chloroquine respectively, invasion was further increased. The reverse pattern was seen in Vero cells alone, where all the above drugs reduced invasion of these forms. Kinetic studies of amastigote transference of G and CL amastigotes to *C. burnetii* vacuoles indicate that amastigote parasitophorous vacuole are more rapidly fused to *C. bunetii* vacuoles in cells treated with Chloroquine

or Ammonium chloride while the reverse was found in cells treated with Bafilomycin A1 or Concanamycin A. Results relating to Y and Tulahuen strains are still being analyzed. Hemolysin and transialidase activities of parasites from the four strains are also being measured. Endosomal-lysosomal sequential labeling with EEA1, LAMP-1 and Rab-7 of the parasitophorous vacuoles formed during the entry of parasite each strain will also be studied and compared to the known patterns of corresponding trypomastigotes.
Financial Support: FAPESP, CNPq and CAPES.

BC40 - Trafficking of trypomastigote forms of the two major *Trypanosoma cruzi* lineages in Vero cells harboring *Coxiella burnetii*

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Recent studies in our laboratory showed that pH plays an important role in modulating the intracellular traffic of parasitophorous vacuoles (PV) of trypomastigote forms of *Trypanosoma cruzi* (CL strain) in Vero cells harboring Coxiella burnetii. These observations lead us to carry out a comprehensive study of the fate of trypomastigote forms (metacyclics- MT, and tissue-culture derived - TCTs) of different strains: CL, Y (*T. cruzi* II) and G, Tulahuen (*T. cruzi* I), after invasion of Vero cells colonized by *C. burnetii*. Our findings revealed that persistent *C. burnetii* infection per se decreased the invasion index of both trypomastigote forms of CL, Y and Tulahuen strains while the presence of the bacteria, per se, did not affect the invasion index of either G strain trypomastigote forms in Vero cells. Alkalynization of the vacuolar or cytoplasmic pH with drugs such as chloroquine, ammonium chloride, bafilomycin A1 and concanamycin A, in Vero cells alone, decreased the invasion index of both trypomastigote forms of all strains studied. Different results were obtained in Vero cells harboring *C. burnetii*, where the four drugs increased the invasion index of both trypomastigote forms of CL strain but did not affect invasion of both trypomastigote forms of Y and Tulahuen strains in Vero cells colonized by *C. burnetii*; surprisingly bafilomycin A1 and concanamycin A decreased the invasion of both trypomastigote forms of G strain. Kinetic studies of trypomastigote transference to the *C. burnetii* vacuole showed that CL strain PV of MTs fuse to *C. burnetii* vacuoles less efficiently transferred in cells treated with all the drugs used, if compared to TCTs. In addition, our results suggest that TCT forms escape more rapidly from the bacteria vacuole. Results of G, Y, and Tulahuen strains are currently being analyzed. Hemolysin and transialidase activities of all trypomastigote forms are also being measured. Fiancial support: CNPq, FAPESP and CAPES.

BC41 - Tc85-11 is involved in trypomastigote adhesion to extracellular matrix and entry into host cells.

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Our laboratory has demonstrated that Tc85-11, a member of the Tc85 family related to the gp85/*trans*-sialidase superfamily, is implicated in host cell invasion by trypomastigotes of *T. cruzi* (Abuin et al. 1989, Mol. Biochem. Parasitol. 35, 229-8). It was shown that a conserved motif at the carboxyl-terminus of the molecule, named peptide J, binds to the host cell surface and is able to enhance the invasion of epithelial cells (LLC-MK2) by the parasite (Magdesian et al. 2001, J. Biol. Chem. 276, 19382-9). Also, Tc85-11 binds to laminin through amino-terminal peptides (Tc85-N). These peptides, in solution, inhibit entry of the parasite by approximately 85% (Marroquin et al. 2004, submitted). It is herein demonstrated that peptide J binds to extracellular matrix (ECM) but does not bind to laminin. Notwithstanding, the addition of peptide J promotes a 5-fold enhancement of trypomastigote adhesion to ECM by an unknown mechanism. Apparently, peptide J is not acting as a bridge between parasites and ECM, since the peptide does not bind to *T. cruzi*. Moreover, peptide J does not compete with trypomastigote binding to ECM. Taken together, these results suggest that the adhesive micro-domains of Tc85-11 may act at the two steps of the trypomastigote cellular infection: parasite anchoring to ECM and promotion of their internalization into host cells.
Supported by: FAPESP, CNPq
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BC42 - THE EFFECT OF ACRIFLAVINE ON THE ULTRASTRUCTURE OF SOME TRYPANOSOMATIDS

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Flagellated protozoa belonging to the Trypanosomatidae family form an ancestral branch of eukaryotes and present unusual biological structures such as the kinetoplast, a specialized region of the mitochondrion which contains DNA (kDNA). The kDNA is composed by circular molecules, which are topologically interlocked constituting a single network. The kDNA arrangement varies according to species and to the stage of development. The majority of trypanosomatids have tightly packed kDNA fibers forming a compact structure (bar-shaped). In endosymbiont-bearing trypanosomatids the kDNA fibers are disposed in a typical looser arrangement through the kinetoplast matrix. The kDNA is sometimes altered or even lost in nature, giving rise to natural dyskinetoplastic (Dk) strains. Dk strains can be obtained by a variety of DNA binding compounds, such as acriflavine and ethidium bromide, and has been employed to investigate the importance of the mitochondrial DNA in the energetic metabolism. Previous studies showed that the kDNA retained in the Dk organism is not organized into networks, appearing dispersed throughout the trypanosomatid kinetoplast. In the present work, we evaluated the action of acriflavine on the proliferation and ultrastructure of trypanosomatids that present distinct kDNA arrangements. This drug inhibited the cell proliferation of *B. culicis*, *C. fasciculata* and epimastigotes of *T. cruzi* at concentrations of 50μg/ml.

Furthermore, electron microscopy analysis showed that the acriflavine treatment resulted in drastic ultrastructural modifications in the kinetoplast. In all species analyzed the acriflavine promoted alterations in the kDNA arrangement, which was seen as a compact network presenting electron-dense patches. In epimastigotes of *T.cruzi* the nucleus was affected and the chromatin was seen less condensed upon prolongued exposure to this drug. The effect of acriflavine in epithelial cells was also analyzed, as well as the redistribution of basic proteins in drug treated trypanosomatids.
Supported by CNPq and FAPERJ

BC43 - Possible mechanisms of invasion of gastric mucosal epithelium by *Trypanosoma cruzi* upon oral infection

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Metacyclic forms of *Trypanosoma cruzi*, which efficiently infect mice by oral route, invade the gastric mucosal epithelium and replicate intracellularly as amastigotes. It has been shown that metacyclic forms (CL isolate) are resistant to pepsin, at pH 3.5, treatment that preserves the gp82 molecule that binds to gastric mucin and is involved in gastric mucosal infection. We have extended these studies to four *T. cruzi* isolates from chagasic patients, two of them (569 and 588) deficient in expression of gp82 but expressing a related molecule gp30, which also induces calcium response in host cells and promotes parasite internalization, and two others (573 and 587) expressing both gp82 and gp30 on the surface. Metacyclic forms of these isolates were compared for the ability to invade epithelial HeLa cells and to infect mice by oral route. Unexpectedly, metacyclic forms of isolates 573 and 587 entered HeLa cells in significantly lower numbers than isolates 569 and 588. When parasites were analyzed for the presence of gp90, a negative modulator of parasite infectivity, isolates 573 and 587 were found to express higher levels of gp90. Although this explained the low infectivity of isolates 573 and 587 in vitro, it could not explain why, when given orally, metacyclic forms of isolate 573 produced much higher parasitemias than the other isolates and invariably killed 40% of Balb/c mice. Treatment of parasites with pepsin at acidic pH extensively digested gp90 of isolate 573 metacyclic forms and significantly increased their ability to invade HeLa cells, but did not affect the infectivity of other isolates. As regards the isolates 569 and 588, which express gp30 but are gp82-deficient, they are not as effective in invading gastric mucosal epithelium because, in contrast to gp82, gp30 binds poorly to gastric mucin. In assays mimicking *T. cruzi*-gastric mucin interaction, the presence of gastric mucin significantly inhibited HeLa cell invasion of gp82-deficient isolates 569 and 588, but not of isolates 573 and 587. These data indicate that efficient *T. cruzi* infection by oral route is associated with expression of surface molecule gp82, and reduced expression of gp90.
Work supported by FAPESP and CNPq

BC44 - The cure of mixed infections with *Trypanosoma cruzi*: more difficult or nor?

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Biochemical and molecular markers have demonstrated the occurrence of mixed *Trypanosoma cruzi* infection in humans, vectors and reservoirs. The clonal evolution model postulated for *T. cruzi* predicts a correlation between the phylogenetic divergence and their biological properties including their drugs sensitivities. The major goal of this study was to verify the influence experimental mixed infections, using different clonal stocks of *T. cruzi* predicts genotypes on the cure rates in comparison with monoclonal infections. For this purpose, groups of 12 BALB/c mice were simultaneously infected with 2 stocks (5,000 blood trypomastigotes of each), from 4 major T. cruzi clonal genotypes, including 19 and 20 (T. cruzi I) and 32 and 39 (T. cruzi II), the former more resistant to benznidazole. A total of 24 combinations were performed. After patent infection, six animals from each group were treated with BZ (100mg//kg) for 20 consecutive days. Treatment effectiveness was assessed through hemoculture and PCR (at day 30), ELISA (at day 60 and 90) and FC-ALTA (at day 120) after treatment. Cure criterion consisted of negative results on parasitological and approaches. Animals with negative results on parasitological and FC-ALTA test were also considered cured. The identification of clone(s) from not cure mice are beginning performed by microsatellites, RAPD and isoenzimes. In 10 mixed infections the rates of cure was higher than the theoretically expected considering the monoclonal infections while in 4 mixtures the cure rates were lower. Curiously, in some cases, clones 100% resistant to BZ in monoclonal infections when combined with clones that showed 100% of cure rates, displayed 100% of cure. On the other hand, mixed infections of clones 100% sensitive displayed partial rates of cure. Mixtures of resistant clones to resistant or partially resistant, showed cure rates higher than the expected. These data suggest that treatment of human infections is more complex that it would be expected.
Financial support: FAPEMIG, CNPq and UFOP

BC45 - Impact of *Trypanosoma cruzi* cruzi mixed infection on biological properties in mice

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Trypanosoma cruzi undergoes a predominant clonal evolution with impact on its biological properties. Biochemical and molecular markers have been demonstrated the occurrence of mixed infection in humans, vectors and reservoirs. The goal of this work is to characterize the mixed infection with clones from different genotypes in comparison with monoclonal infections. For this, 2 clonal stocks of each principal genotypes 19 and 20 (*T. cruzi* I), 32 and 39 (*T. cruzi* II), were combined in pairs. A total of 24 combinations were performed. Groups with 12 BALB/c mice were inoculated via IP, with 5000 blood trypomastigotes of A total of 24 combinations were tested. each clone. The parameters infectivity, mortality, rate of positive hemoculture (30 days after treatment), parasitemia , prepatent period, patent period, maximum of parasitemia and day of peak maximun were evaluated during acute phase. The mortality rates were daily registered for 90 days.The infectivity was 100% for all mixed infections, similar or higher than the observed in monoclonal infections when compared with the most infective clone. The mortality rates showed heterogeneous results in mixed infections with higher, similar or lower than monoclonal infections. The analysis of parasitemia showed different results in 91,66% of the mixed infection. Fifty percent displayed an apparent stimulatory effect and 50% an inhibitory effect among the clones of the same mixture. The positive rates of hemoculture in the mixed infections range from 20% to 100%. A reduction of positivity occurred in 29,1% of them. These data corroborate previous results of our group where a phenomenum of inhibition or stimulation effect of one clone to another was observed in experimental mixed infections in mice and vectors.

Financial support: FAPEMIG, CNPq and UFOP

BC46 - The cytotoxic activity of miltefosine against *Trypanosoma cruzi*: *in vivo* studies

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Miltefosine (1-*O*-hexadecylphosphocoline), a synthetic etherlipid analogue used for the treatment of cutaneous metastasis from mammary carcinomas, is now being considered as a new oral drug for treatment of visceral leishmaniasis in humans (1). Several studies indicated that miltefosine is toxic against *Trypanosoma cruzi in vitro* (2-5). In this work we observed the action of miltefosine (25mg/Kg) and benznidazol (100mg/Kg) orally administered (once a day after infection and for 14 days) in Swiss mice (3 groups of 10) infected with *Trypanosome cruzi* Y-strain (10^5). The treatment of the Swiss mice with miltefosine and benznidazol promoted 100% survival while 60% of the untreated mice died. The animals treated with miltefosine showed a significant reduction of the parasites detected in blood when compared to the untreated animals. Nevertheless, no parasites were detected in the blood of animals treated with benznidazol. After 15 days of infection the animals were sacrificed, and histopathological studies showed a decrease in the inflammatory infiltrated area of the heart and liver and absence of amastigote nests. Spleen follicles sizes were similar between treated and control animals. These results suggest that miltefosine and benznidazol have comparable effects in the treatment of experimental *T. cruzi* infection during the acute phase. References: 1- Guerin *et al.* (2002) Lancet Infect Dis. 8: 494-501; 2- Croft *et al.* (1996) J. Antimicrob. Chemother. 38: 1041-1047; 3- Santa-Rita *et al.* (2000) Acta Tropica 75: 219-228; 4- Lira *et al.* (2001) J. Antimicrob. Chemother. 47: 537-546; 5- Saraiva *et al.* (2002) Antimicrob. Agents Chemother. 46: 3472-3477. Supported by: FUJB-UFRJ, FAPERJ, CNPq and FIOCRUZ

BC47 - Extracellular matrix alterations in experimental murine *Leishmania (L.) amazonensis* infection.

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*Leishmania*is the causative agent of a broad spectrum of human diseases ranging from single self-healing cutaneous lesions to anergic diffuse cutaneous lesions to fatal visceral leishmaniasis. Clinical nanifestations of leishmaniasis de-

pend on a fine interaction between the parasite and the host genetic backgrouds. Several parasites produce proteases that degrade matrix proteins, facilitating the breaching of the dermal barrier to spread the infection. When *Leishmania* promastigotes are inoculated into the host skin, a loosening of the dermis connective tissue matrix occurs enabling then the establishment of infection. In the present study, we describe extracellular matrix alterations in footpad lesions and draining lymph nodes caused by *Leishmania (L.) amazonensis* in mouse strains with distinct susceptibilities to this parasite: BALB/c (susceptible), C57BL/6 (intermediate), and DBA/2 (resistant). Changes in ECM were observed mainly in BALB/c mice that, in general, presented tissue damage associated with high parasite burden. Under polarized light, Sirius Red revealed type I collagen that was predominant in the primary lesion in all strains studied at the early phase of infection, but gradually decreased and was replaced by abundant type III collagen fibres in chronic phase lesions. The presence of type III collagen seemed to provide support to inflammatory cells, mainly vacuolated and parasitized macrophages. Laminin expression was not altered during infection by *L. (L.) amazonensis* in any of the mouse strains studied. Furthermore, the decreased fibronectin expression, in all strains, in areas where amastigotes have been found, indicated that this decline was also not related to the genetic background.

BC48 - Peritoneal Proteins with Yeast Cell Affinity from Mice Infected with *Toxoplasma gondii* Modulates Yeast Phagocytosis of Macrophages

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T. gondii is a parasite capable of deactivating macrophages. *T. gondii* secretes substances during cell invasion that may cause this deactivation and also generate an inflammatory reaction by the host. To determine if the *T. gondii* secretion and host inflammatory proteins could modulate macrophage function, *T. gondii* conditioned medium (CM), and peritoneal washes of normal (SPWn) and infected (SPWi) mice were generated and its effect on macrophage yeast cell phagocytosis determined. To obtain CM, peritoneal *T. gondii* was incubated with DMEM at 37°C for 0 to 30 min and 1, 6, 12 and 24h. Parasites were also washed before addition into DMEM. Mice peritoneal washes with DMEM were performed for SPW. Furthermore, yeasts cells were also incubated with SPWi (SPWi-treated) to remove components with yeast cell affinity. CM and SPW were centrifuged, the supernatants analyzed through SDS-PAGE and added to fetal bovine serum for macrophage cultures. After 24h yeast cell uptake was assayed by light microscopy. Protein profiles of the different CM and SPW were similar. However, protein concentrations increased in the following order: CM where washed parasites were used, CM with longer periods of incubation, SPWn, and SPWi. When macrophages were cultured in CM or SPW a decrease in yeast phagocytosis and increase in adherence was observed; SPWi presented the highest modulation capacity. When SPWi-treated was analyzed by SDS-PAGE one protein band of 140 kDa was reduced and yeast cell uptake was similar to control. In conclusion, proteins from the CM and SPW were not from *T. gondii* secretion, but from the mice peritoneal cavity. They down modulated the uptake of yeast cells and have an affinity for yeast cell components. Further studies are in progress to identify this inhibitory factor.
Supported: UENF, CNPq, FAPERJ.

BC49 - Proteins of the family of articulins are present in the subpellicular network of Toxoplasma gondii

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Toxoplasma gondii withstands different environments during its life cycle, passing through structural and biochemical modifications. During invasion parasite uses its cytoskeleton to actively penetrate host cells, suffering a constriction in the cell body. The association of the pellicle with cytoskeleton elements allows parasite to keep its mechanical integrity and makes possible the gliding motility and cell invasion. Parasite cytoskeleton is formed by the conoid, polar rings, 22 subpellicular microtubules, actin filaments, a myosin motor TgMyoA, and a network composed of 8-10 nm filaments, the subpellicular network, which extends along the entire body of the parasite. As the subpellicular network and the inner membrane complex are predicted to form a membrane skeleton, we used antibodies for proteins of the articulin family from Euplotes aediculatus, a ciliated protozoan, that presents a monolayer of flattened membranous sacs in contact with cytoskeleton proteins, called plateins and cageins. In this work we used different technical approaches (negative staining of detergent-extracted samples, SDS-PAGE, immuno blotting, fluorescence and immunoelectron microscopy, and cDNA library screening) in order to study the structure and composition of this network. Observation of negative staining samples showed that the filamentous proteins extend homogeneously interwoven along the entire length of parasite, ending in a well-delimited circular structure. Analysis with fluorescence microscopy showed a dispersed labeling in the whole cell body of tachyzoites for PL-5 (alpha, beta, and gamma forms of plateins), AP-2 (alpha-platein), and AP-6 (cagein). The labeling pattern is very similar to the distribution of subpellicular network. These results were confirmed in the immunoelectron microscopy, using detergent-extracted samples. We also found in Toxoplasma genome database homology of 54% for beta-/gamma platein and 59% for alpha-1 platein. We are now carrying out an immuno blotting of

these extracted samples with the same antibodies in order to characterize the parasite proteins related to articulins. This work was supported by CNPq, CAPES and Pronex-Faperj

BC50 - *Trypanosoma cruzi* recombinant protein gp82 inhibits enteroinvasive bacterial internalization into human epithelial cells by inducing disruption of actin filaments

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The metacyclic stage-specific surface molecule gp82 of *Trypanosoma cruzi* is implicated in epithelial cell invasion in vitro and in vivo. Metacyclic trypomastigotes inoculated by oral route into Balb/c mice invade epithelial cells of gastric mucosa in gp82 dependent manner and produce high parasitemias. Gp82 is an adhesion molecule that binds to its receptor on epithelial cells and induces calcium mobilization, which is a requirement for parasite internalization. Here we investigated whether purified gp82 molecule, which inhibits HeLa cell entry of metacyclic forms, interferes with cell invasion of enteroinvasive bacteria. Experiments using enteroinvasive *Escherichia coli* (EIEC) showed that the recombinant gp82, fused to GST, significantly inhibited the bacterial uptake by HeLa cells whereas GST had no effect. As inhibiton of bacterial infection is not due to the blockage of host cell receptor, as is the case of *T. cruzi* cell invasion, we examined the possibility that gp82 is interfering with rearrangements of actin cytoskeleton, an event essential for bacterial internalization. HeLa cells were incubated for 30 min in absence or in the presence of GST or recombinant gp82, fixed with acetone before reaction with fluorescein-conjugated phalloidin, and analyzed by confocal microscopy. In HeLa cells incubated with recombinant gp82, but not in cells in which GST was added, not only there was a marked decrease in the number of cells with clearly visible actin filament bundles, but overall they were thinner and shorter. By measuring the length of individual actin filament bundles in approximately 40 cells, we have found that the average length in gp82-treated HeLa cells was approximately 3 fold shorter than in control GST-treated or untreated cells. Experiments of HeLa cell invasion with gp82 are being extended to other enteroinvasive bacteria such as *Shigella flexneri*, which has an invasion plasmid similar to that harbored by *E. coli*, and *Salmonella typhimurium*. This work was supported by FAPESP and CNPq.

BC51 - Influence of iron deficiency or supplementation in cardiac lesions in mice infected with *Trypanosoma cruzi*.

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Iron is essential for all living organisms due to its role in many enzyme systems. Iron levels may affect the host-parasite relationship. The effects of iron deficiency and supplementation on the evolution of experimental Chagas disease were analyzed. Histopathological aspects in the heart were evaluated in Swiss mice infected by Y-strain and submitted to alterations in iron levels. Animals (n=48) were divided into four groups according to the type of treatment they received: control diet, control diet plus iron-dextran, iron deficient diet, iron deficient diet plus desferrioxamine. Twenty four animals were inoculated with 500 blood trypomastigotes of Y strain. The parasitaemia was determined daily and the pick was observed in the same period for all groups. Animals were sacrificed in the 7 and 14 days after inoculation. The hemoglobin dosage was accomplished and none difference was observed among the no-infected groups until the third week. However, in animals treated with desferrioxamine were observed hemoglobinemia only in fourth week. Decrease in the hemoglobin concentrations since the second week were observed in all infected groups. Fragments of the heart were collected to histopathologic analyses and was observed inflammation, degeneration and parasitism in different intensities, depending on the analyzed groups, being more intense in the 14 day of experimentation. In conclusion, the deficiency of iron provoked for diet and treatment with desferrioxamine promoted decrease of the hemoglobin concentration, difficult *T. cruzi* proliferation in vivo, and caused more discreet cardiac lesions. On the other hand, the supplementation with iron favors the parasite and cardiac lesions. Studies of lymphocyte subsets and cytokines in these animals are necessary to better understanding the cellular mechanisms occurring during iron supplementation or deficiency in experimental Chagas infection.

BC52 - Immunosupression and experimental Chagas disease in dogs: impaired cells migration to inflammatory focus in spinal cord

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In the past few years new aspects of immunopathology of Chagas disease have been described in immunosupressed patients. The immediate aim of the present study was investigate the effect of immunosuppression on the spinal cord lesions in acute phase of experimental *T. cruzi*infection in dogs. Sixteen dogs were divided in 4 experimental groups: no-infected (NIG), no-infected/immunossupressed (NIIG), infected (IG) and infected/immunosupressed (IIG) groups. The IG and IIG were inoculated with metacyclic trypomastigotes (Berenice-78 strain) by the intraperitoneal route. The NIIG and IIG were immunosupressed with Azatioprine. Phenotypic analysis show that IG have a progressively in-

crease in THY-1, CD8T cell and decreased in CD 21 and CD5 cells. The expression of CD14 and CD4 is maintained. IIG presented increased in CD4T cells and specific decrease of CD8T cells, with decreased of CD14 and CD21. The spinal cord and myocardium were collected, processed and stained for Hematoxylin-Eosin, Violet Cresil, Glees-Marsland, Weil-Weigert or submitted to immuno-histochemistry. The parasitaemia levels and mortality were larger in IIG when compared to IG. In the heart we observed discreet parasitism in the IG and intense parasitism in IIG. In the spinal cord the glial nodules and perivascular infiltrated was structured in the IG and little structured in the IIG, suggesting any modification in cell migration mechanisms. Thus, immunohistochemistry analysis of these inflammatory cells and endothelium adhesion molecules are necessary. Larger amount of perivascular infiltrated was observed in the cervical, thoracic and lumbar areas of the gray substance in the IG and smaller formation of perivascular infiltrated in the IIG. The absence of macroscopic alterations and parasitism in the spinal cord as well as the discreet histopathologic alterations, without clinical repercussion, found in IG and IIG can be attributed to low virulence of the Berenice-78 strain, the route of inoculation and blood-brain barrier of the dogs.

BC53 - Trypanosoma cruzi invasion is preceded by paxillin recruitment, actin polymerization and tyrosine phosphorilation in mouse peritonial macrophages and epithelial LLCMK2 cell lines.

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Trypanosoma cruzi, the ethiologic agent of Chagas'disease, can invade and proliferate inside a large number of host cells, what includes professional phagocytic - like macrophages - until non professional ones - like epithelial LLCMK2 cell line. During its invasion, trypomastigotes can activate and trigger changes in the pattern of F- actin organization and tyrosine phosphorilation at the invasion site. We have already reported tyrosine phosphorilation importance during T.cruzi invasion and the cytoskeleton participation along the process of adhesion and posterior internalization in macrophages. In the present study we carry out immunofluorescence assays in order to analyze paxillin participation followed by tyrosine phosphorilation and the F-actin recruitment at the invasion site. Both macrophages and LLCMK2 cells reveal a colocalization for paxillin and F-actin during early events of T.cruzi invasion. Dependent phosphatidylinositol 3-kinase (PI3 Kinase) tyrosine phosphorilation also plays an important role in the current process once pretreated cells with Wortmanin, a specific inhibitor for PI3 Kinase, prevented cells to phosphorilate at tyrosine residues in paxillin molecule and, consequently avoid F -actin concentration at the invasion site. Taken together, our results show that T.cruzi invasion is mediated by the presence of phosphorilated paxillin associated with F-actin colocalization. Both events seem to require PI3 kinase participation.
Supported by: CAPES, FAPERJ and CNPq.

BC54 - Cardiac mast cells in *Trypanosoma cruzi* infection isolation and staining

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Mast cells secrete diverse chemical mediators and cytokines pre-storaged in granules that are important for cell recruitment, antigenic presentation and modulation of acquired immunity. These cells are pivotal in inflammatory and fibrotic etiologies such as *Trypanosoma cruzi* induced cardiopathy. However, due to technical limitations such as reduced number of cardiac mast cells, *in situ* and *in vitro* identification and isolation from the tissue, most authors employ peritoneal mast cells to study its cellular relevance in pathological situations.
In this work we tested a rapid method for cardiac mast cells isolation and identification during acute infection with *T. cruzi*. For enzymatic dissociation, histological staining and flow cytometry analysis, we used hearts and peritoneal cells from normal and infected CBA mice. Mast cells were identified by toluidine blue method and with a new mixed solution composed by alcian blue, safranin and toluidine blue. We also evaluated whether the percoll step was indeed enriching or selecting mast cells sub populations.
We always observed significant differences in mast cell numbers when comparing both staining methods, and the mixed solution increased until five times the cellular detection. Using this method, we observed an expansion in peritoneal mast cells after infection but not in the cardiac mast cell population. In addition, we noted that the cardiac population was almost exclusively stained in blue and the peritoneal mainly in brown, indicating that specific staining methods are required for different compartments. Importantly, we observed that percoll gradient enriches the pellet in granule-filled mast cells, but wastes until 50% of degranulated cells, possibly cells responding to infection. Flow Cytometry analysis also showed particularities among mast cells from both compartments. Cardiac mast cells from control or infected mice were CD117- and peritoneal mast cells from infected mice down regulate CD117 when compared to control mice.
Supported by CNPq and Fiocruz.

BC55 - ANALYSIS OF THE BINDING OF *TRITRICHOMONAS FOETUS* AND *LEISHMANIA MEXICANA AMAZONENSIS* TO THE EXTRACELLULAR MATRIX GLYCOPROTEIN LAMININ.

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Extracellular matrix proteins are thought to be relevance

during some primary events in the initiation of the pathogenesis. The Laminins, here named LMN; that is a large multiple domain extracellular matrix glycoproteins; have been described to be recognized by several parasitic protozoa including *T. foetus*, *T. vaginalis* and *L. donovani*. Otherwise, the LMN peptides A13, A208, AGM3 and C16 have already been sequenced and characterized as LMN residing adhesion sequences. Here, we designed experiments looking for the characterization of the interaction of LMN with the extracellular parasite protozoan *Tritrichomonas foetus* and the intracellular pathogen *Leishmania mexicana amazonensis*. Initially, solid phase adhesion assays were carried out with LMN which were adsorbed onto glass slides. We found that the promastigote forms of *L. amazonensis* have its adhesive capacity enhanced in the presence of LMN. In order to identify the presence of a LMN binding proteins at the surfaces of *T. foetus* and *L. amazonensis*, both parasites were lysed and the resulting whole protein extracts were sequentially analyzed by SDS-PAGE and immunoblotting by using LMN-1 monoclonal antibodies. The results demonstrated the presence of a LMN binding protein of approximately 70kDa in *T. foetus* cell extract. Immunoblotting analyses were made by using biotin to demonstrate the possible cell surface localization of the identified LMN binding protein. On the other hand, LMN-coated slides were overlaid or not with each one of the peptides A13, A208, AG73 and C16. Previous treatment of the LMN-coated slides with each one of the peptides resulted inhibition of parasite adhesion at various degrees. Our results clearly demonstrate that both *T. foetus* and *L. amazonensis* recognize LMN. Furthermore, we were also able to identify a specific receptor for LMN, which could be a very important molecule involved in the trigger of the initial process of the pathogenesis caused by each one of the parasites. Supported by PIBIC-UFRJ/CNPq, FAPERJ, FUJB-UFRJ and MCT-PRONEX.

BC56 - Modulation of Malaria parasites cell cycle by changes on Ca^{2+} and cAMP levels induced by Melatonin.

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Host hormone melatonin can induce an increase of cytosolic Ca^{2+} concentration modulating the malaria cell-cycle (Hotta et al, 2000). Here we show that melatonin and other molecules derived from tryptophan, such as N-acetylserotonin (NAS), serotonin and tryptamine, modulate the *Plasmodium falciparum* cell cycle by engendering an increase in cytosolic free Ca^{2+} from intracellular stores. Interestingly cAMP pathway is also involved in the melatonin effect on regulation of malaria cell-cycle. In synchronized *P. falciparum* at trophozoite stage melatonin induces increase of 50% in cAMP levels and about 40% in the activity of cAMP-dependent protein kinase, PKA. When *P. falciparum* or *P. chabaudi* infected red blood cells are treated with the membrane permeable cAMP analogues 8BrcAMP and N6-cAMP there is an increase in parasitemia and this effect appears to depend on activation of PKA. These effects are abolished by the PKA inhibitors 8BrcAMP isomer, PKI and H89. We also investigated the connection between Ca^{2+} and cAMP in the signalling pathways. We found that the increase in cAMP induced by melatonin is inhibited by blockers of phospholipase C activity such as U73122 as well as by addition of Calmodulin inhibitor (Calmidazolium). Moreover the cAMP analogues 8BrcAMP and N6-cAMP induce an increase in cytosolic Ca^{2+} concentration. The present work confirms the importance of melatonin and its precursors in the *Plasmodium* development and contributes to the understanding of the mechanisms by which signalling pathways act to regulate the malaria cell cycle.

BC57 - Observation of the *Toxoplasma gondii* Intravacuolar Network During Ionophore Induced Egress

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Toxoplasma gondii, is an obligatory intracellular parasite that establishes a most unique relationship with its host, actively penetrating and multiplying in any nucleated cell of warm-blooded animals. Three classes of secretory organelles are activated during successive invasion stages: adhesion, penetration and intracellular development. Secretion of dense granules (GRAs) are responsible for the final vacuolar modification and the biogenesis of the intravacuolar network (IVN) of nanotubules. The exact functions of these nanotubules are still unclear but some authors support that it forms a host-parasite channel for metabolite exchange. We used the cell line LLCMK2 infected for 24 hours with *T. gondii* and analyzed the IVN through Field Emission Electron Scanning Microscopy and Transmission Electron Microscopy, gathering evidence that it plays an essential role in maintaining the parasites linked to each other keeping it in the classic rosette structure. *T. gondii* in LLCMK2 cells developed a large PV (up to 8 times larger than the initial vacuole) with at least 12 parasites per vacuole and the intravacuolar network widely distributed over the parasites. When calcium ionophore was added, *T. gondii* immediately started the egress, but for this to be accomplished, the IVN needs to be quickly disrupted, what was observed by both methods (FESEM& TEM). We suggest that the intravacuolar network may act rather as a mechanical support, maintaining the parasites in a compact disposition until egress, which allows the PV to grow in volume up to 8 times and a single host cell to harbor more than 1 PV, than as a nutritional channel. The calcium influx caused by the ionophore, 24 hours after infection, may also trigger mechanisms that rapidly affect the network and allows the parasites to move towards the host cell plasma membrane and complete its intracellular cycle.

BC58 - CHARACTERIZATION OF PLASMINOGEN BINDING TO THE SURFACE OF *Leishmania mexicana*

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In prior studies it was determined that *Leishmania mexicana* interacts with human plasminogen, the zymogen of the plasmatic protease plasmin. This interaction would be able to favor the invasive capacity of the parasite as it has been shown in other pathogens such as *Yersinia pestis* and *Borrelia burgdorferi*. In this work we intend to characterize the interaction between human plasminogen and *L. mexicana* to the molecular level and try to establish what motifs in the plasminogen have the capacity to interact with promastigotes of this parasite, and to identify the receptors of the *L. mexicana* surface responsible for this recognition. Through digestion with porcine pancreatic elastase, and subsequent purification, two fragments were obtained from the digested plasminogen: a fragment that contains the kringles 1-2-3 and the mini-plasminogen (kringle 5-catalytic site). The aforementioned fragments were assayed for their ability to inhibit the union of plasminogen in promastigotes of *L. mexicana* both in solution as with cells immobilized in microtitering plates. It was found that both fragments are capable to inhibit the union of plasminogen, with a preferential recognition through the fragment that contains the kringles 1-2-3. These results were analyzed in a qualitative way with immunocytochemical tests that corroborated our findings. Given that enolase has shown to be a receptor of plasminogen in diverse microorganisms and cells from mammals, binding ability was tested with cytosolic enolase purified from *L. mexicana*, both native and recombinant, through ligand blotting. Plasminogen and enolase were used as ligands in independent assays. These experiments showed that enolase interacts with plasminogen and that this interaction is partially inhibited by EACA and benzamidine. Ligand blotting studies with microsomal fractions of *L. mexicana* showed the presence of enolase in this fraction, which was further corroborated by inmunofluorescence. Purification of this enolase and the identification of other proteins able to bind plasminogen are being carried out through bidimensional gels and ligand blotting followed by mass spectrometry (MALDI-TOF).

BC59 - Effect of several squalene synthase inhibitors on growth and ultrastructure of *Leishmania chagasi*

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Leishmaniasis is an important disease in widely dispersed regions of the world. In South America visceral leishmaniasis (VL) is mainly caused by *Leishmania chagasi*. The morbidity associated with the infection is high, and death may occur in some untreated patients. Treatment has been based upon pentavalent antimonial drugs for more than half a century and problems, including development of resistance to antimonials and lack of efficacy against VL/HIV co-infections, have emphasized the need for new drugs. Squalene synthase (SQS) is an essential enzyme for the biosynthesis of protozoan sterol molecules. In this work, twelve specific inhibitors of SQS were tested against promastigote forms of *L. chagasi*: BPQ-OH, ER27856, WSP889, 890, 891, 892, 893, 894, 895, 896, 897 and 898. We determinated the IC50 values of the compounds and the more efficient drugs were WSP889, 890, 891, 892 and 893 with IC50 of approximately 0.02uM. We carried out growth curves of promastigotes of L. chagasi treated with these five compounds. There was a dose-dependent effect of these drugs on the growth of the promastigotes. WSP889, 890 and 891 inhibited almost 100% of growth and caused cell lysis after 72h at 0.5uM and 1uM. The parasites presented significant ultrastructural changes, which varied from discrete alterations to total destruction of the cell, depending on the drug concentration and the time of incubation. One important change observed was a typical swelling of the unique and highly ramified mitochondrion, which became less electron dense. Changes in the organization of the nuclear chromatin were also observed. This work was supported by European Commission, PRONEX, FINEP, CNPq, CAPES and FAPERJ.

BC60 - The paraflagellar rod of endosymbiont-bearing trypanosomatids: lost but not forgotten

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Kinetoplastids contain a unique cytoskeleton structure called the paraflagellar rod (PFR) which is composed of two major proteins, PFR1 and PFR2, and several minor ones. The PFR is necessary for full motility in these cells and provides support for metabolic regulators that may influence flagellar beating. However, there is an intriguing puzzle: in one clade of trypanosomatids, the presence of an endosymbiont bacterium correlates with the apparent absence of PFR. In spite of this, the flagellum of endosymbiont-harbouring trypanosomatids is as active as the flagellum from species without an endosymbiont. We investigated how these organisms are able to swim despite the apparent lack of PFR and the possible influence of the endosymbiont on this phenomenon. We have identified a single-copy *PFR1* gene in the endosymbiont-

bearing trypanosome *Crithidia deanei*. This gene encodes an expressed protein, *C. deanei* PFR1 that is able to episomally complement a *pfr1* null mutant of *Leishmania mexicana*. *C. deanei* PFR1 was synthesized and transported to the flagellum of *L. mexicana*, where it formed a stable association with the axoneme attachment fibres and recruited other PFR components. The impaired motility caused by *pfr1* disruption in *L. mexicana* was partially recovered by *C. deanei* PFR1, demonstrating that the encoded protein is functional. Moreover, antibodies against PFR1 recognize an extra-axonemal structure in *C. deanei* and careful re-examination of the flagellar ultrastructure reveals that this species does indeed posses a PFR, if somewhat reduced in comparison to non-endosymbiont bearing kinetoplastids. This reduction may reflect the lack of a PFR2 gene in these species. This demonstrates the existence of a cell motility structure that had been missed by previous analyses.

BC61 - The dimension of cargo as limiting factor to endocytosis by *Trypanosoma cruzi* epimastigotes

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Until this moment, the knowledge about endocytosis in *Trypanosoma cruzi* was generated by experiments analyzing specific and non-specific endocytosis of small particles as proteins and lipoproteins coupled to colloidal gold or fluorescent substances. Here, we performed assays aiming to find which is the largest particle size emphT. cruzi epimastigotes are able to uptake. Y strain epimastigotes ingested 0.1 and 0.2-micrometer fluorbeads, but were not able to ingest 0.5 micrometer beads, which remained attached to entry site, without impairing endocytosis of small particles, as transferrin-FITC. Uptake kinetics of 0.1-micrometer beads was similar to that of small particles, being found at cytostome and cytopharynx after five minutes, at perinuclear vesicles and tubules after fifteen minutes, and reaching reservosomes in thirty minutes. Beads of 0.2 micrometers, however, are delayed at the beginning of the pathway, being found in the cytopharynx until fifteen minutes and taking forty five minutes to reach reservosomes. Analysis by fluorescence suggested that the entry site for 0.1 and 0.2 micrometer beads was both cytopharynx and flagellar pocket, which was confirmed by transmission microscopy. Vesicular structures containing several beads were seen in anterior region in early moments of endocytosis. We revested 0.1 and 0.2 micrometer fluorbeads with transferrin and compared their endocytic kinetics with that of uncoated beads. Transferrin coated beads of both sizes were uptaken even more slowly than 0.2 micrometer uncoated beads. Differently from endocytosis of small particles, uptake of fluorbeads did not occur at 4 Celsius degrees. Pre-treatment of epimastigotes with cytochalasin D did not interfere in bead endocytosis, pointing to an event independent from actin. These data indicate that cargo size interferes on uptake kinetics mainly in initial events. The parameters of these uptake events are now under investigation.

Supporting: CNPq, PRONEX-FAPERJ.

BC62 - Role of dibucaine in the endocytic and exocytic pathway of epimastigotes forms of *Trypanosoma cruzi*

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Dibucaine, a local anesthetic, was found to inhibit a calcium independent phospholipase A2 (iPLA2), an enzyme that releases aracdonic acid (AA) from phosphatidylethanolamine. Experiments in different cell types have shown that AA is involved in membrane fusion, degranulation, secretion and phagocytosis. In this study we analyzed the role of dibucaine in the endocytic and exocytic pathway, and in the protein and proteolytic activity expression of *T. cruzi* epimastigote forms (Y strain). Dibucaine treated parasites presented a decrease on the endocytosis rate as observed by flow cytometry. Electron microscopy analysis of the endocytic process was made by a pulse (4h) and chase (20h) assay with BSA-Au complex (15 nm of diameter), with parasites in the absence or presence of dibucaine. After a 20h chase period gold particles were found flocculated, indicating the digestion of BSA. Cells treated with dibucaine presented the gold particles less flocculated, suggesting an inhibition of the proteolytic process. To asses whether dibucaine is interfering in the cruzipain activity, we made an analysis on the direct effect of the drug upon purified cruzipain. Previous results showed an increase on cruzipain activity, suggesting a possible binding between dibucaine and cruzipain. Cellular extract of epimastigotes treated for 4h with dibucaine was incubated with CBZ-Phe-Arg-MCA. Cruzipain activity was dosed in the Fluoroskan. Previous results indicated that in both cases there is an increase in cruzipain activity. Epimastigotes treated for a longer period with this drug (48h) showed a decrease in the cruzipain activity presented in the cellular extract and in the supernatant of these parasites. These results suggest that dibucaine induces the expression of cruzipain in the first hours of treatment in these parasites, but latter it decreases due to a mechanism of down-regulation that remains to be solved. Supported by: CNPq, CAPES, FAPERJ, FUJB-UFRJ.

BC63 - *Trypanosoma cruzi* secrete TcTOX (*T.cruzihemolysin*), a complement 9 factor related molecule within *Coxiella burnetii* vacuoles

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Trypanosoma cruzi metacyclic trypomastigote forms are able to invade a variety of phagocytic and non-phagocytic cells engaging a variety of molecules and signalling pathways. Once

inside the host cells, they are thought to secrete TcTOX (*T. cruzihemolysin*), a complement 9 factor related molecule that at low pH will destroys the parasitophorous vacuole membrane and lead the parasite to the cytosol where trypomastigotes differentiate into amastigotes forms and after approx. 96 hours, amastigotes transform back into trypomastigotes. Disruption of infected mammalian cells occurs by releasing trypomastigotes, prone to infect other cells. Infective extracellular amastigotes also secrete TcTOX . *Coxiella burnetii*, the causative agent of Q fever, is an obligate intracellular bacterium that multiplies within vacuoles of phagolysosomal origin containing LAMP and other lysosomal markers. Vero cells persistently infected with *Coxiella burnetii*form large vacuoles that fuse with protozoan vacuoles. *Coxiella burnetti*, is a well adapted organism that accomplishes all metabolic processes at low pH, as it has been established that their vacuoles maintain an acidic pH during infection. In the last years, we began to examine the behavior of *T.cruzi* trypomastigotes upon invasion of cells that had been previously colonized with *Coxiella burnetii*. In this work, we observed that amastigotes forms seem to escape from *C. burnetii*, vacuoles. After 48 hours, amastigotes could be labeled with polyclonal anti-C9 complement protein antibody (*C9Ab*). We also observed in control Vero cells that intracellular amastigotes showed a strong labeling for C9Ab co-localizing with LAMP-1 labeling, while trypomastigotes invading cells showed a weak labeling for C9Ab. These results could suggest that parasites may use TcTOX to escape from *C. burnetii* vacuoles. Financial support: CNPq, FAPESP, and CAPES.

BC64 - *Trypanosoma cruzi*parasitophorous formation in Vero cells: acquisition of LAMP-1 molecules is not concomitant with vacuole acidificaton.

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Coxiella burnetii, the etiological agent of Q fever, is an obligate intracellular bacterium that resides within acidified vacuoles with secondary lysosomal characteristics. Infective stages of *Trypanosoma cruzi* actively invade a wide variety of cells, a process that may be accompanied by lysosomal recruitment. Recently, we have investigated and characterized early events that occur when Vero cells persistently colonized with *C. burnetii*, are doubly infected with *T. cruzi* trypomastigote forms. We observed that acidification modulates the traffic of trypomastigotes in this system ($AndreoliandMortara, 2003$). Based on these observations, we have investigated the formation and acidification of metacyclic trypomastigote parasitophorous vacuoles in live Vero cells transfected with EGFP-LAMP-1. Vero cells were transfected by eletroporation ($300mV - 500mA$) with pEGFPN1-LAMP-1. Visualization was performed under confocal microscopy with thermo-stable system at 37 °C. Vero EGFP-LAMP-1 cells were infected with metacyclic trypomastigotes ($GandCLstrains$). Images were collected from 30 minutes to 24 hours of infection. Lysotracker Red DND99 was added to the samples ($1mM/ml$) to monitor parasitophorous vacuoles acidification during metacyclic trypomastigotes invasion. It was observed that PV acidification does not correspond to EGFP-LAMP-1 localization. From 30 to 120 minutes there is a prevalence of EGFP-LAMP-1 positive PVs, without corresponding acidification. PV acidification occurs after 2 hours of infection, and between 6 and 12 hours most PVs remained acidic as determined by lysotracker Red and pEGFP-LAMP-1 co-localization. After 18 hours, the number of acidic PV decreased but, surprisingly, EGFP-LAMP-1 labeling remained on T. cruzi parasitophorous vacuole. We concluded that acquisition of LAMP-1 molecules in this system is not concomitant with vacuole acidificaton. Financial support: CNPq, FAPESP, and CAPES.

BC65 - Immunocytochemical Localization of Calreticulin in Trypanosoma cruzi

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Calreticulin, a Ca2+-binding chaperone located in the luminal side of the endoplasmic reticulum (ER) of mammalian cells, presents a carboxi-terminal KDEL retrieval signal involved in its targeting and retention in the ER. It has been shown that calreticulin displays lectin properties; binding to monoglucosylated high-mannose-type oligosaccharides and playing an important role on the process of control of aggregation of partially folded proteins. This protein has been found in vertebrates, invertebrates and higher plants. No calreticulin gene was found in prokaryotes and yeast genomes. Trypanosomatids, which are protozoa belonging to an early branch of the evolution synthesize glycoproteins displaying some special features. It was shown that Trypanosoma cruzi expresses a calreticulin-like molecule which recognises free monoglucosylated high-mannose-type oligosaccharides and that it interacts with monoglucosylated cruzipain, a major cysteine proteinase synthesized by this protozoan. Trypanosoma cruzi calreticulin, formerly known as Tc45, is also a dimorphic immunodominant antigen. In the present study, a polyclonal antibody against calreticulin was used for the immunocytochemical localization of the protein in different developmental stages of Trypanosoma. cruzi fixed under conditions to preserve antigens and embedding in the hydrophilic Unicryl resin. Labelling was observed in the endoplasmic reticulum, Golgi complex, reservosomes, flagellar pocket, cell surface, cytosol, nucleus and kinetoplast. Significant differences in labelling were observed among the three evolutive forms of the protozoan. The functional role of calreticulin in T. cruzi was discussed.

BC66 - Is the TOR signaling pathway present in Trypanosoma?

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TOR (Target of Rapamycin) kinases are essential proteins involved in the control of protein synthesis and regulation of cell growth in response to environmental signals including nutrients, stress and mitogens in eukaryotic cells. Studies with mammalian and yeast cells signaling pathways showed that nutrient starvation inhibits TOR activity, which results in G1 cell cycle arrest, and triggers a stress response program leading to a blockade of translation initiation. The same stress response can be observed in cells treated with rapamycin, an imunosupressant drug, which binds to FKBP12 prolyl-isomerase forming a complex with the TOR kinase through its FRB domain. In addition to the FRB domain, TOR kinases are characterized by the presence of FAT, PI3 Kinase and FATC domains and putative protein-protein interaction HEAT repeats. By searching the released genomic databases, we found two candidates for TOR in Trypanosoma brucei and one in Trypanosoma cruzi. The alignment of these sequences with orthologs of other species revealed that these putative TORs contain the FAT, PI3K, FATC and FRB domains, but not the HEAT repeats. We also found two putative FKB12 in T. cruzi and one in T. brucei, both retaining 9 of the 12 critical residues of the human FKBP12 required for the contact with rapamycin. To study whether a TOR signaling pathway might exist in Trypanosoma, we tested their sensitivity to rapamycin. We found that this drug inhibited T. brucei and T. cruzi growth only at very high concentrations (above 10 mg/ml) when compared to the concentrations used to inhibit yeast cells. At these high concentrations rapamycin induced morphological changes, decreased cell motility and produced death after 3 days. The rapamycin also inhibited protein synthesis but not glucose incorporation in Trypanosoma. Modeling of the FKBP12 rapamycin complex binding with the Trypanosoma FRB domain suggest a weak interaction, explaining the requirement for high drug concentrations. Thus, in spite of the weak inhibitory effect of rapamycin, a TOR signaling pathway, controlling translation could exist in trypanosomes.

BC67 - Comparative analysis of hepatic lesions experimentally induced by Entamoeba histolytica and Entamoeba dispar prior to histopathology and immunohistochemistry.

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The incidence of amebiasis in the world is currently unknown, due to the fact that the species E. dispar was assumed to be morphologically indistinguishable from E. histolytica. Faced with this situation we detected the need for broadening the knowledge on the new species E. dispar specially in relation to its pathogenicity. In this context, we proposed to quantitatively evaluate the pathogenicity of the strains E. histolytica and E. dispar using the area occupied by liquefactive necrosis and the number of trophozoites in different periods of infection as the parameters. Fifteen hamsters were intrahepatically inoculated each with 100,000 trophozoites of the E. díspar MCR and E. histolytica EGG strains. Six animals were inoculated with the respective florae. Groups of three animals were necropsied in the 1st, 2nd, 3rd, 6th and 8th day after inoculation. Liver fragments were processed to obtain semi-serial 4 micrometers thick sections. Slides were stained with HE and immunohistochemically with streptoavidin-peroxidase for detection of trophozoites. All necrosis zones and trophozoites images, as well as the measurements, were obtained through a JVC TK-1270/RGB microcamera and the KS300-Carl Zeiss image analyzer. In the animals inoculated with E. dispar necrosis foci were observed with predominantly neutrophilic infiltrate. Progressive reduction of necrosis zones was observed with a subsequent formation of a single necrosis area with a discreet steatosis. In the 6th and 8th day after inoculation, epithelioid and giant cells in the well-developed granulation tissue were observed. In the animals inoculated with E. histolytica the initial focal hepatitis was more intense than that produced by E. dispar, also evolving along the inflammatory process to more extensive necrosis zones, hemorrhage and steatosis. In the 8th day, extensive calcification areas were observed in the necrosis zones and granulation tissues in some areas. The average of the hepatic necrosis zones induced by E. histolytica was significantly higher than those produced by E. dispar. Correlation between the number of immunohistochemically stained trophozoites and the average area of necrosis zones was observed only in the animals inoculated with E. dispar.
Supported by FAPEMIG, UFMG

BC68 - Activation of Leishmania amazonensis leishporin makes the cytolysin able to bind to cell membrane lipids

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We have previously described in Leishmania amazonensis a pore-forming protein (leishporin) that lyses cells, including macrophages, the parasite's host cells. We have shown that pore formation occurs in two distinct steps: 1) binding of the cytolysin to the plasma membrane and 2) lysis itself, probably by the insertion and/or oligomerization of subunits. Neither surface proteins nor carbohydrates seem to be important for the binding of the cytolysin to cells. Aiming to investigate the role of membrane lipids in binding leish-

porin, hemolytic promastigote extracts were incubated for 5 min at room temperature with liposomes made of cholesterol and dipalmithoilphosphatidylcholine. After removal of liposomes, the lytic activity of the supernatant was determined. We verified that the liposomes totally remove the lytic activity of the parasite extract, indicating the binding of leishporin to one or to both lipids. Knowing that the lytic activity of leishporin depends on the removal of an oligopeptide inhibitor non-covalently associated with the cytolysin, we treated hemolytically inactive supernatant with proteinase K, which destroys the inhibitor. Interestingly, the protease treatment can generate hemolytic activity in the hemolytically inactive extract, indicating that they still contained inactive leishporin. The newly generated hemolytic activity is also removed by the lipossomes, indicating that only the active form is able to bind to the lipids. It is possible that removal of the inhibitor exposes hydrophobic lipid-binding sites. The liposome-pulled down proteins can be shown as two unique bands of Mr of 50 and 66 kDa after SDS-PAGE, which must probably be leishporin.

BC69 - Apoptosis in macrophages infected with *Leishmania amazonensis*

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Macrophages infected with *Leishmania* eventually rupture releasing amastigotes, which are infective for neighbouring cells. Being an amplifying step, the macrophage's death may be a key point in the development of leishmaniosis. In the present work, we monitor the death of *L. amazonensis*-infected BALB/c peritoneal macrophages *in vitro*, investigating whether they die through apoptosis. Using the MTT assay, we have observed a sudden reduction in the viability of the infected macrophages, as compared to non-infected cells. In order to investigate whether the cells were dying through apoptosis, we initially examined the fragmentation of the DNA, using both agarose gels and the TUNEL technique. In agarose gels, we have observed that, in 24 hs after infection, *L. amazonensis*-infected cells presented a DNA fragmentation that appeared as a ladder pattern with fragment sizes multiples of 200 bp, typical of apoptotic cells. The fragmentation of macrophages DNA were confirmed using the TUNEL technique, which allow us to observe that, also in 24 hs after infection, around 40% of the macrophages had their nuclei labeled. These results suggests that programmed cell death occurs in macrophages infected with *L. amazonensis in vitro*. Therefore, it is possible that apoptosis contribute for the progression of the lesions in cutaneous leishmaniasis.

BC70 - GROWTH INHIBITION AND ULTRASTRUCTURAL CHANGES INDUCED BY BARBATIC ACID FROM LICHEN *Cladonia salzmanii* on *Trypanosoma cruzi* in vitro.

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Trypanosoma cruzi is the aethiological agent of Chagas' disease, an illness of considerable morbidity and mortality in Latin America. Nowadays there is no successful treatment. Search of new drugs against *T. cruzi* is therefore an urgent need. Lichen are non-flowering plants which are able to synthesize several metabolites. Some lichen compounds are long known as antibiotic and endowed with several biological and physiological activities including antiparasitic, antimitotic, antiproliferative and anti-inflammatory. In this work we investigated the *in vitro* effects of barbatic acid isolated from lichen *Cladonia salzmanii*, on growth and ultrastructure of epimastigote forms of *Trypanosoma cruzi*. Incubation of culture epimastigotes with different concentrations of barbatic acid resulted in growth inhibition, which was directly correlated to drug concentration. Scanning electron microscopy of barbatic-treated epimastigotes showed a damage to the plasma membrane in all tested concentrations. Barbatic acid also induced abnormal-shaped cells. Signs of cell apoptosis, such as membrane blebbing, and intense lysis of parasites were observed mainly at 20 and 40 μg/ml of barbatic acid. In conclusion, although further studies are needed in order to elucidate the effects of barbatic acid on both the trypomastigote and amastigote forms our results point toward the potential use of this compound against *Trypanosoma cruzi*.

BC71 - Intracellular compartments concentrate acid hydrolases in Trypanosoma cruzi trypomastigotes and amastigotes

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Cruzipain is the major protease of Trypanosoma cruzi. It is a cathepsin L-like cysteine protease synthesized as preproenzime that traffic from Golgi to reservosomes where it is accumulated in epimastigotes. Recently, a lysosomal monomeric glycoprotein serine carboxypeptidase belonging to S10 family was described. Its traffic needs to be better defined, but it was suggested that the serine carboxypeptidase is also accumulated in epimastigote reservosomes. In this work, we performed the localization of serine carboxypepetidase in T. cruzi trypomastigotes and amastigotes derived from tissue

culture supernatant. Indirect immunoflorescence using antibodies arisen against serine carboxypeptidase and cruzipain showed co-localization of these acid hydrolases in compartments confined between nucleus and kinetoplast in trypomastigotes and in the posterior end of amastigotes. Immunoelectronmicroscopy confirmed the co-localization and showed that these enzymes are accumulated in rounded and tubular structures. As the organelles concentrate acid hydrolases in these forms were not described yet, we initiated their morphological characterization. They are constituted by electron lucent tubules and vesicles heterogeneous in size and shape, surrounded by a single membrane. Their acidic character was evidenced by incubating parasites with Lysotracker, a cell permeant acidotropic fluorophore. In T. cruzi the endocytic process was only described in epimastigotes that uptake nutrients by cytostome and flagellar pocket, storing them in reservosomes, acidic organelles found mainly at the posterior region. Endocytic compartments were not detected in trypomastigotes or amastigotes yet. We could not observe the ingestion of bovine albumin, transferring or concanavalin A by these evolutive forms, confirming that they are not able to uptake and store extracellular nutrients. Further experiments are in course to clarify whether these undescribed intracellular compartments of trypomastigotes and amastigotes participate of endocytic or exocytic pathways.
Supported by: PRONEX/FAPERJ, CNPq, CAPES.

BC72 - The cytoskeleton of *Entamoeba histolytica*

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The human intestinal protozoan parasite, *Entamoeba histolytica*, is the causative agent of amoebiasis. The cytoskeleton of this protozoan plays an important role on its penetration through host's intestinal epithelium, connective tissues and portal circulation finally reaching the liver and causing many times fatal abscesses. The ability of the parasite to adhesion, locomotion and phagocytosis of human cells, such as erytrocytes, is directly related to its cytoskeleton. However, the involvement of the cytoskeleton in the maintenance of cellular activities is poorly understood. In the present work we analyzed the ultra-structural organization of *E. histolytica* cytoskeleton attached to a substrate covered by fibronectin and during erytrophagocitosis using light and electron microscopy. Our observations using scanning electron microscopy and deep-etching have revealed a network of filaments all over amoeba cytoplasm and just beneath plasma membrane. In cell surface projections, such as lamellipodia and filopodia, and during phagocytosis this network differs in organization from the rest of the cytoplasm. To confirm the composition of this cytoskeleton network we labeled actin filaments, known as abundant cytoskeleton component. Using fluorescence microscopy, we were able to recognize F-actin by the use of rhodamine-phalloidin and showed the arrangement of microfilaments forming bundles all over the cell, including the projections. However, during erytrophagocytosis, the actin filaments were seen to be concentrated mainly in pseudopods. Amoebic phatogenesis is, in part, based on the ability of the parasite to interact with cells, to its motility and the rapid reorganization of its cytoskeleton, and the present work brings new insights to the understanding of celullar processes involved in amebiasis. Supported by: CNPQ-PIBIC, FAPERJ and CNPQ

BC73 - Spatio-temporal organization of PCNA in the nucleus of Trypanosoma cruzi

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We have found previously that DNA replication in Trypanosoma cruzi is constrained at nuclear periphery (Elias et al. Eukaryotic Cell 1: 944, 2002). The chromosomes that are dispersed through the nucleus in G1 migrate to the nuclear periphery to replicate, remaining there until mitosis. To understand why the chromosomes replicate in the periphery we studied the organization of replication machinery during T. cruzi cell cycle. For this, we prepared antibodies against a recombinant form of the T. cruzi proliferation cell nuclear antigen (PCNA). When these antibodies were used in immunofluorescence against fixed and permeabilized parasites, we observed three patterns of PCNA localization in exponentially growing epimastigotes. A periphery pattern, where PCNA is constrained at nuclear periphery, a dispersed pattern, where molecules are dispersed through the entire nucleus and a labeling pattern with two foci localized in a central part of the nucleoplasm (punctual pattern). By analyzing hydroxyurea-synchronized cultures to obtain parasites at the different stages of the cell cycle we found that these patterns are related to cell cycle. Parasites that are in the G1/S transition presented the punctual pattern, parasites that are replicating their DNA (S phase) displayed a periphery pattern, while parasites after mitosis contain PCNA dispersed through the nucleus. PCNA concentration also varies during the cell cycle, as observed by western blotting assay, increasing when the cell enter in S phase. Together these data strongly suggest that the dynamics of replication machinery is similar to chromosomes dynamics, constraining to nuclear periphery during S phase and dispersing when the cell cycle is complete. Moreover, the focal PCNA accumulation in a central area of the nucleus at the G1/S transition suggests that the replication machinery assembles in specific nuclear domains.
Supported by FAPESP and CNPq

BC74 - Temporal organization and morphological alterations during the cell division cycle of Trypanosoma cruzi

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The cell division cycle of trypanosomes presents unique features as these organisms must replicate and segregate the nucleus, the kinetoplast associated with the single mitochondria and the single flagellar structure in a synchronized manner. Interestingly, the cell cycles and the cellular structures vary considerably among the different species of trypanosomes.

In Crithidia and Leishmania, for example, DNA synthesis in the nucleus starts before DNA synthesis of the kinetoplast, while in Trypanosoma brucei the kinetoplast replicates its DNA before nucleus. These differences seem to be related to the structural organization of these organelles allowing each new cell to assemble adequately. Although the cell structure of Trypanosoma cruzi has been extensively studied, very little is known about the temporal organization or morphological alterations during its cell cycle. Here we show that in epimastigote forms of T. cruzi DNA synthesis last about 3 hours and mitosis occurs almost at the same time in the nucleus and the kinetoplast. Using a monoclonal that recognizes flagellar calcium binding protein and bromodeoxyuridin (BrdU) incorporation to label DNA duplication, we observed that the flagellum emerges only when the nucleus and the kinetoplast completed replication. Therefore, parasites containing two flagellums are in G2 phase of the cell cycle. Moreover, the new flagellum starts to grow before kinetoplast division without physical connection with the cellular body or with the older flagellum, as evidenced by electronic microscopy. We also found that lactacystin, a proteosome inhibitor, blocked the cell cycle in G2 stage, as resulting parasites had all twice the DNA amount, one nucleus, one kinetoplast and two flagella. In contrast, okadaic acid, a PP2A phosphatase inhibitor, inhibited the cytokinesis as most of the cells contained two nuclei, two kinetoplasts and two flagella. These results provide a detailed description of the events occurring during the cell cycle and will be useful for further studies to regarding the cell cycle control in T. cruzi.

Supported by FAPESP

BC75 - Role of lectinic activity of inactive *trans*-sialidase from *Trypanosoma cruzi* in the parasite-host cell interaction

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Trypanosoma cruzi, the causative agent of Chagas' disease, is an intracellular parasite and must invade cells of the vertebrate host in order to survive and establish the infection. Initial communication between *T. cruzi* trypomastigotes and mammalian cells requires contact of soluble or membrane bound parasite molecules with host ligands. *T. cruzi*, expresses on its surface a sialic acid-binding lectin, containing a β-galactoside recognition know as inactive *trans*-sialidase (iTS). Recently we demonstrated that iTS can physically interact with α2,3-binding sialic acid containing molecules on human bone marrow endothelial cells (HBMEC) triggering NF-κB activation and expression of adhesion molecules E-selectin and ICAM-1. Here we demonstrate that HBMEC activation increases *T. cruzi* trypomastigotes attachment to and invasion of endothelial cells. In vitro activation of HBMEC with iTS, leads to a 50% increase in adhesion and infection of endothelial cells by cell cultured trypomastigotes from Y strain. Preincubation of iTS with α2,3-sialic acid containing molecules decrease parasite invasion to control levels. Similar rates of infection were observed when cells were activated with TNF-α. These findings suggest that molecules of *trans*-sialidase family play a role in the host/parasite interaction behaving either as receptors or ligands, resulting in the recognition, attachment to and invasion of host cells, contributing with the inflammatory response observed during infection, enabling parasite replication and evolution of disease. Supported by CNPq (PRONEX), FAPERJ, CAPES/COFECUB, TWAS.

BC76 - Trypanosoma cruzi metacyclic trypomastigotes express a proliferating cell nuclear antigen a protein found normally in replicating cells

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The proliferation cell nuclear antigen (PCNA) is a pivotal protein in DNA replication, DNA repair and cell cycle control. It clamps the replicating DNA acting as the eukaryotic processivity factor for DNA polymerase d/e. It is, therefore, a marker for cell proliferation. In T. cruzi little is known about the proteins involved in the cell cycle and differentiation control. By searching the genome database, we identified the homologous of PCNA and investigated whether this protein is expressed during the differentiation from replicative epimastigotes to non-replicative metaclyclic trypomastigotes forms to understand the relation between the differentiation process and cell cycle control in T. cruzi. We found by western blot analyses using a polyclonal antibody prepared against T. cruzi recombinant PCNA expressed in E. coli that it is present in both forms of parasite in Y and Dm28c strains. In contrast the replication protein A, also required for DNA replication is much less expressed in metacyclic forms. Similar results were obtained by quantitative RT-PCR analysis, suggesting that PCNA is actively synthesized in non-proliferative metacyclic cells. As the metacyclics do not incorporate radioactive thymidine, PCNA is not participating in DNA polymerase processivity in these cells. We are currently investigating the PCNA cellular localization and its possible functions in metacyclic forms of the parasite.

Supported by CAPES and FAPESP

BC77 - HISTOPATHOLOGICAL STUDIES IN THE LIVER OF DOGS NATURALLY INFECTED WITH L. (L.) chagasi.

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In order to study the hepatic involvement in the canine visceral leishmaniasis (CVL) in an endemic area at the North-

west of São Paulo State - Brazil, liver biopsy were collected from animals, with and without clinical signs of the disease and with direct search of parasites positive in lymph nodes smears and/or antibodies detection by ELISA. According to the clinical signs of the disease, 54 dogs attended in the Veterinary Hospital of UNESP in Araçatuba (SP) were divided in symptomatic (n=36) and asymptomatic group (n=18). Histological sections of liver embedded in paraffin were stained by HE and processed for immunohistochemistry to parasite antigens search. The histopathological changes were characterized by peri-portal mononuclear inflammatory infiltrate formed by macrophages, lymphocytes and plasma cells with few polimorphonuclear cells, hyperplasia and hiperthopy of the Kupffer cells, multi-focal inflammatory reaction in the sub-capsular tissue and parenquima, hydropic and fatty degeneration aspects in the hepatocytes and mild fibroblastic proliferation in peri-portal space and in the wall of the big vessels were also present. The hepatic histopathological chages were more intense in the symptomatic when compared to the asymptomatic group. The parasite search was positive in 27,8% (10/36) from symptomatic and in 11,1% (2/18) from asymptomatic dogs by HE and in 55,6% (20/36) and 66,7% (12/18) by immunohistochemistry, respectively. In spite of positive direct search of parasites in lymph nodes from 10/36 (27,8%) animals in the symptomatic and 1/18 (5,6%) in the asymptomatic group, no parasties were observed in the hepatic tissue. Our results showed that the intensity of the liver involvement in canine visceral leishmaniasis in an endemic area at the Northwest of São Paulo State could be related to the clinical signs of the disease. The immunohistochemistry showed high sensitivity for the parasite antigen detection and its correlation with the pathological features.

BC78 - Hepatic damage in caine visceral Leishmaniasis

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Introduction and Objectives: The macroscopic and microscopic hepatic changes has been observed in large number of animals with canine visceral leishmaniasis (CVL). Interestingly, this works related only morphological aspects. However, little is know about immunopathology and histopathologic alterations in liver. Methods and **Results**: In this context, the aim of the present study was to make immunopathological study in liver biopsies from asymptomatic (AD, n=12), oligosymptomatic (OD, n=12) and symptomatic (SD, n=17) CVL-bearing dogs as well as 11 healthy controls (NID). These microscopical results were make association statistical analysis with different histopathological data and parasitism level by immunohistochemistry. In addition, we evaluated the correlation between histopathological picture of liver lesions/parasitism and leukocytes in peripheral blood (CD4+, CD8+, CD5+, Thy-1+, CD14+, CD21+) accessing by flow cytometry. Our data demonstrated in all infected dogs statistical difference in frequency of the capsule and portal inflammation, granuloma formation, Kupffer'cells hypertrophy/hyperplasia, in comparison to NID. SD presented statistical analysis association between capsule/portal inflammation and capsule inflammation/ Kupffer'cells hypertrophy/hyperplasia. Correlation was presented in dogs with clinical signs among peripheral blood and histological parameters in CD5+, CD8+, CD14+ and CD21+. Remarks and Conclusions: These findings suggest leukocytes migration from peripheral blood to hepatic tissue damaged amplifying inflammatory process. Thus, our work indicate the contribute of mononuclear cells in migratory flow into pathological events of the liver in CVL.
Supported by: CNPq, CAPES, FAPEMIG, UFMG, FIOCRUZ& UFOP
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BC79 - *Leishmania (L) amazonensis* PROMASTIGOTES: COOPERATIVENESS BETWEEN PHOSPHATIDYLSERINE POSITIVE AND NEGATIVE FORMS IN THE INFECTIVITY OF METACYCLICS

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Metacyclogenesis of *Leishmania* promastigotes in sandflies is characterized by morphological and molecular changes necessary for parasite survival in the vertebrate host. The role of exposed phosphatidylserine (PS) in amastigote infectivity has already been demonstrated (Curr. Biol.11:1870-1873, 2001). In the present work we analyze PS exposure in purified infective (metacyclic) promastigotes and its role in the recognition by vertebrate host and on the infectivity of these forms of *L. amazonensis*. Metacyclic promastigotes were negatively-selected with a monoclonal antibody (3A1-La-1:3000) specific for non-infective procyclics and then assayed for PS exposure by annexin-V-FITC labeling. We observed that around 50% of /emphL. amazonensis metacyclics promastigotes expose surface PS. Purified metacyclic promastigotes were also used to infect BALB/c mice in vivo and peritoneal macrophages in vitro. The subpopulations of sorted PS-positive (PSpos) and PS-negative (PSneg) metacyclic promastigotes were used, independently or in association, to infect murine macrophages in order to evaluate the infection capacity of each form. PSpos promastigotes are sig-

nificantly more infective than the PSneg population both *in vivo* and *in vitro*. Exposed PS seems to play a role in the infectivity of metacyclic promastigotes since pre-incubation with annexin-V decreased the *in vitro* survival of the parasite and the lesion size in *in vivo* infections. Interestingly, the presence of PSpos metacyclic promastigotes significantly increases the survival capacity of the PSneg population in *in vitro* infections. Our findings implicate PS exposure in the recognition of metacyclic promastigotes by the vertebrate host and suggest that PSpos metacyclics can cooperate with PSneg forms for infectivity. Supported by INCA/FAF, CAPES, PRONEX.

BC80 - Rab 5a involvement during the infection of cardiac cells by Trypanosoma cruzi

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Following endocytosis, the early endosomes represent the major delivery station, where occurs the dissociation of many ligands from their respective receptors, their sorting and delivery to the recycling or degradation pathways. The molecules are transported through the formation of vesicles, which move towards their target compartments where tether and dock with the acceptor membranes. Rabs are small GTPases of the Ras super family that orchestrate the sequence of fusion events. Rab5 is responsible for mediating membrane trafficking events through the fusion of the plasma membrane-derived endocytic vesicles with the early endosome besides also playing a role in early endosome homotypic fusion events. Since Rab 5 and some of its effectors molecules have been shown to regulate aspects of phagosome-early endosome interactions, our aim was characterize the Rab 5a, an isoform of Rab 5, in uninfected and T. cruzi-infected cardiomyocytes, one of the target cells in Chagas' disease. The expression and localization of Rab 5a was performed by both scanning laser confocal microscopy and ultrastructural imunocytochemistry approaches, which showed a punctual labeling throughout the cytoplasm of uninfected cardiomyocytes, mostly at the cell periphery as predicted for mammalian cells. After 24 hours of T. cruzi interaction with trypomastigotes from Y and Dm28c stocks, the infected cardiomyocytes presented an intense labeling near the parasitophorous vacuoles, which lodged the parasite. After longer periods of infection such as 72h, we found an over-expression of Rab5a around the parasites already localized dispersed in the host cell cytoplasm. Our data suggest a recruitment of Rab5 to the nascent T. cruzi-phagosome and its continued high expression maintenance around the intracellular parasite. Biochemical approaches are underway in order to understanding the role of Rab 5a during the interaction of cardiac cells and T. cruzi. Supported by: Capes, FAPERJ, CNPq and FIOCRUZ.

BC83 - The hemoculture sensibility can be related with *T. cruzi* strain and vertebrate host.

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The vast majority of data in literature strongly supports different sensibility of hemoculture methods in Chagas disease diagnosis and the protocols used were considered to be related with this. Apparently several factors may interfere with its hemoculture sensibility, as the group of patients, age, time of infection, conditions of manipulation, growth conditions and period of cultivation. The aim of this study is to evaluate the influence of the inoculum, the vertebrate host and the *T. cruzi* strain in hemoculture sensibility. For this 10 Beagle dogs were inoculated with 2,000 blood trypomastigotes/kg of the Y (*T. cruzi*) and Berenice-78 (*T. cruzi* I) *T. cruzi* strains. The hemoculture were performed at a regular interval of 3 months (during two years) using the protocol described by Chiari *et al.* (1989), modified by Luz *et al.* (1994). The percentage of positive tests during the acute phase ranged from 60 to 100% among animals inoculated with Y and Berenice-78 strains, respectively. In chronic phase the hemoculture was positive in 100% of the dogs infected with Berenice-78 strain. Considering the global results the percentage of positive hemoculture per dog ranged from 37 to 100%. Only one out of five dogs showed positive hemoculture every time. On the other hand, only one out of five infected dogs with Y strain showed positive hemoculture in only one out of eight tests in the chronic phase. To evaluate the influence of the inoculum in hemoculture sensibility other four animals were infected with 4,000 blood trypomastigotes/Kg of the Y strain. No significant differences related to the inoculum were detected. The percentage of a positive hemoculture, considering the global results, ranged from 100 to 25%, in acute and chronic phases, respectively. Our results demonstrate that hemoculture sensibility is dependent on the *T. cruzi* strain, on the vertebrate host. However, the inoculum seems do not influence the percentage of positive hemoculture in chronic phase of the infection.
Financial support: UFOP

BC84 - ROLE OF MANNOSE IN THE IN VITRO INTERACTION OF Trypanosoma desterrensis WITH VERO CELLS

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Studies of Trypanosoma desterrensis, isolated from Epitesicus furinalis bats, have demonstrated the multiplication in vitro of this parasite in Vero cells. Preliminary results suggested the involvement of mannose in the recognition and interiorization of the parasites in this cell line. In order to confirm the role of mannose in parasite-cell interaction, Vero cells (5x104) were cultivated on coverslips in DMEM in 24 wells plates. Culture Trypomastigotes or Vero cells were incubated for 30 min. at 27°C with 10mg/mL of ConA or WGA lectins, washed twice with PBS-BSA 0.5% and kept in DMEM until use. Distinct interaction protocols were used considering the combination of treated and non-treated cell monolayers with the parasite, using T. cruzi Y strain trypomastigotes as control. Interaction assays were performed at 37°C in 5% CO2 using a 5:1 parasites/cell ratio in the presence or absence of mannose and N-acetil D-glucosamine (100 mM). After 72 hours of interaction, infection rate was determined by counting the number of infected cells among 500 randomly chosen cells/coverslip. In order to check the presence of carbohydrates on trypomastigotes and Vero cells surfaces, cells (106 cells/mL) were washed twice in PBS-BSA 0.5%, fixed in formaldehyde 7%, distributed on immunofluorescence (IFA) slides and incubated with different concentrations of ConA-FITC or WGA-FITC conjugates for 30 min. Pre-treatment with WGA or N-acetil D-glucosamine produced no significant alterations on the infection rate for both strains. The pre-incubation of trypomastigotes with ConA increased significantly the intracellular infection of both parasites. On the other hand, when Vero cells were pre-incubated whit ConA the infection was reduced, but it was reverted by addition of mannose. The presence of mannose residues on Vero cells surface, as revealed by IFA and the herein presented results strongly suggest the involvement of manose in T. desterrensis recognition/invasion process in Vero cells.

BC85 - American tegumentary leishmaniasis (ATL) Infection: Analysis of its First Steps.

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American tegumentary leishmaniasis (ATL) is a human cutaneous leishmaniasis (CL), endemic in Brazil, caused by an intracellular flagellate protozoan *Leishmania (V.) braziliensis*. The parasite replicates inside the parasitophorous vacuoles of phagocytic cells. The disease is characterized by one or more cutaneous ulcers, which frequently is associated with satellite lymph-node enlargement. In this work were analyzed the presence of inflammatory cells in active lesions of patients with ATL, with different infection times. Once the initial events in the infection for leishmaniasis determine the course of the infection. Cutaneous biopsies obtained from patients habitants from endemic areas of the Rio de Janeiro State with ATL, were studied after clinical diagnosis, confirmed immunologically by Montenegro test and parasitologically by detection of amastigotes in cutaneous lesion. For conventional transmission electron microscopy, the fragments from cutaneous lesion were fixed in 2.5% glutaraldehyde and 0.1 M Na cacodylate buffer pH 7.2, for 1h at 4°C. Afterwards were post-fixed with 1% osmium tetroxide (OsO_4) in 0.1 M Na cacodylate buffer pH 7.2, dehydrated in acetone, and embedded in Epon resin. Thin sections were examined by routine transmission electron microscopy. Preliminary results demonstrated the presence of a high number of inflammatory cells, mainly mature macrophages in the active lesions. The infiltrated inflammatory variable according with the time of infection, as well as the co-localization of amastigotes of *Leishmania (V.) braziliensis*. The localization this parasites is very difficult because the amastigotas are in a narrow parasitophorous vacuole and the active lesions show few parasites. Immunohistochemical techniques using rabbit anti-*Leishmania (V.) braziliensis* antibodies polyclonal serum were used in this lesion to localize the amastigote forms. Once the first steps in the infection for leishmaniasis are critical, the course of the infection is determined in agreement with the time of infection of the lesion.

BC86 - Characterization of Trypanosoma cruzi strains isolated from sylvatic triatomines collected in Rio Grande do Sul State, Brazil.

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Despite of the Chaga's disease incidence declining (DIAS,

2000), investigations toward Trypanosoma cruzi must continue due to the difficulty in an effective control of its vector. Triatoma infestans, the principal vector of Chaga's disease is practically eradicated from Brazil. However, others Triatominae species are in process of domiciliation as the Triatoma rubrovaria from Rio Grande do Sul, which constitutes a risk factor to the relapse of the chagasic infection. In order to characterize strains of T. cruzi circulating in rural area, we collected triatomines in Quaraí and Santana do Livramento cities from Rio Grande do Sul State, Brazil. Presence of T. cruzi metacyclic tripomastigotes was investigated in feces of the collected triatomines after abdominal compression. Positive feces were inoculated into LIT medium and into experimental animals to perform isolation of T. cruzi. Using this methodology, five strains of T. cruzi were obtained, being denominated, QJI, QJIII, QMI, QMII and QBI. These strains were morphologically and biologically characterized by size determination of bloodstream tripomastigotes in the 15^o day after infection, parasitemic curve and histotropism. Genetic dimorphism of the rRNA 24Sa was also determined by polymerase chain reaction (PCR). All results obtained were notified to the Local Epidemiological Vigilance Service. Supported by FUNDUNESP

BC87 - Infection of mouse dermal fibroblasts by *Blastocrithidia culicis* and *Crithidia deanei*. Role of endosymbiont in the infection process.

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Introduction: Monoxenous trypanossomatid were known as protozoa not able to infect vertebrate cells. However, we have recently reported the infection of mouse dermal fibroblasts (MDF) by *Crithidia deanei* and *Herpetomonas roitmani*, trypanossomatids originally with endosymbiont in contrast to that observed with *Crithidia fasciculate* and *Herpetomonas samuelpessoai*, endosymbiont-free (Santos,D.O.& Bourguignon, S.C. 2004). Thus, our purpose is to investigate the role of the endosymbiont in the process of MDF infection. Materials and Methods: *B.culicis* and *C.deanei* treated or not by cloranfenicol were incubated 5:1 with MDF in RPMI medium 5% FCS in a 28 °C with 5% CO2 for 4h, 24h and 96h in a 24 well plates. After 2h of incubation, the plates were rinsed with PBS and treated by human serum to lyse extracellular parasites. After incubation, the cells were fixed in ethanol, dried and stained with Giemsa, and analyzed by Optical Microscopy. Results: Similarly to the observed with *C.deanei* and *H.roitmani*, *B.culicis* is able to infect MDF. However, these same endosymbiont - free parasites treated with cloranfenicol- were not observed within MDF. In conclusion, this data corroborate our previous results showing that monoxenous trypanosomatids originally without endosymbiont, *C.fasciculate* and *H.samuelpessoa* did not infect MDF. Therefore, the MDF infection by these parasites seems to be determined by the presence of endosymbiont. Financial support: FAPERJ and UFF.

BC88 - Leishmanicidal activity of LLD-3, a nor-triterpenoid from Lophanthera lactescens.

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Leishmaniasis, a disease that alone affects 12 million people worldwide, is found in five continents and is endemic in the tropical and sub-tropical regions. Recently, these numbers are increasing due to the coinfection with HIV. Pentavalent antimonials, still the first choice treatment for this infection, present several side effects and parasite resistance is been reported. All that stimulates the search for new anti-leishmanial agents. Lophanthera lactescens, popularly known in Brazil as "lanterneira", belong to the Malpighiaceae family. A new nor-triterpenoid compound (LLD-3) was isolated from its wood aqueous extract and present effects in vivo like hypothermia, analgesia, reduction of the muscular tonus and increase of bronchial and saliva secretions. A DL50 of 1.4 mg/ml was obtained in mice after oral administration. In this study, we evaluated LLD-3 for its leishmanicidal activity, host cell citotoxicity, as well as, modulation of nitric oxide production by macrophages. The leishmanicidal activity was assayed in mouse murine macrophages infected with L. amazonensis stationary phase promastigotes after treatment with LLD-3 at different concentrations during 24 hs. Our results showed that LLD-3 at 0.1, 1, 10 $\mu g/mlinhibited 49, 57 and 70$ Supported by: CNPq, Faperj.

BC89 - Establishment of polixenic cultures from axenic trophozoites of a non virulent *Entamoeba histolytica*

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Entamoeba histolytica is an intestinal parasite that can cause either non-invasive infection or severe intestinal and extra-intestinal lesions. Synergism with bacteria is one of many factors recognized as influent in the expression of virulence of this parasite. Some studies have described association between amoeba and bacteria by short periods (hours or days) to evaluate virulence. We described herein a definitive re-association of trophozoites of a non-virulent *E. histolytica* with bacterial flora, using a gradual process to change the system of culture from axenic to polixenic. Cytophatic effect upon VERO cells was also performed to compare the behavior of the parasites in the different system of culture. Direct

transference of trophozoites from axenic to polixenic medium resulted in death of amoebas. Trophozoites also perished after the transference to a mixture of TYI-S-33 and Pavlova medium with total bacterial flora. Re-association process was only possible when transference was done to a mixture o TYI-S-33 and Pavlova (1:1) containing a unique bacteria (*Pseudomonas putida*) isolated from the total flora. These bacteria presented a low growth, which allowed survival of trophozoites in the mixture of medium. The rate of Pavlova was gradually increased and when the trophozoites grew relatively well in Pavlova only, we added the total flora. The complete process took 3 months. Trophozoites transferred to polixenic system caused a higher cytopathic effect than those in axenic culture, confirming an increase of virulence.
Financial Support: CNPq

BC90 - ENDOCYTIC ACTIVITY OF *Toxoplasma gondii* TISSUE CYST

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Toxoplasma gondii is a ubiquitous Apicomplexan parasite of birds and mammals, including humans. It is an important opportunistic pathogen of immunocompromised hosts [1]. After infection the *T. gondii* invades an enormous variety of cells and, eventually, forms cysts in several organs particularly brain and muscles. Up to now, few information about the endocytic capacity of *Toxoplasma* tissue cyst has been reported, which raises a number of questions regarding parasite's endocytics processes [2,3]. Cysts isolated from mouse brain were incubated with 200 μg/ml BSA-FITC, a fluid phase endocytic tracer, for 2 and 3h at 37^{o}C, processed as routine and examined with an FV300/BX51 Olympus CLSM. Cysts were also incubated with 1mg/ml native ferritin and 0.25-0.5mg/ml HRP-Au, fluid phase endocytic tracers, at 37^{o}C for 5 min to 24 h. After this period, the samples were processed as routine and then examined with a Zeiss EM10 Transmission electron microscopy. Images from confocal microscopy along with the 3D reconstruction of tissue cysts incubated with BSA-FITC showed the tracer localized at the cyst wall, as well as within the cyst matrix, which after 3h of incubation appeared to be more diffusely than 2h, suggesting a time dependent incorporation. On the other hand, ultrastructural analysis showed a heterogeneous labelling with HRP-Au, displaying areas with the marker in the membrane of the cyst wall and others areas displaying extends regions without any labelling. No HRP-Au complex was seen inside the cyst. Native ferritin was detected distributed all over the cyst wall, predominantly in patches of different sizes and localized inside vesicles and tubules in the cyst matrix, sometimes very close to the bradyzoites suggesting a possible fusion between their membranes. Our present study brings important data related to endocytosis in *T. gondii* contributing in the future design of therapeutic approach during the treatment of toxoplasmosis.
References: [1] Luft, B.J. et al., (1984). JAMA 252: 913-917. [2] Dubey, J. P., D. S. Lindsay, and C. A. Speer (1998). Clin. Microbiol. Rev. 11: 267-299. [3] Guimarães E.V. (2002). Master Thesis. Fiocruz

BC91 - Coupling cytosolic and mitochondrial calcium oscillations in *Plasmodium*

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Mitochondria in mammalian cells take up and release cytosolic Ca^{2+} during agonist-evoked Ca^{2+} increase, but it is not clear whether or how they regulate Ca^{2+} signaling in malaria parasites. We asked whether mitochondria play an active role during agonist-evoked Ca^{2+} release from intracellular stores. Cytosolic Ca^{2+} were measured in Fluo-4 loaded isolated parasites processes using confocal microscopy and spectrofluorimeter. We here demonstrate that Ca^{2+} increases, as elicited by treatment of parasites with sarco-endoplasmic reticulum Ca^{2+} ATPase inhibitors, acid compartment H^{+}-ATPase inhibitor (bafilomycin) or the hormone melatonin, induce rapid and reversible increases of the Ca^{2+} increase concentration in the mitochondria of both human *P. falciparum* and murine parasites *P. chabaudi*. Pre-treatment of parasites with the mitochondrial uncoupler FCCP suppresses mitochondrial Ca^{2+} accumulation. The changes in $[Ca^{2+}]$m appear sufficiently large to induce a rapid activation of mitochondrial dehydrogenases, which can be followed by monitoring the level of NAD(P)H autofluorescence. By using the fluorescent dye Rhod-2 AM, we shown the ability of *Plasmodium* mitochondria to participate in cellular Ca2+ homeostasis. To this end, isolated parasites were simultaneously loaded with the mitochondrial Ca^{2+} probe Rhod-2 and the cytosolic Ca^{2+} dye Fluo-3 AM and their fluorescence intensities were monitored in the same cells by confocal microscopy (Gazarini and Garcia, BBRC, 2004 Aug 321: 138-144). Taken together, these results provide evidence that *Plasmodium* mitochondria reversibly accumulate part of the Ca^{2+} released in the cytoplasm within melatonin signaling pathways.

BC92 - negritoDERMAL FIBROBLASTS - *Herpetomonas roitmani* INTERACTION: DETECTION OF ACID PHOSPHATASE WITHIN OF PARASITOPHOROUS VACUOLE

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Monoxenous trypanosomatids are found in hosts insect and are not considered capable of causing parasitic diseases in vertebrates. However, Herpetomonas roitmani and Crithidia deanei were capable of to invade dermal fibroblasts of primary cultures. Our present aim is to characterize by ultra structural cytochemistry the localization of acid phosphatase in primary cultures of dermal fibroblasts infected by Herpetomonas roitmani. The experiments were carried out mainly using H. roitmani maintained at 28^{o}C in Warrens' medium containing 10The enzyme was revealed by cerium phosphate deposits located within the cisternae of the rough endoplasmic reticule, and especially within of cytoplasmatic vesicles similar the lysosomes. Fibroblasts infected with Herpetomonas roitmani exhibits granules in this cytoplasm with strong enzymatic activity, and apparently is degranulating the acid phosphatase content into parasitophorous vacuole. Enzymatic activity was distributed over the internal surface of membrane of the parasitophorus vacuole. In conclusion, this work shows the infection of mouse dermal fibroblasts by H. roitmani, that is non-pathogenic to mammals and the fusion of the parasitophorous vacuole with lysosomes revealed by acid phosphatase activity.

BC93 - MICROBICIDAL RESPONSE OF MURINE PERITONEAL MACROPHAGES DURING THE INTERACTION WITH *Leishmania (Leishmania) amazonensis* AND *Leishmania (Viannia) shawi.*

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Leishmaniasis is an endemic disease in many parts of the world, caused by different species of protozoan parasites of the genus *Leishmania*. The diseases are characterized by different clinical manifestations in humans, including cutaneous and visceral forms associated with significant rates of morbidity and mortality. This diversity in clinical forms seems to be related with different species of *Leishmania*. This protozoan is an obligate intracellular parasite that developed mechanisms to subvert the microbicidal activity of macrophages, such as inhibition of superoxide and nitric oxide (NO) production. In this work we analyze the microbicidal response of macrophages during the interaction with *L. (L.) amazonensis* and *L. (V.) shawi*. After 72 hours of infection, 92 and 72 percent of activated macrophages (100 ng of lipopolysaccharide and 1mg/ml interferon-gamma), were infected with *L. (L.) amazonensis* and *L. (V.) shawi*, respectively. The number of amastigotes of *L.(L.) amazonensis* by macrophage was significantly higher in relation to *L. (V.) shawi*. *L.(V.) shawi* was not able to inhibit the superoxide production of macrophages. In contrast, *L. amazonensis* could. However, both species inhibit NO production after macrophage infection. Both species were also able to inhibited iNOS activity of infected macrophages as assayed by a diaphorase histochemical method. In addition, the infected macrophages with *L.(L.) amazonensis* showed large parasitophorous vacuoles (PVs) with many amastigotes forms. However, macrophages infected with *L. (V.) shawi* showed tight PVs, presenting only one amastigote. Our data demonstrate that the microbicidal response of macrophage depends on the *Leishmania* species and that the establishment of the infection and survival of the parasite in the macrophages are related with the ability of the parasite to modulate the defense mechanisms of macrophage.
Supported by: CNPQ, UFPA.

BC94 - Extracellular amastigotes of *Trypanosoma cruzi* CL isolate: characterization and analyzes of surface components

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We have raised in our laboratory a panel of monoclonal antibodies ($MABs$) that recognize carbohydrate epitopes in amastigotes of distinct *Trypanosoma cruzi* isolates. However, these MABs do not fully discriminate between the two major phylogenetic lineages; *T. cruzi*I, associated to the sylvatic cycle and *T. cruzi*II that are found mainly in patients and vectors in human dwellings. Here we describe for the first time a MAB ($Ssh-1, IgMisotype$) produced by immunizing Balb/c mice with extracellular amastigotes from CL isolate (*T. cruziII*) that can distinguish from parasites of G isolate (*T. cruziI*). Vero cells infected with amastigotes of CL and G isolates were examined by confocal fluorescence microscopy. Ssh-1 labels CL strain amastigote surface but did not react with G strain parasites. Immunobloting analy-

sis showed a high molecular weight pattern of bands in extracellular amastigotes of CL isolate but not in parasites of G isolate. We performed periodate oxidation on immunoblots and verified that the epitope recognized by Ssh-1 is of carbohydrate nature. We are currently carrying out molecular studies of these carbohydrate epitopes in order to better understand the surface components of amastigotes and verify the role of these components in cell invasion. This study was financially supported by: FAPESP, CNPq, and CAPES.

BC95 - Light and Electron Microscopy Aspects of Cardiomyocytes Gap Junction Behaviour During *Trypanosoma cruzi* Infection

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Gap junctions (Gj) are channels that, in heart, provide a pathway for small molecules from one cell to another, that enables synchronic contractility of cardiac tissue. These channels are formed by hexamers of connexin and, among cardiomyocytes, Cx43 is the most abundant one for its biophysical properties. Several studies about damages and alterations of gap junction's distribution showed that loss or misexpression of connexin 43 led to malfunctions of the heart such as arrythmia and fibrilation.
Trypanosoma cruzi, the agent of Chagas' disease has as it main target cardiomyocytes, which after a period of infection presents myocarditis, fibrosis and arrhytmia. Some studies with cardiomyocytes infected with *T. cruzi* showed that there is a significant loss of these Gj and linked this loss as one of the causes for arrhytmia in chagasic patients.
In the present study, we used indirect immunofluorescence and ultrastructural immunocytochemistry in order to caracterize the loss of Gj throughout diferent periods of intracellular cycle of the parasite. *In vitro* studies were performed by using primary cultures of mouse embryos' cardiomyocytes. Since it was already described that the intracellular form of parasite displayed intense labelling with connexin antibody (Campos de Carvalho et al, 1992) we performed studies with the three forms of the parasite: amastigote, epimastigotes and trypomastigotes. Cells were obtained from 18-20 day old mouse embryos, that were dissociated with a Trypsin-Collagenase sollution. For Laser Scanning Confocal Microscopy, cells were plated on 24-wells dishes and for Transmission Electron Microscopy, on Petri dishes. After 24 hours of plating, cultures were infected with trypomastigote forms of *T. cruzi*, and infection were followed for 24, 48 and 72 hours. For immunofluorescence studies, cells were fixed with 4% of Paraformolaldehyde and for ultrastructural immunocytochemistry, cardiomyocytes and amastigote, epimastigote and trypomastigote forms were fixed with 0,5% of Glutaraldehyde, 4% of PFA and 0,2% of Picric Acid, followed by infiltration on Unicryl resin.
Both Light and Electron Microscopy showed that in non infected cells, connexin staining remained as described previously, as punctual clusters at sarcolemma, while on infected cells, Gj staining were much more reduced and even not detectable, from 24 hours of infection through the whole intracellular cycle of the parasite. The ultrastructural imunocytochemistry for localization of connexin in the amastigote, epimastigote and tripomastigotes forms revealed coloidal gold particles only on the whole membrane of the amastigote form of the parasite but not in the epi and tripomastigote forms. More studies are in progress in order to better understand how these junctions are affected and disappear during *T. cruzi* infection, also try to identify the moment of the infection when gap junction is no longer detectable.
Acknowledgments: This work is supported by FIOCRUZ, PAPES III/Fiocruz, CNPq, FAPERJ.
(We are thankful to Dr. Regina C. Goldenberg for providing polyclonal anti-connexin 43 antibody).

BC96 - Immunocytochemical Localization of MMP-9 in *Trypanosoma cruzi* Infected Mouse Embryo Cardiomyocytes

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Proteinases has been studied in *T. cruzi* and other Trypanosomatids such as Leishmania spp., African Trypanosoma as well as in non pathogenic monoxenic trypanosomatids. Cruzipain the major cysteine proteinase from *T. cruzi* has been identified as a therapeutic target for treatment of Chagas' disease and also the experimental cure for treatment of Chagas' disease using vinil sulphone inihitors has been reported [1]. Recently the proteinase profile in embrionary hepatocytes cultures infected and non infected with *T. cruzi* was analysed by SDS-PAGE-gelatin and we reported an decreased in the matrix metaloproteinase (MMP-9) in the culture supernatant and in *Trypanosoma cruzi* infected hepatocytes. An increase of cysteine proteinases was observed due to parasite's cruzipain (Melo et al.,Hepatology Research,2003).
The present study reports the anti MMP-9 distribution in cardiomyocytes infected or not with *Trypanosoma cruzi*. The cells were processed with Lowicryl K4M and ultrathin sections were obtained from mouse embryos cardiomyocytes and sequencially labeled with rabbit anti-MMP-9 antibody diluted 1:25 in 0,1M TBS, 1% TBS, 1% BSA, 1% Tween. After they were followed by incubation in a goat anti-gold antibody diluted 1:100 (both antibodies from Sigma, St. Louis, Mo, USA). Photographs were taken using a Zeiss EM 10 Electron Microscope.
Immunocytochemistry studies showed that gold immune complexes were distributed in the cytoplasm of infected cardiomyocytes. The parasite showed MMP-9 positive reaction mainly localized in the flagelar pocket and plasma membrane. The observation of one heavily infected cardiomyocyte showing over twenty intracellular parasites display the same reaction.
The presence of MMP-9 in the cytoplasm infected cells may play a role in the breakdown of extracelluar matrix (ECM) either in the spreading and growth and in the invasion process of *T. cruzi*.
Supported by FAPERJ, CNPq, Fundação Universitária José Bonifácio (FUGB) and IOC/FIOCRUZ.
Reference:

[1] Development of cysteine protease inhibitors as chemotherapy for parasitic diseases: insights on safety, target validation and mechanism of action. Mac Kerrow, J. H., 1999. International Journal of Parasitology 29: 833-837, 1999.

Biologia Molecular - Molecular Biology

BM001 - Molecular diagnosis of human cutaneous leishmaniasis by Nested-PCR in the Amazon Region

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Leishmaniases are a group of enzootic and zoonotic diseases caused by protozoan parasites of the genus *Leishmania* (Kinetoplastida: Trypanosomatidae). DNA amplification by the polymerase chain reaction was applied in this investigation for the presence of *Leishmania* parasites in human biopsy. For the diagnosis we have developed a nested PCR (Ln PCR) method of amplification of the mini-exon gene which is unique and tandomly repeated in the *Leishmania* genome. The ability of PCR to detect *Leishmania* cells was compared with those of the conventional methodologies: examination of Giemsa stained biopsy smears, *in vitro* culture and *in vivo* inoculation of biopsy tissue. Biopsies from 14 patients with cutaneous ulcers suggestive of leishmaniasis were analyzed employing these methods. We obtained PCR positivity for all of the parasitologically positive biopsies samples from the patients. The Giemsa stained biopsy smears from 10 of 12 patients were positive (83,3%), *in vitro* culture from 7 of 12 patients (58,3%) and *in vivo* inoculation from 4 of 12 patients (33,3%), although two patients clinically diagnosed as having leishmaniasis were negative for all methods. The Ln PCR may be applied as a new diagnostic technique for the detection of genus *Leishmania* in the biopsy of patients with active tegumentary leishmaniasis and can perhaps be adapted for use in a specific determination of the *Leishmania* species. Supported by INPA/CPqLMD/CNPq/PNOPG.

BM002 - Molecular characterization of *Giardia duodenalis* isolated from clinical samples of the city of Rio de Janeiro

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Giardia duodenalis is one of the main diarrhea agents in human and animals distributed worldwide. Isolates of *G.*

duodenalis present high levels of genetic diversity. Different genotypes have been detected through the genetic analysis. These studies showed the existence of seven genotypes of *G. duodenalis*: the specific mammalians groups (A1, A2, and B), and genotypes for dogs, cats, domestic animals of farm, rats and savages rodents. Molecular techniques are useful in the taxonomy studies, verification of the zoonotic potential of domestic animals isolates, and the correlation between the parasite genetic variability and the sintomatology of giardiasis in humans. The objectives of this work were analyze the genetic diversity of *G. duodenalis* isolated in Rio de Janeiro, determine the giardiasis infection source and dissemination in the studied population, and verify the possible zoonotic potential of this parasite in this epidemiological scenario. For detection and genotyping of *G. duodenalis*, was used nested-PCR of the b-giardina gene and analysis of the restriction fragment lenght polymorphisms (PCR-RFLP). This methodology makes possible an inter and intra-genotypic discrimination (genotypes A and B; A-1 and A-2, respectively). The analyzed population was composed of 310 children (0 and 4 years) and 56 adults from a municipal day-care center of Rio de Janeiro, and 11 domestic animals (10 dogs and one cat) of these children. The general positivity in the parasitologic diagnosis was 60% in the 310 analyzed samples. Of these, 27.9% had been *Giardia* positive. The PCR-RFLP of these samples characterized that all isolates were genotype A, subtype A-1, including 8 dog samples. It was observed a case of child and dog, inhabitants of the same residence, with the same genotype A-1 of *G. duodenalis*. In conclusion, the genotyping of *G. duodenalis* isolates supplied important evidences for the establishment of epidemiologists relations of giardiasis.

BM003 - MULTIPLEX-PCR CHARACTERIZATION OF KDNA MINI-CIRCLES OF *Trypanosoma rangeli* STRAINS ISOLATED FROM DISTINCT HOSTS AND GEOGRAPHICAL ORIGINS

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Trypanosoma rangeli is a hemoflagellate parasite that infects triatomine vectors and different mammalian species, including humans. *T. rangeli* is considered to be harmful to the insect vectors but non-pathogenic to the vertebrate hosts. Distinct biochemical, serological and molecular techniques have shown intra-specific variability in *T. rangeli*. The parasite kDNA mini-circles have been studied and characterized according to the size, number and disposition of conserved and variable sequences. In this study, we have used a multiplex-PCR technique directed to the kDNA mini-circles in order to characterize *T. rangeli* strains isolated from distinct hosts and geographical origins. After DNA extraction by phenol-chlorophorm, a multiplex-PCR using primers S-35/S-36/KP1L was used to characterize the strains as KP1(+) or KP1(-) according to their eletrophoretic profile in silver stained polyacrylamide gels. The strains Choachi and D3493 isolated in Colombia from *Rhodnius prolixus*, R1625 from human in El Salvador and B450 isolated from *Rhodnius sp.* in the Brazilian Amazon, revealed a KP1(+) profile. Strains isolated in southern Brazil from *Panstrongylus megistus* (SC75 and SC76) or *Echimys dasythrix* (SC58 and SC61) presented a KP1(-) profile, as also observed for the Honduran H8GS strain isolated from man. Up to now, no clear relationship was observed between KP1(+) and KP1(-) strains, not allowing to cluster the herein studied strains according to geographical regions or hosts. Considering the parasite intra-specific variability, the reduced number of strains and the recent reports of 4 distinct genetic groups of the parasite circulating in South and Central America, more strains from vertebrate hosts, triatomine vectors and geographical origins will be included in this study in order to verify the usefulness of the kDNA profiles to address the parasite epidemiology.

BM004 - Molecular characterization of *Trypanosoma cruzi* isolates from chagasic patients

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Genetic variability of *Trypanosoma cruzi* populations isolated from 47 chronic Chagas disease patients has been analyzed by Random amplified polymorphic DNA polymerase chain reaction (RAPD-PCR) using three primers M13-40, λ-gt11-F e L15996. *T. cruzi* isolates from patients having the indeterminate, cardiac, digestive and combined forms of the disease presented RAPD-PCR similarity coefficients of 0.83, 0.88, 0.92 and 0.88 with an 0.80 average between isolates from the indeterminate and cardiac forms. High homology was also observed when the profiles of 23 patients having different clinical forms were compared (similarity coefficient average = 0.86). Phenograms representing RAPD-PCR have been constructed using the software Treecon by the UPGMA (Unweighted Pair Group Method using Arithmetic Averages). The isolates have shown distinct grouping on trees topology, not being possible to establish a correlation to the clinical aspects of the Chagas disease. Comparative analysis of RAPD profiles of *T. cruzi* populations from patients with two isolates presented patterns suggesting monoclonal and polyclonal populations. Isolates from patients 1, 2 and 3 obtained before and after treatment presented an average similarity coefficient varying from 0.98 to 1.0; 0.90 to 0.98 and 0.68 to 0.96, respectively, using the three primers. Genetic analysis has shown that isolates from patients 1 and 2 are very similar and suggest that they can

constitute a monoclonal population of the parasite. Isolates from patient 3 have been grouped in different branches of the phenogram and seem to be constituted of different subpopulations (polyclonal structure). RAPD profiles from isolates of patients with clinical evolution have shown low variability with an average 0.91 (similarity coefficient) for patient 5 and 0.81 for patient 13. RAPD profiles from the isolates with characteristic patterns associated to clinical forms of the disease have not been found in any of the cases studied. Financial support: FAPEMIG, CNPq and UFMG.

BM005 - *Trypanosoma cruzi*II in *Leontopithecus* sp: distinct zymodemes in distinct ecosystems of the Atlantic Coastal Rainforest

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Atlantic Coastal Rainforest (ACRF) one of the hotspots of the world includes one of the richest biomes represented by several distinct ecosystems. A high biodiversity may still be found in the only 7% remains of the original forest that extends almost throughout the coastline of Brazil. Several conservation programs that include reintroduction and translocation of the local and endemic fauna have been launched in the last 30 years. One target of such program is the genus *Leontopithecus sp* an endangered **Callitrichidae** (Primate). Lion tamarins (*Leontopithecus* sp) comprise four species that colonize distinct ecosystems of the ACRF. The examination of *L. chrysomelas* (golden faced tamarin), that occupies the northeastern part of ACRF, in the Bahia State, demonstrated that they were positive for the infection by *Trypanosoma cruzi*. We were able to isolate eight *T. cruzi* samples and 3 of them were already typed by mini-exon gene as TCII, corroborating our previous results showing strong association of primates with this genotype. The MLEE analysis showed that the studied isolates belong to the same zymodeme, different from those observed circulating in L. rosalia from ACRF from southeastern Brazil, Rio de Janeiro State. These results showed that these primates are participating from a distinct transmission cycle of *Trypanosoma cruzi* and show the importance of considering the ecological peculiarities of a given area in the modulation of the transmission cycle of parasites in the sylvatic environment.

BM006 - Development of microsatellite markers for strain differentiation of *Eimeria tenella* and *E. acervulina*

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Avian coccidiosis is an enteric disease caused by seven protozoan species of the genus *Eimeria*. Species identification is currently performed using several biological and morphological features, as well as molecular assays based on the amplification of the ribosomal ITS1 and the use of SCAR markers (Fernandez *et al.*, Parasitology 127: 317-325, 2003). The only available molecular tests for strain discrimination are based on DNA fingerprinting (Southern blotting) and RAPD. The former technique is tedious and time-consuming, and the latter presents problems of reliability and reproducibility across different laboratories. The aim of the present work was the development of reliable microsatellite markers for the intra-specific discrimination of two of the most relevant *Eimeria* species: *E. acervulina* and *E. tenella*. In order to select potential candidate *loci*, we employed genomic sequences from the *E. tenella* Genome Project (http://www.sanger.ac.uk/Projects/E_tenella/) and also *E. acervulina* EST sequences generated in our laboratory (http://www.lbm.fmvz.usp.br/eimeria/). The sequences were analyzed with the Tandem Repeats Finder program, and the best candidates were used for designing primers flanking the microsatellite repeats. From a total of 37 markers tested for *E. acervulina*, 12 presented both species-specificity and intra-specific polymorphism, with an average of 2.5 alleles per *locus*. For *E. tenella*, 79 markers were screened, yielding a total of 15 polymorphic *loci*, with an average of 2.5 alleles per *locus*. Taking these data together, the microsatellite markers obtained so far allow for the theoretical differentiation of 129,024 haplotypes for *E. acervulina* and 73,728 for *E. tenella*. We intend to attain a theoretical differentiation of at least 1 million haplotypes for each species, and also extend this survey to *E. maxima*, another relevant pathogenic species. These markers will be useful for population genetics studies and for differentiating highly pathogenic from mild strains, and field from vaccine strains.

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BM007 - Identification of Leishmania species isolated from American Tegumentary Leishmaniasis (ATL) patients from North and Middle West areas of Brazil.

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INTRODUCTION: Leishmaniasis is a zoonosis caused by the protozoan Leishmania. The clinical polymorphism is a result of the host immune response and factors related to the parasite virulence. The identification of the leishmania species and the association among clinical symptoms, epidemic finds and laboratorial data can be helpful to formulate an efficient strategy for the control of the infection. OBJECTIVES: Identify the species of leishmanias responsible for the ATL and associate it with the outcome of infection. METHODS: Patients from North and Middle West areas of Brazil were assisted in the Tropical Diseases Hospital-Anuar Auad-(HDT) of Goiânia -Goiás. The diagnosis of ATL was obtained through clinical, epidemic and laboratorial data (direct exam, IFI, IDR and histopathological analyses). Parasites were isolated from 9 patients, maintained in culture in vitro and in vivo and the species were identified by PCR-RFLP. RESULTS: Seven patients presented localized and ulcerated lesions, one had a disseminated form and the other showed diffuse leishmaniasis. It has been identified Leishmania (L.) amazonensis (L.a., n=4), Leishmania (V.) braziliensis (L.b., n=4) and Leishmania (V.) guyanensis (L.g., n=1). Five isolates were from state of Goiás (2 were L.a. and 3 L.b.), 2 from Mato Grosso (1 L.a. and 1 L.b.), 1 from Tocantins (L.a.) and 1 from Pará (L.g.). We were unable to establish any association among the histopathological analyses, titles of antibodies (IFI), degree of cellular immune response (IDR) and species of the parasites with the clinical evolution during the treatment. CONCLUSION: The high variability of the species and low number of isolates that were obtained make the association between parasite specie and clinical finds very difficult. However the increase of the identified isolate numbers will become a useful tool to diagnose the situation of the leishmaniasis in the North and Middle West area of Brazil.

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BM008 - Polymerase chain reaction (PCR) as criterion of cure on mice infected with different *Trypanosoma cruzi* clonal genotypes and treated with benznidazole

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The aim of this work was evaluate the polymerase chain reaction (PCR) as criterion of cure on mice infected with *Trypanosoma cruzi* belonging to different genotypes and treated with benznidazole (BZ). BALB/c mice infected with twenty *T. cruzi* laboratory-cloned stocks attributed to the 19 and 20 clonal genotypes included in *T. cruzi* I and to the 39 and 32 clonal genotypes included in *T. cruzi* II were previously treated with benznidazole during the acute and chronic phases of the infection. The animals were divided in three groups: i) treated and cured (TC); ii) treated dissociated (DIS) and iii) treated and not-cured (NC), based on results of the fresh blood examination, hemoculture, enzyme linked immunosorbent assay (ELISA) and search of anti-live trypomastigotes antibody by flow citometry. DNA of 90 blood samples was extracted, amplified, visualized by electrophoresis in 4.5% polyacrylamide gel and silver revealed. Considering TC group, the total positivity of PCR was 50.94% (27/53). However, when it was taken into account the *T. cruzi* genotype, the positivity of PCR was 57.89%, 33.33% and 57.89% for 19, 39 and 32 genotypes, respectively. In the DIS group the positivity of PCR was 33.33%, 100.0% and 50.0% for the genotypes 19, 39 and 32, respectively. In the NC mice the positivity was 100.0% (25/25) for all genotypes, including the genotype 20, which is totally drug resistant. These results confirm the higher sensibility of PCR considering others methods used in Chagas disease control of cure, demonstrating the presence of *T. cruzi* DNA in half of the animals considered cured by other techniques. Although statistical analysis is in course, the PCR positivity on treated mice varied with the genotype, suggesting that the phylogenetic diversity of the natural clones of the parasite should be taken into account in studies regarding Chagas disease control of cure.

BM009 - High sensibility of Polymerase Chain Reaction (PCR) to detect *Trypanosoma cruzi* in mice blood independent on parasite clonal genotype and phase of infection

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Trypanosoma cruzi is separated in two phylogenetic lineages: *T. cruzi* I, including the clonal genotypes 19 and 20 and T *T. cruzi* II, including the clonal genotypes 39 and 32. It was demonstrated that cloned stocks pertaining to different

genotypes behave differently regarding blood and tissue parasitism in experimental murine infection. The aim of this work was evaluate the ability of the Polymerase Chain Reaction (PCR) to detect *T. cruzi* in mice blood inoculated with different genotypes. One hundred thirty one blood samples, 70 obtained in the acute phase (60 days post inoculation - d.p.i.) and 61 in the chronic phase of infection (140 d.p.i.), of BALB/c mice inoculated with 5 *T. cruzi* stocks belonging to genotype 19, 5 stocks of the genotype 20, 5 stocks of the genotype 39 and 5 stocks of the genotype 32, were analysed. DNA was extracted from blood samples, amplified, visualised by electrophoreses in 4.5% polyacrylamide gel silver stained. In addition, the results of PCR were compared with the results of fresh blood examination (FBE), hemoculture (HC) and serology (ELISA), performed in these same animals. The positivity of PCR was 100.0% independently of *T. cruzi* genotype and phase of the infection. Although statistical analysis is in course, for the genotype 19 PCR and ELISA displayed 100.0% of sensibility ¿ 89.47% (HC)¿ 76.00% (FBE). For the genotype 20 PCR and ELISA presented sensibility of 100.0% ¿ 92.68% (FBE)¿ 90.20% (HC). For the genotype 39 the sensibility of PCR was 100.0% ¿ 88.89% (ELISA)¿ 65.71% (FBE)¿60.60% (HC). For the genotype 32 the sensibility was: PCR (100.0%)¿ ELISA (86.36%)¿ HC (76.66%)¿ FBE (65.38%). Including in mice infected with stocks that presented sub-patent parasitemia, the PCR in blood eluate was positive, confirming the potential of that technique for Chagas disease diagnosis in murine model independent on the parasite genotype.

BM010 - A multiplex PCR assay that separates *Rhodnius prolixus* from *Rhodnius robustus*

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Rhodnius prolixus and *R. robustus* are the pair of Chagas disease vector species that has received the most attention, in terms of studies on taxonomy, in the last 15 years. This is understandable if one considers the two following facts: (1) *R. prolixus* is presently the most important Chagas disease vector in Latin America; and (2) it is virtually indistinguishable, based on morphology, from *R. robustus*, a vector of secondary importance. The determination of the correct status for these taxa is, thus, of great epidemiological relevance. In order to help clarify this issue, Monteiro and others (2003) have genetically analyzed several populations of these insects and shown that not only are *R. prolixus* and *R. robustus* separate taxa, but also that *R. robustus* is a species complex. We have used the alignment of 20 mitochondrial cytochrome *b* haplotypes (663 bp each; described by Monteiro and others, 2003) to select for amplifiable species-specific regions. We accomplished this by designing one forward primer on a region conserved among all haplotypes, and three reverse primers that anneal to species-specific regions and amplify fragments of different lengths for *R. prolixus* (285bp), *R. robustus* I (349bp), and *R. robustus* II-IV (239bp). Such fragments can be easily separated on regular 1.5% agarose gels. This multiplex assay is simple, objective, cost-effective, and its PCR-based nature makes it applicable to any insect developmental stage, to dried specimens or even to insect remains. It should be particularly useful in certain areas in Venezuela where some of these forms occur in sympatry.

BM011 - *Trypanosoma rangeli* Transcriptome Project: Generation and analysis of expressed sequence tags.

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Trypanosoma rangeli and *T. cruzi* are hemoflagellate protozoan parasites of the Kinetoplastida Order widely distributed in Central and South America, sharing reservoirs, vectors in several geographical areas. *T. cruzi* produces Chagas disease whereas *T. rangeli* infection does not seem to be pathogenic for vertebrate hosts. Nevertheless, these parasites share 60% of their soluble antigenic constitution, which can determine serological cross reactions turning difficult the specific diagnosis as well as complicating the epidemiology of human Chagas disease. Methods routinely used for Chagas disease diagnosis are not capable to distinguish these two species, reinforcing the needs to develop new strategies in order to allow specific differentiation in a fast, easy and economical way. We herein report the results obtained from the construction and sequencing of 3 cDNA libraries of epimastigotes forms of the *T. rangeli* Choachi strain, which resulted on the generation of 656 ESTs. Among these, only 20 showed similarity with *T. rangeli* sequences and 245 returned no hits in any database consulted. Our results reinforce the importance of this strategy to gather new information about the parasite, acting as support for identification of new diagnostic targets or to host-parasite interaction studies. Having described a method to induce *T. rangeli* metacyclogenesis in vitro, our group is currently working on a cDNA library of this parasite form in order to perform an intra and inter-specific comparative study of both epimastigotes and trypomastigote ESTs and ORESTES.

BM012 - Genome size, chromosomal polymorphism and gene synteny in T. cruzi I and T. cruzi II groups

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PFGE and DNA hybridization were used to establish and compare some parameters of the molecular karyotype of nine stocks classified into T. cruzi I and T. cruzi II groups. The

isolates showed a variable number of chromosomal bands (17 to 22) comprised between 0.4 and 3.3 Mbp. Hybridization of 54 genetic markers revealed extensive chromosome size polymorphism. In general, the probes hybridized in larger chromosomes in stocks of T. cruzi II as compared to T. cruzi I. The total number of chromosomes was estimated based on the fluorescence intensity of SYBR Green I-stained chromosomal bands. For this analysis, we have elaborated a mathematical model that takes into consideration that T. cruzi is diploid and the ratio between the fluorescence value of a chromosomal band (F) and its molecular size (M) is a constant (F/M = k). This model was validated with the chromosomal bands of S. cerevisiae, which contain two chromosomes per band. Accordingly, we estimated 52; 65; 72 and 44 chromosomes, respectively, for CL Brener; Esmeraldo cl3, SO3 cl5 and Silvio X10 cl1. The genome size of each stock was calculated by the cumulative sum of the number of chromosomes comprised in each band multiplied by the molecular size of the individual band. Eight putative syntenic groups, encompassing twenty-nine non-redundant genetic markers and distributed in eleven CL Brener chromosomal bands were disclosed. The syntenic groups were conserved in all the stocks. The relative abundance of the 195-bp satellite DNA (120,000 copies in Y strain) was 4 to 9-fold more abundant in T. cruzi II stocks as compared to T. cruzi I. The novel aspects of T. cruzi karyotype contribute to the comprehension of the genome organization of this parasite and will assist the assignment of scaffolds to the CL Brener chromosomal bands. Supported by FAPESP and CNPq.

BM013 - Microarray profiling of gene expression in Trypanosoma cruzi strains sensitive and resistant to benznidazole

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In a previous work, we constructed a prototype DNA microarray that contains predominantly expressed sequence tags (ESTs) of CL Brener and we demonstrated that despite the high genetic diversity of T. cruzi strains, microarrays is a valid tool for comparative genomic studies and for the analysis of gene expression in this parasite (Baptista et al. 2004). Aiming at profiling gene expression in strains susceptible and resistant to drugs, eight strains were selected from our bank. Depending on the strain, the doubling times in LIT medium varied from 35.6 to 101.7 hours. We evaluated the sensitivity of the epimastigote forms to three benznidazole concentrations and concluded that 4 strains were susceptible and 4 strains presented partial or total resistance to the drug. Aiming the construction of a more representative microarray slide, we obtained 1,233 ESTs from CL Brener amastigotes (a kind gift of Dr. A. Gonzalez, CSIC, Granada), 710 ESTs from CL Brener epimastigotes (kindly provided by Drs. W. Degrave and A. Brandão, FIOCRUZ, Rio de Janeiro), 45 ESTs from Tulahuen amastigotes (kindly provided by Dr. S. Teixeira, UFMG, Belo Horizonte) and about 35 cloned sequences of various T. cruzi strains. Sequences were subjected to processing steps including trimming of poor quality regions, vector and spliced leader masking. Clustering of the 1,980 sequences was performed with Cap3 program, indicating 339 clusters with 2 to 20 sequences, 848 singletons and 1,187 (60%) unique sequences. Approximately 1,200 clones representative of singletons and of each cluster were selected to be spotted in the array. PCR amplification of the ESTs (average length 800 bp) was obtained with T3 and T7 primers using DNA polymerase (Biolase). The amplification products were purified with Multiscreen plates. The microarray slide will be spotted in the near future and probed with cDNA of resistant and susceptible strains.
Support: FAPESP; CNPq

BM014 - Shotgun Sequencing of *Trypanosoma cruzi* with ORESTES (Open Reading Frame Expressed Sequence Tags)

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The strategy of EST (expressed sequence tag) sequencing (Adams et al., 1991) represented a qualitative advance in gene discovery. However, high levels of redundancy were observed in the ESTs. Improved efficiency of gene discovery was achieved by using normalized cDNA libraries (Bento Soares et al., 1994). To date, almost 11,000 ESTs have been deposited in databases from cDNA libraries of CL Brener epimastigotes (Urmenyi et al., 1999), amastigotes and trypomastigotes (Aguero et al., 2003). Our laboratory has been developing DNA microarrays to investigate differential gene expression in *T. cruzi* strains (Baptista et al., 2004). In our microarray we have been using predominantly ESTs of CL Brener, generated mostly from the 5' end of the mRNAs. In order to increase the representation of the central portions of transcripts to be spotted in the microarray we have generated ORESTES sequences (Open Reading Frame Expressed Sequence Tags) from epimastigotes of CL Brener strain. This methodology, developed by Dias Neto et al. (1997), involves the generation of short cDNA templates by RT-PCR using arbitrarily selected, non-degenerated primers under low-stringency conditions. To verify the quality of mRNA preparations purified from epimastigotes and possible DNA contamination, RT-PCR and PCR reactions were performed to amplify *T. cruzi* single copy (EF-1) and multicopy (rRNA) genes; kinetoplast DNA; and the 195-bp satellite DNA. After DNAse treatment, cDNA was synthesized in five independent reactions using five arbitrary primers. Analysis of the amplification products by gel electrophoresis indicated that four out of five primers gave the expected results. DNA fragments from 200-400 bp and 400 bp were size-selected from each amplification profile. A total of six profiles were independently cloned using the pGEM TEasy System Vector (Promega). Plasmids were transformed and selected clones were PCR amplified and sequenced. Sequence data are under analysis with bioinformatics tools. Support: FAPESP, CNPq.

BM015 - Functional genomics analysis of *Leishmania amazonensis* macrophage infections in two hosts with different genetic background

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The study of the functional genomics of the host-pathogen interactions is of great importance for the comprehension of the molecular mechanisms controlling the onset of infectious diseases, being the early events of the interaction between pathogens and their hosts decisive steps for the outcome of the subsequent disease. Besides factors directly linked to the pathogen itself, such as pathogenicity and virulence, factors related to the host, such as general state of health and genetic background, are extremely important for the determination of the type and/or severity of the disease. This is especially true when we talk about infection with parasites of the genus *Leishmania*, whose main host cell is the macrophage. The various species of these parasites have the characteristic of producing many different clinical manifestations of leishmaniasis when infecting a host, just as the infection by a single species may elicit very diverse responses in different hosts. With the objective of describing the genetic differences between macrophages of two different strains of inbred mice, one of which is susceptible (CBA/J) and the other resistant (C57BL/6) to infection with *L. amazonensis*, and of evaluating the possible impact of these differences on the course of the infection and on the phenotype of resistance or susceptibility to this infection, the Affymetrix GeneChiptechnology was used. Macrophages from the two strains were compared and 202 differentially expressed genes were selected from this comparison. Many of these genes have already been described as playing some part in the immune response, activating or inhibiting macrophage functions. Among them, genes encoding chemokine and cytokine receptors such as CCR5 e IL-10R can be found, as well as those encoding chemokines like CCL3 e CCL4, complement factor C1q, prostaglandin receptors, and various proteins of the p200 family of IFN-γ induced proteins. Also, genes encoding proteins related to the cytoskeleton, cellular adhesion and phagocytosis, among others, were selected. We also compared infected macrophages of both strains with non-infected controls. In this case, we observed very complex patterns of changes in gene expression of the infected cells, especially of the CBA/J strain, where it was not possible to select genes with a statistically significant decrease in expression during the infection. This work shows a preliminary view of the genetic differences between two mouse strains and of the changes in macrophage gene expression when infected with *L. amazonensis*. More studies must be done in order to confirm these results and to elucidate the molecular mechanisms involved in the resistance and susceptibility to this parasite.

BM016 - Genomic Organization of Telomeric and Subtelomeric Sequences (TAS - "Telomere Associated Sequences") of *Leishmania amazonensis*

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Telomeres are protein-DNA complexes that work as functional complexes that protect chromosomal ends to be recognized as damaged DNA. Telomeric DNA is repetitive, G-rich and protrudes towards the end of the chromosome as a 3'-G overhang. In *Leishmania* spp. sequences adjacent to telomeres form the "*Leishmania* Conserved Telomere Associated Sequences"(LCTAS) which are 100 bp long and contain two conserved sequence blocks (CSB 1 and CSB 2) apart of non-conserved sequences. The aim of this work is to study the genomic organization of *Leishmania (Leishmania) amazonensis* telomeric/subtelomeric sequences. *L. amazonensis* chromosomes were separated in a unique PFGE gel as 25 ethidium bromide-stained bands. All the bands hybridized with the telomeric probe (5'-TTAGGG- 3')3 and with probes generated from the conserved subtelomeric elements (CSB1, CSB2). Terminal Restriction Fragments (TRF) of *L. amazonensis* chromosomes were analysed by hybridizing restriction digested genomic DNA and chromosomal DNA separated in 2D PFGE with a telomeric probe. The results allowed us to estimate that *L. amazonensis* TRF is 3.3 Kb long and that telomeres are polymorphic ranging in size from 0.1 to 1.5 Kb. Using similar approaches we demonstrated that the subtelomeric domains CSB1 and CSB2 are present in low copy number compared to telomeres and are organized in blocks of 0.2 to 1.6 Kb flanqued by *Hinf*I and *Hae*III restriction sites. *Afa*I restriction sites found within the conserved CSB1 elements liberate *L. amazonensis* telomeres from the rest of the chromosome. *Bal*31 sensitive analysis was used to confirm the above results and to differentiate telomeric fragments from intersticial internal sequences. The size of *L. amazonensis* 3' G-overhang was estimated by non-denaturing Southern blotting as being 12 nt long. A model for the organization of *L. amazonensis* chromosomal ends is provided.
Financial support: FAPESP, WHO/TDR - UNDP Bank

BM017 - Identification of ESTs (Expressed Sequence Tags) from metacyclic trypomastigotes of Trypanosoma cruzi (G strain)

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Metacyclic trypomastigotes of *Trypanosoma cruzi* can initiate the infection in the mammalian host by invading a variety of cell types. Analysis of expressed sequence tags (ESTs) of metacyclic trypomastigotes constitutes a useful approach for gene identification and could result in the identification of new targets for chemotherapy and vaccine development. A cDNA library was constructed in vector pCMV-SPORT 6 using poly A+ total (nuclear + polysomal) mRNA isolated from metacyclic trypomastigotes (G strain). We have generated 200 ESTs from randomly selected clones. A total of 55% of the clones had no significant matches to genes from other organisms in the database or were homologous to hypothetical T. cruzi proteins, while 45% had significant matches to sequences in databases. Functional groups of those sequences with matches in the database were constructed according to their putative biological functions. The largest categories were enzymes and proteins of the metabolism (11%), cell surface molecules (7%), retrotransposons and repetitive sequences (6%) and heat shock proteins (4%).
Gene expression in trypanosomatids is regulated essentially at the post-transcriptional level. It has been suggested that mRNA mobilization to the polysomes is a post-transcriptional mechanism of regulation of gene expression in trypanosomatids [Avila et al. 2003, GMR 2:159]. We screened the metacyclic cDNA library with single strand ^{32}P-cDNA synthesized from polysomal RNA of epimastigotes and metacyclic trypomastigotes. Out of the 624 clones isolated, 425 clones (68.1%) only hybridized with the epimastigote probe; 96 clones (15.4%) only reacted with the metacyclic probe; and 103 clones (16.5%) reacted with both probes. This preliminary result suggests that only part of mRNA transcribed by metacyclic trypomastigotes is mobilized to the translation machinery. Sequencing and characterization of these clones are underway.
Support: FAPESP, CNPq.

BM018 - Identification of differentially expressed proteins during Trypanosoma cruzi metacyclogenesis by proteomic analysis

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Trypanosoma cruzi metacyclogenesis involves the differentiation of the replicative non-infective epimastigote forms into the non-replicative infective metacyclic trypomastigotes. The development of a chemically defined medium that supports T. cruzi metacyclogenesis has rendered possible obtaining parasites at various time points of the differentiation process. The regulation of gene expression in trypanosomatids is exerted mainly at the post-transcriptional level. The fact that most trypanosomatid mRNAs result from processing of polycistronic transcripts suggests that stage specific genes must have their expression regulated either through selective transport to the cytoplasm, or through specific degradation/stabilization pathways or, alternatively, through selection of the sequences to be translated in the polysomes. In the case of Trypanosoma cruzi, modulation of gene expression is of great importance because the parasite faces different environments (hosts) during its life-cycle. Studies involving the mRNA population associated to polysomes and characterization of proteins from each developmental stage are of great interest. Thus, microarray and proteomic analyses have been carried out in order to gain further insight into the mechanisms involved in T. cruzi metacyclogenesis regulation. In this work, proteomic maps of epimastigotes, metacyclic trypomastigotes and four intermedia forms during the parasite differentiation were performed to identify proteins diferentally expressed. Peptide mass fingerprints obtained by MALDI-TOF mass spectometry allowed us to identify inequivocally proteins present on the 2-D gels. These proteins might be key factors to understand the parasite biology as well as to be used as markers and therapeutic targets.
Principal Investigators: Carlos Cervenansky and Carlos Robello (Universidad de la Republica), Samuel Goldenberg and Marco Aurélio Krieger (IBMP/FIOCRUZ)

BM019 - The Eimeria ORESTES Project: a preliminary analysis of 33,000 ESTs from *Eimeria tenella*, *E. acervulina* and *E. maxima*

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Coccidiosis of the domestic fowl is an important enteric disease caused by seven different protozoan species of the genus *Eimeria*. As a member of the *Eimeria* Genome Consortium (Shirley et al., Trends Parasitol. 20:199-201, 2004), our laboratory has initiated an EST sequencing project using the ORESTES approach. The initial aim was the generation of at least 10,000 reads of the three most important *Eimeria* species: *E. tenella*, *E. acervulina* and *E. maxima*. All the reads were submitted to an automated sequence processing pipeline (EGene pipeline generator - Durham, Kashiwabara and Gruber, manuscript in preparation). The sequences were masked against vectors and primers, filtered and trimmed. A total of 33,585 high-quality reads were obtained so far, as follows: 12,354 reads of *E. tenella* (3,941 from sporozoites, 3,576 from second-generation merozoites, and 1,419, 2,053 and 1,365 from unsporulated, partially sporulated and sporu-

lated oocysts, respectively), 10,140 reads of *E. acervulina* (1,572 from sporulated oocysts and 8,568 from sporozoites) and 11,091 reads of *E. maxima* (8,840 from sporulated and 2,251 from unsporulated oocysts). Clustering was performed and a total of 3,402, 2,331 and 2,424 distinct events were obtained for *E. tenella*, *E. acervulina* and *E. maxima*, respectively. Joint clustering of *E. tenella* ORESTES reads and 26,955 conventional ESTs from GenBank resulted in circa 6,000 events. These reconstructed transcripts are being mapped on the draft version of the genome sequence using Sim4 program. The clustering and assembly data, as well as the similarity searches are publicly available at the web site of the project, address http://www.lbm.fmvz.usp.br/*Eimeria*/, and can be visualized through the Generic Genome Browser. Sequencing effort is still underway for other developmental stages of *E. maxima* and *E. acervulina*. A semi-automatic annotation pipeline is under development and a comprehensive and curated annotation will be performed.
Financial Support: FAPESP, CNPq and Pró-Reitoria de Pesquisa USP.

BM020 - Gene synteny and amplification of the H locus in /emphLeishmania spp.

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The phenomenon of gene amplification is a common mechanism of drug resistance observed in the protozoan parasite *Leishmania*. A few loci are particularly prone to amplification when the parasite is cultivated in the presence of unrelated drugs. The H locus is a well-known source of amplicons and is widely studied in *Leishmania* spp. In *Leishmania major* and *L. tarentolae* the H region codes genes involved in mechanisms of drug resistance, such as, *PTR1* (Pteridine Reductase-1), *PGPA* (P-Glycoprotein A) and *HTBF* (H region associated terbinafine resistance gene). The amplification mechanisms described in these organisms are primarily based on DNA rearrangment events, which may have an important role in the evolution and organization of the genome. In this work we have focused the amplification-prone H locus to investigate the gene synteny in *Leishmania* spp. We used known genes and repetitive elements as probes in order to draw a comparison between the H locus of *L. major* and the *Viannia* subgenus species *L. braziliensis*. The presence of at least four H region-genes was confirmed in the same chromosome of *L. braziliensis*. Genomic DNA Southern data revealed important differences in the restriction pattern of the locus. We have used partial genomic libraries to isolate two *L. braziliensis* H region genes. Clones bearing the genes *PTR1* and *TTRS* (Tryptophanil tRNA Synthetase) were partially sequenced revealing a considerable divergence between *L. major* and *L. braziliensis*. The sequence divergence was mainly observed in intergenic regions and may explain the difference in the restriction pattern found in Southern analysis. PCR experiments were used to bridge the cloned loci and confirmed that, in spite of the low sequence identity, the gene synteny was maintained in the two species. We also investigated the amplification of the H locus in a terbinafine-resistant *L. braziliensis* cell line. Cross-resistance experiments indicated that *L. braziliensis* terbinafine resistance might not be related to the H locus. Remarkably, terbinafine resistant L. braziliensis cell line did not present amplicons as confirmed in short-run PFGE and by Southern analysis using the *HTBF* gene and H region typical repetitive elements as probes. Gene amplification was observed only after a second selection protocol. The amplicon generated does not seem to contain H region sequences. Altogether, our results suggested that gene amplification is not the favoured event mediating drug resistance in *L. braziliensis*.
Supported by FAPESP and UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases.

BM021 - Functional genomics comparison of two infective forms of *Trypanosoma cruzi* and their corresponding host cell response.

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Metacyclic and blood trypomastigotes are the infective stages of *Trypanosoma cruzi*, sharing common attributes, such as morphological aspect, inability to divide and resistance to complement lysis. However, these forms are adapted to distinct host environments in nature and little information is available concerning the differences between them. In order to study these differences in a high throughput functional genomics approach, we have compared the gene expression profile of these two development forms of the parasite and the differences of the mammalian host cell response towards infection with these two distinct trypomastigotes. A *T. cruzi* microarray containing more than 6,000 probes was used to identify differences in mRNA levels between metacyclic and cell culture derived trypomastigotes. The murine MGU74v2 Genechip (Affymetrix), covering approximately 12,000 gene products, was used to compare the genes expressed by cardiomyocytes infected with these two distinct *T. cruzi* infective forms. Several differentially expressed genes (DEG) from the parasite and from the infected host cell were selected, with stringent statistical control, when comparing the two infective forms. In regard to the parasite, previous known cell markers were present among the aproximately 300 DEG, along with several hypothetical proteins, which constitute aproximately 60% of the total DEG numbers. With regard to the differential host cell response, DEG were observed early in infection (2h), although there was a great increase in these numbers at later times (6h and 24h). Approximately 900 DEG were selected, comprising several functional groups, such as extra-cellular matrix proteins, immune response, apoptosis, cell signaling, etc. These results corroborate the biological distinctiveness between metacyclic and blood trypomastigotes and point to their biological complexity which might lead to the differences in terms of infection process produced by both infective forms. Financial support from NIH, CNPq, Pronex, Fiocruz.

BM022 - PROSPECTIVE SEQUENCING OF THE *Leishmania braziliensis* GENOME

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Leishmaniasis is an endemic disease in 88 tropical and subtropical countries, affecting about 12 million people in the world. The impact of different clinical (visceral, cutaneous and mucocutaneous) forms on public health, and the evolutionary distance between the species *L. major*, *L. infantum* and *L. brasiliensis*, were the main reasons for the initiative of a prospective study of *L. brasiliensis* (MHOMBR75M2903), through GSS (Genomic Survey Sequence) sequencing. Random and semi-random libraries were constructed, using pUC18 as a cloning vector. Plasmid templates from a 2-3 kb insert semi-random library were prepared with Wizard-SV 96 plasmid purification system (Promega). Sequencing reactions were done using Big Dye terminator chemistry with M13 forward and reverse primers and run on an ABI 3730 sequencer (Applied Biosystems). Sequences were trimmed leaving only sequences longer than 150 bases with a PHRED base quality value more or equal of 20 and clustered with PHRED/PHRAP and in-house PERL scripts. An initial set of 1,082 sequences were obtained, with 60.8% G+C content. Those sequences were assembled in 261 contigs, leaving 582 singlets, and were analyzed for similarity against EST, GSS and nt Genbank databases, using E value = 10^{-10} as a cutoff by local BLAST. The results were submitted to FooBLAST, a new tool developed in our laboratory, to organize the BLAST results in a relational database. 50.6% of the contigs showed hits against GSS + nt database while 35.0% of the singlets resulted in positive hits against the same database. On the other hand, only 1.5% of the contigs and 0.5% of the singlets showed positive hits against the EST database. Most of the hits were from trypanosomatid sequences. These results further enrich the *L. braziliensis* GSS database (Laurentino et al., MBP 137, 81-86, 2004) and are being used in comparative analyses between *L. braziliensis* and *L. major*, to predict coding sequences of diagnostic/evolutive/therapeutical interest and infer biological functions for genes involved in metabolic ways.
Financial support: TDR/WHO,FAPERJ/FIOCRUZ,CNPq, PAPESIII/ FIOCRUZ,PDTIS/FIOCRUZ.

BM023 - Chromosomal rearrangements in *Trypanosoma cruzi*: analysis of clones isolated from G strain

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Chromosomal rearrangements have been frequently described in protozoan parasites, including *Trypanosoma brucei*, *Leishmania spp.*, *Giardia lamblia* and *Plasmodium falciparum*. They also are one of the main cause of karyotype variability among the isolates of *Trypanosoma cruzi*. Here, we identified events of inter-specific chromosome rearrangements among clones derived from the G strain. Clones of *T. cruzi* G strain were obtained as described by Lima & Villalta (1989). Briefly, Vero cells were plated and infected with *T. cruzi* metacyclic trypomastigotes to a concentration of 1 parasite per 100 μl R-10 medium. The plates were then observed until the appearance of trypomastigotes in the supernatant which corresponded to the appearance of the first clones. The selected clones were expanded by infecting naive Vero cells. Trypomastigotes from the supernatants were transferred to 25 cm^2 tissue culture flasks containing Vero cells or to LIT medium to grow as epimastigotes. The clones were frozen as epimastigotes in LIT medium containing 7% glycerol. Divergence in the molecular karyotype were evident when we compared the profiles of ethidium bromide-stained chromosomal bands from the clones with the parental G strain. Using standard PFGE conditions it was possible to visualize the absence of chromosomal bands found in the parental strain, and the appearance of new bands in the karyotype of the clones. Only qualitative changes were observed in the DNA content of chromosomal bands when the clones were individually compared. Due to the rearrangements of some chromosomes, we observed rearrangements in the chromosomal location of genes encoding α -tubulin, 24Sα rRNA, hexose transporter, adenylate cyclase and surface glycoprotein genes. On the other hand, H49 and CRA genes remained at the same position in the chromosome, suggesting that the rearrangements occurred only in a certain subset of chromosomes. The characterization of chromosomes involved in the rearrangements is underway in our laboratory.
FAPESP, CNPq

BM024 - Gene trapping strategy for functional genomics of *Leishmania braziliensis*

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We have conducted a survey of the genome of *Leishmania braziliensis* by shotgun sequencing, approximately 15% of the haploid genome of the parasite. This study revealed a low level of similarity between *L.braziliensis* GSSs and *L.major* genomic databank (about 60%) and encouraged a survey of the expressed genome of *L.braziliensis*. We are currently proceeding a functional analysis of the *L. braziliensis* genome using a systematic gene trapping strategy based on Tn5 transposon system. A transposon was engineered into pMOD, a vector for the Tn5 system, and made suitable to be used in *in vitro* transposition reactions for gene trapping. This transposon carries a neomycin phosphotransferase (*neo*), the selectable marker, which will be only expressed if fused in frame with a *Leishmania* gene present in the target DNA. Four cosmid clones from a genomic library from a *L. braziliensis* were

used as targets in transposition reactions and four insertional libraries were constructed containing 2000 clones each. After transfection of insertion pools into *L. braziliensis*, parasites resistant to G418 (neo marker) were rescued. To perform subcellular localisation of fusion products we have used a polyclonal antibody against neomycin phosphotransferase to be detected by immunofluorescence microscopy. Two transfectants are currently being investigated. The fusion proteins of both co-localise with the endoplasmic reticulum marker GRP 78. Database comparison allowed the identification of the endogenous proteins as a microtubule-associated protein and a hypothetical 15.3 kDa protein of *E. coli*. Furthermore, we are mapping the trapped genes within the genome, and evaluating their expression in Northern blotting experiments. The transposon system constitutes a reliable tool for systematic tagging, identification and genetic annotation in this pathogenic parasite.
(Supported by FAPESP and CNPq)

BM025 - A proteomic approach to identify proteins differentially expressed in attenuated *Leishmania* overexpressing the miniexon gene

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The miniexon gene has a central role in the processing of polycistronic pre-mRNAs of Kinetoplastids. The product of this gene (the spliced leader sequence) is the common substrate for the trans-splicing reaction. In this reaction, the capped spliced leader sequence with 39 nucleotides is added to the 5'-end of the majority of the parasites' mRNAs. Antoniazi *et al.*, 2000, showed that the artificial induction of the miniexon overexpression in *Leishmania major* reduced the virulence pattern of a lineage originally virulent (LV39) in BALB/c mice in vivo. Aiming to investigate the mechanism of the observed attenuation we used a proteomic approach, by combining 2D gels to separate and visualize proteins and mass spectrometry to identify those proteins differentially expressed between attenuated *Leishmania* strains (overexpressing the miniexon gene, LVME) and parental line. The 2-D maps were analyzed and spots differentially expressed were excised, digested with trypsin and the resulting peptides were subjected to MALDI-TOF mass spectrometry. Our preliminary analysis suggests that attenuated lineage suffers several metabolic changes due to the overexpression of miniexon. These alterations include differential regulation of proteins involved in response to stress, proteolysis, energetic metabolism, phosphorylation, cell cycle control and proliferation. Our results indicate that the excess of miniexon transcript, or a specific class these transcripts, could be leading to the translation of some transcripts that should be down-regulated in the moment of differentiation from pro- to amastigotes. In parallel, we are currently investigating abundance of a special class of polyadenylated miniexon transcript (Lamontagne& Papadopoulou, 1999) using Real Time PCR to compare their levels on parental and miniexon overexpressor promastigotes, which could be involved in the translation imbalance observed.

Financial support by FAPESP and CNPq

BM026 - Characterization of LSTR378, a repetitive element from chromosomal extremities of *Leishmania* spp.

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We have previously sequenced one extremity of *Leishmania major* chromosome 20 and identified a novel repetitive element, named LST-R378 (Pedrosa et al., MBP 114: 71, 2001). To determine a possible degree of sequence conservation of LSTR378 we have cloned and sequenced PCR products of different species of the genus *Leishmania* from Old and New World. The presence and organization of LSTR378 within the genus was analyzed by PFGE and Southern hybridization: a major signal is detected in chromosome 20 of *L. major* and in a chromosomal band of, approximately, 760 kb in most of the species tested. Fainter signals were observed in other chromosomes of most species tested, however, no hibridization signal was found in *L. tarentolae* and signals are less intense in *L. hoogstraali*. The organization of LSTR378 in different species of the parasite was investigated by RFLP analysis. The results indicate a similar organization in *L. major* species and demonstrate polymorphism in different New World species from subgenus *Leishmania* and *Viannia*. We observed a size polymorphism for fragments amplified by PCR from different species and sequencing revealed regions with insertions Furthermore, BLASTN analyses demonstrate the occurrence of highly conserved regions within LSTR378 among species; within the *L. major* 378 bp element there is an 81 bp region presenting 83 percent identity with the 3'-UTR of a P-Type ATPase from *L. donovani*. This element was further found, with different degrees of identity, in several chromosomes of *L. major*, preferentially in intergenic regions of chromosomes' extremities. Further analysis on LSTR378 structure and distribution is in agreement with the broad shuffling of sequences occurring at chromosome extremities and may represent another tool for species characterization, to be tested in phylogenetic studies.

BM027 - TcruziDB: A *Trypanosoma cruzi* genome database

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TcruziDB.org, an internet-based genomics resource for *Trypanosoma cruzi* went live in April of 2003 as a flat-file

searchable database of sequence data. Historically, the database has contained the unannotated genome and a variety of data mining tools to search this data. Currently, the database contains the annotation released by the TIGR-SBRI-Karolinska Trypanosome Sequencing Consortium (TSK-TSC). The database averages 2,000 hits/month from an average of 150 unique visitors. The database has had several updates. The most recent publicly available release is version 3.0, released on September 10, 2004. The database is now relational and supported by the GUS architecture. This migration, facilitated by the release of a preliminary *T. cruzi* annotation by TSK-TSC, has permitted the integration of several new data types into the database. TcruziDB now contains experimental proteomic data and comparative analyses of other kinetoplastid genomes (T. brucei and L. major). Several new pre-computed data analyses are available (BLAST and motif results, EST mappings and gene comparisons). The new data types permit new queries across all data types, e.g. find all annotated genes with proteomic evidence of expression in at least one stage. Finally, a new graphical interface that displays annotated genes, gene features, and comparative relationships has been added.

BM028 - PlasmoDB: The *Plasmodium* Genome Resource

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Researchers can gain access to the *P. falciparum genome* sequence data, integrate this resource with other relevant data sets, and exploit the resulting information for functional studies, including identification of novel drug targets and candidate vaccine antigens. The *Plasmodium* genome database contains information from multiple sources, including DNA sequences data and curated annotations, automated gene model predictions, predicted proteins and protein motifs, cross-species comparisons, optical and genetic mapping data, information on population polymorphisms, expression data generated by a variety of complementary strategies and proteomics data. Integrating this information at a single site provides "one-stop shopping"for genomics-scale data sets related to malaria parasites. The flexible interface at PlasmoDB enables users to ask complex questions. For example, immunologists trying to develop an antimalaria vaccine might wish to identify potential immunodominant surface antigens. Drug developers might wish to identify enzymes expressed in bloodstream parasites that differ significantly from their human counterparts. Researchers interested in antigenic variation and parasite adherence might wish to identify all gene families in the parasite genome; those interested in genome organization might be interested in the chromosomal location of these proteins; evolutionary biologists might wish to examine all genes for which clear orthologs are known from a variety of species; and so on. PlasmoDB went live in June 2000 - more than two years before the formal completion of the *P. falciparum* reference sequence. The web site receives several thousand hits each day from more than 100 countries. The impact of PlasmoDB can be measured in the scores, possibly hundreds, of publications that have resulted since the database went live and the genome sequence was made accessible.

BM029 - The *Plasmodium falciparum var* gene transcription depends on the acetylation status of chromatin

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Blood stage *Plasmodium falciparum* parasites express the PfEMP1 variant antigens, encoded by the *var* gene family. During intraerythrocytic development, all but one or a few *var* genes are transcriptionally silenced leading to the expression of only one or a few different PfEMP1 antigens on the red blood cell surface. An important feature of the *var* gene expression control is that upon reinvasion, the pattern of activation/silencing is maintained in most of the parasites and only a few parasites change their mainly transcribed *var* gene. This indicates that the transcription pattern is inherited in some way during successive cell cycles. Since DNA methylation apparently does not play a role in this process, we tested if *var* transcription pattern inheritance is controlled by histone modification, such as methylation or acetylation of Histone 3 lysine residues. For this purpose, *P. falciparum* blood stage parasites, previously selected for the transcription of one dominant *var* gene, were exposed to a short treatment with the histone deacetylase inhibitor Trichostatin A. Upon reinvasion, parasites treated with Trichostatin A lost their initial transcription pattern and transcribed a number of different *var* genes instead of a dominant transcript, as observed before treatment or in control cultures. This indicates that *var* transcription is controlled by histone modification events and shows directly, for the first time, that *var* gene expression is controlled by epigenetic processes. Support: FAPESP

BM030 - Genomic localization and evolution of DnaJ-like genes in different species of Trypanosomatids

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Trypanosomatids are unicellular parasites belonging to a very old evolutionary lineage of eukaryotes that emerged around 500 million years ago. In this family, *Trypanosoma brucei*,*Trypanosoma cruzi* and *Leishmania major* are human pathogens causing distinct diseases. The parasite genome sequencing projects currently underway showed that despite a relative high level of sequence divergence between these organisms, the three species have maintained a striking conservation of gene order (sinteny). Similarly, it was reported in

Leishmania donovani,*T. cruzi* and in three Salivarian species (*T. brucei*, *T. vivax*,*T. congolense*), that the overall organization of the region flanking the glucose transporter (THT) locus is conserved. Unlike in prokaryotes, where genes functionally related are grouped in operon units, those associated to the THT gene cluster are not functionally related and thus it is not clear how the gene order has been conserved during evolution. In this work, we aimed to characterize whether the synteny level of the genomic flanking region downstream the THT gene cluster is conserved in lower trypanosomatids (*Crithidia*,*Herpetomonas*,*Phytomonas*,*Blastocrithidia*), using different gene markers associated to the THT locus in other members of the family. We showed that the DnaJ-like gene localizes in the megabase range of all trypanosomatids, but in the case of *L. major* and *T. rangeli* some copies were also detected in smaller chromosomes. This might reflect gene duplications in these latter species. We also carried out preliminary phylogenetic analyses of DnaJ-like protein sequences from ten different trypanosomatid species which revealed tree-topologies consistent with those already described using some other genes and proteins.

BM031 - CHARACTERIZATION OF SERINE-, ALANINE-, AND PROLINE-RICH PROTEINS (SAP) OF *Trypanosoma cruzi*

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In a previous work we described the isolation and characterization of genomic and cDNA clones encoding a Serine-, Alanine-, and Proline-rich protein (SAP) of *Trypanosoma cruzi* metacyclic trypomastigotes. SAP peptides display several repeats (P2-4, S2-3, A2-3, AS, SA, PA, AP, SP, PS, and TP) that are partially homologous to the serine-, alanine-, and proline-containing motifs of *Leishmania major* and *Leishmania mexicana* proteophosphoglycans. Genes coding for SAP are part of a polymorphic family whose members are linked to members of gp85/sialidase and mucin-like gene families. Here we present the functional characterization of SAP peptides.

T. cruzi native proteins that share epitopes with SAP were identified using mouse anti-sera against the SAP recombinant protein. By immunoblotting and 2D gel electrophoresis, we identified five proteins of metacyclic trypomastigotes that strongly reacted with anti-SAP antibodies. Indirect immunofluorescence associated to confocal microscopy showed that some of these epitopes are located on the surface of metacyclic trypomastigotes.

Biological assays were performed to investigate the functional role of SAP protein. Recombinant SAP bound to HeLa cells in a dose-dependent manner, suggesting a ligand-receptor interaction. Cell invasion assay showed that SAP was able to inhibit 44 % the internalization of metacyclic forms of CL strain into HeLa cells. SAP also triggered the host cell Ca^{2+} response required for parasite internalization. Taken together, these results suggest that SAP could be involved in the invasion of mammalian host cells by metacyclic trypomastigotes.

Supported by: FAPESP.

BM032 - Identification and characterization of new members of metacyclic trypomastigote stage-specific protein GP82 of *Trypanosoma cruzi*

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Metacyclic trypomastigotes of *Trypanosoma cruzi* express a developmentally regulated surface glycoprotein (GP82) which has been implicated in host cell invasion. GP82 is encoded by a polymorphic multigene family whose members are distributed in several chromosomes and can be divided into subsets on the basis of hybridization patterns with specific probes. Here we present the identification of new members of *GP82* gene repertoire. We constructed a representative cDNA library with polyA+ mRNA from metacyclic trypomastigotes of G strain. Screening of 3,072 clones using *GP82* gene as a probe resulted in 100 positive clones (3%), confirming that GP82 mRNAs were preferentially accumulated in metacyclic trypomastigotes.
Twelve clones were selected for further characterization. We identified at 5'-UTR of six cDNAs (clones 2B5, 2C5, 4C13, 4G6, 4D14 and 5G22) a 300-500 bp extension that shared sequence identity with *GP85* and *ASP2* genes. All of 12 clones displayed poly (A) tail, and clones 2C5, 3N8, 4D14, 4C13, 2L11 and 4G6 showed a stretch of polypyrimidine at the 3'-UTR region. The sialidase domain and the motif VTVxN-VxLYNR were present in ten clones. The peptide P4, which contains a specific epitope of GP82 (MoAb 3F6 epitope), was found 100% conserved in five clones. These results indicated that many variants of *GP82* gene repertoire are transcribed simultaneously in a trypanosome population.
Supported by FAPESP.

BM033 - Expression of GP82 and GP90 surface glycoprotein genes during *in vivo* metacyclogenesis of *Trypanosoma cruzi*.

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Metacyclic trypomastigotes of *Trypanosoma cruzi* express two major stage-specific surface glycoproteins called GP82 and GP90 which have been implicated in the invasion of mammalian host cells. The differentiation of epimastigotes into metacyclic trypomastigotes (metacyclogenesis) occurs in the digestive tract of the triatomine vector. Expression of *GP82* and *GP90* genes during in vivo metacyclogenesis

was analyzed by RT-PCR (reverse transcriptase and polymerase chain reaction) and immunofluorescence microscopy using monoclonal antibodies (MoAb).
We employed trypanosomes found at digestive tract of naturally-infected kissing-bug *Rhodnius prolixus* after 5 days intervals elapsed after feeding on a infected mouse with *T. cruzi* (G strain). RNA was extracted from parasites collected at different sections of digestive tract, and cDNA synthesized by RT-PCR. Levels of transcripts were determined in a real-time PCR reaction with SYBR-Green I chemistry employing specific primers for *GP82*, *GP90* and glyceraldehyde 3-phosphate dehydrogenase(*GADPH*).
Epimastigotes were detected in the rectal ampulla after 15^{th} day, while metacyclic trypomastigotes were only detected from 25^{th} day (5%) to 40^{th} day (62%). Transcripts of *GP90* and *GP82* were detected after the 15^{th} day reaching their maximum levels at 40^{th} day. The levels of *GP90* and *GP82* transcripts from parasites collected at 40th day were 4.0- and 10.9-fold higher, respectively, than those collected at 15^{th} day.
Expression of surface proteins was analyzed with MoAbs 3F6 and 1G7 that are specific for GP82 and GP90, respectively. No fluorescence signals were detected in epimastigotes, suggesting that *GP90* and *GP82* transcripts were not translated in this stage. Positive reaction with 3F6 was coincident with appearance of metacyclic forms on the 25^{th} day. GP90 became detectable with 1G7 on the 30^{th} day. Altogether, our data suggest a coordinated mechanism linking transcription and expression of stage-specific *GP82* and *GP90* genes during *in vivo* metacyclogenesis.
FAPESP, CAPES, BIOLAC

BM034 - Post-transcriptional control of levels of mRNA encoding the metacyclic trypomastigote stage-specific protein GP82 of *Trypanosoma cruzi*

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Trypanosoma cruzi uses post-transcriptional mechanisms to regulate gene expression. Thus, we are investigating the mechanisms involved in the control of expression of *GP82* gene, which encodes a developmentally regulated 82-kDa surface glycoprotein expressed by metacyclic trypomastigotes.
There are evidences that mRNA mobilization to polysome is an alternative mechanism of post-transcriptional gene expression regulation in *T. cruzi* (Ávila *et al.*, 2001, *Mol. Biochem. Parasitol.* 117). Thus, polysomal fraction of metacyclic trypomastigotes and epimastigotes were loaded on a linear 15-45 % sucrose gradient to separate mRNA associated with different number of ribosomes. Using a density gradient fractionator, the polysome profiles of the two stages were recorded and collected. The RNA from each fraction was extracted, transferred to nylon membrane and hybridized with the *GP82* and *α-tubulin* gene, the later used as housekeeping control. *GP82* probe hybridized with a single 2.7 kb band in the polysomal fractions of metacyclic trypomastigote.
To confirm these results, the levels of *GP82* transcript in polysomal mRNA were determined in metacyclic trypomastigotes and epimastigotes by real-time PCR, using SYBR-Green I chemistry and relative quantitation method. The primers were designed to reach the maximum polymerase efficiency. After normalization with *α-tubulin* gene, we detected 9 units of difference in cycle threshold between the two stages, being *GP82* transcripts 512-fold more abundant in metacyclic trypomastigote than in epimastigote form. Together these data suggest that the addressing of *GP82* transcripts to polysomal compartment is developmentally regulated and may be an important post-transcriptional mechanism to regulate protein expression.
Supported by FAPESP, CNPq and CAPES.

BM035 - Overexpression of a membrane protein from the ABC transport family (LmABC-CR1) confers Cyclosporin-A resistance to *L.(L.) major* cells after gene transfection.

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Cyclosporin-A (CsA) is a cyclic hydrophobic oligopeptide naturally produced by fungi. Besides this compound is usually known as a potent imunossupressor, it also presents a couple of other physiologic activities as anti-inflammatory and anti-allergic, or even acting as fungicidal or antiparasitic. Starting from a *L.(L.)major* Friedlin A1 strain (LmFA1) genomic library constructed into the cLHYG shuttle vector, DNA was transfected on promastigotes, and cells submitted to overexpression / selection experiments in the presence of increasing concentrations of CsA. One out of the two selected cosmids capable to render CsA resistance to LmFA1 was chosen for further analysis. The cosCip I insert (with approximately 35kb) was mapped and deleted based upon two restriction patterns, aiming at localization of the gene-locus related with CsA resistance. Deleted DNAs were then transfected into LmFA1 wild type cells and submitted to functional tests in the presence of CsA, after DNA copy number amplification. In spite of the low (but significant) values of IC50, we identified one locus related with CsA resistance after partial nucleotide sequencing and comparative analyses in the leishmania genomic database. This locus contains an LmFA1 gene that codified for a 1,844 aminoacids ORF expressing a membrane protein from the ABC transporter family (LmABC-CR1), which seems to be related with CsA resistance in leishmania. This protein presents high identity (95,5%) with a *L.(L.)tropica* ABC transporter protein (LtABC-A1), and low identity (27,2%) with another LmFA1 ABC transporter (PRP-1) isolated from our group, and related to Pentamidine resistance. Previous molecular analysis shown an evident polymorphism of LmABC-CR1 among *L.(L.)amazonensis, L.(V.)braziliensis* and *L.(L) chagasi*, that could be related with the different response of these species in cultures containing CsA. The role of this protein on the CsA resistance in different leishmania species will be further studied. Support: FAPESP,CNPq,LIM-48

BM036 - Study of the role of the ABC transporter PRP1 of *Leishmania (L.) major* in Pentamidine resistance

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Pentamidine (PEN) is a second line compound used in treatment of leishmaniasis especially in cases of resistance to pentavalent antimonium. Little is known about resistance and parasite targets in the treatment using PEN. Recently, we have characterized the gene *PRP1 (Pentamidine Resistance Protein 1)* able to mediate PEN resistance in *Leishmania (L.) major* when overexpressed (Coelho et al., 2003). *PRP1* gene is a single copy gene located in the chromosome 31 of *L. (L.) major* Friedlin strain and belongs to the conserved ABC transporter superfamily. Previous studies by Southern blotting indicated that *PRP1* gene is present in different species of the *Leishmania* genus and primers based on the sequence of the first nucleotide binding domain of *PRP1* gene are able to amplificate DNAs related to this ABC transporter. For characterization of this ABC transporter, the recombinant PRP1 was expressed in fusion to the Glutathione-S-Transferase in *Escherihia coli* and purified by affinity chromatografy. The recombinant protein was used to immunize rabbit in order to obtain anti PRP1 polyclonal serum. Previous studies by immunoblotting using rabbit anti PRP1 indicated that PRP1 has around 180 KDa and is expressed in transfectants containig the *PRP1* gene and in wild type parasites. Similar results were obtained at mRNA level using internal primers of *PRP1* gene by RT-PCR in transfectants and in wild type *L. (L.) major*. Further analysis aiming at cellular localization of PRP1 in *Leishmania* will be held using anti PRP1 serum in immunofluorescence experiments, as well as PRP1-GFP constructs to study the role of PRP1 in *Leishmania* PEN resistance.
Supported by FAPESP, CNPq and LIM-48.

BM037 - Characterization of *meta 2* and *meta 2/GFP* overexpressing mutants of *Leishmania amazonensis*

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Both *meta 1* and *meta 2* genes of *Leishmania amazonensis* are single copy genes upregulated in metacyclic promastigotes and conserved in all *Leishmania* species analyzed to date and in some other trypanosomatids. Previous studies showed that the meta 1 protein, which encodes a 12 kDa peptide localized to the flagellar pocket, is related to the virulence of the parasite, but its function is still unknown (Uliana et. al., 1999). The meta 2 protein, characterized as a 444 amino acid peptide (48 kDa), contains three copies of the META domain, which is defined as a conserved domain present in secreted proteins of bacteria and in the meta 1 protein of *Leishmania*, followed by a sequence similar to calpain-like proteins of *Leishmania* and *Trypanosoma*. Immunofluorescense experiments using the anti-meta 2 sera produced in BALB/c mice showed that the meta 2 protein localizes to the anterior end of the flagellum and is also dispersed in the cytoplasm. In order to study the possible role of the meta 2 protein during infection, overexpressing mutants of the *meta 2* gene and of the fusion *meta 2/GFP* were obtained using the vector pSSU-int (MiBlitz et. al., 2000), that promotes the integration of the cassette into the SSU rRNA locus. The integration sites were confirmed by PCR and Southern blots. *Meta 2* mRNA expression levels in the mutant lines was shown to be increased by Northern blots and real-time PCR. *In vitro* infection experiments, carried out in J774 macrophages, showed no differences in the percentage of cells infected by control or mutant parasites. In contrast, the infection of BALB/c mice with both *meta 2* and *meta 2/GFP* mutants resulted in a 2 to 3-fold increase in the size of the lesions as compared with the wild type parasite.
Supported by FAPESP and CNPq.

BM038 - Iron superoxide dismutase (TcFeSOD) is up-regulated in *Trypanosoma cruzi* populations with *in vitro*-induced but not *in vivo*-selected resistance to benznidazole.

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Superoxide Dismutase (SOD) is a central component in oxidative defense in most organisms and functions removing excess superoxide radicals via dismutation to oxygen and hydrogen peroxide. Murta *et al.* (2002), using the Representation of Differential Expression methodology selected the TcFeSOD gene, which was overexpressed in the *T. cruzi* population resistant to benznidazole (BZ). In this work, TcFeSOD was characterized in six *T. cruzi* populations and clones susceptible and with *in vitro*-induced (17LER) or *in vivo*-selected resistance to BZ (BZR). The northern blot profile of total RNA from *T. cruzi* samples, hybridized with TcFeSOD probe revealed two transcripts, one of 1.2 Kb and other of 1.6 Kb. The *T. cruzi* drug-resistant population 17LER expressed 3-fold more TcFeSOD mRNA than 17WTS. This difference of expression was confirmed by real-time RT-PCR. The other *T. cruzi* populations and clones expressed the same level of TcFeSOD independent of their drug resistance phenotype. The results of real-time PCR and southern blot, showed that 17LER has 2-fold more TcFeSOD gene copies than 17WTS. The TcFeSOD gene can be located either in one or two chromosomes depending on the *T. cruzi* strain. Its location is drug-resistance independent but zymodeme strain dependent. It is present in the 775 and 880 Kb chromosomal bands of the 17WTS/17LER *T. cruzi* populations. Both chromosomal bands were more intense in 17LER than in 17WTS. Western blot analysis of *T. cruzi* protein extracts probed with a rabbit anti-recombinant TcFeSOD polyclonal serum revealed a unique band of 23 KDa for all *T. cruzi* strains. The intensity of this band was similar in all samples,

except 17LER, which displayed a band 2-fold more intense. Our data suggest that TcFeSOD is up-regulated in *T. cruzi* populations with *in vitro*-induced but not *in vivo*-selected resistance to benznidazole. Supported by CAPES, CNPq, FAPEMIG, PADCT, PRONEX and PAPES/FIOCRUZ.

BM039 - Identification and molecular characterization of a NOD-like gene in *Leishmania*

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NODs are members of a recently identified family of cytosolic proteins that have been implicated in the intracellular recognition of pathogens. Many of these proteins contain a leucine rich repeat (LRR) region, a nucleotide-binding domain known as NOD (nucleotide-binding oligomerization domain) and a third domain involved in a variety of functions including apoptosis regulation, activation of the nuclear factor κB and regulation of MHC class II expression (Inohara and Nunez, Nature Rev. Immunol. 3: 371-381, 2003). In the process of characterizing the genomic region of the *Leishmania amazonensis meta*1 gene (Ramos et al., FEMS Microbiol Lett, *in press*), we identified an ORF that presented similarity with the human NOD3 protein. Southern blots of *L. amazonensis* and *L. major* genomic DNA probed with a fragment containing part of the NOD-like ORF indicated that this is probably a single copy gene in both species. This fragment was also used to probe Northern blots containing RNA purified from *L. amazonensis* log and stationary phase promastigotes and amastigotes. A 3 kb transcript upregulated in amastigotes was identified. The complete nucleotide sequence of the *L. amazonensis* ORF and 3 untranslated region were obtained and compared with the orthologous *L. major* sequence (*L. major* Friedlin genome project, *LmjF17.0900*, www.genedb.org), revealing high degrees of similarity. A PCR amplified fragment encoding the NOD-like *L. major* ORF was cloned into the histidine tagged expression vector pAE. The 68-kDa recombinant protein will be purified and used to raise specific antibodies. A sequence search in the unfinished *L. major* Friedlin genome project data (http://www.genedb.org) allowed the identification of 5 further possible NOD-related genes The sequences encoding these 5 ORFs were cloned and their patterns of expression will be determined. The characterisation of these gene products will produce the necessary tools to investigate the participation of these proteins in the macrophage-parasite interaction and in the host cell invasion.
Supported by FAPESP, CAPES and CNPq.

BM040 - Overexpression of the LPG2 Golgi GDP-mannose transporter Involved in the Synthesis of Leishmania Phosphoglycans in the integrating pIR1SAT Vector

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Leishmania phosphoglycans, including the major surface glycoconjugate lipophosphoglycan (LPG), are important in interactions with both the sand fly vector and vertebrate host. The biosynthesis of LPG and related glycoconjugates takes place mainly in the Golgi apparatus, and nucleotide-sugar transporters are required to transport activated sugars from the cytosol to the lumen for their incorporation by LPG glycosyltransferases. The GDP-mannose transporter was identified in L. donovani and is encoded by the gene LPG2, and deletion mutants (lpg2-) are unable to assemble all phosphoglycans, including LPG. While the LPG2 transporter has been studied in situ, for detailed characterization we have pursued reconstitution studies of purified LPG2 in liposomes. One of the drawbacks hindering the study of these transporters is their low abundance. Here we made use of a highly active expression vector, pIR1SAT, which integrates into the rRNA genomic locus where expression is very strong. A C-terminal His-tagged LPG2 gene was inserted into pIR1SAT, and integrated into the genome of an L. major lpg- line following cleavage with SwaI to expose the rRNA locus targeting sequences. These colonies (termed L. major lpg2-/SSU:IR1SAT-LPG2-HIS) showed restoration of LPG synthesis. For comparison episomal transfectants of pXG-LPG2HIS were also generated (L. major lpg2-/pXG-LPG2HIS). Microsomes were purified from early log, log and stationary phases from both trnasfectants, and subjected to Western blot analysis with an India-His probe. With either construct, expression of microsomal LPG2-HIS was maximum in log phase and decreased in stationary phase. However, the expression of LPG2-HIS was 10-fold higher in L. major lpg2-/SSU:IR1SAT-LPG2-HIS. This result confirms the ability of pIR1SAT integration vectors to yield high levels of protein expression. This will facilitate the study of proteins whose expression requires a homologous eukaryotic and/or Leishmania system for activity, such as transporters and enzymes involved in glycoconjugate synthesis.

BM041 - *Trypanosoma cruzi* strains with a less efficient mismatch repair respond abnormally to oxidative stress

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Recent evidences have shown that the cell cycle response to oxidative stress is associated with DNA mismatch repair (MMR) efficiency. The interaction between reactive oxygen species and DNA causes a variety of lesions including 8-oxo-7,8-dihydro-2-deoxyguanosine (8-oxo-dG), which is a highly mutagenic product known to cause AT to CG trasnversions. Also, the MMR pathway appears to be associated with the repair of 8-oxo-dG oxidative lesions. Since *Trypanosoma*

cruzi strains have variations in MMR efficiency and strains with a less efficient MMR are more susceptible to oxidative stress, we decided to investigate the susceptibility of different strains to hydrogen peroxide. Subsequently, the strains were submitted to treatment with cadmium, which is known to inhibit the MMR. Higher cisplatin resistance confirms this inhibition. After MMR inhibition, all strains were equally more susceptible to hydrogen peroxide suggesting that MMR efficiency is associated with the cell survival during oxidative stress. Therefore, after hydrogen peroxide treatment we performed the quantification of 8-oxo-dG in the genome of these strains. The increase of 8-oxo-dG was more significant in the strains that were more sensitive to the hydrogen peroxide treatment. Finally, we observed that strains with less efficient MMR have a higher frequency of tranversion mutations, possibly associated with oxidative DNA damage. Taken together, our results suggest that the oxidative stress is an important source of mutagenesis in *T. cruzi*.

BM042 - Squalene Mono-Oxygenase overexpression is related to Terbinafine resistance in *Leishmania (L.) major*.

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Different mechanisms can be related to Terbinafine (TBF) resistance, a sterol biosynthesis inhibitor. We demonstrated by transfection of genomic libraries constructed in the multicopy episomal cosmid vector (cL-HYG) that leishmania resistance to TBF could be achieved by the expression of at least 6 different loci. Two of them (cTbf 2 and cTbf 4) were also capable to act synergistically with some azoles as Itraconazole or Ketoconazole (CTZ). To identify the gene(s) present in these resistant and/or synergistic loci, insert deletion were originated by partial digestion and further self-ligation, followed by DNA transfection. Functional tests in the presence of TBF and CTZ were performed on transfected wild type cells after gene over expression. Using this strategy, we were able to isolate small fragments from original inserts cTbf 1, cTbf 2 , cTbf 3 and cTbf 4, related with CTZ hypersensitivity or TBF resistance. Nucleotide sequencing reactions followed by comparative analysis with the leishmania genome project database, allowed us to determine that inserts cTbf 1, cTbf 2, cTbf 3 and cTbf 4, belong respectively to chromosomes 16, 27, 13 and 14 of *L. (L.) major* Friedlin A1. We also identified in a fragment of cTbf 4 insert that render TBF resistance after transfection on wild type cells an ORF of 569 aminoacids that codifies for Squalene Mono-Oxygenase (SqMO). Preliminary molecular analysis indicated that *SqMO* gene seems to be present in single copy in many species of the parasite as *L.(L.)amazonensis, L.(V.) brazieliensis or L.(L.) chagasi*. So far, all others loci did not shown any identity with proteins of known function. We hope soon that future biochemical and/or molecular analysis can elucidate the role of these loci on leishmania resistance. Supported by: FAPESP, WHO-TDR, CNPq and LIM-48.

BM043 - Primary structural features of the 20S proteasome subunits of *Trypanosoma cruzi*

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The 20S proteasome is the proteoliytic complex that is involved in removing abnormal proteins, and it also has other diverse biological functions. Its structure comprises 28 subunits arranged in four rings of seven subunits, and exist as a hollow cylinder. The two outer rings and two inner rings form an $\alpha 7\beta 7\beta 7\alpha 7$ structure, and each subunit, α and β, exists as seven different types, thus giving 14 kinds of subunits. In order to identify gene products in *T. cruzi* sharing similarities with proteasome subunits we queried the data set of the reference clone of *Trypanosoma cruzi* Genome Project, the CL-Brener. By using the in silico analysis we were able to design the molecular organization of the 20S proteasome from this parasite. Through the analysis of a collection of cDNA and genomic clones, we identified a superfamily of 14 genes encoding proteasome alpha and beta subunits. Amino acid sequence homology of the subunits within the *T. cruzi* family (23-38%) were lower than those corresponding orthologs from yeast and human (65-88%). Structural features observed in eukaryotic proteasome subunits, like β type signature at the N-termini, Thr active sites in $\beta 1$, $\beta 2$ and $\beta 5$ subunits, and nuclear localization signal-like sequences in some β type subunits, were shown to be conserved in *T. cruzi*.

BM044 - Identification of components of the ubiquitination machinery in *Trypanosoma cruzi*.

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Ubiquitin is a small protein, highly conserved among eukaryotes, that becomes covalently attached to both itself and a variety of celullar proteins. The conjugation of ubiquitin to other cellular proteins regulates a range of eukaryotic cell functions, like 26S proteasome degradation pathway, chromatin remodeling, DNA repair, transcription regulation, receptor modulation and immune response. The high efficiency and seletive ubiquitination reactions reflect the properties of enzymes known as ubiquitin activating enzymes (E1), ubiquitin- conjugating enzymes (E2s) and ubiquitin-protein ligases (E3s). The main objective of this work was in silico analysis of the *Trypanosoma cruzi* Genome Project and the identification of genes that coding enzymes of ubiquitination pathway. Based on this analysis, we have identified 126 clusters that presented an alignment to known GenBank or-

tholog proteins through BLASTx covering at least 95% of our sequence with the ubiquitination enzymes. Among these, 3 contig has high homology with E1 enzyme (covering of 95%), 20 had a conserved core domain consisting of $\geq$ 150 amino acids and 14 contigs had 100% coverage of the E2-17 kDa, E2-17kDa v.1, UBC7 and UBC13, specific for ubiquitin conjugation. Also were identified 2 contigs with 100% coverage to E2 ubiquitin like protein specific (SUMO and Nedd8). A total of 8 clusters have high similarity with HECT-domain E3s and RING E3s. The results reported here demonstrate that *T.cruzi* express several orthologues genes related with the ubiquitination machinery. Given that the physiological pathways regulated by ubiquitin and biological roles, our future research will address of expression patterns studies and the analysis of developmentally regulation of this gene family not understanding in trypanosomatid parasite.

BM045 - Single allele knockout and Over-Expression of *Leishmania (L.) amazonensis Glucose-6-Phosphate Dehydrogenase*

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Glucose-6-Phosphate Dehydrogenase (G6PD) is the key enzyme in the pentose shunt, which, among other products, reduces NADP to NADPH. This nicotinamide coenzyme takes part in cellular detoxification of reactive oxygen and nitrogen species. G6PD is a valuable agent in the regeneration of its reduced form and important for cell survival in situations of oxidative stress in different models, such as bacteria, fungi and mammalian cells. We have recently characterized the coding region of *L. (L.) amazonensis G6PD* that showed to be an interesting target in the identification of *Leishmania* (Castilho *et al* ,2003). The nucleotide sequence obtained could then be used to generate *Leishmania* mutants with depleted or increased activity of *G6PD*, providing tools to evaluate its physiological importance to the parasite.
The increase of activity was obtained by episomal expression of *La*G6PD. Initially a fragment containing the *L. (L.) amazonensis* ribosomal promoter (Uliana *et al* , 1996), a synthetic *trans*-splicing site and the *hygromycin* resistance marker were cloned upstream of the previously characterized *arginase* (da Silva *et al* , 2003) trans-splicing site and 5' UTR. That construction (pLaPribhygargss) was successfully used to select transfected *L.(L.) amazonensis* resistant to hygromycin. Then, *LaG6PD* ORF was cloned downstream the *arginase* fragment (pLaPribhygargssG6PD construct). RT-PCR, northern blot, and activity assays indicated that the mutants express the episomal *G6PD* correctly, reaching to 3 fold activity.
The depletion of G6PD activity is being attempted by knocking out the *L. (L.) amazonensis G6PD*. To obtain the first allele knockout, the homologous recombination was conducted to introduce the *neomycin* gene replacing, in frame, the catalytic domain of the enzyme. The analysis of a northern blot indicates the existence of a second mRNA containing *G6PD* bigger then the wild type *G6PD* mRNA, that correlates with the insertion of *neomycin* gene.
Supported by CNPq and FAPESP

BM046 - ATPase ACTIVITY OF MSH2 PROTEINS FROM *TRYPANOSOMA CRUZI* MAY CORRELATE WITH DIFFERENCES IN MISMATCH REPAIR EFFICIENCY OBSERVED AMONG VARIOUS PARASITE STRAINS

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Recent studies from our group based on *MSH2* SNPs showed that the *Trypanosoma cruzi* species can be divided into three haplogroups, denominated A, B and C. Furthermore, studies of microssatelite instability and cisplatin resistance assays indicated that strains belonging to MSH2 haplogroup A have a more efficient mismatch repair (MMR) activity than haplogroup B and C strains. PCR amplification and sequencing of a multi copy *T. cruzi* antigen, TcAG48, showed that strains belonging to haplogroups B and C display greater TcAG48 genetic variability when compared to haplogroup A strains. It should be noted that strains belonging to haplogroups B and C are responsible for the chronic cases of Chagas disease in Brazil. Since MSH2 plays a central role in MMR, we decided to investigate whether aminoacid differences found between the haplogroups could result in functional differences in this protein. Hence, the *MSH2* of two *T. cruzi* strains, Colombiana (haplogroup A) and CL Brener (haplogroup B) was amplified and sequenced. We found that 10% of aminoacid substitutions are located in regions described as important for the protein structure or its functional activity. Recombinant MSH2 from both strains has been expressed and purified from *E. coli* cultures and their *in vitro* activity was compared using ATPase assays. Our preliminary results suggest that the MSH2 from Colombiana possesses a higher intrinsic ATPase activity compared to the CL Brener isoform, indicating that differences in MMR efficiency found between *T. cruzi* haplogroups are related to differences in MSH2 ATPase activity. Other experiments need to be carried out with different protein samples in order to confirm this. These findings are consistent with our hypothesis that predicts that polymorphisms in the *MSH2* gene may determine metabolic differences in the MMR pathway among *T. cruzi* strains and that this could be an important source of antigenic diversity in this parasite.

BM047 - Characterization of a *Trypanosoma cruzi* RAB-Like protein with GTPase activity

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RAB GTPases regulate exocytic and endocytic pathways of eukaryotic cell, controlling vesicle docking and fusion. Here we report the characterization of TcRABT, a RAB-Like of *Trypanosoma cruzi* that was shown to bind and hydrolyze GTP at higher levels when compared to other *T. cruzi* RABs. The TcRabT ORF predicts a 183 amino acid polypeptide and a 20.5 kDa protein that shares the highest values of similarity and identity with human and rat RAB23, presenting all the five GTP-binding domains. However, the cystein residues at the C terminus, carried by all members of RAS superfamily, essential to isoprenylation, an prerequisite for the membrane association, are not present in TcRABT. This finding may indicate that TcRABT may represent a novel member of a small GTPase family as it does not seem to be a typical RAB, neither an obvious member of another family. TcRabT presents an open reading frame of 552 bp, is present in a discrete number of copies, as shown by Southern Blot, located on a XIII chromosomal band (1,43 Mb chromosomal DNA) by PFGE. TcRabT is transcribed as a single 1.2 kb mRNA in epimastigotes. Its transcript, observed by RT-PCR, was equally abundant in epimastigotes and spheromastigotes forms and much less in metacyclic trypomastigotes. Polyclonal antibodies raised against a polypeptide of the TcRABT C-terminus were able to recognize a 20 kDa band in *T. cruzi* strains as well as a 49 kDa band corresponding to the GST fusion protein on Western Blotting. Polyclonal antibodies against TcRABT are being raised in mice and the purified serum will be used in immunolocalization assays. TcRABT fused to GFP will be superexpressed in epimastigotes in order to localize the protein. All the differences between TcRABT and other RABs make TcRABT a particular and good model of study of the *T. cruzi* endocytic pathway.

This work was supported by CNPq and FAPERJ

BM048 - Characterization of a Trypanosoma cruzi gene encoding an antigen involved with cellular trafficking

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In this study we propose to characterize two cDNAs isolated from a Trypanosoma cruzi library of amastigote forms screened with sera from chagasic patients. Sequence analyses of these cDNAs, denominated TcAG38 and TcAG49 show that they present significant homology with VIP36 and ERGIC 53, which are eukaryotic proteins involved with cellular sorting or recycling. TcAG38 cDNA contains an ORF of 550 amino acids, in which a microssatelite element encodes a poli-Glu region located at the C-end of the protein. PCR amplifications with DNA from different strains of parasite revealed a polymorphism encompassing the microssatelite region of the gene. Southern blot and sequence analyses from the T. cruzi genome database (www.geneDB.org) indicated that this antigen is encoded by a single copy gene in the genome of the parasite. Thus, the two distinct cDNA isolated from the library correspond to two polymorphic alleles. Northern blot analysis revealed that TcAG38 is constitutively transcribed into a 2,5 kb mRNA, which is slightly more abundant in amastigote than epimastigote and trypomastigote forms. After expressing the recombinant protein as a GST fusion in E.coli, using the pGEX vector, the GST::pAG49 protein was purified and it was used to immunize mice. The antibody obtained recognizes the two isoforms of this protein in parasite protein extracts. Interestingly, sera from chagasic patients was shown to recognized the purified fusion proteins only in ELISA and immunoprecipitation assays, but not in Western blot assays. The localization of this antigen in the parasite was also investigated using the anti-pAG49 antibodies we generated in mice as well as by transfecting epimastigote with a vector encoding the protein tagged with GFP (pAG38::GFP). In both experiments, a distribution near the flagellar pocket, co-localized with the kinetoplast and Golgi region, was determined.

BM049 - The Putative Telomerase Reverse Transcriptase Component of *Leishmania* spp. : Gene Cloning and Characterization

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Telomeres are the protein-DNA complexes that protects the ends of linear chromosomes. In *Leishmania* spp., the telomeric DNA consists of the conserved TTAGGG repeated sequence and is replicated by the action of telomerase. Telomerase is a multisubunit complex composed by a reverse transcriptase component (TERT), an intrinsic RNA molecule (TER) and associated proteins. It ensures the complete DNA replication adding new telomeric sequences to the G-rich strand. It also works as part of the higher order complex that regulates the capped/uncapped telomere states. A search for sequences sharing similarities with conserved TERT motifs at the public *Leishmania* Genome database resulted in a sequence whose translation contained an open reading frame of $\sim$ 1101 amino acids with all the canonical TERT motifs. A PCR-based cloning strategy was used to obtain products spanning the entire ORFs of putative *L. major*, *L. amazo-*

nensis, *L. braziliensis* and *L. donovani* TERTs. Analysis of similarities by ClustalW multi sequence alignment revealed that they are $\sim$ 85% identical, suggesting that in *Leishmania*, telomerase is very conserved. In addition, the size and sequence of *L. amazonensis* genomic and cDNA PCR products were identical. Southern blotting analysis indicated that this putative TERT sequence is present in all parasite species in a low copy number and that in *L. major* and in *L. amazonensis* it seems to be located in a band corresponding to chromosome 36. RT-PCR products were amplified with different primer sets and confirmed that *Leishmania* TERT is expressed in log phase cells. Phylogenetic analysis of separate portions of known TERTs, including the putative *Leishmania* TERTs, based on the N-terminal and reverse transcriptase motifs follows the evolutionary relationships already described. Financial support: FAPESP, WHO/TDR - UNDP Bank.

BM050 - Differential survival of *Trypanosoma cruzi* in heat shock temperatures after gamma irradiation

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Trypanosoma cruzi is the human parasite that causes Chagas' disease. Only a few genes involved in DNA metabolism have been described in this species. Recently our group has found evidences indicating the capacity of the parasite to perform DNA break repair by homologous recombination. In these studies, epimastigote cultures of the CL-Brener strain were gamma irradiated at 500 Gy in order to induce breaks in DNA and their genome was analyzed through "Pulsed Field Gel Electrophoresis"(PFGE). The gel data indicate loss of chromosomes and the occurrence of recombination events. We also observed that the irradiated cultures showed higher sensitivity under warmer temperatures. To better investigate these changes, we submitted the irradiated culture and the wild type strain to heat shock conditions. The cultures were maintained for at least five days at the following temperatures: 28°C, 30°C and 37°C, 28°C being the standard growth temperature, while 30°C and 37°C were considered to be heat shock conditions for epimastigotes. Despite the fact that both cultures presented similar growth at 28°C, at 30°C and 37°C the number of parasites of the irradiated culture was lower than the wild-type. These results suggest that the irradiated strain may have changed the pattern of gene expression, most likely for genes related to the heat-shock (HS) response. We speculated that, in irradiated cultures, altered expression of HS genes could have happed as a consequence of translocation events within these loci in their genome. To test this hypothesis, Northern Blot analyses to evaluate differences in the amount of transcripts of HS genes in the irradiated cultures are currently under way.

BM051 - Phylogenetic evidence of introgression in the *T. cruzi* I and *T. cruzi* II complex using direct sequence comparison of five genetic markers of hybrid subgroups " rDNA 1/2 " and " Z3 "

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Trypanosoma cruzi has sufficient genetic diversity to be considered a complex of at least two different species. These two main "groups" named *T. cruzi* I and *T. cruzi* II were defined by phylogeny and not merely by typing studies. Additional subgroups of strains have been described, such as "1/2" (rDNA 1/2) and "Z3"(zymodeme 3), whose positioning within the *T. cruzi* I and II complex is still controversial. In this study, we cloned and sequenced five genes that encode the EF-1α (Elongation Factor-1 alpha); actin; DHFR-TS (dihydrofolate reductase-thymidylate synthase); TR (trypanothione reductase); and 18S rRNA from 25 *T. cruzi* strains. Sequences were used to infer phylogenies and polymorphism. Our phylogenetic study shows that *T. cruzi* I and *T. cruzi* II groups are monophyletic, different from 1/2 and Z3 subgroups, which cluster with either *T. cruzi* I or *T. cruzi* II strains as also observed for CL Brener. These results suggest that these strains are hybrids of *T. cruzi* I and *T. cruzi* II parental lineages. The localization of the hybrid group haplotypes in most of phylogenies and their identity with sequences of *T. cruzi* I and *T. cruzi* II suggest that these groups have resulted from introgression of *T. cruzi* II into *T. cruzi* I. Also, our study shows a higher polymorphism in subgroups "1/2", "Z3" and CL Brener. The hybrid nature, high polymorphism and wide geographic distribution of subgroups "1/2" and "Z3" suggest that the hybrids, generated by sporadic introgression, have a selective advantage in the *T. cruzi* species complex.
Financial support: FAPESP, Howard Hughes Medical Institute, CNPq.

BM052 - FUNCTIONAL ANALYSIS OF TWO PUTATIVE eIF4A HOMOLOGUES IN Trypanosoma brucei.

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In higher eukaryotes initiation of protein synthesis starts with the binding of the translation initiation complex eIF4F (eIF4E, eIF4G and eIF4A) to the methylated cap present on the 5'end of mRNA. In trypanosomatids the presence of a highly modified cap (cap4) plus the spliced leader sequence on the 5'end of all mRNAs, suggests the possibil-

ity of differences in the way mRNA is recruited for translation. To investigate translation initiation in trypanosomatids, we have identified two putative eIF4A homologues in the Trypanosoma brucei genome sequence, here called TbEIF4A1 and TbEIF4A2, with 63% and 56% identity to human eIF4A respectively. In order to study the functional role of both T. brucei eIF4A homologues, these genes were amplified and cloned into different plasmid vectors allowing: protein expression in Escherichia coli/antibody production; expression of fluorescent YFP fusion proteins in transfected parasites for intracellular localization; RNA interference (RNAi). **Quantification**: Both proteins are present in procyclic and bloodstream parasite stages at constant concentrations, however TbEIF4A1 is at least a hundred times more abundant than TbEIF4A2. **Intracellular localization**: Despite the high homology between both homologues (69% identity), TbEIF4A1 is localised to the parasite cytoplasm whilst TbEIF4A2 is confined into the nucleus. **RNAi**: After 24hs of RNAi induction the cells expressing TbEIF4A1-dsRNAs begin to die in large numbers and after 48hs translation is completely abolished, confirming the essential function of TbEIF4A1 in parasite protein synthesis. In contrast, cells expressing TbEIF4A2-dsRNAs do not show any growth retardation or cell death effect. Interestingly, they exhibit a huge reduction in motility and change in morphology after some weeks of maintenance under RNAi induction. We are currently determining the extent to which these factors are associated with actively translating mRNAs in different stages of trypanosomatid species and developing Tap-Tag technology to isolate a putative parasite translation initiation eIF4F complex in vivo.

BM053 - Signal transduction pathways involved in host cell invasion by *T. cruzi* amastigotes of strains from the two major phylogenetic lineages

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Mammalian cell invasion by *Trypanosoma cruzi* requires the activation of signal transduction pathways that result in a Calcium response both in the parasite and host cell. Recent studies with different T. cruzi isolates have grouped the parasite in two major lineage phylogenetic lineages: *T. cruzi* I linked to the sylvatic cycle of the parasite and other mammalian hosts, and *T. cruzi* II associated with the domestic cycle and human disease. Besides genetic markers *T. cruzi* I and II infective forms engage characteristic signaling pathways upon invasion of cultured cells that correlate with distinct infectivities. By using drugs that interfere with specific signaling processes, we investigated if the difference in the ability of *T. cruzi* strains to invade host cells was associated to activation of specific signaling routes in the parasite. Experiments were performed with Extracellular Amastigotes (EA) derived from axenic differentiation of tissue-culture trypomastigotes, using G ($T.\ cruziI$) and CL ($T.\ cruziII$) strains. Treatment of parasites with adenyl cyclase activator forskolin increased the infectivity of both G and CL EA towards HeLa cells. On the other hand genistein, a specifc protein tyrosine kinase inhibitor reduced by approximately 17% the invasion of G but not of CL strain EA into HeLa cells. Treatment with: phospholipase C inhibitors (i) U73122 and (ii) neomycin; with drugs such as (iii) caffeine that affects calcium realease from inositol-1,4,5-triphosphate-sensitive stores, or (iv) thapsigargin, an inhibitor of intracellular calcium transport ATPases, did not significantly affect the infectivity of either strain. Treatment of parasites with ionomycin plus amonium chloride or nigericin that release calcium from acidic vacuoles containing a calcium/proton exchange system (*acidocalcisomes*) reduced the infectivity of AE of both strains. Taken together, these data suggest that for host cell invasion G and CL strains amastigotes engage signaling pathways that lead to an enhancement of cAMP whereas calcium would be mobilized from acidocalcisomes through a hypothetical channel regulated, in theory, through a cAMP-dependent protein kinase. Financial support: FAPESP, CNPq, and CAPES.

BM054 - QUANTIFICATION OF MULTIPLE HOMOLOGUES CODING FOR eIF4F SUBUNITS IN Leishmania major.

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In eukaryotes protein synthesis starts with the binding of the translation initiation complex eIF4F to the cap present on the 5' end of the mRNAs. This complex allows the recognition of the mRNAs by the small ribosomal subunit and the initiation of translation. eIF4F is composed by three subunits: eIF4A, a RNA helicase; eIF4E, the cap binding protein; and eIF4G, a large scaffolding protein. In trypanosomatids the presence of a modified cap plus the spliced leader on the 5' end of the mRNAs suggests the existence of differences in the way the mRNA is recruited for translation. However little is known about the translation machinery in these pathogens. Our research has as its major objective the study of the initiation stage of protein synthesis in the trypanosomatids, focusing on the characterisation of subunits of the eIF4F complex. First we identified within the databases generated by the *Leishmania major* genome sequences homologues to the three eIF4F subunits. We found four eIF4E sequences (LmEIF4E1-4), two eIF4As (LmEIF4A1-2) and five eIF4Gs (LmEIF4G1-5), some of which have been reported previously. Here we describe the expression analysis and quantitation of LmEIF4E1-3, LmEIF4A1-2 and LmEIF4G3 in *L. major* promastigotes. Their genes were amplified, cloned and expressed in *E. coli*. Isoform specific antibodies were then produced in rabbit

and tested in Western blots against the various recombinant proteins. They were then used to analyse the expression and to estimate the intracellular levels of the various homologues in extracts of *L. major* promastigotes. With the exception of LmEIF4A2 all the other proteins were detected, however their levels varied considerably. LmEIF4A1 and LmEIF4E3 are very abundant (more than $5x10^4$ molecules/cell), LmEIF4G3 is moderately abundant (about $5x10^3$-10^4 molecules/cell) and LmEIF4E2/LmEIF4E1 are rare (less than $5x10^3$ molecules/cell) . These results are consistent with LmEIF4A1, LmEIF4E3 and possibly LmEIF4G3 playing relevant roles in translation at least in the promastigote stage.

BM055 - Cloning and characterisation of a gene enconding a cathepsin B-like protease from *L. (L.) amazonensis*.

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The amastigote stage of *Leishmania* expresses high levels of cysteine proteinase (CP). Most of the cysteine proteinase activity in *Leishmania* is related to the developmentally regulated expression of multicopy genes encoding cathepsin L-like proteinases well characterised in *L. (L.) mexicana*, as *CPA* and *CPB* genes (Mottram et al., Curr. Opin. Microbiol. 1: 455, 1998). In contrast, few genes encoding cathepsin B-like proteinases from *Leishmania* have been described. These genes are constitutively expressed as single copy genes in *L. (L.) mexicana*, *L. (L.) major*, *L. (L.) chagasi*, and *L. (L.) donovani*. The cathepsin B-like proteinases from *L. (L.) chagasi* and *L. (L.) donovani* have been shown to increase the parasite's survival within the host macrophages (Somanna et al., J. Biol. Chem. 277:25305, 2002). In this report we describe the cloning and characterisation of a gene encoding a cathepsin B-like protease from *L. (L.) amazonensis*. Primers derived from the *L. (L.) major* cathepsin B were used to amplify a fragment used to probe a genomic cosmid library from *L. (L.) amazonensis*. Positive clones were mapped, subcloned and sequenced. Southern blot analysis revealed that the *L. (L.) amazonensis* cathepsin B gene is present in a few copies in the parasite genome. The nucleotide sequence analysis showed a high degree of identity to cathepsin B-like from *L. (L.) mexicana*, *L. (L.) donovani* and *L. (L.) major*. A 1023 nt ORF was predicted encoding a 341 amino acid putative protein. Expression of the gene resulted in a recombinant protein of 15 kDa lacking the signal peptide. Plasmid constructs to overexpress cathepsin B have been obtained and transfected in *L. (L.) amazonensis*. Selection of stable mutants is in progress and their characterisation will be performed by *in vitro* and *in vivo* infection experiments. Supported by FAPESP and CNPq.

BM056 - Gene characterization and expression of the Universal Minicircle Sequence Binding Protein of *Trypanosoma cruzi*(PDZ5)

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Kinetoplast (kDNA) is a network-like structure that represents the mitochondrial DNA of the order kinetoplastidae, which encloses a variety of unicellular parasites, such as *Trypanosoma cruzi* and *Crithidia fasciculata*. This DNA network is composed of two different molecules called maxicircles and minicircles. Minicircles replication starts at the Universal Minicircle Sequence (UMS), a conserved nucleotide sequence represented by the dodecamer 5'-ggggttggtgta-3'. In *C. fasciculata* there is a well characterized protein that binds to the UMS and is thought to recruit the replication machinery. This Universal Minicircle Sequence Binding Protein (UMSBP) localizes in the kinetoflagelar zone. We have characterized the *T. cruzi* UMSBP, which we have named PDZ5. It is a 134 aminoacids zinc finger protein, homologue to *C. fasciculata* UMSBP. Western blotting analysis using anti-*C. fasciculata* UMSBP antibodies revealed that the *T. cruzi* endogenous PDZ5 is about 14 kDa. Eletrophoretic mobility shift assay indicated that recombinant PDZ5 binds specifically to the Universal Minicircle Sequence. We have investigated pdz5 gene organization and expression. PDZ5 is present as a single copy gene in the chromosomal band XX of *T. cruzi* genome, downstream to the proteasome beta 5 subunit gene and upstream the pzfp1 gene. We have mapped the trans-splicing and polyadenylation sites of pdz5 1 kb mRNA, which is transcribed as an polycistronic mRNA along with the proteasome subunit gene. There is a great allelic polymorphism in this locus represented by a 62 bp deletion on the proteasome-pdz5 intergenic region and an approximately 2 kb deletion, which localizes upstream to the proteasome subunit gene. This allelic polymorphism is present in some, but not all, *T. cruzi* strains. We are now analyzing the possible participation of these polymorphisms in differential control of alleles expression and the possible correlation of this polymorphism distribution pattern among *T. cruzi* strains and their division in two different lineages.

BM057 - Evaluation of the Genetic Polymorphism in *Leishmania amazonensis* Isolates.

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Parasites of the species *Leishmania amazonensis* have been associated with cases of cutaneous, mucosal, visceral and diffuse cutaneous leishmaniasis. Previous studies have demonstrated that mice infected with isolates from visceral leishmaniasis develop a pattern of disease characterized by small lesions with a large number of lymphocytes, granuloma formation, few infected macrophages and no metastases, contrasting to the disease caused by infection with isolates from

cutaneous and mucosal clinical manifestations. These observations have led us to investigate the genetic polymorphism of *L. amazonensis* strains. In order to do so, *L. amazonensis* strains isolated from patients with different clinical manifestations of leishmaniasis were analyzed using molecular techniques such as random amplification of polymorphic DNA (RAPD), simple sequences repeat anchored PCR (SSR-PCR), pulsed field gel electrophoresis (PFGE) and by in vitro growth rate analysis. The RAPD, SSR-PCR and PFGE profiles were used to generate dendrograms based on a distance matrix using unweighted pair-group method with arithmetic averages (UPGMA). Results presented here show that visceral and mucosal leishmaniasis isolates were grouped into different clusters in relation to the strains obtained from patients with other clinical manifestations of leishmaniasis. Moreover, we also observed a significant difference in the in vitro growth rate of visceral isolates when compared to isolates from cutaneous leishmaniasis. Collectively, these results indicate that there is an association between genetic fingerprints and clinical manifestations of disease caused by *L. amazonensis*.

Supported by CNPq, FAPESB and PAPES-FIOCRUZ.

BM058 - Structural organization of TcTREZ0 (*Trypanosoma cruzi* Tandem Repetitive Element Z0) in the genome of *Trypanosoma cruzi*

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The repetitive DNA fraction accounts for 44% of the nuclear genome of *Trypanosoma cruzi*. Repetitive elements, such as retrotransposons and tandem repeated sequences, may play an important role in the control gene expression and genomic rearrangements. In a previous communication [Souza et al. 2003, *Rev Inst Med Trop* USP 45 (Suppl. 13):178] we described the isolation and partial characterization of the TcTREZ0 (*T. cruzi* Tandem Repetitive Element Z0), a 1.6-kb repeated element arranged in tandem arrays in the genome of *T. cruzi*. Here we present further structural characterization of this element.

The copy number of TcTREZ0 was estimated to be 2,110 copies per haploid genome (50 Mb), representing 4% of the nuclear genome of clone CL Brener. TcTREZ0 was mapped to 13 chromosomal bands of clone CL Brener. However, strong hybridization signals were detected with megabase bands (3.5 to 1.5 Mb) suggesting that these bands carry most of TcTREZ0 elements in the genome. Distribution of TcTREZO elements in megabase chromosomes was analyzed using YAC clones derived from chromosomes XVI, XVII and XX of clone CL Brener. TcTREZ0 elements were distributed over a 1.3-Mb region of chromosome XX, and a 670 kb-region from chromosome XVI. This result was confirmed by fiber FISH (Fluorescent *In Situ* Hybridization) analysis using TcTREZ0 as a probe. Moreover, this experiment showed that the average size of blocks carrying tandem arrays of TcTREZ0 is 5 to 15 kb.

The 1.6-kb *Eco*RI fragment containing TcTREZ0 was found in several *T. cruzi* strains (Sylvio X-10, G and Y) indicating that this element is conserved among isolates of lineages I and II. BLAST search of sequences in GenDB database (http://www.genedb.org) showed the presence of partial homologous sequences to TcTREZ0 in other trypanosomes (*Trypanosoma brucei* and *Trypanosoma vivax*) and *Leishmania major*.

Supported by FAPESP.

BM059 - CHARACTERIZATION OF 14-3-3 SIGNALING MODULE PROTEINS IN THE HUMAN PARASITE *TRYPANOSOMA CRUZI*

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The protozoan parasite *Trypanosoma cruzi* is the causative agent of Chagas Disease- a serious illness that leads to potentially fatal heart or digestive pathology in up to one-third of cases. The World Health Organization estimates that 16-18 million people in Latin America are currently infected and another 100 million people are at risk of infection. Although diagnostic tests have been developed, there is currently no effective drug to treat chronic infections. This is due in part to the fact that little is understood about the complex and unique molecular biology of the parasite and the pathways and events associated with host cell invasion. One approach to clarify events leading to pathogenesis is to study signaling pathways in trypanosomes. Because this parasite's genome is currently being sequenced and reporter constructs are now available, it is possible to use molecular biology techniques to study this organism. Since 14-3-3 genes are known to function in a wide variety of cellular processes in other parasites as well as in all eukaryotes, we feel that studying the function of these genes in *Trypanosoma cruzi* will lead to a better understanding of how this parasite adapts to its host environments. We have identified, fully sequenced, and cloned two 14-3-3 genes in this organism. Currently we are determining if these genes can complement a 14-3-3 yeast knockout strain. Preliminary results indicate that the trypanosome genes can partially restore growth in the yeast 14-3-3 deletion mutant. We also plan to observe responses to stressors such as caffeine, sorbitol, and rapamycin. Future research will include identification of interacting proteins and construction of *T. cruzi* knockout strains in order to determine the role of 14-3-3 in adaptation to host cells.

BM060 - RNA binding protein genes and an intercalated gene of unknown function are differentially expressed in *Trypanosoma cruzi* life cycle

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Trypanosomes are a group of eukaryotic organisms with

many unusual characteristics in their molecular biology; so the identification and characterization of RNA binding proteins in *Trypanosoma cruzi* is particularly relevant as they play key roles in the regulatory mechanisms of gene expression. In this work, we have identified coding sequences for the proteins, named TcRRM1 and TcRRM2, in the EST database generated by the *T. cruzi* Genomic Initiative. TcRRM1 and TcRRM2 contain two RNA binding domains (RRM) and are very similar to two *Trypanosoma brucei* RNA binding proteins previously reported, Tbp34 and Tbp37 and to a not yet annotated ORF in *Leishmania major* genome project. The *T. cruzi* RRM genes are organized in a tandem of at least 7 copies, alternating with copies of Tcp28, a gene of unknown function. However TcRRM transcripts accumulation is higher in the spheromastigote stage, while Tcp28 transcripts accumulate more in the trypomastigote stage suggesting developmental regulation. Preliminary imunofluorescence assays localize the RBP genes in the cytoplasm of the parasite cells, unlike the *T. brucei* orthologs, which localize in the nucleus. Both TcRRM and Tcp28 genes were cloned in pGEX vectors and expressed in *E.coli* strains as fusion proteins. We are now, producing these proteins in large-scale for functional assays.

BM061 - IDENTIFICATION OF THE HEAT SHOCK ELEMENT(S) IN THE POST-TRANSCRIPTIONAL REGULATION OF HSP70 GENES OF Trypanosoma cruzi.

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Kinetoplastid show unusual mechanisms of RNA processing, such as trans-splicing, polycistronic transcription and RNA editing, and a predominance of regulation of gene expression at the post-transcriptional level. Post-transcriptional control of gene expression have been shown in kinetoplastids, as in other eukaryotes, to be mediated by sequence elements present in untranslated regions (UTRs) of mRNAs and/or intergenic regions. The HSP70 genes of *Trypanosoma cruzi* are organized as 7-10 copies arranged in tandem, and the protein is synthesized at normal temperatures. Upon heat shock, both HSP70 synthesis and mRNA levels are increased in a transcription-independent manner. As a first step to identify the heat shock responsive elements, the HSP70 trans-splicing acceptor and clevage/polyadenylation sites were identified. We found a major and a minor trans-splicing acceptor site, and three distinct polyadenylation sites. In addition, analysis of several 3UTR sequences cloned by RT-PCR shows polymorphism of the length of a central TTA repeat region. In an attempt to find the heat-shock elements in the HSP70 UTRs, plasmids constructs containing the chloranfenicol acetyltransferase reporter gene flanked by segments of the HSP70 intergenic region that contain the 5UTR and 3UTR together with their respective processing signals were constructed. Rab7 UTR-containing reporter plasmids are being used as control. In all constructs transcription is directed by a sequence containing an active 18S rRNA promoter. CAT enzymatic activity and mRNA levels resulting from transfection of the plasmid constructs are being determined. We are also determining the half-life of the endogenous *T. cruzi* HSP70 mRNA under stress and non-stress conditions.

Supported by CNPq, FAPERJ and FUJB.

BM062 - Molecular characterization of the gene that codifies a ribosomal protein in *Blastochritidia sp*

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The ribosomes, as well as spliceosomes, are formed by ribonucleoproteins. The structural organization of the two ribosomal subunits, which are formed mainly by RNAs and 70-80 different ribosomal proteins, reflects a complex mechanism of action. The ribosomal proteins, L17 and L27, are localized in the base of the 50S subunit central protuberance, in the peptidil site of the ribosome, being necessary during the junction of the subunits and formation of the peptide linking during the process of protein synthesis. In attempt to clone genes related to ribonucleoproteins by using immunoscreening of cDNA *Trypanosoma cruzi* library and anti-Sm affinity-purified antibody, surprisingly it was possible cloning and expression of a ribossomal protein (TcrL27) that still had not been described in *T.cruzi*. By RT-PCR it was possible to confirm the presence of this gene in different strains of *T. cruzi* and *Leishmania major*. The same approach has been used to clone the gene in *Blastocrithidia sp* using the total RNA in 3'RACE System with oligonucleotide directed to 5'-UTR region designed from TcrL27 protein. However, a 550bp-PCR product was obtained being cloned and sequenced and the analysis in NCBI database showed high identity (88%) with the L17 protein of *Leishmania infantum* and 79% of identity with the same protein of *T.cruzi*. RAPD analysis with genomic DNA using TcrL27 primer and the three parasite species demonstrated no amplification with *Blastochritidia sp*as expected. These results may be explained by phylogenetic distance among these Trypanosomatids. The Bl-L17 gene will be subcloned in an expression vector for better characterization of the protein in these parasites. LRM is supported by FAPESP (proc. No. 03/013830-0). The work was partially supported by PADC/FCF-UNESP

BM063 - Origin of clonal diversity in natural isolates of *Plasmodium falciparum*

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Selectively neutral genetic markers are the most appropriate tools for analyses of genetic diversity and molecular epidemiology of parasite populations. Here we assessed pat-

terns of clonal diversity in sympatric isolates of *Plasmodium falciparum* to test whether most clones recovered from a single isolate, in areas of low to intermediate levels of malaria transmission, are genetically related. A total of 117 clones were obtained, through limiting dilution experiments in vitro, from eight *P. falciparum* from Ariquemes, Rondônia and typed for eleven microsatellite markers. A PCR strategy with end-labeled primers was used to amplify each single copy locus; PCR products were analyzed for size and fluorescence intensity on an ABI377 automated sequencer using the GENESCAN software. Data from samples with poor amplification of particular loci (peak height $\prec$ 200 fluorescent units) were discarded. Six of eight isolates contained multiple clone infections, containing 2 to 5 genetically distinct parasite populations. However, within-isolate diversity was relatively limited, with most clones sharing 9 to 10 alleles. These data suggest that clones co-infecting the same host do not represent a random sample of clones circulating in the area; most multiple clone malaria infections in this area seem to have originated from co-inoculation, by infected mosquitoes, of genetically related parasite populations. Support: FAPESP and CNPq.

BM064 - Characterization of snRNAs and Sm proteins in *Trypanosoma cruzi*

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Some important factors in functioning of the eucariotic cells are the small complexes of RNA and proteins; these particles of ribonucleoproteins (UsnRNPs) have an essential role in the pre-mRNA processing, mainly during splicing (cut of introns and union of exons). UsnRNPs present a common protein core associates between itself and with the snRNA, called Sm proteins, and specific proteins of each snRNP. Seven proteins (Sm B/B', - D1, - D2, - D3, - E, - F and - G) are joined forming ring-like structure into the snRNPs. Even though they are well defined in mammals, snRNPs are still not characterized in certain Trypanosomatids as *Trypanosoma cruzi*. Our preliminary results had demonstrated that the use of DEAE-Sephacell column allowed to concentrate these proteins of the nuclear extract from *T. cruzi* epimastigotes forms and after Western-blotting with antibodies anti-Sm (unidirectional electrophoresis) bands range of 15 to 60 kDa as expected were showed. These proteins have been analyzed by two-dimensional electrophoresis and Western-blotting with anti-Sm antibody to localize the common protein core, that later will be characterized by mass spectrometry. Also, other related studies are being carried through the laboratory with the purpose to study *T. cruzi* UsnRNAs (U1, U2, U4, U5, U6) using RT-PCR with primers designed after BLAST of *T. brucei* sequences with the available *T. cruzi* genome database. Until this moment, *T. cruzi* (Y strain) U2 and U5 snRNA were cloned and sequenced. Supported by FUNDUNESP proc. 850/03

BM065 - Standardization of in vitro trans-splicing reaction using permeable cells system with three different *T. cruzi* strains

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Splicing is the process of mRNA maturation in which introns (intergenic regions that not codifying polipeptides) are removed of primary transcripts and exons (coding regions) joined to form a contiguous sequence specifying a functional polypeptide; such process can occur in the same transcript (as cis-splicing) or between two independent transcripts (trans-splicing). Since there is some difficulty to reproduce the trans-splicing reaction in vitro, the use of a permeable cells system for analysis of the mRNA transcription and trans-splicing may serve as a model of study to assist in the inquiry of synthesis, processing and regulation of mRNA in trypanosomes. Thus, in this work, initially, a PCR with genomic DNA from Y, NCS and BOL *T. cruzi* strains have been done for SL sequence amplification which was cloning in a vector, in such way that, after in vitro transcription of these clones, the anti-sense SL RNA was able for using in the reaction of RNases protection; this allow to confirm the trans-splicing reaction with permeable cells for each strain. It was also standardized the analysis of trans-splicing reaction using silver stain instead radioactive material and autoradiography, which made possible its use on the evaluation of drug interference in the mRNA processing on these parasites. The results had allowed to verify that this stain methodology made possible the analysis of the band patterns which appeared after the trans-splicing reaction, but it was not as sensitive as necessary for detecting the newly-synthesized mRNAs after RNases treatment. In the experiments which were used oligonucleotides designed for the 5' and 3' SL regions, respectively, incubated with mRNAs deriving of *T. cruzi* (Y strain) trans-splicing reaction and RNase H, the silver stain analysis was satisfactory for evaluation of the profile of the remaining bands. Studies about drug interference in the mRNA processing are in progress in the laboratory and the preliminary results seem to indicate that this model can be useful for checking anti-trypanosome activity. This work was supported by PADC/FCF-UNESP. MTO and ESO are sponsored by PIBIC-CNPq (2003/2004 and 2004/2005, respectively).

BM066 - Regulated expression of exogenous genes in Trypanosoma cruzi

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We have expressed the green fluorescent protein (GFP) gene in *Trypanosoma cruzi* using a tetracycline-inducible T7 promoter system. A *T. cruzi* cell line named CL-Brener 13, in which the bacteriophage T7 RNA polymerase and the tetracycline repressor from *E. coli* are stable expressed, was generated by DaRocha and cols (2004). We tested the ability of regulating the GFP expression in this cell line by constructing a plasmid pROCKPT7:Otet/GFP, which contains the T7 RNA polymerase promoter and the tetracycline operator sequence upstream the GFP reporter gene. In this construction, β-tubulin sequences have been inserted flanking the GFP expression cassette in order to direct integration of this plasmid into the parasite genome. A hygromycin selectable marker has also been placed in the pROCKPT7:Otet/GFP plasmid. After selecting transfected parasites, we added tetracycline to the cultures and measured GFP expression by flow cytometry. We detected an increase in the number of parasites expressing GFP in the presence of 10μg/ml tetracycline as early as 6 hours, and after a month, the number of fluorescent parasites has reached 50 per cent. We concluded that this is an efficient system to induce regulated expression genes in *T. cruzi* and may constitute an useful tool for functional genomic studies in this parasite.

BM067 - Auto-regulated expression of alpha and beta tubulin genes of Trypanosoma cruzi

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We have been studying the molecular mechanisms responsible for expression of alpha and beta tubulin genes in *Trypanosoma cruzi* to understand the control of stage-specific gene expression in trypanosomatids. In higher eukaryotes, tubulin mRNA levels are subjected to auto-regulatory mechanisms affecting mRNA stability: when the pool of free tubulin increases in the cytoplasm, polysomal tubulin mRNAs are desestabilized by binding of protein factors to nascent N-terminal tubulin tetrapeptide. This binding results in recruitment of RNAses that degrade polysomal tubulin mRNAs. Using vinblastine and taxol, drugs that disrupt tubulin dynamics by opposite mechanisms, we found evidences indicating similar regulatory process operates in *T. cruzi*. Vinblastine causes morphological alterations in *T. cruzi* epimastigotes whereas taxol does not. This result parallels with the effects of these treatments on the levels of microtubule-associated tubulin: vinblastine causes significant despolimerization of microtubule-associated tubulin whereas taxol maintains the microtubule structure unchanged. In accordance with these effects, only vinblastine treatment was found to alter the levels of alpha and beta tubulin mRNAs. Two hours after addition of 50μM vinblastine, treated parasites present a significant reduction in the levels of tubulins mRNAs. Experiments using actinomycin D showed that this reduction is due to a decrease in the half-life of tubulins mRNAs. Western blot analyses are in agreement with auto-regulatory model: epimastigotes, which have higher levels of tubulin mRNA, contain less amount of free tubulin subunits compared to amastigotes and trypomastigotes. To investigate the involvement of the sequences within tubulin mRNAs, plasmids containing the luciferase reporter gene associated with these sequences have been constructed and are presently being used in transient and stable transfections assays of epimastigotes.

BM068 - POLYMORPHISM STUDY OF APICAL MEMBRANE ANTIGEN 1 (AMA-1) OF Plasmodium vivax ISOLATES IN BRAZIL

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Malaria causes annually about 300 thousand clinical cases in Brazil, being the Plasmodium vivax the most common species. Several molecules of this parasite have been characterized and they constitute important targets to compose an antimalarial vaccine. Apical Membrane Antigen (AMA-1) is highly conserved among the different Plasmodium species, suggesting that this molecule presents essential biological role. Recent studies propose that AMA-1 is involved in the merozoite contact and reorientation processes during the erythrocyte invasion. Few studies characterize the diversity of AMA-1 in populations exposed to malaria endemic areas. Thus, in this study, we characterized the diversity of AMA-1 of P. vivax (PvAMA-1). For this aim, 57 blood samples from 27 patients residing in Municipality of Cuiabá, state of Mato Grosso were selected. Of these, 23 patients were infected with P. vivax infection comprising 32 positive samples obtained in distinct periods of blood collection. The gene that codifies PvAMA-1 was amplified by a nested PCR, resulting in 22 positive samples. The amplified fragments showed sizes range 320-738pb. The amplification products of nine isolated was sequenced and analyzed. DNA polymorphisms specific to Brazilian samples were demonstrated through the comparison of the DNA sequences obtained by us with the PvAMA 1 sequences deposited in data bank. Indeed, such nucleotide substitutions seemed to be specific to P. vivax subpopulations harboring each patient, once they have not been observed in stocks isolated from distinct individuals.

BM069 - PROBING THE POLYCLONAL NATURE OF Trypanosoma cruzi STRAIN BERENICE-78 WITH POLYMORPHIC MICROSATELLITES OF DNA

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Chagas disease, caused by the protozoan Trypanosoma cruzi, is an endemic disease that affects 18 million people in Latin America. The first human case of Chagas disease was described by Carlos Chagas (1909) in a two-year old girl, called Berenice, in Lassance, Minas Gerais, Brazil. Two isolates of T. cruzi were obtained from this patient at different times: 1962 and 1978, respectively called Berenice-62 (Be-62) and Berenice-78 (Be-78). Although both strains were obtained from this same patient, they showed significant diferences when they were compared using biological and biochemical criteria. Earlier studies by Veloso et al. (2000), based on RAPD and isoenzymes profiles suggested the presence of different subpopulations in Be-78. The major goal of the present work was to clarify the structure population of the strain Be-78 using microsatellite markers. For that, Be-78 was inoculated in four dogs (A, B, C and D), reisolated and then reinoculated by 25 successive passages in mice. The DNA obtained from the parental strain Be-78 and mice passages Be-78 1A, 1B, 1C, 1D, 25A, 25B, 25C and 25D were submitted to PCR for analysis of five microsatellite loci. The microsatellite analysis of strains Be-78 1B, 1D and 25B were genetically distinct from the Be-78 parental. This fact confirms that strain Be-78 is polyclonal and that the successive passages in laboratory animals had propitiated the preferential growth of subclones of the strains with different characteristics. To confirm these findings, single cells of the Be-78 25B population were sorted by FACScan Cell Sorter. The microsatellite PCR in single cells of was able to detect the presence of two distinct genetically subpopulations, corresponding Be-78 and Be-62 confirming our previous data and showing that Be-62 and Be-78 were indeed originated from the same infected person.

BM070 - Molecular characterization of the genes encoding two proteins that associate with the telomeric G-rich strand of *Leishmania (L.) amazonensis*

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Telomeres are the physical ends of linear chromosomes and play a crucial role in maintaining genome stability by protecting the chromosome termini from degradation and end fusions. The telomere structure is formed by repetitive DNA associated with proteins. Proteins that interact with the double-stranded telomeric DNA are generally negative regulators of telomere elongation by telomerase. In contrast, proteins that bind the G-rich single-strand DNA normally act as positive regulators, although they can also play dual roles, such as the case of Pot1p and Cdc13p. Two *Leishmania (L.) amazonensis* proteins that bind the telomeric G-rich strand and share features with other known telomeric proteins were identified and characterized using biochemical approaches. Mass spectrometry analysis and sequence *de novo* showed that they are homologues of *L. major* replication protein A (Rpa-1) and of *L. major* Rbp38p. In yeast, Rpa-1 is implicated in telomere maintenance. The present study shows the molecular cloning and characterization of genes encoding *L. amazonensis* Rpa-1 and Rbp38. Both ORFs were amplified by a PCR-based strategy using genomic DNA and cDNA as templates. BLAST sequence analysis confirmed the similarities among *L. amazonensis* genes and other sequences available in the public database. The genomic organization and chromosomal location of both genes suggested that they are present in low copy number in the parasite genome. The long-range goal of this project is to disturb the organization of *Leishmania* telomeres as means of induce parasite growth arrest. In order to reach this goal *LaRpb38* and *LaRpa-1* were cloned in *Leishmania* pX63Neo expression vector. A transfectant lineage overexpressing LaRpb38- pX63Neo is being analyzed for changes in phenotype, plasmid copy number and transcription level. Comparative estimations of telomere lenght between transfectants and wild type will give us a clue about the function of LaRpa-1p and LaRbp38p in parasite telomeres. Financial support: FAPESP, WHO/TDR - UNDP Bank.

BM071 - Preliminary Analysis of Gene Expression During Infection of Mouse Skeletal Muscle Cells from Primary Cultures by *Toxoplasma gondii* tachyzoites

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Toxoplasma gondii, which is an obligate intracellular apicomplexan parasite that can infect most nucleated cells, causes devastating disease in immunocompromised humans. Recently, the study of host-parasite interactions has been

greatly aided by large-scale gene expression analysis using microarray technology. The goal of our study was to analyse global changes that occur when a protozoan intracellular parasite infects a mammalian host cell. We examined the transcriptional profile of mouse skeletal muscle cells from primary cultures in response to *T. gondii* infection using the Genechip platform. For this work, total RNA was extracted from uninfected cells (control) and from cells with different times of interaction (30 min, 4h and 24 h). Hybridization results were analyzed by the RMA method. During infection, 127 genes were modulated and are involved in diverse cellular processes, such as: transcriptional regulation, cell signaling, protein transport, protein synthesis, immune responses, cellular adhesion and cytoskeleton and cell cycle progression. The cellular processes and the genes involved were similar to those seen by Blader *et. al.*, 2001, except for enzymes associated with glucose metabolism. They have described up regulation of the glycolysis pathway, but we have seen no differential expression what might be explained by high amount of available glucose in muscle cells. A more detailed microarray study of this host-parasite interaction is under way.

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BM072 - Gene characterization and predicted protein structure of the Trypanosoma cruzi mitochondrial chaperonin HSP10

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The HSP10 is a component of a multimeric apparatus composed of HSP60 and HSP10 proteins. This chaperone machine is responsible for helping cellular proteins to reach their stable three-dimensional conformation and also reduces protein denaturation in stressing conditions and prevents the formation of protein aggregates. Both HSPs are inducible proteins and must be coordinately regulated, and are, therefore, good models for studying kinetoplastid gene regulation. The *Trypanosoma cruzi* HSP60 gene has been previously isolated and characterized in our laboratory. As a first step to characterize the coordinated regulation, we begun by studying the other component of the *T.cruzi* chaperone machine, HSP10. The complete coding sequence was obtained from the EST sequencing effort of the *T.cruzi* genome project (Verdun *et al*, 1998, Infect. Immun. 66(11):5393-5398). The comparison of the predicted amino acid sequence of *T.cruzi* HSP10 with other HSP10 sequences shows small conserved regions spread over the entire length of the protein. We also detected a 5 residue deletion conserved only in tripanosomatids. When molecular modeling methods were performed, this deletion leads to a two fold increase in the size of the orifice located on the upper surface of the HSP10 structure when compared with the *Escherichia coli* ortholog protein complex. This information could help to elucidate the still obscure role of this orifice on the chaperone machine function. A phylogenetic tree built from the sequences mentioned above point to a vertical evolution of the HSP10 protein. Southern blot and gene equivalent experiments, in agreement with preliminary genome sequence (tcruzidb.org) suggest that HSP10 is present as a multicopy gene arranged in tandem, which contain 3 copies. The HSP10 genes are separated by 2 unusual long intergenic regions with no significant similarity between each other. The genes are located at chromosomal band XVIII, which was verified by PFGE. The level of HSP10 mRNA, of about 0.5 Kb, does not increase upon heat shock at 37 C and 40 C. However, a smaller mRNA is induced at higher temperatures. RT-PCR experiments suggest this is due to alternative polyadenilation. Supported by CNPq, FAPERJ, FUJB

BM073 - Characterization of a novel 664-bp repetitive element (DR664) closely associated to TS (trans-sialidase) genes of Trypanosoma cruzi

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Trypanosoma cruzi contains a large number of repeated DNA sequences which may be responsible for the genome plasticity and control of gene expression. In the course of studying genes encoding surface antigens of *T. cruzi* (clone Sylvio X10.6), we fully sequenced a 30-kb genomic region (cosmid C2) and identified a novel 664 bp repetitive element flanking the trans-sialidase (TS) gene. Here we report further characterization of this repetitive element named DR664. Sequence comparison showed that the DR664 is closely related to the ORF-3 from the non-LTR retrotransposon L1Tc (Olivares M. et al. 1995, J. Mol. Biol. 247:49). ORF-3 codes for a gag-like protein with unusual cysteine motifs similar to the C2H2 zinc finger family of transcription factors. Association of DR664 element with TS gene was confirmed by hybridization of *T. cruzi* genomic DNA digested with several restriction enzymes and chromosomal bands separated by pulsed field gel electrophoresis with DR664 and TS probes. Four chromosomal bands hybridized with both probes, suggesting that TS and DR664 are associated in different regions of the genome. This organization was confirmed by screening of *T. cruzi* Genome Project database (http://www.genedb.org/genedb/tcruzi/). Among 17 contigs carrying a complete copy of TS gene, three contigs (TcBr 00 10470053509495 TcBr 00 10470053506923 TcBr 00 10470053507085) showed two DR664 elements flanking the TS gene, exactly as described in cosmid C2. DR664 can also be found flanking mucin genes. PCR assay using primers derived from TS and DR664 showed that they can be associated in four different strains of *T. cruzi*, including isolates from *T. cruzi* lineages I and II. Our data indicated that DR664 is derived from 3'end of the non-LTR retrotransposon L1Tc. Association with TS gene suggests that this novel element could have participated in the recombination and dispersion of TS gene in *T. cruzi* genome.
Supported by FAPESP.

BM074 - Analysis of the phosphoglucomutase (PGM) gene among different stocks of *T. cruzi*

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The considerable molecular heterogeneity among different *Trypanosoma cruzi* stocks was first demonstrated by multilocus enzyme electrophoresis (MLEE). The studies performed on isoenzyme variability among parasite stocks employing six enzymes: ALAT, ASAT, glucophosphate isomerase (GPI), glucose 6 phosphate dehydrogenase (G6PDH), malic enzyme (ME) and phosphoglucomutase (PGM), allowed their division in three main groups, named zymodemes 1, 2 and 3. The genetic polymorphism was subsequently confirmed by the analysis of MLEE and random amplified polymorphic DNA (RAPD) data, which have shown that *T. cruzi* has a clonal population structure. The *T. cruzi* population is distributed into two major phylogenetic lineages (group 1 and group 2), each retaining considerable heterogeneity, and therefore separated from each other by evolutionary distances that are four times greater than the distance separating men from chimpanzees. We have previously reported the cloning and characterization of the *T. cruzi* pgm gene in Dm28c. Here, we extended this characterization at the molecular level to 5 additional stocks. Oligonucleotides directed to the extremities of the coding region of pgm were used in PCR performed by a proof reading polymerase, using genomic DNA as template. The sequences obtained from the PCR fragments, together with the CLBrener pgm sequence (TIGR chr.7811 and 8825) were aligned using CLUSTALW. As expected, a very high degree of conservation was observed among the tested stocks (98-99,8% identity of predicted amino acid). However, among the minor variations observed, the nature of the predicted amino acid residue at position 16 was of particular interest to us. While the residue at this position was consistently alanine in CLBrener and Tulahuen (Tul), an aspartic acid was present at position 16 of Dm28c, G, Silvio X10/6 and Brazil (group 1). The potential charge change introduced by the modification of residue 16 may account for the differences in enzyme profiling described in the past. While both CLBrener and Tul are suspected to be hybrid genotypes, a recent MLEE analysis demonstrated that the migration of PGM of these stocks is identical. The molecular analysis of pgm will be further investigated in other *T. cruzi* stocks. Supported by CNPq, FAPERJ.

BM075 - Characterization of *Trypanosoma cruzi TcRjl locus* and analysis of its transcript

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The Ras superfamily of GTP binding proteins encompasses several gene families that regulate a plethora of events in the eukaryotic cell. We have previously described a novel branch of this superfamily which we have named RJLs. These genes are present in many unicellular organisms and also in deuterostomes but apparently missing in some intermediary phyla, suggesting an intriguing possibility of lateral gene transference between lower and higher eukaryotes. Here we describe the *Trypanosoma cruzi* orthologue, *TcRjl* which was isolated and had its *locus* characterized in a region of almost 5 Kb. Its 660 bp ORF, predicting a protein of 24.13 kDa, is present as a single copy gene in *T. cruzi* I lineage, and from one to two copies in *T. cruzi* II lineage. TcRjl shares 73% aa sequence similarity with its closest identified orthologue, *T. brucei* TbRjl. RT-PCR experiments reveled that *TcRjl* is transcribed in mRNA in the three main life forms of the parasite, while northern hybridization demonstrated that *TcRjl* is transcribed in *T. cruzi* epimastigotes as at least two transcripts, one of around 950 nt and other of 1500 nt. Splice-leader addition was mapped to a single site at - 69 bp upstream of *TcRjl* orf indicating that the two mRNA types may derive in differences at the 3' of *TcRjl* mRNA. *TcRjl* locus presents considerable synteny with *Rjl loci* from *Trypanosoma brucei* and *Leishmania major* as available from their respective genome projects. Financial Support: CAPES, FAPERJ and PRONEX

BM076 - Recombinant expression of a novel RasGEF up-regulated in cells exposed to T. cruzi-derived GPI mucin.

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The guanine nucleotide exchange factors of the Ras protein super family (RasGEF) are essential cellular components in the process of Ras activation in response to diverse extracellular stimuli. Various GEFs associated to Ras proteins have been identified. However, their functional characterization remain to be demonstrated. It has been recently identified in our laboratory a new gene which encodes a RasGEF, designated GPIγ4. Its expression was significantly induced in murine macrophages upon stimulus with glycosylphosphadylinositol (GPI) derived from the protozoan parasite T. cruzi, the causing agent of Chagas disease. Furthermore, other inflammatory stimuli, such as LPS, were capable of triggering its expression in macrophages. To begin the functional characterization of GPIγ4, we decided to clone its coding sequence in to prokaryotic vector in order to express the recombinant protein in bacteria. A 1422 base pairs cDNA fragment was amplified by RT-PCR from a population of cDNAs derived from total RNA of murine macrophages stimulated with GPI mucins of T. cruzi. Following purification of the amplified cDNA the fragment was cloned in frame with the 6X- Histidin sequence of the pQE30 vector. The expression of the recombinant protein was induced by the addition

of isopropyl-β-D-thiogalactopyranoside (IPTG) to the bacterial cell culture. Total cellular extracts were collected at 0, 4 and 18 hours. The samples were then fractionated on to SDS-PAGE and stained with Coomassie blue dye. Alternatively, samples were also transferred to a nitrocellulose membrane and hybridized with anti- histidin polyclonal antibody. The results indicate the expression of a polypeptide with an expected molecular weight of 52 KDa.

BM077 - Post-Genomic Analysis of the cAMP Signal Transduction Pathway During *Trypanosoma cruzi* Life Cycle

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The cellular differentiation from epimastigote to metacyclic trypomastigote in *Trypanosoma cruzi*, as well as some genes and factors involved in this process, has been extensively studied by our group including the role of cAMP as an important secondary messenger in inducing metacyclogenesis. We searched for genes that encode different proteins related to cAMP transduction pathway in the sequence data banks, allowing us to select different members of these gene families (Adenylate Cyclases - AC, ADC1, ADC4 and TczAC , Phosphodiesterases and the genes for the holoenzyme cAMP dependent Protein Kinase - PKAr1, PKAr3, PKAc1, PKAc2 and PKAc3 -). In order to quantify the expression of these genes during the life cycle of *T. cruzi*, we have developed primers and probes for Real time RT-PCR analysis. The results indicate that PKAc3 and PKAr3 showed a similar association pattern with the polysomal mRNA during the life cycle of T. cruzi that can be explained by their interaction to form the holoenzyme PKA. In addition the level of the AC polysomal mRNA is increased in the metacyclic forms in agreement with the increase in the levels of cAMP in metacyclic trypomastigotes previously observed by Rangel-Aldao et al. (1987). Our data strongly suggest that genes related to this pathway have a complex and coordinated gene expression regulation.
Financial support from PRONEX, CNPq, and Fundação Araucária

BM078 - UNIQUE FEATURES OF *T. BRUCEI* eIF2 α AND eIF4E, THE MAIN MEDIATORS OF TRANSLATIONAL CONTROL IN EUKARYOTES.

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Little is known regarding translational control in trypanosomatids despite its relevance in regulating gene expression. The phosphorylation of translation initiation factor eIF2α signals for programs of cell recovery from a variety of stresses in eukaryotes. In yeast eIF2α is phosphorylated by protein kinase GCN2, which is activated by amino acid starvation. The genomic sequence of *T. brucei* (Tb) eIF2α suggested a protein larger than the known orthologues, with an extension of 117aa at the N-terminus with homology only to the other trypanosomatid's eIF2α. Tb-eIF2α contains a threonine in place of the Ser51 that is phosphorylated in all other eukaryotes. We confirmed that this sequence is expressed in *T. brucei* by sequencing the complete cDNA and by immunoblot using antibodies raised against recombinant Tb-eIF2. This is the only form of eIF2α found in this parasite. The Tb-eIF2α protein lacking the N-terminal extension was functional in yeast. We showed that Tb-eIF2α is a very poor substrate for both GCN2 and PKR*in vitro*. We constructed a Tb-eIF2α protein with a mutation changing the potential phosphrorylation residue Thr to Ala and another mutant where the same residue was altered to Asp. Both Thr and Ala containing proteins allowed yeast cells to grow on 3-aminotriazole, which signals for amino acid starvation, suggesting that Tb-eIF2α forms a defective eIF2 complex. The Asp mutant imparted a defective growth to yeast cells; the corresponding mutation in yeast eIF2α leads to inviable cells. Another important point of translational regulation is provided by eIF4E, the cap-binding protein. By cDNA analysis, we certified genomic data indicating that Tb-eIF4E contains an internal 18aa insert relative to its orthologues. Structural modeling indicated important differences in the cap-binding pocket. We showed that Tb-eIF4E does not bind efficiently to the cap m^7GpppG. Functional analysis of Tb-eIF4E is now in progress.
Supported by FAPESP

BM079 - Isolation and characterization of calmodulin-like gene of *Phytomonas serpens*.

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Flagellates of the Trypanosomatidae family are parasites of a wide range of animals and plants. Chagas'disease is caused by the protozoan *Trypanosoma cruzi*. The infection is endemic in Latin America and serological data indicate that over 20 million people are infected. *Phytomonas serpens*, a trypanosomatid parasite of plants, can causes diseases of economic significance in plantations. It has been demonstrated that serum from patients with Chagas'disease presented a strong reactivity to *P. serpens* antigens. The common antigenic determinants shared by *T. cruzi* and *P. serpens* induced a significant reduction of parasitemia and mortality in mice challenged with a high number of metacyclic trypomastigotes of *T. cruzi*. Western blot analysis of a two-dimensional gel electrophoresis showed that a estimated 17 kDa protein reacted with the sera from Chagas'

disease patients. Preliminar analysis of the aminoacids sequence showed that this protein has similarity of calmodulin of *T. cruzi*. Based on a conserved sequence of calmodulin gene of *T. cruzi* it was construct two primers (CalmF and CalmR) in order to amplify a fragment of approximately 0.35 kb in size. This fragment was used as a probe to screening a *P. serpens* lambda gt11 genomic library and we are presently isolating positive clones in order to characterize for calcium-binding genes in this microorganism once a few is known about *P. serpens* genome. Principal investigators: Carlos Cervenansky (Universidad de la Republica). Supported by CNPq, Capes and PROPPG-UEL.

BM080 - *Phytomonas serpens*: analysis of antigenic reactivity with *Trypanosoma cruzi*.

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Phytomonas serpens, a trypanosomatid parasite of plants, is responsable for various agricultural crop diseases and it is transmitted between plant hosts by phytophagous insects. Previous studies in our laboratory showed that *P. serpens* shares antigenic reactivity with *Trypanosoma cruzi*, the causative agent of Chagas'disease. Sera from Chagas' disease patients cross reacted with total protein extracts of *P. serpens*. In addition, oral immunization with *P. serpens* induced a significant reduction of parasitemia and mortality in mice challenged with a high number of metacyclic trypomastigotes of *T. cruzi*. This study aims to identify proteins involved in the cross reactivity between *P. serpens* and *T. cruzi*. For Western blot analysis, parasites were and total proteins extracts were separated by electrophoresis in two-dimensional SDS polyacrylamide gel. The proteins were then electrotransferred onto Hybond-C membranes following standard procedures and the blot was assayed with serum of patients with Chagas'disease. Proteins with estimated molecular masses of 15 to 100 kDa, in a range of pI from 3 to 10 showed cross reactivity with these serum. The recognized proteins will be analised by mass spectometry (MALDI TOF) and the results will be submitted to MASCOT SEARCH (www.matrixscience.com) in order to search homology with known proteins. In addition, a *P. serpens* lambda gt11 genomic library was construct and approximately 275.000 clones were obtained, with inserts with sizes around 1.6 kb. The immunoscreening with sera of Chagas' disease patients of this library has being realized in order to isolate and characterize genes involved in antigenic relationship between these parasites and to verify the cellular events that these proteins could be involved.Principal investigators: Carlos Cervenansky (Universidad de la República).
Supported by CNPq, Capes and PROPPG-UEL.

BM081 - *In vitro* shuttle mutagenesis and functional analysis in *Leishmania*. Gene disruption and complementation of a telomere-located RNA Polymerase III

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Transposon-based mutagenesis is a powerful approach for gene identification and analysis. These self-replicative elements can bring together genetics and biochemistry because of their inherent ability to insert molecular tags and/or inactivate endogenous phenotypes. As the *Leishmania* genome project has been completed, the development of tools for high-throughput targeting mutagenesis and trapping protocols is now required. We have used the mariner *in vitro* transposition system as a source of reagents for integration into the genome. Genomic libraries of *Leishmania* spp. were constructed into a shuttle vector and targeted with specialized transposons, which were designed for gene disruption and/or reporter expression. The information generated by primer-island sequencing of insertion libraries was compiled on the Artemis format, providing a graphical display of the data. The anchoring of the insertion information across the annotated genome allowed the design of linear fragments suitable for gene disruption experiments. The locus-specific integration of such fragments was validated in two sub-telomeric single-copy genes: a 5,2 Kb ABC transporter-like gene on chromosome 11 and a 4,4Kb DNA-directed RNA polymerase III identified on chromosome 20. Following the transfection of linear fragments, the specific integration into the parasite genome was confirmed by Southern analyses of PFGE-separated chromosomes and digested genomic DNA. *Bal* 31 nuclease assays confirmed telomere integration. As expected for essential genes, the inactivation of the RNA polymerase second allele required the complementation of the intact gene, which was transfected in an episomal context. The transcription status of the target gene was confirmed in Northern blotting experiments. The ready-to-use collection of reagents generated is a valuable tool for functional and comparative studies of this protozoan parasite. In addition to the prompt generation of mutants and the identification of essential genes, this strategy also allows gene-reporter expression from a chromosomal context. Supported by FAPESP and UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases.

BM082 - Characterization of heat shock protein 70KDa (TcHSP70) in *Trypanosoma cruzi* populations susceptible and resistant to Benznidazole.

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Heat Shock Proteins (HSPs) are synthesized under different chemical or physical stress. Murta *et al.* (2002) investigated the differential gene expression in *T. cruzi* populations and clones susceptible and with *in vitro*-induced (17LER) or *in vivo*-selected resistance (BZR) to benznidazole (BZ). The authors used the Representation of Differential Expression methodology selected the TcHSP70, which was overexpressed in the *T. cruzi* populations resistant to BZ. In order to confirm the variations in the levels of mRNA transcribed from the TcHSP70 genes of different parasite populations, northern blot analysis was performed. The northern blot profile of total RNA from drug-resistant and susceptible *T. cruzi* samples, hybridized with TcHSP70 probe, revealed one transcript of 2.1 Kb for all *T. cruzi* samples. The *T. cruzi* drug-resistant population 17LER expressed 4-fold more TcHSP70 mRNA than 17WTS. The other *T. cruzi* populations and clones expressed the same level of TcHSP70 mRNA independent of their drug resistance phenotype, similar to that of 17WTS. Based on the differences observed in the levels of TcHSP70 mRNA, we decided to determine whether different populations of parasites might also be expressing different amounts of protein. Western blot analysis of *T. cruzi* protein extracts probed with a rabbit anti-recombinant TcHSP70 polyclonal serum revealed two bands, one of 70KDa and another of 73KDa for all *T. cruzi* strains. Each band had the same intensity and was common to all *T. cruzi* samples independent of their drug resistance phenotype. The TcHSP70 mRNA is overexpressed in the 17LER population compared to the 17WTS, but the protein level is similar in both populations. These data suggest that TcHSP70 gene is regulated either at the level of primary transcript processing, mRNA stability or the protein stability.
Supported by CNPq, FAPEMIG, PRONEX, PADCT and PAPES/FIOCRUZ

BM083 - A new annotation of trypanosomatids hypothetical proteins searching motifs and domains as functional patterns

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Trypanosomatids protozoans has a complex gene regulation. Many peculiar events as RNA editing, policistronic transcription, trans-plicing and presence of specialized organell like glicossome, acid calcisome represent a challenger for scientist and genome program open new possibilities to find ways to understand the different and interesting process of the parasite life. However, in many cases no homologies can be identified in databanks, and these sequences have been called unknown genes or hypothetical proteins. According to Sanger Centre Institute 2004, a number of more than 11.000 sequences in trypanosomatids are annotated as unknown genes. Given the complexity of the kinetoplastid biology is possible that many of these genes develop a crucial role in parasitism influencing both survival and virulence in host and vector. To study the function of 'orphan gene' is necesary dissect the informations contained in tripanosomatid genome searching in hypothetical protein for pistes to re-annotate genes and obtain a new group with probably a better defined concept of orphan gene. The aim of our work was to develop a software able to re-annotate hypothetical protein considerated as unknown genes in tripanosomatid programs. We developed a software *Proto-GIM* (Protozoan Gene Identification Motifs) using mysql and java program based in the use of known regular expression present in different available software with the possibility to added new regular expression related with post-translational modification, organelle targeting or other cellular implications. The principal characteristic of this software is the possibility to analyse a high number of gene crossing motifs database with sequences database and found clues to understand the function of the gene at one time. 1194 sequences with complete coding regions from *T. brucei*, *T. cruzi* and *eishmani sp.* were analysed by *Proto-GIM* and we used several bioinformatics tools to compare the efficiences. BLAST analysis resulting in 70 homologies with known function. Thus, using *Proto-GIM*, PROSITE and e-MOTIF to search motives and domains shows that *Proto-GIM* and PROSITE present simillar eficience identifying 1172 and 1169 sequences, whilst e-MOTIF identify 86 sequences. Several sequences analysed from *Proto-GIM* were found targeting to nucleous (167), glicosome (57) and endoplasmic reticulim (87). Moreover, 1172 sequences were identified containing post-translational modification site being the more expressive to GPI-anchor site present in 261 sequences. Finally, 14 sequences did not have functional patterns and were annotated as orphan genes. Taking together all results we were able to classify 231 sequences with putative functions such as enzymes, receptors, structural proteins, DNA binding proteins and others functions. The understanding of the function of re-annotated and orphan genes as well as its role in parasite biology through biochemical, molecular and celular approaches may help to elucidade new specific parasite targets to development vaccines and chemotherapics to control parasite diseases.
Support: IOC-Fiocruz, CpqAM-Fiocruz, CNPq.

BM084 - Mutation analysis of a novel P.falciparum ABC superfamily protein in quinine resistant parasites from the Amazon Region.

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The emergence of Malaria parasites resistant to a wide array of antimalarial drugs, is being one major problems to control the disease in the Amazon region. Quinine (QN), another quinolein-based drug, has been effective against malaria parasites for centuries, but clinical failures and the increase of the parasite clearance time have been reported in the Amazon Region. Two ABC transporters (pfmdr1 and pfmdr2) from the P. falciparum has been implicated in responses to Chloroquine (CHL) and QN, as well other drugs, but until now, none of the two markers were proved to be directly involved on quinine resistance. We hypothesized that other transporters may play a role in P. falciparum response to QN, and the responses are result from additive and/or interacting contributions of multiple proteins. In this work, we propose to identify SNPs in a novel ABC protein gene that was previously described by Su et al. as being a potential drug transporter in the P. falciparum. Mutations found in culture stable isolates showed a correlation between high levels of QN resistance and number of SNPs. Isolates from the Brazilian and Colombian malaria endemic areas were collected and genotyped for the ABC transporter. In addition, we measured the dose response in vitro to QN and CHL and correlated with the mutations found in the gene.

BM085 - TcSof1 a protein of the U3snoRNP complex of *Trypanosoma cruzi* is differentially expressed during metacyclogenesis.

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T. cruzi life cycle involves at least three distinct developmental stages: epimastigotes, trypomastigotes and amastigotes. The epimastigotes and amastigotes forms are replicative forms of *T. cruzi*, while the trypomastigotes are the infective and non-replicative forms. Using the technique of Differential Display, we isolated a fragment of 901 nt which is differentially expressed during the metacyclogenesis process. The complete sequence of this gene in *T. cruzi* Dm28c was obtained showing an open reading frame of 1335 nt. This sequence showed significant similarity with the Sof1p protein of *Arabdopsis thaliana*, a nucleolar protein associated with the U3 small nucleolar ribonucleoprotein complex (U3snoRNP), involved in rRNA processing. Like its yeast orthologue, the putative protein which was named TcSof1 display seven WD40 repeat motifs. In order to express this protein in *E. coli* the complete sequence was cloned in the expression vector pQE31 and the recombinant protein was purified from inclusion bodies. Rabbits were immunized and the specific polyclonal antibody was obtained. Western blot analysis using the anti-TcSof1 serum showed that the protein is present in *T. cruzi* epimastigotes forms, nutritional stress forms, parasites after 24 hs of differentiation and amastigotes forms but not in metacyclic trypomastigotes. Our group has previously shown that a protein named TcImp4 associated with U3snoRNP is also expressed in epimastigotes forms but not in trypomastigotes suggesting that the proteins associated with this complex might be co-regulated by the same mechanism. Imunolocalization assays showed the presence of TcSof1 in the nucleus as expected. Furthermore, to confirm that the protein is a component of the U3snoRNP complex we are currently using a TAP-tag technique to determine which proteins interact with TcSof1.
Financial support from PRONEX, PROSUL, CNPq, Fundação Araucária, FIOCRUZ.

BM086 - Pyrophosphatase (TcPPase) is up-regulated in a *Trypanosoma cruzi* benznidazole resistant population selected *in vivo*.

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Infections caused by *T. cruzi* are among the most widespread parasitic diseases in Latin America and are responsible for heavy socioeconomic losses. There is therefore considerable interest in developing novel chemotherapeutic approaches, based on unique aspects of metabolism of this parasite. The *T. cruzi* pyrophosphatase gene (TcPPase) encodes a vacuolar-type proton-translocating pyrophosphatase (V-H+-PPase), associated with the acidic vacuoles acidocalcisomes. These organelles contain polyphosphate and calcium storage extremely important for parasite survival inside the host cell. Recently, using DNA microarray methodology, Murta et al, (2002) have identified a TcPPase mRNA expression 2.8-fold higher in a *T. cruzi* benznidazole resistant population selected *in vivo* (BZR) when compared to its susceptible counterpart (BZS). In the present study we have investigated the TcPPase mRNA expression levels as well as its gene amplification in these two populations. To precisely determine the variations in mRNA transcription levels northern blot and quantitative real time RT-PCR (qRT-PCR) analysis were performed. The northern blot profile of total RNA probed with the TcPPase revealed two transcripts; one of 2.0 Kb and another of approximately 3.8 Kb in both populations. Comparative densitometric and qRT-PCR analyses have shown a TcPPase mRNA expression 2-fold higher in the population BZR than the BZS. Aimed at verifying whether mRNA expression is mediated, at least in part, by gene amplification, real time PCR was performed using different concentrations of genomic DNA. The results showed that BZR population presented twice as much TcPPase copies as the BZS. The importance of V-H+-PPase to the parasite metabolism and survival, together with the fact that TcPPase gene was up-regulated in a *T. cruzi* population resistant to benznidazole

and the apparent lack of such a enzyme in mammalian cells make it a potential target for the development of new drugs. Suppoted by CNPq, FAPEMIG, PRONEX, PADCT and PAPES/FIOCRUZ.

BM087 - Molecular characterization of TcPUF6, a pumilio-like protein from Trypanosoma cruzi

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In trypanosomes regulation of gene expression occurs mainly at the post-transcriptional level. Mechanism regulating stage specific gene expression could involve either changes in stability, localization or translation of mRNAs. Pumilio-like proteins are well-known translational repressors that have been described in lower and higher eukaryotes. In Trypanosoma cruzi the sequence of different members of the Pumilio family, which has at least nine reported members, have been recently deposited in the GenBank. TcPUF6 is a member of this protein family and the homologue of the Trypanosoma brucei PUF1 protein. TcPUF6 is localized in the cytoplasm of epimastigote forms of the parasite showing a characteristic pattern of cellular localization also seen in other Pumilio-like proteins. The cytoplasmic signal observed is not uniform but appears to be concentrated in multiple discrete foci. The protein is expressed in similar levels in the different forms of the parasite showing no clear stage specific regulation. Western blot analysis of polysomal gradients showed that TcPUF6 is present in the polysomal fraction. A recombinant TcPUF6 fusion protein is able to bind specifically to the hunchback NRE (nanos response element) suggesting that the target sequence of this protein family is conserved throughout evolution.
Financial support from PADCT, PRONEX and CNPq. Proyecto Clemente Estable 7108, DYNACIT

BM088 - MOLECULAR MODELLING OF SUBUNITS OF THE TRANSLATION INITIATION COMPLEX eIF4F FROM *Leishmania major.*

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Our work aims the study of the initiation stage of protein synthesis in the protozoan parasite *Leishmania major*. In this process the complex eIF4F, formed by the subunits eIF4A, eIF4E and eIF4G, plays a relevant role by recruiting the 40S ribosomal subunit to the 5' end of the mRNA. eIF4A is a helicase that unwinds secondary structures on the mRNA, eIF4E binds the cap on the 5' end of the mRNA and eIF4G acts as a scaffold to the assembly of the complex and also interacts with other proteins. Through primary sequence analysis we have previously identified in *L. major*, two homologues of eIF4A, four of eIF4E and five of eIF4G. Biochemical analysis with selected homologues showed that some of them, but not all, have functional properties reminiscent of eIF4F subunits. Here we describe the results obtained with the 3D molecular modelling of homologues of each eIF4F subunit from *L. major*. These are named *Lm*EIF4A1, *Lm*EIF4E2 and *Lm*EIF4G3. The models were created using the program MODELLER6a based on 3D structures, solved by X-ray diffraction, from the respective vertebrate/yeast homologues. All models were validated and appear to be of good overall quality. The model of *Lm*EIF4A1 is similar to the original structure, most of the characteristics were preserved, so was the surface electrostatic potential. The model of *Lm*EIF4E2 has some punctual differences in the cap binding slot, but analysis of these mutations show that they could perform the cap binding activity. Modelling of the eIF4G central HEAT domain from *Lm*EIF4G3 shows a remarkable difference when its surface electrostatic potential is compared with that of the original human eIF4G, but the overall folding and important aminoacids were conserved. These results represent the first concrete structural proposal for the whole complex eIF4F and shed some light on how they may interact *in vivo*.

BM089 - CLONING AND CHARACTERIZATION OF THE GENES ENCODING THE NUCLEOLAR PROTEINS NOP10 AND N0P58 OF TRYPANOSOMA CRUZI

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Trypanosoma cruzi, the causative agent of Chagas disease, shows several peculiarities related to the mechanisms of gene expression regulation. This parasite regulates its gene expression predominantly by a post-transcriptional process, involving either the processing of long polycistronic transcripts by trans-splicing and poly-A tail addition or mechanisms based on stage-specific changes in mRNA stability and translation. Less attention has been payed, however, to the role of the ribosomal RNA processing as a stage-specific gene expression control mechanism. Most of the steps of ribosome

biogenesis in eukaryotic cells take place primarily in the nucleolus and are performed by small nucleolar ribonucleoproteins (snoRNPs). Experimental data from our laboratory show that some proteins of U3 snoRNP, the snoRNP particle involved in the processing of the 18S rRNA, are not expressed in the trypomastigote metacyclic stage of T. cruzi. These data suggest that the absence of some snoRNPs might be part of a mechanism that contributes to shut down the translation of mRNAs, by blocking the synthesis of new ribosome particles. This observation associated to the fact that the T. cruzi nucleolus is not well characterized, leaded us to study this nuclear subcompartment. Recently, we have identified two other genes that share homology with those encoding Nop10 and Nop58 proteins, which are involved in the rRNA processing in yeast. T.cruzi homolog proteins, named TcNop10 and TcNop58, were expressed in E.coli. Antisera were raised against the recombinant proteins in order to immunolocalize and study the expression pattern of these proteins during T.cruzi life cycle. Analysis of the expression of these genes by Northern blot showed that TcNop10 and TcNop58 transcripts display 0.5 kb and 2.3 kb mRNAs, respectively. Immunolocalization assays showed that TcNop10 is dispersed within the nucleus, whereas TcNop58 is restricted to the nucleolus. We are currently using a bacterial two-hybrid system to investigate whether Nop10p and Nop58p can physically interact with other nucleolar proteins of the snoRNPs complex.

Financial support from Pronex, CNPq, Fundação Araucária and Fiocruz

BM090 - IDENTIFICATION OF TWO DIFFERENT PABP MOLECULES FROM Leishmania major.

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The 5' and 3' ends of most eukaryotic mRNAs are defined by the cap structure and the poly(A)-tail respectively. Both elements are important for mRNA recognition in translation. The cap is recognized by the complex heterotrimeric eukaryotic initiation factor eIF4F and the poly(A)-tail is bound by the poly(A) binding protein (PABP). In protein synthesis binding of eIF4F to the cap allows the recruitment of the 40S ribosomal subunit to the mRNA. This process is enhanced by the poly(A) bound PABP, presumably mediated by a direct eIF4F/PABP interaction. Little is known about protein synthesis in trypanosomatid protozoans but single PABP homologues have been described from *Leishmania major*, *Trypanosoma cruzi* and *T. brucei*. Strikingly the *L. major* protein was seen to be only 45% similar to both Trypanosoma PABPs. Our group is interested in characterizing homologues to relevant proteins required in translation initiation in trypanosomatids. To study PABP we began by searching *L. major* genome sequences for possible homologues. We found not only the previously described protein (LmPABP1) but also a second homologue which is 65% similar to the PABP described from *T. cruzi* and *T. brucei* (LmPABP2). Both genes were amplified, cloned and transcribed/translated in vitro generating polypeptides of 57 and 63 kDa, respectively. The two proteins were then expressed in *Escherichia coli*, as His and GST fusions, purified by affinity chromatography and used to immunize rabbits. Working only with the LmPABP2 antisera we found it to be isoform specific and capable of detecting a 63kDa band in *L. major* promastigote extracts. Preliminary quantitation analysis indicates that it is a very abundant protein with about $1.3x10^5$ molecules per cell. We are currently performing comparative assays with the LmPABP1 antisera. The recombinant full-length proteins and various deletions will also be used in protein-protein interaction experiments aiming to investigate their role in translation.

BM091 - Identification of the genes differentially regulated by mutants forms of the GTPase TcRho1 of Trypanosoma cruzi using microarray technology.

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Rho family proteins are members of the Ras superfamily of small GTPases. In higher eukaryotes, these proteins play critical roles in the cell mobility, phagocytosis, intracellular transport, cell adhesion and maintenance of cell morphology. Other cellular processes under Rho family control are the regulation of activity of the NADPH oxidase complex, progression of the G1 phase of cell cycle, transcription regulation and metastasis induced by different oncogenes. The TcRho1 gene identified in our previous work is the only Rho family member in Trypanosoma cruzi. We have generated different lineages of T. cruzi Dm28c overexpressing TcRho1 mutants in order to characterize TcRho1 function. The lineages are: TcRho1ΔCaaX (a mutant not farnesylated with poor metacyclogenesis levels in TAU-3AAG medium), and mutants obtained by site-directed mutagenesis TcRho1G15V, TcRho1Q76L (constitutive active proteins), and TcRho1T20N (constitutive inactive protein, which demonstrated decrease on proliferation and poor adhesion ability during metacyclogenesis process). To elucidate the pathways regulated by TcRho1 we carried out experiments of competitive hybridization using T. cruzi DNA microarray (biochip - version 4.0.1). So that, polysomal RNAs were extracted of epimastigotes at log phase of the control lineage transfected with vector alone (pBS:CnFc), and of the different mutant lineages (TcRho1ΔCaaX, TcRho1G15V, TcRho1Q76L, and TcRho1T20N). RNAs were reverse transcribed and labeled with fluorescent dyes. The analysis of the different hybridizations realized on dye-swap conditions, demonstrated specific sets of mRNAs differentially mobilized to the polysomes, such as sequences of ESTs encoding kinase proteins and metabolic proteins. These results will be further confirmed by real time RT-PCR analysis. This work was supported by CAPES, CNPQ, PRONEX and Fiocruz.

BM092 - BCTOP2 GENE ENCODES AN EXCLUSIVELY NUCLEAR TOPOISOMERASE II IN BLASTOCRITHIDIA CULICIS

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DNA topoisomerases are involved in cellular processes as diverse as replication, transcription, recombination and chromosome segregation. Such enzymes solve topological problems related to DNA double helical structure by breaking and rejoining DNA strands. Considering this aspect, there are two major classes of topoisomerases known as type I enzymes (topoI), which break and reseal one strand of DNA, and type II enzymes (topoII) that break and rejoin both strands of the DNA helix. DNA topoisomerases are inhibited by antimicrobial and antitumoral agents and migth be promising target for antitrypanosomal. We have cloned and characterized the gene encoding the topoII from the monoxenic trypanosomatid B.culicis (BcTOP2), as this enzyme might be used as a prototype for topoII enzymes from pathogenic trypanosomatids and consequently a good model for future structural and functional studies.In order to gain further insight into the expression and localization of topoisomerase II, a polyclonal antiserum was raised against a portion of BctopoII (11,3 kDa), that includes its carboxy domain (CDTOPO), the less conserved region among trypanosomatid topoisomerases II. This portion of B. culicis topo II was fused with GFP in order to improve its solubility in E.coli. The recombinant protein (38,7 kDa) was purified on nickel-NTA resin and used for antiserum production. Western blot analysis of B. culicis extracts with the antiserum raised against recombinant BcTopoII showed a polypeptide of 138 kDa, compatible with the expected size based on the deduced amino acid sequence of BcTOP2 gene. The antiserum against BctopoII was used to investigate the site of expression of the enzyme in B. culicis. Immunolocalization assays showed that the antiserum recognized the nuclear topoII but failed to detected the enzyme in the kinetoplast of the protozoan.

BM093 - Antigen components of ribonucleoprotein complexes of Trypanosoma cruzi: cellular localization and reactivity with chagasic sera

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Several cDNAs were isolated by immunoscreening an amastigote cDNA library of T.cruzi with sera from chagasic patients. Partial sequence analysis revealed that proteins that are part of ribonucleoprotein complexes and proteins that contain repetitive amino acids are frequently immuno selected. Two cDNAs were chosen for further characterization, regarding their function in the parasite and their reactivity with sera from chagasic patients. One cDNA, named TcRBP48, presents high homology with two RNA binding proteins from T.brucei, and also showed high reactivity against sera from infected individuals. To evaluate the expression of the native protein in the parasite, we generated antibodies against the recombinant form expressed in E.coli. Western blot showed reactivity with a 28 kDa protein in extracts of three different T.cruzi lineages. The second cDNA, named TcRpL7a, is homologous to the L7a ribosomal protein of eukaryotes and also contains an extension of repetitive amino acids in its N-terminal region. The entire protein, as well as the N-terminal (repetitive) fragment and C-terminal (non-repetitive) fragment, were obtained as GST fusion proteins. Purified recombinant proteins had their reactivity evaluated by Western blot and ELISA assays with chagasic sera. The results indicated that the reactivity with antibodies from infected individuals occurs exclusively against the repetitive segment. We also found that the repetitive region reacts, by ELISA, with 60 percent of a large panel of individual sera from chagasic patients. To localize the TcRpL7a protein in the parasite, the cDNA sequence was cloned as GFP fusion in the pTREX vector. The transfected epimastigote showed , GFP::TcRpL7a expression in the nucleus/nucleoli, which is compatible with the human L7a protein localization.

BM094 - Molecular Diagnostic of *Leishmania chagasi* in peripherical blood

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Visceral leishmaniose is an important public health problem in Brazil, specially in the northeast of the country where it configures an endemic illness. The laboratory diagnosis is done by visualization of the parasite in the bone marrow, being the collection of the sample painful and uncomfortable for the patient. Molecular biology methods to detect leishmanioses could be interesting to avoid invasive procedures and hospital costs related to internalization of the patients in a poor area without hospital conditions. The main objetive of this work is to establish a PCR diagnostic for visceral leishmaniose in peripherical blood. We use DEB8 and PIA3 primers which amplify 809 bp minicircle and 260 bp repetitive sequences from *Leishmania chagasi* (described by Lachaud et al., 2000) to set up individual and multiplex PCRs. The PCR reaction conditions were optimized: $MgCl_2$ concentrations, annealing temperatures and number

of cycles. The ideal conditions for a good amplification were established: for DEB8, 2 mM of $MgCl_2$, a annealing temperature of 58^{o}C and for PIA3 the concentration of $MgCl_2$ was 1 mM and 48^{o}C. We established the sensibility of the method using DNA from promastigotes of *Leishmania chagasi* obtained from axenic cultures using 1pg of DNA for DEB8 and 10pg for PIA3. The experimental infection *in vitro* was made using 10^6 promastigotes forms incubated with blood at 37^{o}C for 2 hours. After incubation, a DNA extraction was performed from each phase defined in a ficollpaque method. PCR was done using DEB8 primers and a 809 bp band was observed in the leucocytary phase. A multiplex PCR was defined to improve the diagnostic of leishmaniose. We used DEB8 and PIA3 primers and defined the multiplex conditions in $MgCl_2$ 1,5 mM with an annealing temperature of 58^{o}C. We got a sensibility of 1 pg similar to individual PCRs and no amplification was observed when DNA from *T. cruzi*, *L. major* and *L. brasiliensis* was used. We have started the validation of the method with patients with clinical suspect of Kalazar from bone marrow with the multiplex PCR from peripherical blood. We hope to establish a rapid and non-invasive molecular biology method to diagnose Leishmanioses routinely in northeastern hospitals.

BM095 - RNA polymerase II transcription of spliced leader genes occurs in a defined nuclear compartment of Trypanosoma cruzi

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It is well known that RNA polymerase II (Pol II) of trypanosomes transcribes long polycistronic messages, which are not capped, but are processed by trans-splicing and polyadenylation to form mature mRNAs. In these organisms, no classical promoters have been recognized and gene expression is controlled post-transcriptionally. The same RNA polymerase also transcribes the genes coding for the spliced leader (SL) RNA, which are capped, exported to the cytoplasm, processed, and re-imported into the nucleus, before they are used as splicing donors to form mRNAs from pre-mRNA polycistronic transcripts. The uncoupling between SL transcription and splicing is reflected in the absence in trypanosomes of the typical heptapeptides repeats found in the carboxy-terminal domain (CTD) of most eukaryotic RNA polymerases II, and suggests that SL and pre-mRNA transcription are spatially separated in the nucleus. Here we provide evidence that transcription of SL RNAs occurs in a particular domain in the Trypanosoma cruzi nucleus, separated from other pre-mRNA transcription. We generated specific antibodies that recognize the RNA polymerase II unique T. cruzi CTD. Using these antibodies, we demonstrated that a large amount of the RNA Pol II is found concentrated in a domain close to the parasite nucleolus coincident with the localization of SL genes. The remaining RNA Pol II is diffusely distributed in the central part of the nucleoplasm, probably representing transcriptional sites of the polycistronic messages. This SL-associated RNA Pol II localization is dependent on the transcriptional state, being abolished by actinomycin D in intact cells and by alpha-amanitin in permeable cells, We thus have identified a new nuclear compartment that recruits large amounts of RNA Pol II for the transcription of SL genes, to a new distinct from the pre mRNA transcription events.
Supported by FAPESP

BM096 - TbNT8 Transporters are the Major Nucleobase Permeases in African Trypanosomes

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Parasites cannot synthesize purines de novo and therefore must salvage these compounds from their hosts. The transport of purine nucleobases and/or nucleosides through the plasma membrane is the first step in the uptake of these compounds, and several purine permeases are expressed in *T. brucei*: TbAT1, an adenosine/adenine permease; TbNT2-TbNT7, encoded by a cluster of related genes; TbNT9, encoded by a single copy gene; and the TbNT8 family encoded by several very closely related genes organized in a tandem repeat. All these genes encode nucleobase/nucleoside transporters with different purine substrate specificities. TbNT8 is expressed at high levels in procyclic forms. Expression in yeast and *Xenopus* oocytes demonstrated that this permease mediates the uptake of purine nucleobases and is expressed in the plasma membrane. Considering the diversity of purine transporters expressed in *T.brucei*, our aim was to investigate the contribution of the *TbNT8* gene family to purine salvage using the method of RNA interference (RNAi). The *TbNT8* ORF was subcloned in the pZJM vector, containing opposing tetracycline-inducible T7 promoters and electroporated in procyclic *T.brucei* strain 19-13. When RNAi was turned on by tetracycline, the cellular growth rate was very similar to the control. However, transport assays demonstrated that uptake of the nucleobase hypoxanthine was reduced 6-fold in T.brucei procyclics when TbNT8 expression levels were reduced by RNAi. The K_m for the residual hypoxanthine uptake was slightly increased in RNAi induced parasites (1,56 $\pm$ 0,56 μM (n=3)) compared to control parasites (0,58 $\pm$ 0,199 μM (n=3)). These results demonstrate that TbNT8 transporters are responsible for the majority (85%) of purine nucleobase uptake in procyclic trypanosomes. Nonetheless, parasites deficient in TbNT8 expression are able to grow at a rate similar to wild type parasites, presumably because other nucleoside and nucleobase permeases allow salvage of essential purines. Financial support: CNPq fellowship, NIH grant AI44138 and AI25920.

BM097 - Determining the *var* gene repertoire size in Amazoniam *Plasmodium falciparum* isolates

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In *Plasmodium falciparum*, a highly polymorphic multi-copy gene family termed *var*, encodes the variant surface antigen *P. falciparum* erythrocyte membrane protein 1 (PfEMP1), which has an important role in cytoadherence and immune evasion of infected erythrocytes. With the final aim to construct a DNA microarray containing most of the var genes in circulation, we first tried to measure the global repertoire size of *var* genes in parasites of a defined area. Using previously described universal PCR primers for the first Duffy binding-like domain (DBLα) of *var* we are analyzing the DBLα repertoires of ten isolates from the Western Brazilian Amazon (Porto Velho, RO). In order to describe repertoires, we first obtained clonal parasite populations, which were then characterized by microssatellite analysis. All isolates showed a single allelic type at each locus and all had a unique genotype combination, suggesting that they were clonal populations. Sequence analysis of the first 4 isolates showed 16-25 unique DBLα sequences per isolate, indicating a repertoire size between 35-50 *var* genes per genome. The number of shared sequence between each of the isolates was 5,33 +/- 2,42, ranging from 3 to 10 shared sequences. These data indicate that the overall repertoire size may be significantly smaller than in previously examined South-East Asian isolates.
Supported by Fapesp.

BM098 - Old yellow enzyme (TcOYE) is down-regulated in *Trypanosoma cruzi* populations with in *vitro*-induced but not *in vivo*-selected resistance or naturally resistant to benznidazole

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Old Yellow Enzyme (OYE) is an NADPH oxireductase that, in *T. cruzi* (TcOYE), catalyzes prostaglandin PGF2alpha synthesis in addition to the reduction of some trypanocidal drugs. Using the DNA microarray methodology, we identified TcOYE, which was 8-fold less expressed in the *T. cruzi* population that showed *in vitro*-induced resistance to benznidazole (BZ) (17LER), as compared to the susceptible counterpart population 17WTS. TcOYE was characterized in fifteen *T. cruzi* strains and clones susceptible and naturally resistant to BZ and nifurtimox and with *in vitro*-induced (17LER) or *in vivo*-selected (BZR) resistance to BZ. Quantification of TcOYE mRNA levels in *T. cruzi* strains by northern blot and real-time RT-PCR analyses confirmed the low level of TcOYE gene expression in 17LER as compared to 17WTS. All the other *T. cruzi* strains and clones expressed the same level of TcOYE independent of their drug resistance phenotype. Real-time PCR and southern blot results showed four TcOYE gene copies in 17WTS and a single copy in 17 LER. TcOYE gene is located in chromosomal band of 1,800 Kb for all strains and, additionally in band 1,900 Kb on zymodeme B strains. The 1,800 Kb band from 17LER was 4-fold less intense than 17WTS. Western blot analysis of *T. cruzi* protein extracts probed with a rabbit anti-recombinant TcOYE polyclonal serum revealed a unique band of 42 KDa for all *T. cruzi* strains. The intensity of this band was similar in all samples, except 17LER that displayed a band nearly 7-fold less intense. Our data show that 3 out of 4 TcOYE gene copies were deleted in *T. cruzi* population with *in vitro*-induced but not *in vivo*-selected resistance or naturally resistant to BZ. These data suggest that the drug resistance mechanism seems to be different between the *T. cruzi* populations *in vitro*-induced and either *in vivo*-selected resistance or naturally resistant to BZ. Supported by CNPq, FAPEMIG, PRONEX, PADCT and PAPES/FIOCRUZ.

BM099 - The Rab/GTPase YPT of Leishmania spp and its role in terbinafine resistance.

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The H region of Leishmania major is a locus that undergoes amplification when the parasite is subjected to drug pressure. We have previously shown that the HTBF gene of L. major is responsible for the H-region mediated resistance to terbinafine. This anti-fungal drug is capable of inhibiting squalene epoxidase, which is a key enzyme in the biosynthesis of ergosterol, an essential component of these parasites' cell membrane. The predicted aminoacid sequence of HTBF showed significant homology to the YIP1 protein of Saccharomyces cerevisae. In the yeast, YIP1 interacts with YPT, a Rab/GTPase related to vesicle docking and trafficking. Therefore, through its interaction with a YPT-related protein, HTBF could mediate resistance to terbinafine using a mechanism that involves the increased formation and/or redirection of vesicles. HTBF could also counteract the interference in ergosterol biosynthesis by activating the membrane

repairing machinery. We are currently investigating the role of L. major YPT gene in terbinafine resistance. The YPT homolog of L. major is a 603 pbs gene coded in chromosome 27. The gene was amplified from the genome by PCR and confirmed by sequencing. It was then cloned into the pGEM vector and into vectors pELHYG2 and pXG1, which are capable of mediating replication and expression within the protozoan parasite. A transfectant bearing pXG1-LmYPT was obtained and is being tested for terbinafine resistance. A possible synergistic action between LmYPT and HTBF is also being investigated by co-transfection of pXG1-LmYPT into terbinafine-resistant cell lines overexpressing HTBF.

BM100 - Polymorphism of the old yellow enzyme (TcOYE) gene in *Trypanosoma cruzi* strains susceptible and resistant to benznidazole

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Murta et al. (2004) observed that the old yellow enzyme (TcOYE) is down-regulated in *T. cruzi* populations with *in vitro*-induced but not *in vivo*-selected resistance or naturally resistant to benznidazole (BZ). OYE is an NADPH oxireductase that, in *T. cruzi*, catalyzes prostaglandin PGF2α synthesis in addition to the reduction of some trypanocidal drugs. In this work, we investigated the polymorphism of the TcOYE nucleotide and amino acid sequences from nine *T. cruzi* strains and clones susceptible and naturally resistant to BZ and nifurtimox and with *in vitro*- induced (17LER) or *in vivo*-selected (BZR) resistance to BZ. The *T. cruzi* samples were previously classified as zymodemes Z1, Z2 and ZB according to their isoenzyme patterns. The TcOYE amplified 1,140 bp ORF fragment from *T. cruzi* samples was cloned, sequenced and analyzed using Phred, Phrap, Consed and Multialine softwares. The multi-alignment of the TcOYE nucleotide and amino acid sequences showed 20 nucleotide mutations with substitution of 6 amino acid residues. None of mutations happened on the TcOYE active site. No association of the nucleotide mutations or amino acid substitutions with resistance phenotype was observed. On the other hand, 12 nucleotide mutations with 3 substitutions of amino acid residues are associated to zymodeme of the *T. cruzi* strains. The others 8 nucleotide mutations with 3 substitutions of amino acid residues are associated to specific *T. cruzi* strains. The TcOYE from *T. cruzi* populations susceptible (17WTS) and with *in vitro*-induced resistance to BZ (17LER) presented the same nucleotide and amino acid sequences. On the other hand, *T. cruzi* clone 16R with *in vivo*-selected resistance to BZ, presented one nucleotide and one amino acid mutation compared to its counterpart clone 4S. In conclusion, our data show that the polymorphism of the TcOYE nucleotide and amino acid sequences are associated with the zymodeme of the *T. cruzi* strain, but not with drug resistance phenotype. Supported by CNPq, FAPEMIG, PRONEX, PADCT, PAP/CPqRR and PAPES/FIOCRUZ.

BM101 - Analysis of histopathological, immunocytochemical and polymerase chain reaction for canine visceral leishmaniasis diagnosis on skin biopsies of naturally infected animals

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Parasite detection is necessary for definitive diagnosis of Canine Visceral Leishmaniasis (CVL). Histological (HE) methods have shown low sensitivity. However, the immunocytochemical (ICQ) and polymerase chain reaction (PCR) have been more accurate than HE. In this work we carried out HE, ICQ and PCR for CVL diagnosis on skin biopsies from naturally infected dogs. Skin biopsies (ears, nose and abdomen) were obtained from twenty nine animals from Zoonosis Department of Belo Horizonte City Hall. These animals with positive serological exams to *Leishmania* (IFAT and ELISA) were grouped in asymptomatic (n 10), oligosymptomatic (n 10) and symptomatic (n 09). For histopathological exams paraffined skin sections stained with Hematoxilin-Eosin (HE) were analyzed by optic microscopy in order to evaluate the parasitism. For ICQ studies the streptoavidin peroxidase immunohistochemistry method was carried out for *Leishmania* detection in canine paraffined tissues (Tafuri et al., 2004). For PCR, primers 13A and 13B were used to amplify a fragment of the conserved region of Leishmania kDNA minicircles. PCR was positive in 82,8%, ICQ 62,1 % and HE 51,7 % of the total animals number. Considering each defined clinical animal group, PCR and ICQ methods were more positive in 77,8 % and the HE method was positive in 66,7 % of the symptomatic animals. Oligosymptomatic animals PCR method was positive in 80 % and the ICQ and HE methods were positive in 40 %. On the other hand, 90 % of asymptomatic animals showed PCR positive reactions, 70 % ICQ positive reactions and 50 % in HE positive analysis. Based on these results we can conclude that PCR was the best parasitological method for the detection of canine visceral leishmaniasis in skin biopsies. In addition, PCR has been shown to be very important to detect the parasite in

asymptomatic animals. Financial support by: FAPEMIG, CNPq and UFMG

BM102 - PRELIMINARY CHARACTERIZATION OF TRANSCRIPTS ABUNDANT IN AMASTIGOTE FORMS OF *LEISHMANIA (L.) MAJOR.*

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Previous work conducted in our laboratory (Iribar et al, 2003) to characterize a *Leishmania major* gene with uncommon features (*ODD1* gene) led to a preliminary study of its genomic region. The analysis revealed two transcripts present in higher levels in amastigotes when compared to promastigotes. Our aim is the characterization of both transcripts, named EB4.4 and EE3.4, localized in a 4.4 kb *Eco*RI-*Bgl*II and in a 3.4 kb *Eco*RI-*Eco*RI genomic fragments, respectively. Subclones from EB4.4 and from EE3.4 have been generated (named pUCEB4.4 and pUCEE3.4) and sequenced using a transposon-based strategy (Tosi & Beverley, NAR, 28:784, 2000). The assembly was accomplished with Phred/Phrap/Consed (Wilson et al, Nature, 368: 32, 1994). The *in silico* analysis revealed two putative genes for pUCEB4.4; a class I Fructose-biphosphate aldolase and a RNA-dependent helicase (Rev.Inst.Med.Trop.S.Paulo 44-suppl.12, 2002). On the other hand, for pUCEE3.4, two Open Reading Frames (ORFs) were predicted to be coded by the same strand but the similarity search in the NCBI databank (http://www.ncbi.nlm.nih.gov) indicates that one of the ORFs did not match to any previously classified gene and the second ORF seems to be a ribosomal protein coding gene (Rev.Inst.Med.Trop.S.Paulo 45-suppl.13, 2003). The levels of transcripts present in pUCEB4.4 and pUCEE3.4 were analysed in Northern experiments. The results indicated that the transcript preferentially expressed in amastigotes corresponds to the ORF coding for the putative ribosomal protein, which was chosen for further functional analyses. For this purpose, a selectable marker for *Leishmania* (the NeoKO-mariner transposon) has been inserted within ORF1 of pUCEE3.4, allowing exclusively the overexpression of the second ORF. The transfectant carrying pUCEE3.4NeoKO did not show any apparent phenotypic change, under high concentrations of G418. Furthermore, to identify this possible overexpressed protein we are currently investigating the expression of this putative gene, comparing protein patterns from the wild type *Leishmania major* - CC1 with the mutant, using 2D-electrophoresis. To investigate subcellular localization of this protein, we submitted pUCEE3.4 to a transposition reaction using a transposon bearing GFP gene (green fluorescent protein gene) to be expressed only if in frame with one of the episomal genes. Sequencing of transposed plasmids allowed choosing a clone with the expected insertion into ORF2. Transfection of this construct (pUCEE3.4GFP) has been conducted.

Supported by FAPESP and CNPq

BM103 - Investigating the role of untranslated regions of phosphoglycerate kinase genes in episome maintenance and transcription control in *Leishmania*

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Glycolisis is compartmentalized in organelles called glycosomes in Trypanosomatids. Among the enzymes involved in the pathway, phosphoglycerate kinase is peculiar for the presence of cytosolic and glycosomal forms. In *Leishmania* species there are two forms of the enzyme: one is present in the glycosome, the PGKC, an enzyme of 51kDa and the second one is cytosolic, PGKB, a 45kDa protein. We are investigating some aspects of the regulation of expression of the PGK genes. Genomic fragments bearing either *PGKB* or *PGKC* genes were subcloned in pX63Neo and transfected in *Leishmania*. PGKB and PGKC transfectants (CC1[pX63Neo*PGKB*] and CC1[pX63Neo*PGKC*], respectively) showed remarkable differences in episome copy number and transcript levels when submitted to high levels of drug pressure (G418 - Azevedo, MS thesis, 2004). Our data supports the speculation that an element present either in the 3' or 5' untranslated regions (UTR) of *PGKC* controls the episome replication and interferes with the level of the *PGKC* transcript. To investigate such regions, chimeras of *PGK* clones were constructed, in which the 5' UTR of *PGKC* was cloned to replace the corresponding region of *PGKB* and vice versa. The same procedure has been used to generate 3'-UTR chimeras. The expected fragment sizes were confirmed by restriction pattern analysis and the chimeras were transfected in *Leishmania* for molecular and phenotypic analyses including evaluation of the episome copy number, transcript and protein levels. Furthermore, to investigate a possible correlation between replication control and stability of the exogenous molecule, we are currently investigating all the transfectants for episome stability in a comparative study using limiting dilution essays.

Supported by FAPESP and CNPq.

BM104 - Molecular characterization of two *Leishmania major* tryptophanyl-tRNA synthetases.

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The fidelity of protein synthesis is dependent on the correct charging of tRNAs with their cognate amino acids. This process is catalyzed by aminoacyl tRNA synthetases (aaRSs) specific for each particular tRNA. From the comparison of sequence homologies and crystal structures, aaRSs

could be divided into class I and II. Tryptophanyl-tRNA synthetase (WARS) belongs to a class I aaRS, which share the consensus sequences "HIGH"and "KMSKS"and the Rossman fold domain. We have characterized two *L. major* tryptophanyl-tRNA synthetase (LmWARS), a cytoplasm localized WARS (LmWARS1) and a WARS with mitochondria localization, (LmWARS2). The mature mRNA transcript for the Lmwars2, is 2347 nucleotides long as defined by 5' and 3'RT-PCR. Southern blot experiments showed that Lmwars2 is a single copy gene. According to the *L. major* genome sequence, this gene is localized in the chromosome 23. In a similar way, Lmwars1 gene transcript was identified by 5'and 3'RT-PCR experiments and Southern blot analysis. Lmwars1 ORF is 1191 base pairs long and is also a single copy gene located in the chromosome 29 of this parasite. These characteristic allows future knockout experiments. The gene Lmwars2 with mitochondrial targeting signal sequence were cloned into a Leishmania specific expression vector (pNUS-HCn) an *L. major* culture has been established. Both enzymes were cloned into the expression vector pET28a and introduced into *Escherichia coli* BL21 (DE3). These constructs resulted in the overproduction of the recombinant proteins. LmWARS1 resulted insoluble and LmWARS2 soluble expression after induction with IPTG. A purification protocol has been developed to LmWARS2 protein, however, the resulting LmWARS2 is unstable, precipitating shortly after purification. Mouse polyclonal antibodies against LmWARS2 were obtained and LmWARS1 will be produced for further imunolocalization and imunoprecipitation experiments Molecular modeling for LmWARS1 has been constructed using as model the citoplasmatic Human triptophanyl tRNA sinthetase.

Work supported by: FAPESP, PRONEX and the University of São Paulo

BM105 - Role of the Leishmania (Viannia) braziliensis gene PGPA in heavy metal resistance

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The study of the ability of Leishmania spp. to evade chemotherapy is important to help the design of effective leishmaniasis treatments. Leishmania major submitted to pentavalent antimonials and other unrelated drugs amplifies a locus of 48kb, the H region. One of the H-region genes, PGPA, confers resistance to antimonials and is associated to vesicular elements of the exocytic and endocytic pathways. We have previously shown that the HTBF gene mediates resistance to terbinafine and may be related to vesicle docking and trafficking. We have also found that in spite of a marked sequence divergence between L. major and L. (Viannia) braziliensis, gene synteny is maintained across the H locus of the two species. The aim of this study is to investigate the activity of PGPA in L. (Viannia) braziliensis and its role in heavy metal resistance. A L. major probe allowed the isolation of part of the LbPGPA gene from a partial genomic library. The cloned fragment of PGPA was further used to isolate the intact gene from a L. braziliensis genomic library constructed into the shuttle vector pELHYG. The role LbPGPA in antimonial resistance is also being investigated by subjecting this species of the Viannia subgenus to drug pressure. Using a stepwise selection protocol we isolated L. braziliensis cell lines resistant to 12 and 20mg/ml of glucantime. Southern analysis revealed that PGPA was not amplified in the cell line resistant to 12mg/ml of glucantime and PFGE analysis showed that the resistant line did not carry amplified episomal molecules. As for cell lines selected in other unrelated drugs such as terbinafine and different from other Leishmania spp., L. (V.) braziliensis does not seem to favor gene amplification as a mechanism underlying drug resistance. Our current efforts are focused on the identification of the molecular mechanism used by the glucantime-resistant L. braziliensis.

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BM106 - CLONING AND EXPRESSION OF PROTEIN TARGETS IDENTIFIED FROM 2D-GELS OF A *Leishmania major* SUBCELLULAR FRACTION.

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The promastigote form of *Leishmania* parasites infects macrophages within which the parasite differentiates into the non-motile amastigote form. Proteins secreted by promastigotes are involved not only in the communication between mammalian host cells and the infective parasite but also in the escape of amastigotes from the phagolysossome of infected macrophages. With the aim of identifying secreted proteins in both Old World and New World Leishmania species, intracellular vesicles from cell lysates of promastigote forms of *L. major* and *L. amazonensis* were extracted by centrifugation. and were analysed by 2D-SDS-PAGE. The peptide mass fingerprint and the sequence tags of tryptic peptides of protein spots from MALDI-TOF-MS analyses were compared with a predicted protein database from *L. major*. Four proteins that were identified using this strategy were selected for further study; Nucleoside diphosphate kinase b (NDKb), GTP-binding protein, calpain-like protease and a carboxypeptidase. In each case, the coding sequence was amplified from total genomic DNA by PCR using oligonucleotides complementary to the 5'and 3'regions in the genomic DNA. Restriction sites for BamH1 were incorporated into both oligonucleotides, and after amplification all fragments were successfully cloned into the vector pT7T3, and subsequently subcloned into the *Leishmania* expression vector pX63NEO. For expression in *E. coli*, restriction sites for NdeI and BamHI were incorporated into oligonucleotides and used to amplify the target sequences already cloned in pT7T3. After digestion with restriction enzymes, these fragments were ligated into the expression vector pET28a. Expression trials in *E. coli* BL21(DE3)pLysS transformed with the resulting constructs showed that of the 4 proteins stud-

ied, only the carboxypeptidase failed to produce detectable levels of recombinant protein. Supported by FAPESP, CNPq and PRONEX.

BM107 - Non-coding RNAs from *Leishmania major*: looking for targets

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On the last decade novel classes of untranslated RNA molecules have been described. These noncoding RNAs (ncRNA) affect a large variety of processes including transcriptional regulation, chromosome replication, RNA processing/modification, mRNA stability and translation. We are currently investigating three putative *Leishmania* ncRNAs genes: *ODD1*, *ODD2* and *ODD3*, whose secondary structure presents hairpin-like motif resembling those found in microRNAs. Based on the knowledge that ncRNAs can cause degradation of its target mRNA or block its translation by sequence complementarity, we designed sense oligonucleotides corresponding to the hairpin region found in the *ODD* genes. To identify putative mRNA targets, these oligonucleotides were used as probes in Northern blot and primers in reverse transcription-polymerase chain reaction (RT-PCR) experiments. In Northern experiments we detected transcripts of 1.7 to 2.2 kb with sense oligos ODD1-2s, ODD2-3s, ODD3-1s and ODD3-2s, while the remaining probes, ODD1-1s, ODD2-1s and ODD2-2s were unable to detect transcripts. The same oligonucleotides were used in RT-PCR experiments in conjunction with a primer designed to anneal to the splice leader region of any trans-spliced RNA. The template used in these experiments was total RNA extracted from promastigotes in mid-log phase of the culture. The products were cloned, sequenced and analysed *in silico* using the *L. major* Friedlin databank. One of the amplified RNA molecules, a 141bp fragment and potential *ODD2* target, is present twice within a 44kb region of chromosome 33 for which no gene was annotated. Curiously, another amplified RNA, potential *ODD1* target, is also present as a duplication of 83 bp within the same region of chromosome 33. The Northern blot and RT-PCR results suggest that this region might be transcribing for other ncRNAs possibly interacting with *ODD1* and *ODD2* final products. Other amplified RNAs were found disperse in the genome. We are also conducting some functional studies to understand the possible role of *ODD* genes and their correlation with the described RNA targets. We generated constructs of each of the *ODD* genes and they were transfected into *Leishmania* to obtain parasites overexpressing each *ODD* gene. Transfectants were recovered and will be analysed for phenotypic changes and to investigate potential changes of the target RNA levels compared to parental strains. Our studies suggest that ODD genes may transcribe for functional ncRNAs and could act as riboregulators.

Financial support from: FAPESP, CAPES e CNPq

BM108 - ANALYSIS OF GENETIC POLYMORPHISM OF N-TERMINAL REGION OF THE P126 PROTEIN IN Plasmodium falciparum FIELD ISOLATES FROM THE BRAZILIAN AMAZON

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

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BM109 - *In silico* and functional analysis of recombination between non-homologous chromosomes of *Leishmania*

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We aim to contribute to the understanding of recombination processes that happen between non-homologous chromosomes of *Leishmania* spp and its correlation to genetic diversity gain. The complete genomic sequence data of *Leishmania major* reference strain allowed us to perform in-house *in silico* analyses comparing the left and right of about 44Kb extremities of the 36 chromosomes of *L. major*. Our aim is to address the extension of recombination between non-homologous chromosomes in the parasite. These analyses revealed that *Leishmania* chromosomes present a high degree of sequence shuffling taking place at the chromosomal ends. We decided to investigate the function and expression profile of one of the genes involved in the shuffling process. The chosen gene is a Peptidyl dipeptidase (DCPA) or oligopeptidase A, the most extreme gene located at the ends of chromosomes 1, 2 and 27 of *L. major*. Local alignment searches (BLASTX) between these chromosomal ends revealed that the *L.major* DCPA gene duplication is part of a 16kb region shared between chromosomes 2 and 27. Alignment of *L. major* DCPA gene sequences revealed differences suggesting that they may be playing different functional roles. We are currently performing experiments aiming the evaluation of the expression profile and function diversity of these genes. In addition, comparative pulsed field gel electrophoresis (PFGE) revealed that DCPA is a single copy gene in *L. braziliensis*. Furthermore, an analysis of the observed shuffling between non-homologous chromosome is under way to compare chromosomal ends of *L.major* and *L.braziliensis*.
Supported by FAPESP and CNPq

BM110 - Participation of the serine-rich *Entamoeba histolytica* protein in amebic adhesion to and uptake of dead cells

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Cytotoxicity and phagocytosis characterize invasive infection with the enteric protozoan *Entamoeba histolytica*. In our preliminary studies, host cell killing was caspase 3-dependent and preceded amebic engulfment of killed cells. Cytotoxicity required contact via the amebic galactose-specific lectin, but only partial inhibition of phagocytosis by D-galactose implicated a second receptor. Here, we show that the serine-rich *E. histolytica* protein (SREHP), a polymorphic surface antigen with multiple tandem repeats and no known function, is a potential phagocytosis receptor. To identify receptors for engulfment of apoptotic host cells, monoclonal antibodies against *E. histolytica* membrane preparations were screened for inhibition of phagocytosis. Of 40 antibodies tested, one (10D11Ab, IgG1 isotype) blocked uptake of apoptotic Jurkat lymphocytes by 89 ± 8% (20 mg/ml, mean ± SD vs. isotype control for all data, n=9, p=0.001)). Adherence inhibitory IgG1 monoclonals to the galactose-specific lectin heavy and intermediate molecular weight subunits had no effect. 10D11Ab only reduced adherence to healthy cells by 25 ± 14% (n=9, p=0.001) and cell killing (^{51}Cr release) by 26 ± 8% (n=3, p=0.05). In contrast, 10D11Ab reduced adherence to apoptotic cells by 59 ± 8% (n=3, p=0.002); 10D11Ab and D-galactose (11 mM, inhibits adherence ≈50%) together reduced adherence to apoptotic cells by 84 ± 5% (n=3, p=0.0003). 10D11 antigen was partially purified by immunoaffinity chromatography and mass spectrometry sequencing identified peptides from SREHP. Exogenous expression in Eschericia coli (BL21(DE3)pLysS) and immunoblots confirmed recognition of SREHP by 10D11Ab. These experiments suggest that SREHP participates in amebic phagocytosis of apoptotic host cells, perhaps by facilitating adherence to killed cells. Since SREHP is an acylated membrane protein with no intracellular domain, it likely functions as part of a larger signaling complex. To further investigate SREHP's role in adherence, we are testing the ability of Chinese hamster ovary cells stably transfected with SREHP to bind apoptotic cells and bacteria.

Bioquímica - Biochemistry

BQ01 - The presence of free D-alanine in *Leishmania amazonensis*

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Protozoan parasites of the genus *Leishmania* are responsible for a spectrum of diseases. Leishmania amazonensis causes the diffuse cutaneous leishmaniasis in humans in South America. This poorly understood condition presents with numerous disseminated lesions and is often irresponsive to chemotherapy. Alanine is the most prominent free amino acid present in these parasites, and of several other species of protozoa. The presence of free D-amino acids has been implicated in several biological functions. The aim of this study was to examine the presence of the D-form of alanine in promastigotes forms of Leishmania amazonensis. Measuring the chiral amino acid contents by high performance liquid chromatography we detected a significant amount of free D-alanine, in parasites promastigotes. The concentration of free D-alanine in the cellular extract was estimated to be 514+/-84 pmol per mg dry cell weight. This content represents 8.9 per cent of total free alanine, being highly concentrated in the soluble fraction (80.6 per cent). Furthermore, we were able to decrease this pool of D-amino acid, without affecting the total alanine amount, by treating our cultures with D-cycloserine, a known alanine racemase inhibitor. The fact that D-cycloserine could specifically decrease D-alanine concentration in Leishmania amazonensis cultures suggests a specific biosynthesis pathway. Supported by: Pronex, CNPq, CAPES and FAPERJ.

BQ02 - Glucose transport in *TRYPANOSOMA RANGELI* trypomastigotes

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TRYPANOSOMA RANGELI is a hemoflagellate parasite that affects humans as well as domestic and wild animals in Central and South America. This parasite has an overlapping distribution with *T. cruzi*, allowing the occurrence of single and or mixed infection in both vertebrate and invertebrate hosts in same geographical region. Little is know about membrane transport systems in this parasite. In this study, we characterized the glucose transport activity present in tripomastigotes parasites using D-[U-^{14}C] glucose. *T. rangeli* tripomastigotes were obtained by cultivation of epimastigotes in DMEM medium at 28^0 C according Koerich et al ⟨2002⟩. Parasites were washed three times with phosphate buffer saline ⟨ PBS, pH 7.4 ⟩ by centrifugation, and ressuspended in the same buffer at a concentration of 1 x 10^8 cells/ml. Transport assays were performed using 50 μ l of cells, and initiated by the addition of 10μl of the desired concentration of D-[U-^{14}C] glucose. After incubation at 25^{0}C for 5 s, the cells were diluted in 1 ml of ice-cold PBS containing 100 μM $HgCl_2$, centrifuged, and washed with same buffer. Our results indicate that these tripomastigote cells transported glucose by means of a facilitated transporter with a high affinity for D-glucose ⟨ Km 80 μM, Vmax 0.6 nmol/min/ 10^7 cells ⟩. Competitive experiments using glucose analogues and other carbohydrates showed a higher inhibition with 2-Deoxy-D-glucose, D-mannose and D-glucosamine. No inhibition was observed in the presence of D-fructose, pentoses and D-galactose, differently that it was observed respectively in other tripanosomatids and in *T. rangeli* epimastigote form. *T. rangeli* epimastigote glucose transport are under investigation.
Supported by: CNPq and Funcitec

BQ03 - CLONING, EXPRESSION AND CHARACTERIZATION OF A Plasmodium falciparum OCTAPRENYL PYROPHOSPHATE SYNTHASE

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The isoprenoid biosynthesis of intraerythrocytic stages of P. falciparum has been studied and pointed out as an important target for the development of new antimalarial drugs. End products of the isoprenoid metabolic pathway are prenylated proteins, dolichols and ubiquinones. The isoprenoid chains derive from condensation reactions of linear 5-carbon prenyl diphosphates. A family of enzymes known as prenyltransferases catalyses the condensation. Searching for novel drug targets in the pathways leading to isoprene molecules in Plasmodium falciparum, we analysed a putative polyprenyl synthase from the genome sequence. The corresponding gene is transcribed in ring and trophozoite stages of Plasmodium falciparum, but not in schizont blood stage parasites. After expression in Escherichia coli, the recombinant protein as well as a partially purified protein from Plasmodium falciparum extracts exerts clearly an octaprenyl synthase activity, revealed by mass spectrography; and a partially purified fraction from parasites, with the similar Michaelis constants. Polyclonal antibodies raised against the recombinant protein also confirmed the presence of the native protein in trophozoite and schizont stages. The enzyme is inhibited by the terpene Nerolidol an isoprenoid analogue. Due to its absence in humans, the MEP pathway of isoprenoid precursor biosynthesis and the identified enzyme and its inhibitors may therefore be exploited for Malaria therapy.
Supported by FAPESP

BQ04 - THE METABOLITE 1-DEOXY-D-XYLULOSE-5-PHOSPHATE IS NOT ONLY AN INTERMEDIATE OF THE NON-MEVALONATE PATHWAY FOR THE BIOSYNTHESIS OF ISOPRENOID BUT IS ALSO INVOLVED IN de novo BIOSYNTHESIS OF PYRIDOXAL IN *Plasmodium falciparum*

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Two genes encoding the enzymes 1-deoxy-D-xylulose-5-phosphate synthase and 1-deoxy-D-xylulose-5-phosphate reductoisomerase have been recently identified, suggesting that isoprenoid biosynthesis in the major malaria parasite *Plasmodium falciparum* depends on the methylerythritol phosphate (MEP) pathway, and that fosmidomycin could inhibit the activity of 1-deoxy-D-xylulose-5-phosphate reductoisomerase. The metabolite 1-deoxy-D-xylulose-5-phosphate is not only an intermediate of the MEP pathway for the biosynthesis of isopentenyl diphosphate but is also involved in the biosynthesis of thiamin (vitamin B1) and pyridoxal (vitamin B6) in plants and many microorganisms. Herein we report the first isolation and characterization of most downstream intermediates of the MEP pathway in the three intraerythrocytic stages of *Plasmodium falciparum*. These include, 1-deoxy-D-xylulose-5-phosphate, 2-C-methyl-D-erythritol-4-phosphate, 4-(cytidine-5-diphospho)-2-C-methyl-D-erythritol, 4-(cytidine-5-diphospho)-2-C-methyl-D-erythritol-2-phosphate, and 2-C-methyl-D-erythritol-2,4-cyclodiphosphate. These intermediates were purified by HPLC and structurally characterized via biochemical and electrospray mass spectrometric analyses. We have also investigated the effect of fosmidomycin on the biosynthesis of each intermediate of this pathway and isoprenoid biosynthesis (dolichols and ubiquinones). An active biosynthesis of pyridoxine (vitamin B6) in parasite metabolically labeled with [2-^{14}C]1-deoxy-D-xylulose-5-phosphate is also demonstrated. For the first time, therefore, it is demonstrated that the MEP pathway is functionally active in all intraerythrocytic forms of *Plasmodium falciparum*, and de novo biosynthesis of pyridoxal in a protozoan is reported. Its absence in the human host makes both pathways very attractive as potential new targets for antimalarial drug development.

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BQ05 - Subcellular distribution, purification and kinetic characterization of native and recombinant enolase from *Leishmania mexicana*

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Enolase is a glycolytic enzyme in *Leishmania mexicana* promastigotes, which catalyzes the interconvertion of 2-phosphoglycerate in phosphoenolpyruvate. Subcellular distribution studies by differential and isopicnic centrifugation and digitonin treatment showed that enolase activity was exclusively found in the cytosol. Native enolase was partially purified, with a specific activity of 18 U/mg. The Michaelis-Menten constant for 2-phosphoglycerate was 80μM and 215 μM for phospho*enol*pyruvate. The recombinant enzyme was overexpressed in *Escherichia coli* and purified to homogeneity, with a specific activity of 38 U/mg. The Michaelis-Menten constants for the purified recombinant enolase were 50 μM and 200 μM for 2-phosphoglycerate and phospho*enol*pyruvate, respectively. Native enolase was inhibited by pyrophosphate, fluoride and fluoride in presence of inorganic phosphate. K_is for these compounds were 30 μM, 2 mM and 40 μM for the glycolytic reaction, and 237 μM, 6 mM and 133 μM for the gluconeogenic reaction, respectively. Similar results were obtained for the recombinant protein. Enzyme assays with cytosol from*Leishmania mexicana* promastigotes revealed that enolase was activated in presence of dithiothreitol, KCl and decreasing enzyme concentrations. These results show the possible formation of oligomeric states, product of feasible ionic and redox interactions, which may well be modulating enolase activity and regulating the different pathways phospho*enol*pyruvate follows in the cell.

BQ06 - SUBCELLULAR LOCALIZATION, PURIFICATION, CHARACTERIZATION AND PROTEIN-PROTEIN INTERACTION STUDIES OF THE ENOLASE FROM *Trypanosoma cruzi* EPIMASTIGOTES

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Enolase is a glycolytic enzyme and a potential drug target for the treatment of Chagas disease. We determined that the activity of this enzyme is exclusively localized in the cytosol

of *Trypanosoma cruzi* epimastigotes, according to differential and isopicnic centrifugation and digitonin permeabilization. The enzyme was purified to homogeneity with a specific activity of 182 U.mg^{-1} and an approximate molecular weight of 49 kDa. The enzyme has a pH optimum between 8 and 8.5 and a Mg^{++} optimum of 2 mM. Other divalent cations, Mn^{++}, Co^{++} and Ca^{++} in the presence of Mg^{++} inhibit the enzyme. The K_m is 55 μM for 2-phosphoglycerate and 147 μM for phospho*enol*pyruvate. The k_{cat} value is 4766 min^{-1} for the glycolytic reaction and 2038 min^{-1} for the gluconeogenic reaction. Fluoride, both in absence and presence of inorganic phosphate competitively inhibits the enzyme, with K_i of 2570 μM and 45 μM respectively. Pyrophosphate also inhibits the enzyme, with a K_i value of 200 μM. The partially purified enzyme is activated by decreasing protein concentration. We found that enolase from *Trypanosoma cruzi* interacts with other parasite proteins that co-purify with it, as shown by ligand blot, gel filtration chromatography and native and bidimensional electrophoresis. We performed MALDI-TOF peptide mass fingerprinting in order to identify these proteins.

Work supported by: EEC, CDCHT

BQ07 - Biochemical characterization of the glutamate transport system in *Typanosoma cruzi.*

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Amino acids and glucose are the main carbon and energy sources in trypanosomatids. The relevance of L-proline transport in *Trypanosoma cruzi* intracellular stages was previously demonstrated (Silber et al. 2001, J. Eukaryot. Microbiol. 49, 441-446; Tonelli et al. 2004, Cell. Microbiol. 6, 733-741). Proline, as well as glutamate and aspartate, are involved in the epimastigote to metacyclic trypomastigote differentiation process (Contreras et al. 1985, Mol. Biochem. Parasitol. 4, 83-96). However, little is known about the transport of glutamate by this parasite. In the present work, the kinetic parameters and mechanism of the the glutamate transporter in *T. cruzi* epimastigotes have been biochemically characterised. It consists of a single transport system with a Km = 0.35 mM and a Vmax = 0.095 nmol/min x $20x10^6$ cells. The transport activity remains constant along a period of starvation of 3 hours. This transport process showed to be insensitive to the ions Na^+ and K^+, but it was sensitive to the extracellular pH, with optimum activity at pH = 5. The inhibition of glutamate transport by FCCP suggest that this system is powered by the cytoplasmic membrane H^+ gradient. Further studies on this system under the light of the available information on proline and glucose transport will facilitate the understanding of the energy sources used by *T. cruzi* in the different life cycle stages.

Supported by FAPESP and CNPq.

BQ08 - *Leishmania (Viannia) braziliensis* - macrophage interactions: Involvement of a flagellar glycoprotein containing α-GalNAc residue in the glycan structure

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Glycoconjugates are known to be involved in *Leishmania*-macrophage interactions as described by several authors. In our lab it was produced a monoclonal antibody (mAb), termed SST-3, which recognizes specifically high molecular weight glycoproteins (180 - 200 kDa) present in *Leishmania (Viannia) braziliensis* promastigote flagellar surface. The mAb SST-3 was able to inhibit significantly macrophage infectivity by promastigotes. The characterization of the 180 kDa flagellar glycoprotein and its role in macrophage infection has been investigated. By Western blotting it was demonstrated that *Helix pomatia* lectin recognizes the gp180 and by indirect immunofluorescence it was verified that this lectin binds only in the flagella of *L. (V.) braziliensis* promastigotes. Binding competition of mAb SST-3 and *H. pomatia* lectin to gp180 was evaluated by indirect immunofluorescence. A dose-dependence inhibition was observed when promastigote forms of *L. (V.) braziliensis* were pre-incubated with *H. pomatia* lectin and then with mAb SST-3 and anti-mouse IgG conjugated to fluorescein. On the other hand, when the parasites were pre-incubated with mAb SST-3 and then with biotinylated *H. pomatia* lectin no fluorescence was detected. This data could be due to the mAb SST-3 reactivity to the triplet glycoprotein, 180-200 kDa, while *H. pomatia* lectin recognizes only the major component corresponding to gp180. In order to identify the macrophage receptor which recognizes the 180 kDa flagellar antigen of *L. (V.) braziliensis*, mouse macrophage (glyco)proteins were fractionated by SDS-PAGE and transferred to nitrocellulose membrane. It was shown that flagella bind specifically to a 30 kDa macrophage protein and pre-incubation of flagella with mAb SST-3 inhibits almost completely the flagellar-macrophage receptor interaction. The competition verified between mAb SST-3 and *H. pomatia* lectin suggests that this mAb recognizes a glycan structure containing α-GalNAc residue and that this epitope may be involved in *L. (V.) braziliensis* flagella-macrophage interaction.

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BQ09 - Purification and characterization of glycolipids from *Leishmania (Leishmania) amazonensis* promastigotes

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The cutaneous leishmaniasis in the New World is caused by several species of *Leishmania*, such as *L. (L.) amazonensis*. The *Leishmania* during his life cycle alternates between non-motile amastigote inside mammalian macrophage and flagellated promastigote in the phlebotomine vector. Several authors have described the parasite-macrophage interaction is dependent on the presence of surface membrane glycoconjugates. Our lab have already established the role of *L. (L.)*

amazonensis amastigotes stage-specific glycosphingolipids in the interaction of these forms with macrophages. Purification and characterization of *L. (L.) amazonensis* promastigote glycolipids can contribute to elucidate the mechanisms of promastigotes-macrophage interaction. *L. (L.) amazonensis* promastigotes were grown in LIT (Liver Infusion Tryptose) medium supplemented with 10% heat inactivated fetal calf serum, at 23^{O}C and the lipids/glycolipids were extracted with a mixture of isopropyl alcohol/hexane/water (55:20:25, v/v/v) and chloroform/methanol (2:1, v/v). The organic extracts were combined and dried in rotary evaporator. On a first step total lipids extract were fractionated by Folch partition. The lower phase, after dialysis, showed the presence of glycolipids and phospholipids as identified by orcinol/H_2SO_4 and Dittmer Lester reagent by High Performance Thin Layer Chromatography (HPTLC). The lower phase was fractionated by chromatography in DEAE-Sephadex. The neutral fraction was chromatographed on a Silica gel 60 column. The fraction eluted with chloroform/methanol (3:7; v/v) was further purified by High Performance Liquid Chromatography (HPLC) on Iatrobeads 8010 column using a gradient of isopropyl alcohol/hexane/water (55:43:2 to 55:30:15; v/v/v). The structure of at least three components are under study by GC-MS. The acidic fraction eluted from DEAE-Sephadex with 0.05 M sodium acetate in methanol showed the presence of inositolphosphoceramide, as characterized after alkaline hydrolysis and GC/MS analysis. Studies are currently been carried out in order to establish differences in glycolipids expression between *L. (L.) amazonensis* amastigotes and promastigotes.

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BQ10 - Heparin-receptor like present in *Leishmania braziliensis* promastigotes and its involvement in the interaction with the phlebotomus midgut

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Leishmania is a dimorphic parasite, whose life cycle is dependent on the recognition of molecules from the host, on the surface of mammalian phagocytic cells and sand fly midgut components. Nowadays, there is some evidences that glycosaminoglycans and probably their receptors may influence the parasite life cycle in both hosts (Volf *et al.*, 2001; Butcher *et al.*, 1992; Love *et al.*, 1993). However, the description and structure of the *L.braziliensis* glycosaminoglycan-binding receptors are not clearly understood, as well as the phlebotomus midgut related proteins. In this work, we propose a strategy to obtain the heparin-binding receptors from infective *L.braziliensis* promastigotes. Also, we have described the potential of such receptor to recognize molecules at the sand fly midgut. The approach to isolate the heparin-binding receptor consisted of an association of the Triton X-114 method with heparin-sepharose and DEAE-cellulose chromatography. SDS-PAGE analysis of the parasites hydrophobic protein extracts revealed proteins bands of 63kDa and 55kDa, while a single protein, eluted in one fraction at the final chromatograph step, was detected with the native electrophoresis assay. Further, radiolabel analysis were performed to verify the ability of such isolated heparin-binding receptor to recognize *Lutzomyia* sp midgut proteins. In this experiment, the solubilized midgut proteins were resolved in SDS-PAGE, transferred to 0.2 μm nitrocellulose membranes and incubated with the isolated radiolabeled heparin-binding receptor. As controls, the nitrocelulose membranes were previously incubated with non labeled *L.braziliensis* promastigote proteins or PBS. The autoradiograph image revealed that radiolabeled promastigote hydrophobic proteins bind to several polypeptides of the vector midgut while that the heparin-binding receptor interact with specific polypeptides. Our data demonstrate the presence of a heparin-binding receptor in infective *L.braziliensis* promastigotes and it represents a physicochemical property to attach on the midgut well defined proteins from sand fly vector. Supported by CAPES/FAPERJ/CNPq.

BQ11 - Detection of calpain-like proteins in a wide range of trypanosomatids by antibody cross-reactivity

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Calpains are a family of neutral Ca+2 dependent cysteine proteases ubiquitously expressed in mammals. Several homologues of this enzyme have been detected in lower organisms such as insects, nematodes, fungi and yeast. In trypanosomatids, a cytoskeleton-associated protein (CAP5.5 or TbCALP1) has been shown to display similarity restricted to the catalytic region of calpain-type proteins. The discovery of calpain homologues will further the study of calpain and provide a powerful genetic tool. In addition, there are several lines of evidence indicating that calpain-catalyzed proteolysis is involved in the regulation of various cellular functions. Therefore, the identification and characterization of calpain-like proteins in trypanosomatids may reveal an interesting new alternative for drug design. In the present study we have screened several trypanosomatids for the presence of calpain-like enzymes by western blotting analysis using seven different antibodies: C21, C23 and C24 (raised against the whole molecule, the cysteine active site, and the histidine active site, respectively, of human brain m-calpain); anti-Dm-calpain (raised against the *Drosophila melanogaster* calpain); anti-CAP5.5 (raised against the *T. brucei* calpain-like protein); anti-CDPIIb and anti-Ha-CalpM (raised against the whole molecule of a lobster calcium dependent protease and the N-terminal region of a muscle specific calpain from the American lobster *Homarus americanus*). Our results indicate that trypanosomatids from the genera *Trypanosoma*, *Leishmania*, *Phytomonas*, *Leptomonas*, *Herpetomonas*, *Blastocrithidia* and *Crithidia* possess a protein migrating at 80 kDa that shares common epitopes to calpains, although

quantitative differences have been detected. The striking difference was detected between the endosymbiont-harboring and endosymbiont-free strains of *Crithidia deanei* and among *Leishmania amazonensis*, *Leishmania braziliensis* and *Leishmania chagasi*. A combination of the basic characterization of each molecule and comprehensive comparative studies will help in disclosing both the general and specific functions of calpains in trypanosomatids.
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BQ12 - Proteolytic activities of *Bodo* sp., a free-living flagellate

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In this study, we report the characterization of cell-associated and extracellular proteinases of *Bodo* sp., a free-living flagellate of the family Bodonidae, order Kinetoplastida. Cells were grown for 7 days at 28in BHI medium with feeder bacteria. After centrifugation, 10^8 cells were lysed by the addition of SDS-PAGE sample buffer (Laemmli, 1970, Nature, 227:680-685). Alternatively, 10^8 cells were incubated in PBS for 8 hours, the cells were removed by centrifugation and the supernatant was concentrated against polyethyleneglycol before the addition of sample buffer. The proteolytic activity was determined by SDS-PAGE containing co polymerized gelatin as substrate (Heussen and Dowdle, 1980, Anal. Biochem., 102:196 202). Following electrophoresis, the gels were soaked in Triton X-100 and then incubated for 24h at 20, 28and 37in different pH values, in the presence or absence of the proteinase inhibitors. The SDS-PAGE-Gelatin analysis revealed that the proteolytic activity was better detected at 28in pH 5.5, although there was no qualitative difference among the different pH values and temperatures tested. Four cell-associated bands migrating at 120 kDa, 100 kDa, 80 kDa and 70 kDa were detected. In the bacterial pure culture, obtained by plating in BHI-agar, only the 100 kDa band was observed. The higher molecular mass proteases belong to the serine-proteinase class, based on its inhibition by 1mM PMSF, as well as the 100-kDa band detected in the flagellate and in the bacterium. On the other hand, the 80 kDa and 70 kDa were not inhibited by PMSF, E-64, 1,10 phenanthroline or pepstatin. Both the bacteria and the protozoan released a 100 kDa band, which was inhibited by PMSF. The biochemical characterization of proteases in non-parasitic kinetoplastid flagellates may help to determine their relationship to the already characterized trypanosomatid proteases.
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BQ13 - INFLUENCE OF ANTILEISHMANIAL GLUCANTIME ON THE LABELING OF RED BLOOD CELLS WITH TECHNETIUM-99m

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Leishmaniasis, a disease caused by protozoa of the genus *Leishmania* is distributed worldwide. Despite the tremendous advances made in understanding the immunology, biochemistry and molecular biology of *Leishmania*, there is still no efficient vaccine against this disease. The pentavalent antimonial glucantime is the elected drug for the treatment of all the clinical forms in humans. The influence of drugs on the labeling of red blood cells and plasma proteins with technetium-99m (^{99m}Tc) has been reported. The labeling of blood elements with ^{99m}Tc is employed in nuclear medicine. In this work is reported result of studies involving the influence of glucantime, which has potent antileishmanial activity, on the labeling of red blood cells, plasma and cellular proteins with ^{99m}Tc using an *in vitro* study. Blood was withdrawn from *Wistar* rats (n=10) and incubated with various concentrations of glucantime. Solution of stannous chloride (a reduction agent) and ^{99m}Tc were added. Blood was centrifuged and plasma (P) and red blood cells (C) were isolated. Samples of P and C were also precipitated with trichloroacetic acid, centrifuged and insoluble (IF) and soluble (SF) fractions were separated. The results showed that the high concentration (100%) of glucantime was capable to reduce significantly (p menor que 0.05) the uptake of ^{99m}Tc (%ATI) on C and the fixation on the IF-C. The %ATI decreased on C from 96.99±0.89 to 71.17±5.01 and on IF-C from 92.65±10.44 to 72.14±7.17. It was also observed a slight crenation and hypocromia on the red blood cells. Once in blood elements labeling procedure with ^{99m}Tc depends on the presence of stannous (+2) ions, the substances present in the glucantime probably could increase the valence these ions to stannic (+4). This fact would decrease the %ATI on blood elements and would indicate the presence of oxidant agents in the glucantime.

BQ14 - Identification of proteins that specifically associate with the double-stranded *Leishmania (L.) amazonensis* telomeres.

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Telomeres are specialized structures located at the end of linear chromosomes. They are composed of proteins that form complexes with the telomeric repetitive DNA, and are essential to maintain genome stability and cell viability. Telomeric proteins can associate with the single-stranded or the double-stranded DNA, in sequence or structural manners. The im-

portance of telomeric binding proteins for telomere architecture and telomerase regulation has lead to their identification in diverse species ranging from yeast to human. *Leishmania (L.) amazonensis* is the causative agent of cutaneous leishmaniasis, a debilitant disease occurring in the tropical areas of Brazil. *Leishmania* spp. telomeres are composed by the conserved TTAGGG sequence, which is maintained by telomerase. Proteins that associate to the G-rich telomeric strand were identified in affinity purified extracts of *L. amazonensis*. However, there are no descriptions of proteins that associate with the double-stranded form of *Leishmania* spp. telomeres, which is our present goal. Using biochemical approaches (chromatography and Electrophoretic Mobility Shift Assay), two *L. amazonensis* complexes were identified. They interact with the double stranded telomeric DNA and were named LaTAF1 and LaTAF3 (*Leishmania amazonensis* telomere associated factor 1 and 3). Other complexes were also formed but they seem to be the result of a supershift or protein multimerization (LaTAF2, LaTAFa and LaTAFb). The *in vitro* characterization of the complexes indicated that they are specific for *Leishmania* spp. telomeres and that they need more than three telomeric repeats to be formed. Footprinting analysis is being used to determine the specific binding site. In order to purify and biochemically characterize these proteins we are currently performing complementary purification using two affinity chromatographic columns. The purified proteins will be fractionated in Coomassie stained protein gels followed by fingerprinting analysis and sequence *de novo* by mass spectrometry (MALDI-TOF and ESI-MS/MS). Financial support: FAPESP, WHO/TDR-UNDP Bank

BQ15 - Proteolytic activity of the proteasome in Plasmodium falciparum.

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The ubiquitin-proteasome pathway is the principal mechanism in the cell for control protein degradation (Glickman et al, 2001, Physiological Reviews 82, 373-428). The degradation of a protein by ubiquitin-proteasome pathway involves two distinct and successive steps: (a) covalent attachment of multiple ubiquitin molecules to the target protein, and (b) degradation of the targeted protein by 26S proteasome. 26S proteasome is a complex divided into three sub-complexes: 20S, 19S, and 11S. Several studies have also demonstrated that proteasome dysfunction induces apoptosis in various types of cells (Drexler et al, 1997,Proceedings of the National Academy of Sciences USA 94, 855-860; Lopes et al., 1997 Journal of Biological Chemistry 272, 12893-12896). The existence of a Plasmodium proteasome has been shown indirectly by using inhibitors as lactacystin (Li et al., 2000, International Journal for Parasitology 30, 729-733), and directly by cloning of the gene of the 20S proteasome b-subunit (Certad et al., 1999, Experimental Parasitology 93, 123-131). During erythrocytic stages the parasite undergoes radical morphological changes and many rounds of replication, events that likely require proteasome activity (Gantt et al., 1998, Antimicrobial Agents Chemotherapy 42, 2731-2738). In our laboratory, we have demonstrated that proteasome activity was presented in schizont stage, suggesting that activity is probably related with erythrocytic schizogony where occurs the highest turnover of proteins and nucleic acid synthesis. Studies reported in our laboratory have demonstrated that terpenes (farnesol, limonene, linalool or nerolidol) inhibit the development of the intraerythrocitic stage of P. falciparum in vitro, probably by interfering in the elongation of the isoprenic chain attached to benzoquinone ring of coenzyme Q, dolichols, and inhibiting the isoprenylation of proteins in P. falciparum (Macedo et al., 2002, FEMS Microbiology Letters 207, 13-20; Moura et al., 2001, Antimicrobial Agents Chemotherapy 45, 2553-2558; Rodrigues Goulart et al., 2004, Antimicrobial Agents and Chemotherapy 48, 2502-2509). Preliminary results, have demonstrated that farnesol, as lactacystin, inhibited the proteolytic activity of the proteasome in cultures of the P. falciparum. However, the activity proteolytic of the proteosome increased when cultures of the P. falciparum were treated with limonene. Nerolidol has not demonstrated effect upon the activity proteolytic of the proteasome. Additional events as decrease of the mitochondrial membrane potential (Dym) have been observed when cultures of the P. falciparum were treated with lactacystin or farnesol. This suggests that inhibition the proteolytic activity of the proteasome in P. falciparum could be acting as starter of a cascade of events similar to apoptosis.
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BQ16 - Comparative study of the effect of derived of the lapachol in *Crithidia deneai* with and without endosymbiont.

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Monoxenous trypanosomatids were known as protozoa not able to infect vertebrates. Recently, Santos DO and Bourguignon, SC (2004) demonstrated that *C.deanei* is able to infect murine fibroblast whereas *C.fasciculate* endosymbiont-free) does not. *C.deanei* presents a bacterium-like endosymbiont in their cytoplasm (Faria-e-Silva et al., 1996). Our laboratory also reported that the presence of the endosymbiont represents the resistance of *C.deanei* to the lyses by complement (Santos DO and Bourguignon SC, 2004). Thus, the goal of the present work is to investigate whether the endosymbiont within *C.deanei* can influence trypanosomatid-resistance to the Lapachol-derived substances. Materials and Methods 1- Parasite-*C.deanei*, with and without endosymbiont were kept in liver infusion BHI-medium. 2-Trypanocidal Assay and substances- A stock so-

lution of substance α-lapachone, β-lapachone, epoxide of nor-α-lapachone, epoxide of -α-lapachone, alil-nor-β-lapachone, LAPAC2O, Epoxilau and 1,2-sodium sulfonate- was prepared in DMSO (1.0%). 3-Analyze of the cytotoxic effect was done by counting in a optical microscopy. The final concentration of all the drugs tested was 50μM. Results- When *C.deanei* with endosymbiont were treated by β-lapachone, epoxide of nor-α-lapachone, LAPC20, we observed a slight reduction in the parasite proliferation. Interestingly, the same tripanossomatid whithout endosymbiont were almost completely killed (90%) at the same experimental conditions. Surprinsing, the following substances: alil-nor-β-lapachone and Epoxilau were able to kill both parasites (*C.deanei* with and without endosymbiont). Conclusion- Taken together our results showed that the strongest resistance to the substances used here was determined by the presence of the endosymbiont in the *C.deanei*.

BQ17 - Dimethylsulfoxide induces aberrant cellular division, differential expression of cell-surface polypeptides and enzymatic activities, and apoptosis in the insect trypanosomatid *Herpetomonas samuelpessoai*

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Dimethylsulfoxide is a widely used agent in cell biology. It is well known as a cryoprotectant, cell fusogen and a permeability enhancing agent. These applications depend, to a greater or lesser extent, on the effects of DMSO on the stability and dynamics of biomembranes. DMSO at 4% triggers the process of cellular differentiation in *Herpetomonas samuelpessoai*. Little information is known about the signal transduction pathways that could be involved in the effects of DMSO on the morphology and physiology of these flagellates. In the present study, we showed that 4% DMSO promoted an inhibition by approximately 50% on the cellular growth of *H. samuelpessoai* when cultivated in defined medium for 48 h at 26C. It is known that DMSO induces apoptosis in several cellular systems. In this sense, we extracted the DNA from *H. samuelpessoai* after DMSO treatment and apoptotic DNA nucleosomal fragmentation was observed after agarose gel, which shows that DMSO triggers apoptosis pathway in this monoxenous trypanosomatid. DMSO also seemed to induce perturbation during the cell division in *H. samuelpessoai*, as detected by Giemsa stained smears, in which approximately 10% of parasite population produced aberrant division forms, including cells with dissimilar distribution of nucleus and kinetoplast. Since DMSO could be altering some *H. samuelpessoai* membrane properties, we tried to identify some alteration in the cell surface protein composition as well as ecto-protease and ecto-phosphatase activities in non treated and DMSO treated parasites. For instance, DMSO induced a markedly down regulation in the synthesis of 97 kDa polypeptide. Ecto-metalloprotease activity, sensitive to the zinc chelator *O*-phenanthroline, had its activity reduced by approximately 20% in DMSO treated parasites when compared with non treated ones. On the other hand, ecto-phosphatase activity was majority expressed in DMSO treated cells, as judged by cytochemical analysis. SUPPORTED BY - CNPq, FAPERJ, FUJB.

BQ18 - Leishmanolysin (gp63 Metalloprotease)-like activity extracellularly released by Herpetomonas samuelpessoai

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*Herpetomonas samuelpessoai*produces great amount of a cell surface metalloprotease that presentes similar biochemical properties with the gp63 metalloprotease of Leishmania spp, which is a well known virulence factor expressed by these digenetic parasites. We aimed to study the secretory proteolytic enzymes in *H. samuelpessoai* after grown on BHI medium for 48 h at 26C. The cells were collected by centrifugation and incubated in PBS for 1 to 4 h. The supernatants were obtained by centrifugation and filtration. The results demonstrated that during PBS incubation, *H. samuelpessoai* secreted at least 12 polypeptides, some of them presenting proteolytic activity. Three proteases were evidenced on gelatin SDS PAGE with apparent molecular masses of 85, 63 and 50 kDa. The major extracellular 63 kDa protease had its activity significantly diminished by chelating agents. Cations also influenced the 63 kDa metallo-enzyme activity. This metalloprotease was active in pH values ranging from 4 to 10.The optimum temperature of the 63 kDa enzyme was 37C. Casein was cleaved by this proteolytic enzyme, while BSA and hemoglobin were not degraded. *O*-phenanthroline was able to inhibit the secretion of the 63 kDa enzyme in a dose dependent manner. Fluorescence microscopy and flow cytometry using anti-gp63 antibody demonstrated the presence of similar molecules in the cell surface of *H. samuelpessoai*. Western blotting analysis showed the presence of a polypeptide of 63 kDa in the cellular extract and in the supernatant fluid of *H. samuelpessoai*, which shows immunological similarities between these two distinct trypanosomatids. Anti-CRD antibody failed in recognizing any secreted polypeptide from *H. samuelpessoai*. However, when fixed parasites were previously treated with PLC, the anti-CRD and anti-gp63 antibodies recognized a 63 kDa released polypeptide, which strongly suggests that gp63 molecule is anchored to the *H. samuelpessoai* surface via glycosylphosphatidylinositol anchor, being released by proteolysis and not by phospholipolyis.

BQ19 - Further investigation on the interaction of *Phytomonas serpens* with salivary glands of *Oncopeltus fasciatus*

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Trypanosomatids parasitize all classes of vertebrates, as well as some invertebrates, preferentially insects from the orders Diptera and Hemiptera, and also plants. Often recognized trypanosomatid genera, only two (the pathogenic *Trypanosoma* and *Leishmania*) have been actively investigated for any length of time while the plant flagellates, *Phytomonas* species, have recently begun to attract attention due to their role as agricultural parasites. *Phytomonas* species are parasites of both plants and insects. The hemipteran *Oncopeltus fasciatusis* is not only the natural host for *Phytomonas elmasiani*, but it is also capable of hosting different species of trypanosomatids by experimental infection. The invasion of the vectors salivary glands is one of the most important events for the life cycle of *Phytomonas* species. In the present study we observed, by means of scanning electron microscopy, the *ex vivo* interaction between *Phytomonas serpens* and the external face of salivary glands of *O. fasciatus*. This binding seems to occur through a 130 kDa protein receptor for the parasites. The association of the parasites with the salivary glands was strongly inhibited by N-acetyl-D-glucosamine, N-acetyl-D-galactosamine and α-methyl-D-mannoside, as well as by the 130 kDa purified protein. Polyclonal antibodies raised against human laminin were able to recognize this protein, through immunoblotting. The mass spectrometry of the trypsin-digest of this protein matched 23 % of human laminin beta-3 chain precursor sequence by the digested peptides.
Supported by: CNPq, FAPERJ, CNPq/PIBIC-UFRJ and PRONEX (0885).

BQ20 - Platelet-activating factor (PAF) modulates proteolytic activities in *Trypanosoma cruzi*

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Trypanosoma cruzi is a protozoan parasite that exhibits a complex life cycle that includes two intermediate hosts (triatomine insects and mammals, including man) and four morphogenetic forms: epimastigote, metacyclic trypomastigote, amastigote, and bloodstream trypomastigote. In the transmission from the vector to the mammalian host, the parasites have to trespass barriers, such as the extracellular matrix, and reach susceptible host cells. Surface or secreted proteases have been implicated in parasite infections by their role in degrading proteins of the extracellular matrix, surviving the intracellular environment, evading the host immune response, and in preventing blood coagulation. Platelet-activating factor (PAF) is an alkyl-phospholipid mediator produced and released from various inflammatory cells including neutrophils, monocytes, platelets and endothelial cells. Although PAF was initially recognized for its potential to induce platelet aggregation and secretion, subsequent investigations have elucidated a broad range of biological actions, including cellular differentiation, chemotaxis and induction of inflammatory response. We have shown that PAF triggers in vitro metacyclogenesis of *T. cruzi*. The present study reports a proteinase activity in epimastigote forms of *T. cruzi* cultivated in LIT medium in the absence or in the presence of 10^{-6} M PAF. Proteinases were analyzed on SDS-PAGE with gelatin incorporated into the gel, as substrate. We detected two cell-associated proteolytic activities, both in the parasites cultivated with and without PAF. The proteolytic activities were partially inhibited when PAF was included in the digestive gel buffer. This set of results suggests an important role for PAF-modulated *T. cruzi* proteinases in parasite-host cell interaction. Supported by: CNPq, FAPERJ, CAPES

BQ21 - *Vernonia rupestris* and *V. fruticulosa* extracts exert a trypanocidal effect against trypomastigote forms of *Trypanosoma cruzi* in *in vitro* biological assays

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Trypanosoma cruzi trypomastigotes forms are capable of invading and replicating in different types of cells causing frequently a chronic and multifocal disease. Benznidazole is the only drug employed in the treatment of this infection in Brazil but it is only partially effective in acute infections and mostly inefficient in chronic stages. Herein, we evaluated the trypanocidal potential of different plant extracts of the genus *Vernonia* using *in vitro* assays, against two strains of *T. cruzi* (Y and Bolivia). The *in vitro* assay was carried out using blood with 10^5 trypomastigotes forms/mL and extract solutions (leafs of *V. fruticulosa* and *V. rupestris* extracted in different solvents and prepared in dimethylsulfoxide 5% - DMSO - saline; 1:20) at different concentrations 2.000, 1.000 and 500 μg/mL. The bioassays were performed on microtiter plates; negative and positive controls were run in parallel. The plates was incubated at 4^{o}C during 24 hours and the number of parasites determined according to the Brener method. The extracts were also tested on intracellular amastigote forms cultured in P388D1 cells. P388D1 cells were grown in Dulbeccos Modified Eagle Medium supplemented with 10% of FCS in a microtiter plate (2 x 10^4 cells/well). The cells were then infected with 2 x 10^5 parasite forms/well. The biological system was incubated in 5% CO_2

at 37^{o}C and after 24 hours the respective extract was tested (32, 16 and 8 μg/mL). The efficacy of the drugs was measured using the MTT method. The extracts demonstrated a significant action on trypomastigote forms (more than 50% lysis of parasites in Bolivia strain and with slightly less lysis in Y strain trypomastigotes, using non-polar *V. fruticulosa* extracts) but not against amastigote forms. Our data showed the difference of susceptibility between strains and that the active principle against *T. cruzi* is a non-polar compound. Supported by CNPq

BQ22 - THE ACTIVATION OF PRECURSOR FORMS OF THE T. cruzi MAIN CYSTEINE PROTEASE, CRUZIPAIN, IS MODULATED BY GLYCOSAMINOGLICANS AND BY MILDLY ACIDIC pH.

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The major cysteine protease of *T. cruzi* - cruzipain - is a key enzyme for parasite survival and establishment of the infection in the mammalian host. Cruzipain belongs to the papain-like family of cysteine proteases, enzymes that are synthesized as inactive precursors that require the removal of their inhibitory N-terminal domains (i.e., prodomains) in order to become fully active. The 3-D structures of the precursor forms of some members of this family revealed that the association of the prodomain with the enzyme's catalytic site occurs in an inverse orientation that unfavors auto-catalytic processing. Studies on the maturation of recombinant cruzipain (Tulahuen) *in vitro* suggested that this enzyme is processed by auto-catalysis at pH 5.5. This is in contrast with *in vivo* studies, which suggest that zymogen maturation occurs in the Golgi complex, a compartment bearing a neutral to mildly acidic environment. The aim of the present study is to systematically investigate the requirements for *in vitro* maturation of recombinant cruzipain precursors. The proform of cruzipain (Dm28c) was cloned and expressed with a poli-histidine tag, purified under denaturant conditions and sucessfully refolded. Activation of refolded cruzipain was carried out in pH values ranging from 5 to 7 and in the presence of glicosaminoglicans (GAGs). Contrasting with mammalian papain-like enzymes, efficiently activation of cruzipain precursors was achieved solely at pH 6, and to a minor extent, at pH 5.5. Heparan sulfate, but not condroitin sulfate, negatively interferes with cruzipain maturation. Pepsin and dextran sulfate, which were sucessefully used to activate many papain-like enzymes, failed to promote activation of cruzipain. We previously described that the intact pro-domain of cruzipain potently inactivates mature enzyme (K_i=30 pM) at pH 6. We observed that the tight complex formed between cruzipain and the prodomain is not disrupted even in the presence of 100-fold excess of biot-Mu-Phe-hPhe-VSPh - an irreversible cysteine protease inhibitor, that displays high affinity for cruzipain. *In vivo*, it is possible that after zymogen processing, intact pro-domain re-associates with mature enzyme, rendering it inactive until delivered to latter acidic compartments of the secretory pathway. Taken together, our results suggest that in *T. cruzi*, the release of fully active cruzipain may be a tightly regulated event. Supported by CNPq, FAPERJ.

BQ23 - Modulation of the cysteine protease of *T. brucei brucei* (brucipain) by glycosaminoglicans and enzyme inactivation by the N-terminal domain (pro domain) of cruzipain.

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Cysteine proteases (CPs) of the papain superfamily expressed by pathogenic protozoa have been strongly implicated in their survival and interaction with the host. Potent synthetic irreversible inhibitors to the main CP of *T. cruzi*, cruzipain, have been successfully tested in animal models of *T. cruzi* infection. It was demonstrated that such inhibitors are also capable of killing the African trypanosome, *Trypanosoma brucei*, when supplemented to the culture medium of procyclic forms. Although structurally different inhibitors to CPs have been repeatedly shown to drastically affect the survival of *T. brucei brucei* their primary target, brucipain, has never been studied in detail. This prompted us to characterize this enzyme at the molecular and biochemical level. We have previously reported the expression of functional brucipain and the primary characterization of its biochemical properties. Similarly to cruzipain, brucipain displayed strong substrate inhibition when assayed with Z-F-R-MCA at 25^{o}C and this inhibition was reverted at 37^{o}C. This suggests that brucipain also bears a second substrate-binding site at the central domain, which can be modulated by temperature. Here, we report that the substrate inhibition displayed by brucipain is also modulated by glycosaminoglicans (GAGs), such as: heparan sulfate, chondroitin sulfate and dermatan sulfate. In the presence of GAGs, the kinetic (K_M and k_{cat}) parameters for the hydrolysis of Z-F-R-MCA were affected when assayed at 25^{o}C and this modulatory effect was potentiated at 37^{o}C. GAGs may be interfering in the phenomenon of substrate inhibition by binding directly to a putative allosteric site and preventing the binding of a second molecule of substrate or by inducing a conformational change in brucipain that hides the allosteric binding site. Brucipain was also strongly inactivated by the N-terminal domain (i. e., pro domain) of cruzipain (k_i= 27pM), revealing its striking functional similarity to the *T. cruzi* enzyme . Interestingly, contrasting with cruzipain, the inhibition of brucipain by the pro domain of cruzipain presented slow-binding kinetics at pH 6.5. These results suggest that compounds capable of modulating the putative allosteric site of these enzymes and the pro domain of cruzipain may serve as models for the design potent inhibitors to the cysteine proteases of both *T. cruzi* and *T. brucei*. Supported by CNPq, FAPERJ.

BQ24 - PROTEIN PROFILES OF *Trypanosoma cruzi* CULTIVATED WITH DIFFERENT CARBON SOURCES BY BIDIMENSIONAL ELECTROPHORESIS

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Chagas disease (American trypanosomiasis) continues to be an important public health problem in South America. The etiological agent of the disease is the hemoflagellate protozoan *Trypanosoma cruzi*, and the occurrence of the protozoan and the respective insect vectors encloses an area from the south of the United States until the north of Argentina. The objective of this work is to analyze the proteomic expression of *T. cruzi* when using different sources of carbon and to characterize key molecules in the metabolism of the parasite or in its interaction with the host. Initially, axenic cultures of epimastigote forms of *T.cruzi* (strain CL Brener) were used for the assays of bi-dimensional electrophoresis from culture conditions supplemented with either L-proline or glucose. Cells were lysed by freezing and thawing and proteins were precipitated with trichloroacetic acid. The samples were submitted to bidimensional electrophoresis, which separates proteins by isoelectric focussing (different pH ranges were tested) and molecular weight. Gels were stained by silver or comassie blue and some spots were sent for mass spectrometry analysis. The gel patterns were analyzed using the PDQUEST/BIORAD program. Using a pH range of 3,0 - 10,0, 137 well stained spots were found common both conditions, while 35 spots were only found in cells grown in culture supplemented with glucose and other 41 spots were found in cells grown in culture supplemented with L-proline. Densitometric analysis demonstrated that 27 spots obtained from cells grown in L-proline were two times more intense than cell grown in glucose, suggesting that the expression level of some protein was higher in under that condition. Other conditions of pH range and culture parameters are under investigation. Metacyclic, amastigote and trypomastigote forms are under analysis with the same procedure. This study is of considerable relevance for the study of the parasite metabolism, and subsequently for the development of new strategies of imunotherapy and drug design against this pathogen.
Supported by: FIOCRUZ-PAPES III, FIOCRUZ- PDTIS, CNPq, FAPERJ

BQ25 - Proton transport in Herpetomonas sp.

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In this work, we show that Herpetomonas sp. possess an electron dense organelles with structural, chemical and physiological properties similar to the acidocalcisome. Pyrophosphate-driven proton uptake was measured in cells permeabilized by digitonin using acridine orange. The proton-pyrophosphatase activity was inhibited by sodium fluoride (NaF) and imidodiphosphate (IDP), the proton-pyrophosphatases inhibitors. Proton was released with addition of 125 uM calcium, suggesting the presence of a calcium/proton antiport in the acidic compartment. However, sodium was unable to release protons from these organelles. The proton-pyrophosphatase activity was optimal in the pH range of 6.5 to 7.2. These activity was completely dependent of ion potassium. The maximal activity occurred with 130 mM of KCl. The pyrophosphate-driven proton uptake was dependent on the PPi concentration. However, due to the fast substrate consumption, we were not able to obtain a value for half-maximal activation. Maximal values for proton rate were obtained at concentrations of PPi above 100 uM. In addition, X-ray elemental mapping associated with energy-filtering transmission electron microscopy showed that most of the cations, namely Na, Mg, P, K, Fe, Zn and Cu are located in the acidocalcisome matrix. These results suggest that Herpetomonas sp posses an organelle that is able to acumulate proton using the energy coupled from pyrophosphate hydrolysis. This organelle posses a calcium/proton antiport and an proton-pyrophosphatase which is inhibited by IDP and NaF, as described in acidocalcisomes. In addition, electron-dense organelles with structural properties and elemental composition similar to the acidocalcisomes were identified. However, in contrast to the other trypanosomatids so far studied, we found neither a proton-ATPase sensitive to bafilomycin A (V-proton-ATPase) nor a sodium/proton antiport in Herpetomonas sp.

BQ26 - Ouabain-Insensitive Na^+-ATPase activity in *Leishmania amazonesis* promastigote

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Parasites of genus Leishmania present two evolutive forms in your life cycle, amastigote and promastigote. A striking characteristic common to all species of *Leishmania* is the capacity to establish intracellular parasitism preferably in macrophages. The understanding of how this parasite manages to disturb macrophage function has pivotal relevance to our knowledge of the disease and will allow the identification of specific molecules or protein involved in the crosstalk between parasites and host cells, as well as the effectors of these cascades that could further provide candidate chemotherapy targets. In *Trypanosoma cruzi* two Na^+

pumps have been described, the classic ouabain-sensitive ($Na^+ + K^+$)ATPase (Caruso-Neves et al, Z. Natuforsch. 53c, 1049 - 1054,1998) and the ouabain-insensitive, furosemide-sensitive Na^+-ATPase (Caruso-Neves et al, Z. Natuforsch. 54c, 100 - 104, 1999). It has been described the presence of a classic ouabain-sensitive ($Na^+ + K^+$)ATPase in *Leishmania mexicana* (Felibert et al, Mol. Biochem. Parasitol. 74, 179 - 187, 1995). In the present work, Na^+-ATPase activity has been characterized in *Leishmania amazonesis* using promastigote homogenate. Na^+ stimulates the ATPase activity in *L. amazonensis* homogenate in a dose dependent manner reaching a maximal activity at 120 mM ($253{,}53 \pm 9{,}95 nmol Pi x h^{-1}$ x mg^{-1}). Furthermore it can be seen that 30 mM K^+ had no effect on the Na^+ stimulated ATPase activity in the presence of 1 mM ouabain. The addition of 1mM ouabain in the absence of Na^+ and K^+ did not change the basal Mg^{+2}-ATPase activity and was observed that 1mM ouabain, in the absence of K^+ did not change the Na^+ stimulated ATPase activity in *L. amazonesis*. It is known that the Na^+-ATPase is inhibited specifically by furosemide. The addition of 2mM furosemide was capable to completely inhibited the Na^+ stimulated ATPase activity and did not change the basal Mg^{+2}-ATPase activity. Our studies suggest the existence of an oubain-insensitive furosemide-sensitive Na^+-ATPase in *Leishmania amazonesis*.

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BQ27 - Biodistribution of neutron activated meglumine antimoniate in healthy or *L. (L). chagasi* infected BALB/c mice

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Pentavalent antimony, as meglumine antimoniate (Glucantime) or sodium stibogluconate (Pentostam), is the main treatment for leishmaniasis, an endemic and neglected threat in Brazil. Despite over half a century of clinical use of these antileishmanial agents, their mechanism of action, toxicity and pharmacokinetics data remain mostly unknown, probably due to established clinical protocols. Recently, some new protocols, using low doses, have been proposed at clinical grounds, without pharmacological approach. Here we present data of meglumine antimoniate activated by neutrons (NAG), for tracing its fate and metabolism in healthy mice or with experimental visceral leishmaniasis. Radiotracer was prepared by neutron irradiation of Glucantime (Aventis, S.Paulo, Brazil), inside the IEA-R1 nuclear reactor, which produced two pure radioisotopes Sb-122 and Sb-124. NAG presented slightly color change, probably due to the meglumine polymer formation, but the same antileishmanial activity as the native compound, either in vitro *L. (L.) chagasi* infected macrophage or in in vivo treatment of mice infected with *L. (L.) chagasi*. NAG biodistribution in organs from healthy or infected mice showed higher uptake in the liver. Liver and kidney from healthy mice showed a slightly but significant higher NAG uptake when compared to infected mice. NAG was mostly eliminated by biliar excretion, after liver processing, reaching the intestinal lumen, with a small and fast renal excretion. Others reported this finding, but is quite diverse from the main renal excretion usually assumed by most authors. The serum kinetics was biexponential, with two compartments: a distribution in the central compartment and other associated to drug excretion. The use of the radiotracers, easily created in nuclear plants by neutron activation, is an interesting instrument for pharmacokinetics studies, helping to elucidate some questions about pentavalent antimony action, and offering an alternative methods for tracing drugs, specially those compounds that presents elements with high atomic numbers.

BQ28 - Searching for the mechanism of hemozoin formation in *Rhodnius prolixus*

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Heme, the prostetic group from hemoglobin (Hb), can be highly toxic due to its ability to act as a catalyst of the formation of reactive oxygen species. Hematofagous arthrophods ingest large quantities of blood in each meal and the digestion of Hb produces high amounts of free heme in their digestive tracts. The formation of the pigment called hemozoin (Hz), an insoluble crystalline aggregate of heme, is a mechanism for heme detoxification that occurs in triatominae bugs. Hz is formed in the perimicrovilar membrane, synthesized by the intestinal epithelium. Our goal is to find the molecular entity responsible for Hz formation in the blood-sucking bug *Rhodnius prolixus*. We first established a solubilization protocol for perimicrovilar membrane proteins using Igepal. Following protein extraction we performed a DEAE-Toyopearl column where we achieved preliminary fractionation, visualized by eletrophoresis. Some of those fractions exhibited Hz formation activity, but the material was not enough for further purification. We used also a hemin-agarose affinity column that indicated the presence of a 60kDa polypeptide that bound specifically to the affinity gel. As an alternative, we are testing the involvement of 3 putative perithrophins and mucins found in a cDNA library from *Rhodnius* midgut, which showed heme-binding motifs in their sequences.

BQ29 - *Trypanosoma rangeli*: Differential Expression of Cell-Surface Polypeptides in Short and Long epimastigotes

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Previous reports of our research group demonstrated great

differences in the expression of cell-surface enzymes, such as ecto-phosphatase and ecto-ATPase activities, in the short and long epimastigote forms of *Trypanosoma rangeli*. As well-known, the parasite proteins are involved in host cell recognition and penetration. Moreover, the majority of the surface antigens on trypanosomatids are glycosylated, and some of them function as receptors involved in a variety of process including signal transduction. In this context, a comparative study of cell-surface protein composition in short and long epimastigote of *T. rangeli* was carried out using biotinylation method. After grown in LIT medium for 7days to short and 14 days to long epimastigotes forms, the cultures were centrifuged and washed with PBS. Then, live parasites were added with 0.5 mg/ml sulfo-NHS-LC-biotin (an impermeable biotin derivative) for 30 min at 4 Celsius degrees, followed of extensive washed with PBS, to remove the unreacted biotin. Biotinylated parasites were lised by 0.1% SDS and polypeptides were analyzed on 10% SDS-PAGE. The separation of whole-body proteins revealed by Coomassie blue staining showed the presence of about 25 bands ranging from 150 to 20 kDa in both developmental stages of *T. rangeli*. There were generally minor quantitative differences in the whole protein profile in both systems, such as the 97 and 95 kDa polypeptides that were preferentially expressed in the long epimastigote forms. Accentuated differences were detected when the cell-surface biotinylated polypeptides were analyzed. Long epimastigote forms produced a great amount of biotinylated bands (ranging from 70 to 20 kDa) when compared with the short one. These differences may be a reflection of changes in the nutritional requirements and/or supplies during life cycle stages of the parasites. In addition, our findings suggest a differential protein expression between the two epimastigote forms, which could be related with the cellular differentiation process in *T. rangeli*.

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BQ30 - Biochemical Characterization and Cytochemical Detection of Acid Phosphatase in *Trypanosoma rangeli*.

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Trypanosoma rangeli is transmited through triatominae vectors, during a blood meal on man and other mammals. It develops as short epimastigotes forms in the gut of the insect, invades the haemocoel and a few days after infection they disappear to be replaced by a massive colonization by long epimastigotes forms. In several protozoa parasites ecto-phosphatase has been described, although the physiological role has not been well established, it seems involved with cell differentiation. Previous studies demonstrated that *p*-nitrophenilphosphate (*p*-NPP) and β-glycerophosphate dephosphorilation was more effective in short than long epimastigotes. The aim of the present study is to verify the presence of acid phosphatase in short and long epimastigotes of *T. rangeli* from H 14 strain. Between pH values of 6.0-8.5 phosphatase activities were decreased when these values increased. The effects of different inhibitors on the ecto-phosphatase activity showed a higher inhibition with the classical acid phosphatase inhibitors: ammonium molybdate and sodium vanadate were 100% respectively. For the cytochemical detection they were fixed for 30 min in 1% purified glutaraldehyde in 0.1M cacodylate buffer, pH 7.2 with 5% sucrose, at 4°C. The parasites were rinsed in 0.1M tris-maleate buffer and incubated for 1 h in 1mM Cerium Chloride plus 1mM β-glycerophsphate plus 5% sucrose in 0.1M tris-maleate buffer (pH 5.0). After incubation, the cells were rinsed in 0.1M cacodylate buffer, pH 7.2, dehydrated in acetona and embedded in Epon. Ultrathin sections were obtained and observed by transmission electron microscopy. The results showed a high positive reaction to acid phosphatase in short but not in long epimastigote forms. These findings suggest a different membrane surface in each epimastigote form of *T. rangeli*.

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BQ31 - Standardization of two dimensional gel electrophoresis for Proteomic Analysis of *Leishmania (Viannia) braziliensis*

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Leishmania (Viannia) braziliensis, the main causal agent of Tegumentary Leishmaniasis in South America, produces a wide variety of clinical manifestations that may be related both to the host immune response and parasite gene expression. Regulation of gene expression in kinetoplastids differs from higher eukaryotic species, being the control at the post-transcriptional level, the major mechanism of gene regulation in these protozoans. This consideration makes proteomic approach a valuable tool to investigate the relationship between gene expression patterns in parasites and clinical phenotypes. This work describes the standardized

protocols we used to obtain reproducible extraction and separation of *L. (V.) braziliensis* proteins by two dimensional gel electrophoresis (2-DE) using axenic promastigotes, and a protein map for different strains of this parasite. The best condition of protein extraction used $3x10^8$ parasite cells lysed by freeze/thawing in PBS buffer pH 7.4. The extracts were precipitated with TCA and washed with cold acetone. Finally, the pellet was resuspended in a buffer containing 8M Urea, 2% CHAPS, 20mM DTT and 0.2% ampholytes. The best quantity of proteins for isoelectrofocusing in gel strips of 7 cm and 17 cm was 40mg and 200mg, respectively. Proteins were separated over pH gradients 3-10 and 4-7, resolved in 12% polyacrylamide gels and visualized by silver staining. An average of 350 and 400 spots in pH gradients 3-10 and 4-7, respectively, were observed. These conditions yield well reproducible gels suitable for image analysis and further identification by mass spectrometry.

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BQ32 - Post-translational modifications of Trypanosoma cruzi histone H4

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Histone tails provide sites for a variety of post-translational modifications, including acetylation, phosphorylation and methylation. These modifications profoundly affect transcription, chromatin assembly, DNA replication and repair, recombination, and chromosome segregation. We have previsously found that Trypanosoma cruzi histone H4 and H2A undergo post-translation modifications as observed by tritiated acetic acid incorporation and Q-TOF analysis. To further characterize these modifications, we raised polyclonal antibodies against multi-acetylated peptides corresponding to the histone H4 N-terminal. We obtained and immunopurified antibodies that recognized specifically the K15. By Western blotting assay we showed that these antibodies in epimastigotes, but not in trypomastigotes, recognized a small percentage of the total histone H4. By immunofluorescence analysis, these antibodies recognized foci in the parasite nucleus, showing that K15 acetylation occurs at specific nuclear domains. We also investigated the post-translation modifications of histone H4 by mass spectrometry. By MALDI-TOF analysis, we found that peptide 10-23 of histone H4 has several 14 Da additions. Peptide 1-9 was not detected. By IT-MS-MS, we detected a 42 Da addition in K5 and K11, which could be attributed either to trimethylation or acetylation. We conclude that a small percentage of histone H4 is acetylated at K15, while the K5 and K11 lysines can be either acetylated or trimethylated.

Supported by FAPESP

BQ33 - Use of heme/hemoglobine as growth factor by insect-form of T. cruzi

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Trypanosoma cruzi, the ethiologic agent of Chagas disease, is transmitted through insects vectors during the blood-meal on vertebrate host. These insects ingest about 10 mM heme bound to hemoglobin (Hb). It was hypothesized that parasite requires heme as nutrient because of its biosynthesis deficiency and it has been reported that globin-derived peptides produced during Hb digestion in the hindgut of the insect are involved in the cruzi metacyclogenesis process. Herein, we have been investigating the effects of hemin, Hb and globin-derived peptides on T. cruzi growth. Addition of hemin or Hb increased significatively the parasite proliferation in a dose-dependent manner. The same stimulating effect on growth is observed when globin-derived peptides were added to the medium. In all cases, no differentiation into metacyclics was observed. Moreover, albumin-derived peptide stimulated growth as well, indicating that the effect on growth is not only related to heme. In order to fellow the fate of heme and hemeprotein during the endocytotic process, we decided to use globin-Pd-Mesoporphyrin IX (globin-Pd) and globin-rhodamine. We followed the time of internalization of fluorescent tracers in parasites. In the early time of incubation (0-5 min), in both cases, the fluorescence signal was associated with vesicles in the vicinity of nucleus and kinetoplast (including possibly cytostome), reaching reservosomes after longer time of incubation (5-20 min). By using albumin-Pd-Mesoporphyrin IX as fluid phase marker, a similar internalization pathway was observed. In addition, an excess of free Hb was not able to dislocate the fluorescent of globin-Pd during its internalization. Taken together, our data suggest that heme and hemoglobin might be essential as growth factors to the development of cruzi. The different ligands are taken up in a similar time curse and labeled the same organelles in a similar way as reported for transferrin-gold and BSA-gold. Supported by FAPERJ, CNPq, PADCT, and HHMI (to PLO) and PRONEX.

BQ34 - Oligomeric, neutral aminopeptidase of *Trypanosoma cruzi*

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Proteases are implicated in many aspects of the physiology of microorganisms, as well as in host-pathogen interactions. Aminopeptidases are emerging as novel drug targets in infectious agents. In this study, we characterized an aminopeptidase from the protozoan *Trypanosoma cruzi*, the causative agent of Chagas disease. The aminopeptidolytic activity was identified in cell free extracts from *T. cruzi* using the substrate leucine-7-amido-4-methylcoumarin (L-AMC). The protein displaying this activity was purified to homogeneity from *T. cruzi* epimastigotes by a three-step chromatographic procedure. SDS-PAGE analysis revealed that the purified enzyme has a molecular mass of about 200 kDa. It also showed that this aminopeptidase of T. cruzi (APTc) is an oligomeric enzyme formed by monomers of 50 kDa. Gel enzymography experiments indicated that enzymatic activity depends on the oligomeric structure of the protease, but does not involve inter-chain disulfide bonds. The purified APTc showed to be highly specific since it did not hydrolyze a set of other fluorogenic substrates tested. The activity of this aminopeptidase depends on Zn^{2+} and is sensitive to bestatin and 1,10-phenanthroline. In contrast, inhibitors of serine, cysteine and aspartic proteases did not affect the activity of APTc. These data indicate that APTc is a metallo-aminopeptidase. This enzyme could play a role in supplying required amino acids and/or be involved in peptide/protein processing.
Sponsored by CNPq.

BQ35 - The methylthioadenosine phosphorylase (MTAP) of *Trypanosoma cruzi*

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Trypanosomatids have an absolute requirement for purines because they lack the machinery to synthesize their own purine ring, *de novo* synthesis. One of the sources of purines comes from (MTA), a byproduct from the production of polyamines, which is cleaved by a specific MTA phosphorylase (MTAP) into adenine and methyl-thioribose-1-phosphate (MTR1P). Adenine is converted to adenine nucleotides (purine salvage pathway) while MTR1P intermediate can be recycled into methionine. This pathway is potentially exploitable for chemotherapy target in trypanosomatidic parasites because of the needs of pre-formed purines and the high cost of methionine synthesis. The *mtap* gene of *Trypanosoma cruzi* was isolated in our laboratory and the active recombinant enzyme was obtained. It shares significant identity to other MTAPs, is insensitive to tubercidin, has a neutral pH optimum, and displays maximal activity at 50^oC. Although MTAP hydrolyzes adenosine, deoxyadenosine, guanosine and xantosine, its best substrate was found to be MTAP. Western blotting experiments using a specific antibody raised against the recombinant enzyme revealed that MTAP is expressed by epimastigotes, amastigotes and trypomastigotes of *T. cruzi*. Further molecular and functional studies will elucidate the relevance of MTAP to the parasite's physiology and show the potential of this enzyme as a drug target.
This research is sponsored by CNPq.

BQ36 - Tomatine and tomatidine are toxic to the plant trypanossomatid *Phytomonas serpens*

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Some plants produce substances to their own defense against pathogens and predators including fungi, bacteria, viruses, and insects. The solanaceous plant species as potatoes and tomatoes synthesize glycoalkaloids possibly for this end. The tomato plant Lycopersicon sculentum synthesizes the glycoalkaloid tomatine, that consist of a mixture of two glycoalkaloids: alpha-tomatine and dehydrotomatine. Tomatidine is producted by the loss of saccharide side chain of Tomatine. Studies have shown that tomatine strongly react with cholesterol of cell membranes, promoting disruption of membrane causing cell death. In contrast, the products of degradation of tomatine, including tomatidine, do not react with cholesterol. Flagellates of the genus Phytomonas are etiologic agents of diseases affecting plants of great economical importance including coffee and coconut and infecting edible fruits such as tomato, orange and many others. In the present study we intend to investigate the effects of tomatine and tomatidine in Phytomonas serpens. To investigate the effect of these compounds in different stages of cell growth, we made cellular growth curves adding 50 micromolar of tomatine or tomatidine in days 0, 1 and 3 after inoculum. Our results shown that when tomatine or tomatidine were added at day 0, no cellular growth was observed. In contrast, when they were added at day 1, we observed an inhibition by around 10 percent caused by tomatine and 20 percent caused by tomatidine. Moreover, we observed that, when added at day 3, tomatine caused death of all cells with a drastic morphology change. This effect was not observed with tomatidine. We also observed that this effect was dose dependent. Our results indicated that tomatine and tomatidine are toxic to this trypanosomatid, possibly with different molecular targets. More experiments are being performed to investigate the molecular target of these two tomato compounds and possibly use them as chemotherapeutic agent against trypanosomatids.

BQ37 - Lipid Biosynthesis Inhibitors Reveal New Aspects of the Endosymbiosis in Trypanosomatids

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In some trypanosomatids an endosymbiotic bacterium coevolves with the host-protozoan, contituting a valuable model to understand the origin of organelles, as the mitochondrion and the chloroplast. *Crithidia deanei* is a trypanosomatid which presents an endosymbiotic bacterium in the cytoplasm. Since the origin of the endosymbiont envelope is controversial, its lipid composition was investigated in order to verify if it is related to the host trypanosomatid or to an ancestral bacterium. Alkyl-lysophospholipids (ALP), have shown a significant antiproliferative activity against trypanosomatids. The effects of these compounds have been related to perturbation of the alkyl-lipid metabolism and to the biosynthesis of phospholipids, as well as damage to cellular membranes. In previous studies we verified that edelfosine had a dose-dependent effect on cell growth and also promoted ultrastructural alterations in *Crithidia deanei*, as mitochondrion swelling and disruption of the endosymbiont envelope. Furthermore, this ALP also interfered in protozoan phospholipid biodynthesis with a decrease in phosphatidycholine (PC) and cardiolipin (CL) content. Miltefosine, which is also an ALP, acts *in vivo* as inhibitors of mitogenic signaling and presents antiproliferative activity. As miltefosine is a phospholipid (PL) analogue, it can interfere with enzymes that regulates phospholipid biosynthesis. In a previous work we showed that PC, which is rarely found in bacteria, is a major component of the endosymbiont envelope and also suggested that the symbiont presents more than one pathway to synthesize PC. In this work we found that the miltefosine had a dose-dependent effect on *C.deanei* proliferation and also promoted ultrastructural modifications in this trypanosomatid. Interestingly, cell containing the symbiont were more resistent to the drug effect than aposymbiotic protozoa.This result reinforces the idea that the symbiont improves cell growth and metabolism.
Supported by: CNPq and FAPERJ

BQ38 - Casein kinase 2 (CK2) activities in *Leishmania braziliensis*: a comparative study between infective and non-infective strains

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Protozoa of the genus *Leishmania* cause cutaneous, mucocutaneous or visceral diseases in man depending on the species of the parasite and the host immune response. Phlebotomine sandflies are the vectors of *Leishmania* spp. in at least 88 countries. Leishmaniasis is distributed worldwide and 12 million people are estimated to be infected with about 1.5 million new cases each year (WHO). Approximately 50% of these patients are children. In Brasil mucocutaneous leishmaniasis are associated with infection by species of the Leishmania (Viannia) braziliensis complex; also, *L. (V.) braziliensis* parasites have been identified in cases of disseminated cutaneous leishmaniasis. *Leishmania* metacyclogenesis is associated with changes in morphology, signal transduction pathways, gene expression and structural alterations in the surface molecules of these parasites. Therefore, studies related to enzymes responsible for phosphorylation and dephosphorylation of proteins present on the external surface of these parasites, are extremely important. Several ecto-enzymes have been described in trypanosomatids, including ecto-phosphatases and ecto-kinases. Casein kinase 2 activities have been described both on cell surface and as secreted enzymes in *Leishmania major, L. amazonensis* and *L. tropica*. These enzymes seem to be involved with cell growth, differentiation and infectivity. In the present study we demonstrate the influence of CK2 activities in the infectivity of *Leishmania braziliensis* infective strain (MHOM/BR/2002/EMM-IOC-L2535) in mouse peritoneal macrophages. The infective strain CK2 activities were at least 30 fold higher than those present in the non-infective strain. This set of results indicates that CK2 activities are likely to be involved with the virulence of this protozoan parasite. When specific CK2 inhibitors (TBB and DRB) were added during the L. braziliensis-macrophages interaction assays, a drastic inhibition (60%) of this process was observed. When only the parasites were pre-treated with these drugs, the same pattern of inhibition was verified (58%). The infective strain secreted CK2 activity was able to promote the phosphorylation of a secreted 55kDa protein. This phosphorylation was strongly inhibited by the CK2 inhibitors heparin and TBB. Monoclonal antibodies raised against mammalian CK2 were able to recognize, through immunoblotting, a high molecular weight protein obtained from L. braziliensis incubation medium, both when these parasites were induced or not to secret CK2.
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BQ39 - Influence of Casein Kinase 2 in *Trypanosoma cruzi*

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Chagas disease is wide spreading throughout Latin America where nearly 20 million people are infected by *Trypanosoma cruzi* and 90 million are at risk in endemic areas (WHO 2000). There are about 5 million people infected with *T. cruzi* in Brasil. *T. cruzi* is transmitted by the faeces of haematophagous insects of the subfamily Tri-

atominae (Hemiptera, Reduviidae). *T. cruzi* parasites undergo complex morphological changes through their life cycle both in the insect vector and in the vertebrate host. *T. cruzi* cell differentiation is highly regulated and includes significant changes in biochemical pathways, which allow the adaptation to different environments. This process is associated with transformations in morphology, signal transduction pathways, gene expression and structural alterations in the surface molecules of these parasites. Therefore, studies related to enzymes responsible for the phosphorylation and dephosphorylation of proteins present on the external surface of these parasites are extremely important. Several ecto-enzymes have been described in trypanosomatids, including ecto-phosphatases and ecto-kinases. Casein kinase 2 activities were depicted on the cell surface and as secreted enzymes of *Leshmania major, L. braziliensis, L. tropica* and *L. amazonensis*, where these enzymes seem to be involved with cell growth, morphology and infectivity. In the present study we have identified a kinase activity (CK2) in *T. cruzi* (Colombiana strain CTC-IOC 004). This enzyme seems to be present on the surface of these parasites, in the cytoplasm content and as a secreted form. The addition of dephosphorylated casein promoted a 53% increase in the secreted CK2 activity of *T. cruzi*, but it had no effect on the other activities. We have also tested the specificity of induction of CK2 secretion by *T. cruzi* by the addition to the reaction buffer of some possible substrates for this enzyme. We used Balb/c peritoneal macrophage protein extract, BSA, FCS and inactivated human serum. Only macrophage protein extract and human serum were able to promote an enhancement on the secreted CK2 activity by 67% and 36% respectively.

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BQ40 - Phosphatidylcholine and lysophosphatidylcholine effects in cell differentiation of *Herpetomonas samuelpessoai*

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Herpetomonas samuelpessoai is a trypanosomatid parasite of the insect *Zelus leucogramus*. During its life cycle, this parasite presents three evolutive forms: promastigote, paramastigote and opisthomastigote. Lysophosphatidylcholine (LPC) is a major bioactive compound of plasmatic lipoproteins like LDL. This molecule is involved in atherosclerosis and inflammatory diseases, being produced in physiologic and pathologic conditions. In earlier studies, it was demonstrated that LPC is present in *Rhodnius prolixus* saliva, acting as an anti-haemostatic molecule. Platelet-activating factor (PAF) is a phospholipid that is a potent mediator of many cellular functions in diverse biological and pathophysiological processes, including cellular differentiation, inflammation and allergy. Recently we showed that PAF triggers cell differentiation of *Herpetomonas muscarum muscarum* and *Trypanosoma cruzi*. In the present work we describe the effects of LPC and phosphatidylcholine (PC) in cell differentiation of *H. samuelpessoai*. Parasites were grown for periods ranging from 1 to 4 days, in Roitman's complex medium, in the absence or in the presence of the following lipid modulators: PAF (10^{-7} M) as a positive control, PC and LPC (both at 10^{-8} M and 10^{-9} M). The percentage of non-differentiated (promastigotes) and differentiated (paramastigote and opisthomastigote) forms was daily determined by using Giemsa-stained preparations. At least 200 cells were examined by phase-contrast microscopy in each preparation. The best results were obtained after 48 hours of growth. Control parasites: 65% promastigotes, 35%differentiated; parasites grown in the presence of PAF: 25%promastigotes, 75% differentiated; 10^{-8} M PC-treated parasites: 23% promastigotes, 77% differentiated; 10^{-9} M PC-treated parasites: 22% promastigotes, 78% differentiated; 10^{-8} M LPC-treated parasites: 40% promastigotes, 60% differentiated; 10^{-9} M LPC-treated parasites: 27% promastigotes, 73%differentiated. These results demonstrate that not only PAF, but also LPC and PC, which are other phospholipids commonly present in plasma lipoproteins, are able to trigger cell differentiation of *H. samuelpessai*. The signaling pathways involved with this process are currently under investigation.

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BQ41 - Platelet-activating factor (PAF) modulates proteolytic activities in *Trypanosoma cruzi*

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Trypanosoma cruzi is a protozoan parasite that exhibits a complex life cycle that includes two intermediate hosts (triatomine insects and mammals, including man) and four morphogenetic forms: epimastigote, metacyclic trypomastigote, amastigote, and bloodstream trypomastigote. In the transmission from the vector to the mammalian host, the parasites have to trespass barriers, such as the extracellular matrix, and reach susceptible host cells. Surface or secreted proteases have been implicated in parasite infections by their role in degrading proteins of the extracellular matrix, surviving the intracellular environment, evading the host immune response, and in preventing blood coagulation. Platelet-activating factor (PAF) is an alkyl-phospholipid mediator produced and released from various inflammatory cells including neutrophils, monocytes, platelets and endothelial cells. Although PAF was initially recognized for its potential to induce platelet aggregation and secretion, subsequent investigations have elucidated a broad range of biological actions, including cellular differentiation, chemotaxis and induction of inflammatory response. We have shown that PAF triggers in vitro metacyclogenesis of *T. cruzi*. The present study reports a proteinase activity in epimastigote forms of *T. cruzi* cultivated in LIT medium in the absence or in the presence of 10^{-6} M PAF. Proteinases were analyzed on SDS-PAGE with gelatin incorporated into the gel, as substrate. We detected two cell-associated proteolytic activities, both in the parasites cultivated with and without PAF. The proteolytic activities were partially inhibited when PAF was included in the digestive gel buffer. This set of results suggests an important role for PAF-modulated *T. cruzi* proteinases

in parasite-host cell interaction.
Supported by: CNPq, FAPERJ, CAPES

BQ42 - Determination of mitochondrial hydrogen peroxide release in *Trypanosoma cruzi* different strains

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Reactive oxygen species (ROS) are constantly generated in mitochondria and can damage lipids, proteins and DNA. These organelles contain antioxidant thiol enzymes that remove mitochondrial H_2O_2, in addition to cytosolic ones, which can prevent mitochondrial oxidative stress by removing membrane-diffusible ROS. These enzymes play an important role in protecting mitochondria from oxidative damage since thiol oxidation is the main event affecting mitochondrial membrane proteins in this process. In *T. cruzi*, H_2O_2 is mainly detoxified by thiol antioxidants proteins, i.e. tryparedoxin peroxidase located either in the cytosol (TcCPX) or in mitochondria (TcMPX). In this study, mitochondrial membrane potential and H_2O_2 release were evaluated in two *T. cruzi* strains with different resistance to oxidative stress. Cells (2,5 x 10^7 cells/ml) were incubated in 150 mM KCl, 10 mM Hepes, pH 7.2, 2 mM $MgCl_2$,1 mM EGTA, 5mM succinate,2 mM KH_2PO_4, 1 mg/ml BSA, 30μM digitonin and mitochondrial Ψ was estimated through fluorescence changes of safranine O. Safranine O uptake was different among strains. Mitochondrial ROS generation was determined spectrofluorimetrically using amplex red (50μM) and horseradish peroxidase (HRP - 1U/ml) under the same conditions, but in the presence of oligomycin (1μg/ml). It was possible to monitor an increase and a decrease in ROS production, in the presence of antimycin A (2μM) or CCCP (1μM), respectively and the influence of phosphate in this process. ROS generation was higher in the strain with less resistance to oxidative stress (Y strain). Previously we have shown that Tulahuen 2 cells have a higher resistance to the oxidative stress generated by H_2O_2 and a higher TcCPx content than Y cells. The results shown herein reinforce the marked diversity that exists within the population of *T. cruzi* and show that a higher content of TcCPX and probably TcMPX can protect mitochondria from oxidative damage.
Supported by: FAPESP

BQ43 - A characterization and comparative study of protease activities in extracts and extracellular secretions of *Leishmania (Viannia)braziliensis* strains

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Leishmaniasis is a worldwide parasitic disease with two million new cases occurring each year, and one-tenth of the world's population at risk of infection (World Health Organization, 2000). In South America, *Leishmania (Viannia) braziliensis* is the main causative agent of tegumentary leishmaniasis producing different clinical manifestations that may be related both to the host immune reponse and parasite gene expression. Proteins expressed are of special interest in the case of Leishmania parasites whose life cycle is developed in very different enviroments within the vertebrate and invertebrated hosts. In this context, several molecules has been identified as potencial virulence factors of this genus i.e. the zinc-metalloproteinase gp63, secreted acid phosphatase and cysteine proteinases. This work reports the characterization of the proteases expression profile among *L. (V.) braziliensis* strains isolated from different locality and clinical forms. For zymograph studies of proteases activities of extracts, 1x109 promastigotes were washed in PBS 0.01M pH 7.2 and disrupted in 10 mM Tris-HCl pH 6.8 containing 1% of Triton X-100. For secreted proteases analyses, 1x10^9 promastigotes were ressuspended in 1ml of RPMI 1640 media plus 25mM HEPES and incubated by one to three hours at 25°C. After, cells were centrifugated and filtered in a 0.22 mm pore membrane filter. Proteases activities were detected in 7,5% polyacrylamide gel co-polymerized with 0.1% gelatin. Zimographies of whole-cell extracts proteases showed a differential electrophoretic migration profile between strains of distinct clinical and geografical origin. Likewise, analysis of secreted enzymes demonstrated a distinct profile among them. Inhibition assays using 5 μM E-64, 10mM EDTA, 500 μM PMSF, 10mM o-phenanthroline, 10 μM TLCK and 5μM pepstatin failed to hamper the enzymatic activity of *L. (V.) braziliensis* strains. Proteases activities detected at pH 5.5 were different from that detected at pH 7.0 for both extract and extracellular secretions.
Financial Support: CNPq, FIOCRUZ, FAPERJ.

BQ44 - Differential protease expression in extracts and extracellular secretions of *Trichomonas vaginalis*: a comparison between a fresh isolate and a long-term cultured parasite.

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Trichomonas vaginalis is a parasitic protozoan that is the ethiological agent of human trichomonosis, a worldwide spread sexually transmitted disease. Several molecules of *T. vaginalis* had been identified as virulence factors involved in host-tissue injury: adhesins, cell-detaching factor, pore-forming proteins and proteases. Among them, proteases parasitic proteases are involved in nutrient acquirement, immune response evasion and cytotoxicity to host cells. In this work, it was characterized both the constitutive and secreted protease profile of two *T. vaginalis* strains, one fresh isolate (FMV1) and a long-term cultured strain (FF28JT-Rio). For zymography studies of proteases activities of extracts, $3x10^9$ parasites were washed with PBS 0.01M, pH 7.2 and disrupted in 10mM Tris-HCl buffer pH 6,8 with 1% Triton X-100. For secreted proteases analyses, $3x10^9$ cells were ressuspended in 1ml of RPMI 1640 media plus 25mM HEPES and incubated by one to three hours at 37^oC being centrifugated and filtered in a 0.22 mm pore membrane filter. Proteases activities were detected in 7,5% polyacrylamide gel co-polymerized with 0.1% porcine gelatin. Zimographies of whole-cell extracts proteases show a differential electrophoretic migration profile between the strains. Likewise, analysis of secreted enzymes demonstrated a distinct profile among them and different from that produced by their constitutive proteases. Only E-64, a cysteine protease inhibitor, was able to hamper the enzymatic activity of both strains of *Trichomonas vaginalis*.

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BQ45 - Preliminary proteomic analysis of *Triatoma infestans* saliva

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A major function of the salivary secretion of blood-feeding arthropods is to inhibit homeostasis and inflammation in host tissues. The peptide masses resulting from in-gel tryptic digests of saliva fractions eluted from the Oligo-dT column were subjected to LC-MS/MS. The entire DTA files containing MS/MS data from LC-MS/MS runs were subjected to database search via Mascot software. The genome sequence database of *Anopheles gambiae* was chosen due to its phylogenetic proximity to *T. infestans*. Only proteins that matched to a minimum of two peptides identified with highly significant database matching scores ≥ 13 indicated by the software as significant hits were considered as positive identification. The 162 different proteins resulting were grouped according to their function using the Gene Ontology for molecular function and biological process classification and bibliographic references. We report that 25% of the proteins in the triatomine's saliva are involved in the elicitation of the insect innate immune response (system of activation of prophenoloxidase, besides cecropins, defensin antimicrobial peptides, serine proteases and its inhibitors); 8% of metabolic enzymes involved in digestion (serine proteases, trypsins, alpha-amylases, maltase, etc); 7% of proteins involved in the facilitation of blood feeding; 10% of proteins involved in insecticide resistance, and proteins involved in the facilitation of lipids and iron transport and other mechanisms of transport that assist in the nutrition. The remaining proteins belonging to various families are related to diverse functions crucial to the understanding of the insect physiology and biochemistry. Considering insect transmitted blood-born diseases are the greatest human killers and that some are incurable, many investigators believe that the study of the proteins in the salivary glands of hematophagous insects could generate the knowledge required for prevention of these illnesses.

BQ46 - A Screening for Inhibitors of Adenine Phosphoribosyltransferase from *Leishmania tarentolae*

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Leishmaniasis is a serious disease caused by parasites of the order *Kinetoplastida*. According to the World Heath Or-

ganization (WHO, 1998), 88 countries are affected, with 12 million people infected. The need for new drugs for the treatment of leishmaniasis comes from a lack of safe drugs. Knowing that the Leishmania parasites are purine auxotrophs, our purpose is to explore the salvage pathway as a target for the development of new drugs that may come as an alternative treatment for leishmaniasis. We used recombinant APRT from *L.tarentolae*as model system, to evaluate the inhibitory capacity of organic extracts from marine invertebrates collected along the Brazilian coastline. The screening of 330 extracts from marines invertebrates was performed using an easy and fast spectrophotometric enzymatic assay, which allowed the identification of 7 crude extracts as promising APRT inhibitors. Six of the seven extracts were fractionated guided by the APRT inhibition assay. Up to present, two substances were isolated: PcS2d from the sponge *Petromica ciocalyptoides* and A1g from *Ptilocaulis walpnersi*. The compounds showed inhibitory activity of Leishmania APRT at 2.87 μM and 1.43 μM, respectively. Compound PcS2d was subsequently identified as a halistanol A sulfate, previosuly isolated from other different sponges. The sulfate groups of halistanol A sulfate were removed by hydrolysis and the inhibitory activity on APRT was no longer observed. Compound A1g inhibits not only APRT but also HGPRT and XPRT, important PRTases from Leishmania. The triple PRTases inhibition indicates compound A1g as a promising Leishmania inhibitor.

Supported by: FAPESP (CEPID), PRONEX.

BQ47 - Analysis of Miconazole effect on evolutive forms of *Leishmania (L.) amazonensis* as a model for synergistc studies with inhibitors of the Ergosterol pathway.

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The search for effective regiments against leishmania became extremely necessary, once leishmaniasis chemotherapy is based upon the use of pentavalent antimonial compounds; drugs that are toxic and involve long-term treatment and high cost. Combination therapy of drugs, which exhibit synergism in their action, must be considered. In this study we tested the in vitro response of *Leishmania* species/strains to Miconazole (MCZ), an imidazole with effective antifungal action that inhibits the Ergosterol pathway at 14-demethylase level. It is well known that MCZ inhibit amastigotes and promastigotes proliferation of both *L.(L.)donovani* and *L.(L.)amazonensis* (Haugham et al.,1992, Biochem Pharmacol.44:2199-2206). Considering that response to treatment varies either with the parasite species involved or with the clinical form of the disease, we decides to test MCZ against same Brazilian species (as model *L.(L.)amazonensis*). Our main goal were: (1) determinate the IC50 value to MCZ for promastigote cultures of *L.(L.)amazonensis*, (2) evaluate the MCZ effect directly in amastigote extracted from mice lesion, as well as in interaction with peritoneal macrophages from BALB/c, and (3) verify the viability of infected and non infected macrophages in the presence of MCZ. As expected, initial results for both promastigotes and amastigotes analysis are quite similar than the observed for Haugham (1992). Using a range of MCZ concentration from 0 to 10μg/mL, we also verified leishmanicid activity on infect macrophages in the presence of MCZ concentration bigger than 5μg/mL (cell viability 75% - 3 parasites per cell, against cell viability 77% -12 parasites per cell, in the control without MCZ). With this infective model working well, we will carried out similar analysis with *L.(L.)major* and *L.(L.)chagasi*, aiming at synergism effects of these and same other imidazoles compounds tested with a couple of lineages of leishmania that over-express gene-loci related to the resistance of Ergosterol inhibitors. Support: FAPESP,CNPq,LIM-48.

BQ48 - Potent leishmanicidal action of S-nitrosoglutathione against *L. major* and *L. amazonensis*

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Nitric oxide (NO) is a key endogenous molecule that mediates a wide range of physiological and pathophysiological processes. NO has also a central role in the immune response against leishmania protozoa. Cytokine-activated macrophages have been shown to kill intracellular *Leishmania* mainly through the production of NO. S-nitrosoglutathione (GSNO) is an endogenously found S-nitrosothiol which releases NO spontaneously and behave as a NO reservoir, due to its greater stability in comparison with free NO. In order to investigate the potential of utilizing this NO-donor in the treatment of leishmaniasis, we tested the in vitro activity of GSNO against *Leishmania* promastigotes. *L. major* and *L. amazonensis* promastigote cultures incubated with GSNO in concentrations ranging from 10 to 500 μmolL^{-1} have confirmed the leishmanicidal action of this NO donor and allowed determining IC50 values of 118 and 58 μmolL^{-1} for *L. major* and *L. amazonensis*, respectively. Interestingly, the CC50 of GSNO against macrophages was found to be ca. 10-fold greater, relative to its toxicity against promastigotes. The evaluation of GSNO activity against intracellular amastigotes is under way. Based on the IC50 values found, one can consider that GSNO is a potent leishmanicidal agent. Moreover, the much higher CC50 displayed against host macrophages, indicates that GSNO has potential to be used in the treatment of cutaneous leishmaniasis.

Supported by FAPESP.

BQ49 - BISPHOSPHONATES AS ALLOSTERIC INHIBITORS OF *Trypanosoma cruzi* HEXOKINASE: STUDIES ON INTERMEDIARY METABOLISM

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Recent work from our laboratories has shown that glycosomal hexokinase (HK) from *Trypanosoma cruzi* is inhibited by inorganic PPi, which acts as a heterotropic allosteric regulator. In this work we investigated the potential inhibitory activity of a series of bisphosphonates (metabolically stable PPi analogs) against both native and recombinant HK, overexpressed in *E. coli*, both purified to homogeneity. The compounds 3-A (2-(4-pyridyl)-1-hydroxyethane-1,1-bisphosphonate) and 14-A (N-(2-(3-pycolyl)) aminomethylene-1,1-bisphosphonate) showed strong and selective inhibition of the native enzyme. Lower inhibitory activity was observed towards the recombinant HK, suggesting structural differences between the two proteins. 3-A showed mixed type inhibition with respect to ATP, with $K_s = 3$ μM and $K_{ii} = 4$ μM; these values were significantly smaller than those for PPi ($K_{is} = 500$ μM and $K_{ii} =$ 10.46 mM). 14-A displayed a competitive inhibition mechanism respect to ATP and a K_i of 16 μM. We also performed kinetic studies of the inhibitory activity of these compounds towards HK present in the matrix of digitonin-permeabilized glycosomes and we observed differences in both the PPi inhibition mechanism and the values of inhibition constants for bisphosphonates, when compared with the soluble enzyme. The effect of these compounds on D-glucose consumption was investigated in both permeabilized epimastigotes and intact cells growing in LIT medium. The results were consistent with the kinetic data obtained against pure and glycosome-associated HK and indicate that in the presence of bisphosphonates, epimastigotes compensate the reduction in the rate of D-glucose consumption by increasing amino acid degradation. In agreement with this, studies with ^{13}C-NMR showed that succinate excretion was diminished in treated cells. These results suggest a novel approach for rational chemotherapy against this parasite.

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BQ50 - Biochemical and ultrastructural changes induced by 20-hydrazone-imidazoline-2-yl-5α-pregnan-3β-ol on *Leishmania(L) mexicana*

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In the search for new effective chemotherapeutic alternative for leishmaniasis we evaluated the antiproliferative effects and biochemical and ultrastructural changes induced by 20-hydrazone-imidazoline-2-yl-5α-pregnan-3β-ol, synthesized as possible Δ^{24} sterol methyltransferase (24-SMT)inhibitor. *Leishmania (L.) mexicana* (NR strain) promastigotes were maintained in Schneider's insect medium supplemented with 5% fetal bovine serum at 26 $\circ$C. 10^7 parasites/ml at exponential phase of growth were exposed to 0.5, 1, 3, 6 and 10 μM of 20-hydrazone-imidazoline-2-yl-5α-pregnan-3β-ol during 72 hours; growth was followed by direct counting in Neubauer chamber. Sterols analysis of parasites were carried out using gas chromatography and ultrastructural observations by transmission electron microscopy employing conventional methods. The title compound induced an antiproliferative dose dependent effect on *L.(L.) mexicana* promastigotes. We found 48h after treatment 20% and 50% growth rate reduction with 1 and 3 μM, respectively (IC_{20} and IC_{50}) and 9.5 μM the corresponding estimated minimal inhibitory concentration (MIC). Associated with progressive growth arrest the parasites showed loss motility, swelling, vacuolization and cluster formation. Ultrastructural alterations with MIC basically consisted of mitochondrial swelling showing inner membrane loss integrity and the appearance of electron dense bodies containing concentric membranes. Ergosterol and episterol (ergosta-7,22-dien-3β-ol) were the mainly sterols present on parasites not treated during 48h whilst treatments IC_{20} and IC_{50} conducted to disappearance of ergosterol and episterol, and accumulation of colesta-7,24-dien-3β-ol and colesta-5,7,24-trien-3β-ol, possible as an effectively 24-SMT inhibition. These findings suggest that potent antiproliferative effects of 20-hydrazone-imidazoline-2-yl-5α-pregnan-3β-ol and ultrastructural alterations described could be related to ergosterol biosynthesis inhibition by an specific Δ^{24} sterol methyltransferase blockage, constituting a promising alternative to be evaluated *in vivo* for the leishmaniasis treatment.

Key words: Leishmaniasis, sterol hydrazone, C-24 sterol methyltransferase

BQ51 - Effect of Aureobasidin A in *Leishmania (Leishmania) amazonensis*

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Aureobasidin A (AbA), produced by *Aureobasidium pullulans*, is an antifungal antibiotic that inhibits the growth of a wide range of pathogenic fungi. The effect of AbA has been related with an inhibition of fungal inositolphosphoceramide synthase. Glycoinositolphospholipids, inositol phosphorylceramides and glycosylinositolphosphorylceramides have been described in trypanosomatides. In the present work we analyzed the effect of AbA in *Leishmania (Leishmania) amazonensis*, one of causative agent of American tegumentary leishmaniasis. This protozoan is a digenetic parasite that synthesizes specie-specific glycolipids that have been considered potential targets for anti-*Leishmania* drugs.

When promastigote forms of *L. (L.) amazonensis* were incubated with AbA 10μM, although it was not observed any effect of on cell viability, AbA was able to inhibit significantly the parasites' growth. In order to analyze the effect of AbA in *L. (L.) amazonensis* infectivity *in vivo*, procyclic and metacyclic promastigote forms were treated or not with AbA 10μM for 72 hours, inoculated in BALB/c mouse footpad and the lesion growth was followed up for nine weeks. It was noted a significant delay in the lesion development either for metacyclic or procyclic promastigote treated with AbA. These data suggest that AbA may alter/modulate the expression of molecules related with parasite division or parasite-macrophage interaction.
On the other hand, when amastigote forms isolated from BALB/c lesions were maintained with AbA 10μM for 24 hours, a significant decrease of parasite viability was observed (almost 50%) probably by disruption of cell membranes. In order to better understand the effect of AbA in amastigotes during macrophage infection, AbA was added to the cultures at 2 and 24 hours post-infection, and a decrease of 97% and 47% on the number of macrophages infected with amastigotes was observed, respectively. These results suggest that AbA is more toxic to amastigote forms in the beginning of infection.
The metabolic pathway studies of glycolipids in *L. (L.) amazonensis* by incorporation of ^{3}H-serine and ^{3}H-palmitic acid are under investigation. The exploration of the role of ceramide-based glycolipids can provide information to development of new chemotherapeutic anti-*Leishmania* drugs, since the treatment available nowadays has shown a high incidence of adverse effects and drug-resistant cases.
Supported by FAPESP and CNPq.

BQ52 - Analysis of the Tubercidin antiparasite action in association with the nucleoside transport inhibitor S-(4-Nitrobenzyl)-6-Thioinosine in *Leishmania spp.* cultures.

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The research of new therapeutic approaches for leishmanisis is important once the treatment is restricted to compounds with a certain toxicity degree. Tubercidin (TUB), a purine analog, possesses a potent anti parasite action, however with high toxicity for host cells. The decrease of TUB toxicity can be reached associating S-(4-Nitrobenzyl)-6-Thioinosine (NBMPR), an inhibitor of the purine nucleoside transport, specifically target to mammals cells, protecting them of the TUB toxicity. These compounds were tested in cultures of promastigotes forms of *Leishmania (Leishmania) amazonensis, L. (L.) chagasi, L. (L.) major, L. (Viannia) braziliensis*, as well as in amastigotes forms of *L. (L.) amazonensis*, seeking to evaluate the individual action and in association with both compounds in the parasite cells. It was also analyzed the effect of TUB and NBMPR in interactions macrophage-*L.(L.) amazonensis*. So far, our results demonstrate the maintenance of the anti parasite action of larger concentrations of TUB (1μM) in association with NBMPR inhibitor in both cultures, evidencing that NBMPR does not alter the anti parasite effect of TUB. When macrophages were infected with *L. (L.) amazonensis* in the presence of high concentrations of TUB and NBMPR, we did not observe significant alterations at the macrophages structure level, demonstrating that the NBMPR protect cells for TUB toxicity. Starting from these results, we will further analyze the TUB action in association with NBMPR in vivo after mice infection with *L. (L.) amazonensis* cells. Supported by: FAPESP, CNPq, LIM-48

BQ53 - Effect of glycolipid synthesis inhibitors on *Trypanosoma cruzi* growth

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Trypanosoma cruzi is the etiologic agent of Chagas' disease which causes a significant morbidity and mortality and it is a major problem due to the limited therapeutic choices and their adverse reactions. The different life stages of *T. cruzi* expresses a variety of glycoconjugates on their cell surface as glycosylphosphatidylinositol (GPI) anchored glycoproteins, as well as glycosilphosphorylceramides (GIPCs). Most of these glycoconjugates present ceramide as their lipid moieties. The inositol phosphorylceramide (IPC) synthase, is a common enzyme to fungi and plants, which catalyzes the transfer of phosphoinositol from phosphatidylinositol to ceramide to synthesize IPC. Inhibitors of IPC synthase, such as Aureobasidin A (AbA), blocks the growth and also can act as a fungicidal drug in a large number of fungi. Recently, Salto et al (Eukaryot Cell 2, (2003); 756) reported that AbA also impaired the differentiation of *T. cruzi* trypomastigotes at acidic pH. On the other hand, Fumonisin B1 (FB_1), produced by several *Fusarium* species, is a competitive inhibitor of ceramide synthase, resulting a disruption of cellular sphingolipid synthesis. In this work, we analyzed the effect of ceramide synthesis inhibitors FB_1 and AbA in *T. cruzi* epimastigotes and amastigotes growth. Epimastigotes (strain CL) were cultivated in LIT medium in presence of different concentrations of AbA and FB_1 (2 to 50 μM). A significant growth inhibition (50%) was observed at 24 hours, in presence of AbA (10μM). The inhibition was more drastic when parasites were analyzed after five days of culture, where it was verified an inhibition of 93% for epimastigotes growth in presence of AbA 10μM, although 97% of these parasites were still viable. Conversely, no effect in epimastigote growth was detected with FB_1 up to a concentration of 50μM. The effect of AbA and FB_1 was also tested in Vero cells infected with trypomastigotes (strain CL). A significant reduction of the number of amastigotes infected cell was observed in presence of 20μM of AbA or FB_1 (respectively 90% and 65%) after 3 days of incubation. Nevertheless, no alteration of the infectivity index was detected. Our data suggest that ceramide/IPC synthases may be essential at different steps for epimastigotes and amastigotes growth. The precise mechanism of inhibition of these inhibitors is under investigation.
Supported by FAPESP and CNPq.

BQ54 - Activity of tamoxifen against *Leishmania* sp. *in vitro*

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The treatment of leishmaniasis relies mainly on the administration of pentavalent antimonials. Alternative chemotherapeutic compounds include amphotericin B and pentamidine. All these drugs are administered by parenteral routes, require long courses of treatment and present high toxicity and costs. Tamoxifen (TMX) is the most commonly used drug for the treatment of breast cancer (JAIYESIMI et al., 1995) and it is a known estrogen receptor modulator that acts as an antagonist and partial agonist. But it has also been reported to have many pleiotropic effects both *in vivo* and *in vitro* that cannot be explained by an interaction with the estrogen receptor (KELLEN, 1996). Modulation of calmodulin, caspases and kinases, interference in ceramide metabolism and inhibition of the acidification of intracellular organelles have all been proposed as possibly related to TMXs action. To investigate whether TMX was an inhibitor of *Leishmania* growth, *in vitro* cultured promastigotes and amastigotes purified from lesions were incubated with different concentrations of the drug and cell viability was assessed by measuring MTT cleavage. Our results show that TMX is active against promastigotes of *L. amazonensis* (IC50: 11,1 μM), *L. major* (IC50: 9,3 μM), *L. braziliensis* (IC50: 10 μM), *L. chagasi* (IC50: 17,7 μM) and *L. donovani* (IC50: 19,9 μM). Moreover, the growth of *L. amazonensis* amastigotes was also inhibited by TMX with an IC50 value of 13,3 μM. TMX was also active against intracellular amastigotes. The reduction of infection was determined by comparison of infection in treated and untreated macrophages with different TMX doses. The treatment of *L. amazonensis* and *L. major* infected macrophages with 10 μM TMX resulted in 50 to 80% decrease in infection rates. Our data show that TMX is effective against *Leishmania* sp. parasites and it may represent an important alternative in the treatment of leishmaniasis.
Supported by FAPESP

BQ55 - TRIPANOCIDAL EFFECTS OF β-LAPACHONE-DERIVATES ON *T.CRUZI* AND CELLS VERO.

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The tripanocidal activities on *T.cruzi* of the naphthoquinones and derivatives α and β-lapachone, have been intensively studied. In the present work we describe the effect of the new substances on epimastigote form of *T.cruzi*. Materials and Methods 1-*T.cruzi* Dm28c epimastigotes was raised in BHI-medium. 2-Trypanocidal Assay- A stock solution of substance- β-lapachone (β-lap), β-hidroxi-lapachone (β-OHLap), epoxide of nor-β-lapachone (EpoxiNb), alil-nor-β-lapachone (AlilNorb) and lapachol acetate (LAPAc2O)-was prepared in DMSO (1.0%). 3-Analyze of the drugs effect was done after quantification of active parasites on the 72h of the culturing, by counting in a optical microscopy. The final concentration of all the drugs tested was 50μM and group control was treated with DMSO 1.0%. 4- Analyze of the drugs effect on the Vero cells was done how reported by Margis et al, 1989. Results and Conclusion - The b-lap, b-OHLap, EpoxiNb, AlilNor-bLap and LAPAc2O were lethal for *T.cruzi*. These substances killed the parasites, epimastigotes foms, in a period of 72 hours. We have also tested the cytotoxity of these drugs on the Vero cells. We investigated the toxic effects of these above-described substances [2,5; 5,0; 12,5; 25 and 50 μM] on the Vero's cells. The substances b-lap, and AlilNor-bLap were toxic to the Vero's cells even in the short concentrations. Interestingly, b-OHLap, EpoxiNb and LAPAc2O were poorly cytotoxic to the Vero cells (the effect was reduced to zero at the smallest concentration of these substances). The trypanossomacidal effect (dose-dependent) of these substances is now being investigating by our laboratory.

BQ56 - A SIMCA model built for megazol and 13 derivatives with biological activity against *Trypanosoma equiperdum*

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The protozoan *Trypanosoma equiperdum* (*T. equiperdum*) is the causative agent of the disease known as dourine (or durine). It is a contagious disease that happens in horses and others equines, and it takes place mainly in Africa. It is transmitted from host to host during the copulation, becoming usually chronic. Acute signs are characterized by augment (inflammation) of genital organ. Furthermore, it appears edematous subcutaneous plaques, light and intermittent fever, progressional paralysis of posterior members, emaciation and death. In 1995, Professor Cristina Northfleet de Albuquerque, by occasion of her doctorate, synthesized some analog compounds of the megazol, chemically 1-methyl-2-(5-amino-1,3,4-thiadiazole)-5-nitroimidazole. Her main intention has been *Trypanosoma cruzi* and Chagas disease, but some of her compounds were tested against *T. equiperdum* too. The biological activity was obtained from *in vitro* tests when the median lethal dose (MLD) of the compounds was

determined. SIMCA (Soft Independent Modeling of Class Analogy) is a statistical method built with the aim to relate descriptors (or variables) of analog compounds with their biological activity. This structure-activity relationship (SAR) study was performed by using the Pirouette 2.02 computational program. The quantum chemical AM1 (Austin Model 1) semi-empirical method of the AMPAC 6.55 computational package was employed to optimize the chemical structures of the megazol and its 13 derivatives with activity against *T. equiperdum*. Around 50 descriptors, shared in electronic, polarizability, steric and lipophilic descriptors, were calculated on the optimized structures. The SIMCA model selected 5 variables which were able to discriminate the compounds studied into two groups (active and inactive compounds): molecular electronic energy (Eelet), charge on the first Nitrogen at substituent 2 (qN'), dihedral angle (D3) and bond length between atom C4 and its substituent (L4).
Financial Support: CAPES

BQ57 - NEROLIDOL INHIBITS THE BIOSYNTHESIS OF ISOPRENOIDS IN *Leishmania amazonensis*

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Biosynthesis of isoprenoids in eukaryotes is initiated from acetyl-coenzyme A as the main substrate. Most organisms synthesize hydroxymethylglutaryl-CoA which is reduced to mevalonate by HMG-CoA reductase. Mevalonate is converted into isopentenyl pyrophosphate which generates geranyl pyrophosphate and farnesyl pyrophosphate (FPP), the main precursors of polyisoprenoids such as dolichol, ubiquinone, cholesterol or ergosterol and prenyl groups transferred to prenylated proteins. The inhibition of isoprenoid biosynthesis leads to suppression of cell growth and death. Nerolidol is a sesquiterpene produced by the isoprenoid pathway in plants, found in a variety of foods and fruit oils and possessing antibactericidal, antitumoral and antiprotozoal activity. In *Plasmodium falciparum* nerolidol inhibits the biosynthesis of dolichol and ubiquinone (Macedo et al., FEMS Microbiol. Let., 207:13, 2002). In a previous work, we showed that nerolidol inhibited the growth of promastigotes (IC50 $\sim$ 85,22 $\pm$ 5,45 μM) and amastigotes (IC50 $\sim$ 67,73 $\pm$ 3,79 μM) of *Leishmania*. In the present study, we show that nerolidol inhibits the proliferation of intracellular amastigotes *in vitro*. Nerolidol was also used to treat BALB/c mice infected with *L. amazonensis*. Parenteral or topic administration of the drug resulted in decreased lesion size during the treatment. However, mice were not cured and the disease progressed after interruption of the therapeutic scheme. To investigate nerolidol s mechanism of action, *L. amazonensis* promastigotes were treated with 30 μM nerolidol and labelled with [^{14}C]-acetic acid, [2-^{14}C]-mevalonate or [^{3}H]-FPP. The hexane extract of these parasites was analysed by HPLC or HPTLC and we verified inhibition of the dolichol and ubiquinone biosynthesis after labelling with [^{14}C]-acetic acid and [2-^{14}C]-mevalonate but not with [^{3}H]-FPP. These results indicate that nerolidol is probably an inhibitor of the farnesyl pyrophosphate synthase or of an enzyme responsible for the synthesis of FPP precursors.
Supported by FAPESP and CNPq.

BQ58 - New 3-Substituted Quinolines in The Therapy of Visceral Leishmaniasis

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The antileishmanial efficacy of four novel quinoline derivatives was determined *in vitro* against *Leishmania (L.) chagasi*, using extracellular and intracellular parasite models. When tested against promastigotes, all synthesized compounds showed significant antiparasitic effect, with IC50 values in the range 0.091 - 18.78 μg mL^{-1}. The relationship between the chemical structure and the biological activity showed that the 4-substitution of Cl^- by OH^- in the quinoline ring, seems to have significantly improved the antiparasitic effect. The 3-substitution of allyl by cinnamyl also contributed to a better antileishmanial effect, once a considerable decrease in the IC50 values was found through the cinnamyl introduction. When tested against *L. (L.) chagasi*-infected macrophages, compound 3b demonstrated a considerable efficacy being 8.3-fold more active than the standard pentavalent antimony. No significant antileishmanial activity was found for compounds 3a, 4a and 4b. The killing effect of compound 3b was driven specifically to the parasite, without host cell activation, as demonstrated by nitric oxide production. Ultrastructural studies of promastigotes treated with compound 3b showed mainly enlarged mitochondria, with matrix swelling and crista reduction. New study and developments on established antiparasitic structures as quinoline rings could be useful and low-cost models for new pharmacological compounds, especially for neglected diseases as Leishmaniasis.

BQ59 - The search for novel inhibitors of *L.(L.)amazonensis* arginase

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Arginase is an essential enzyme of the parasitic protozoa *Leishmania* involved in the polyamine pathway. The impor-

tance of this enzyme in the infection has been shown using an arginase inhibitor, N^{ω}-hydroxy-L-arginine (Iniesta et al., 2001), as well as by genetic manipulation that resulted in the production of a knocked out mutant with a single allele of the arginase gene that consequently reduced the enzyme activity (Alves et al., 2003). In both cases, the infectivity rate was reduced. Exploring the potential of arginase as a chemotherapeutic target, we used the *Leishmania* arginase structural model. Chemical similarity analysis and 2D-substructure searches of data bases using the interface SciFinder, that resulted in the selection of a small set of inhibitor candidates. The selected molecules possess a variety of moieties that may allow the establishment of important structure-activity relationships between enzyme and inhibitors (e.g. N^{ω}-nitro-L-arginine) or could cause steric blockage around the active site (e.g. NG-metil-L-arginine). The *Leishmania* recombinant enzyme was expressed with the pRSET vector in *Escherichia coli* resulting in a construct that lacks the hexa-histidine tag. Due to the presence of internal histidines, we were able to purify the recombinant enzyme by a two-step chromatographic purification method including a nickel-chelating Sepharose and an anion exchange column. Enzyme assays are underway to determine the kinetic constants of the purified arginase itself, or in the presence of the inhibitor candidates. The leishmanicidal potential will then be evaluated by *in vitro* infection of macrophages cultures. Financial Support: FAPESP and CNPq.

BQ60 - The anti-parasitic activity of furamidine analogues against Trypanosoma cruzi and Leishmania (L) amazonensis

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Parasitic protozoa are unicellular pathogens with a complex life cycle displaying distinct morphological stages during their development in both vertebrate and invertebrate hosts. Among the protozoa, members of the Trypanosomatidae family include a large number of species that cause human diseases such as Chagas disease and Leishmaniasis. In this respect, diphenylfuran diamidines represent an important promising class of DNA-targeted antiparasitic agents. The best-known member of the series is the DB75 commonly referred as furamidine and related unfused aromatic diamidines that have proven useful for the treatment of parasitic infections. Since recent literature points to the need of finding more efficient and less toxic chemotherapeutic approaches for both Chagas' disease and Leishmaniasis, our present aim was investigated the in vitro effects of DB75 and its phenyl-substituted analog DB569 on both intracellular amastigotes as well as trypomastigotes of Trypanosoma cruzi, Y and Dm28c, stocks and upon promastigotes of Leishmania L amazonensis. Our results showed that both compounds have anti-parasitic effect against T. cruzi and Leishmania parasites. However, the DB569 was more potent than DB75 at reducing the proliferation of intracellular forms of T. cruzi besides exhibiting higher trypanocidal dose-dependent effects against trypomastigotes of T. cruzi from both stocks and promastigotes of L. amazonensis. Fluorescence microscopy experiments indicated that both diamidines were localized within the nuclei and kinetoplast of the parasites. Electron microscopy studies showed that the treatment of the parasites with DB75 and DB569 induces important alterations of the parasite nucleus and kinetoplast, at sites where their DNA target is localized. Altogether, the data show that furamidine displays anti-parasitic activities against T. cruzi and L. L amazonensis, and the phenyl substitution of its amidine termini significantly enhances the anti-parasitic activity. The furamidine analogue DB569 appears as a promising candidate for further evaluations in vivo. Supported by: Faperj, CNPq, INSERM and PAPESIII/FIOCRUZ.

BQ61 - Antigiardial activity of the essencial oil from *Ocimum basilicum*

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The giardiasis is an infection that affects more than 200 million people all over the world, being one of the most frequent causes of infection in humans. The ethiological agent is the protozoan *Giardia lamblia*. Transmission occurs by the ingestion of water or occasionally food containing *G. lamblia* cysts or is acquired by person-to-person contact. Symptomatic infection in humans is characterized by diarrhea, epigastric pain, nausea, vomiting, and weight loss. *Ocimum basilicum* L. (Lamiaceae) comprises annual and perennial herbs and scrubs native to the tropical and subtropical regions of Asia, Africa and Central South America. It is also considered to be a source of aroma compounds and essencial oils containing biologicaly active constituents that possess insect repellent, nematocidal and antibacterial activity. Although essencial oils in different basil cultivars are variable, prevalent components are monotherpenes and phenylpropanoids. Prompted by these findings, we decided to investigate the effect of the essential oil on *G. lamblia*. The essential oil was used to analyze the modulation of peritoneal mouse macrophage infection by *G. lamblia*. The nitric oxide production by the infected macrophages was also determined. *G. lamblia* cells were incubated in culture medium (TYI-S-33, suplemented with 10% of fetal bovine serum), in the presence of the essential oil (2mg/mL), at 37C for 2 hours. Parasites survive

and cell morphology were evaluated under transmission electronic microscopy. Besides, the protein's profile determined by SDS-PAGE revealed the expression of some different proteins when the cells were incubated in the presence of the essential oil (2mg/mL) for 1 and 2 hours. Pretreatment of peritoneal mouse macrophages with 1,5mg/mL essential oil dilution reduced in 56% the association index between these macrophages and *G. lamblia*, as compared to the control ones.

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BQ62 - α-TOMATINE 50% LETHAL DOSE ON CULTURE FORMS OF TRYPANOSOMATIDS

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Secondary metabolites accumulated by plants have important functions on plant protection against pathogens. The glycoalkaloids are involved in this protection, one of them,α-tomatine, exists in high concentration in green tomato fruits (*Lycopersicon esculentum*), whereas in the ripe fruits it is changed to its aglycone form, tomatidine, inoffensive to the pathogens. A tomato parasite, the trypanosomatid *Phytomonas serpens*, could develop only in ripe fruits, with evidence of involvement of α-tomatine in the tomato plant defense. To investigate the sensibility of the Trypanosomatids to the action of α-tomatine, we used logarithmic phase culture forms in GYPMI medium of 20 lower trypanosomatids and *Trypanosoma cruzi*. The 50% lethal dose (LD50) were determined mixing equal volumes of 10^7 cells of the various culture forms with solutions between 10^{-3} to 10^{-9} M of α-tomatine in PBS (150mM, pH7.2 with 10% ethanol) during thirty minutes at room temperature. The same tests realized with the aglycone form of tomatine (tomatidine) as control showed not detectable toxicity on the same trypanosomatids culture forms. The tests were evaluated by the % of motility of the protozoan culture forms observed with optical microscopy in a Newbauer chamber. The results after the LD50 determination (Reed& Muench) showed the LD50 oscillating between 10^{-4} to 10^{-6} M of α-tomatine. Little differences were observed between the LD50 of the analyzed samples, and none of them showed evidences of resistance, due to action of tomatinidase, as demonstrated in some pathogenic fungi.

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BQ63 - Identification of peroxide molecules with antimalarial activity against Plasmodium falciparum aiming treatment of chloroquine-resistant human parasites

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Treatment of human malaria has become increasingly complicated due to the emergency and spread of mutants resistant to the available antimalarials. It relies mainly on quinine combined with other drugs, especially the artemisinines (artemether and artesunate) of high cost and non-curative if used alone. This new antimalarial prototype, a peroxide bond in a trioxane heterocycle, was recently synthesized (Vennerstrom et al, Nature 2004). There is no resistance to artemisinines which makes the peroxide drugs ideal antimalarials. Aiming at new antimalarial molecules we have: (i) tested hundreds of compounds from medicinal plants used against fever and found 28/50 species active; (ii) characterized molecules responsible for their activity; in Bidens pilosa, Asteraceae we found flavonoids and polyacetilenes; (iii) tested several benzophenazines and chalcones, some highly active; and (iv) synthesized and tested one stable molecule based on artemisinine isolated from Artemisia annua (Asteraceae). This new functional peroxide group (with esther, epoxide and ozonide) was produced and tested against P. falciparum (Pf). In vitro it caused total hemolysis at high doses ($\geq 25\mu$g/ml); lower doses were less toxic (6 to 1.5μg/ml) inhibiting Pf growth by up to 90%; 0.78μg inhibited 50% Pf growth; lower doses were inactive. Treatment of P. berghei infected mice with 50 and 100 mg/kg reduced 60 and 80% the parasitaemia. Thus, this new synthetic peroxide, partially active against malaria, is a promising compound to be further modified and tested against drug resistant malaria parasites.

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BQ64 - Molecular characterization of a Plasmodium berghei Chloroquine (CQ) resistant strain (PbCR) and its use for tests of new antimalarials in vivo

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The overspread of human malaria in the tropics and high mortality of children caused by *Plasmodium falciparum* (Pf) has made the search of new antimalarial drugs an urgent need. This is especially important since most Pf strains in the endemic areas have developed resistance to

CQ and to other quinoline derivatives (like mefloquine, amodiaquine and quinine). We have tested new drugs based on ethnopharmacology using *P. berghei* parasites sensitive to CQ (Pb-CS), some benzophenazines and chalcones were highly active. Our present aim was to test antimalarials against parasites CQ resistant (Pb-CR), a phenotype selected in mice infected with Pb-CS, treated with increasing doses of CQ, up to 150mg/kg, and consecutive blood transfers. The Pb-CR strain kept in liquid nitrogen for decades was defrosted, transferred to mice which submitted to 15 mg/Kg of CQ showed a partial reduction of parasitaemia. This suggested that CQ resistance was not a stable character, a data confirmed by genotypical characterization. The Pb-CR parasites were negative for the mutation in the gene *cg10*, homologous to *pfcrt*, responsible for Pf-CR (collaboration with V. do Rosario group, University of Lisbon). Thus Pb-CR has a physiological adaptation to CQ or has another gene responsible for CR, to be clarified. Maintained under new CQ pressure the Pb-CR parasites tolerated maximum CQ doses, thus they may be useful for in vivo tests of new antimalarials aiming Pf-CR, the case of artemisinine derived molecules under tests, of practical importance.

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