BQ.01 – *TRYPANOSOMA CRUZI* Y STRAIN EPIMASTIGOTES PROTEOMIC MAP: NEW INSIGHTS IN POST-TRANSLATIONAL MODIFICATIONS

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Chagas' disease caused by protozoan *Trypanosoma cruzi*, is an endemic neglected illness in Latin America, responsible for considerable human mortality and morbidity. Parasite's life cycle involves vertebrate and invertebrate hosts, and three evolutive forms. In this study, the proteomic profile of *T. cruzi* epimastigotes (Y strain) was evaluated, to identified post-translational modifications. 2D-PAGE was employed, and the gels were stained with colloidal Coomassie for total protein or ProQ-Emerald or Diamond for glyco- or phospho-proteins, respectively. All peptides identification was performed in MALDI-TOF/TOF. Epimastigotes' proteins were extracted by freezing-thawing in a lysis solution (8M urea, 4M thiourea, 4% CHAPS, 40 mM Tris, 60 mM DTT, 1% ampholytes and protease inhibitor cocktail). Two-dimensional electrophoresis (500 μg) was performed using 4-7 IPG strips followed by 12% SDS-PAGE. Approximately 617 spots were detected, being 160, 24 and 35 identified in the gels stained with Comassie, Pro-Q-Emerald and Pro-Q-Diamond, respectively. Glycoprotein content embrace chaperones, beta-tubulin, prostaglandin F2a synthase, ATPase, tyrosine aminotransferase, sterol 24-c-methyltransferase, tryparedoxin peroxidase, among others. Phosphoproteins included glutamine synthetase, pyrroline-5-carboxylate synthetase, 14-3-3, spermidine synthase, initiation factor 5a, chaperones, cytoskeleton proteins, as well as five hypothetical proteins. Our data could provide new insights in the signaling networks in *T. cruzi*, supplying additional information for the development of alternative drugs for Chagas' disease.
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BQ.02 – A PROTEOMIC APPROACH TO UNDERSTAND TGF-β EFFECT IN *T. CRUZI* BIOLOGY

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TGF-β is a molecule involved in the development and maintenance of acute and chronic chagasic cardiopathy. This cytokine is also intimately associated with *Trypanosoma cruzi* as a regulator of different stages of the parasite´s life cycle, such as parasite survival, invasion, proliferation and differentiation. This raises the question about which *T. cruzi* molecules could be involved in these cellular processes stimulated by TGF-β. This work aims to characterize TGF-β responsive molecules through a phosphoproteomic approach using two-dimensional non-linear pH 3-10 gels, differential fluorescent protein staining and mass spectrometry (MALDI-TOF-TOF). We determined that 5 ng/ml was the optimal TGF-β dose for the induction of phosphorylation events, under our experimental conditions. A kinetic study was performed by incubating or not *T. cruzi* epimastigotes (Y strain) with TGF-β in four different time periods (1, 5, 30 and 60 minutes). Phosphoproteins were stained with ProQ Diamond and total proteins with Sypro Ruby (both from Invitrogen), images captured using Typhoon Trio (GE Healthcare) and differential profiles analysed with PDQuest software (BioRad). A number of 30 proteins had their phosphorylation and/or expression pattern altered in response to TGF-β and were identified through mass spectrometry. Their putative functions were correlated with their possible roles in intracellular signaling triggered by TGF-β, in agreement with the phosphorylation and/or expression behavior observed in our analysis. Moreover, we assessed the effect of TGF-β on parasite proliferation. We found that TGF-β addition to epimastigote cultures led to a 36% increase in parasite growth after 72 hours ($p<0,05$). The data presented here contributes to the elucidation of the molecular mechanisms related to TGF-β signaling in *T. cruzi*, providing a source of new potential therapeutic targets against Chagas disease.
Supported by IOC (Fiocruz), CNPq and PAPES V.

BQ.03 – PROTEOMIC ANALYSIS OF POPULATIONS OF *Leishmania braziliensis* AND *Leishmania infantum chagasi* RESISTANT TO POTASSIUM ANTIMONY TARTRATE.

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The emergence of drug-resistance *Leishmania* species is a relevant problem in several countries. Although several mechanisms of drug-resistance are known, there are few studies concerning drug resistance on New World *Leishmania* species. Recently, we selected *in vitro* populations of *Leishmania braziliensis* and *L. infantum chagasi* that are 20 and 4-fold more resistant to potassium antimony tartrate Sb III, respectively (LbSbR/LcSbR) than theirs susceptible counterparts (LbWTS/LcWTS) (Liarte & Murta, 2010). In the present study, proteomic analysis of *L. infantum chagasi* and *L. braziliensis* susceptible (WTS) and SbIII-resistant (SbR) populations was performed using two-dimensional gel electrophoresis (2-DE). The protein extracts (600µg) from four *Leishmania* samples were applied to immobilized pH gradient (IPG) strips (17cm, pH 4-7 linear) and then submitted to isoelectric focusing. Subsequently, IPG strips were transferred to a 12% polyacrylamide gel and after electrophoresis, the gels were stained with colloidal Coomassie Blue G-250. For each pair of samples, proteins from three independent experiments were obtained, and gels in duplicate wer analyzed for each cultivate. The differential expression analysis was performed comparing the quantity of matched spots in each pair of sample. A protein was considered differentially expressed when the ratio between the intensities of S (susceptible) and R (resistant) spots were ≥ 2.5-fold. The analysis of the gel images showed significant difference of the protein profiles between the two *Leishmania* species analyzed. The average number of spots from *L. braziliensis* (353 spots) was higher than *L. infantum chagasi* (320 spots). Analysis of the gel images showed that *L. braziliensis* presented 92 and 26 spots more expressed in the susceptible and resistant populations, respectively. On the other hand, *L. infantum chagasi* presented higher number of spots overexpressed in the resistant population (101 spots) than in its susceptible pair (26 spots). Further, these spots will be submitted to mass spectrometry for protein identification. Financial Support: CNPq, FAPEMIG, CPqRR, UNIMONTES and UNICEF/UNDP/World Bank/WHO/TDR.

BQ.05 – *TRYPANOSOMA CRUZI* REDOX- INTERACTOME: *IN VIVO* IDENTIFICATION OF PROTEINS TARGETED BY TRYPAREDOXIN I

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During infection, *Trypanosoma cruzi* is subjected to the host oxidative environment. Its antioxidant responses are critical for successful infection. Trypanosomatids have a unique hydroperoxide detoxification system dependent on the thiol trypanothione. Tryparedoxin peroxidases catalyze the reduction of peroxides and they are regenerated by tryparedoxins (TXN), which in turn are reduced by trypanothione. In previous work we have functionally and structurally characterized the *T. cruzi* tryparedoxin peroxidases (cytosolic and mitochondrial) which detoxify hydroperoxides and peroxynitrite in a very efficient way. Moreover, when parasites are transfected with these peroxidase genes, they become more infective. Tryparedoxins belongs to the thioredoxin superfamily of proteins, which are oxidoreductases involved in antioxidant defences. They have been implicated in distinct cell functions such as hydroperoxide detoxification cascades, DNA synthesis and kinetoplast replication due to their ability to reduce target proteins. However, only a few target proteins of tryparedoxins are currently known. The aim of this work was to extend our knowledge of *T. cruzi* tryparedoxin interactome *in vivo*. Tryparedoxin reaction mechanism is based in two conserved cysteines. Cys40 residue and the oxidized target form a transient disulfide bridge, which is further resolved by Cys43 of the tryparedoxin, releasing the oxidized tryparedoxin and the reduced protein target. Based on the reaction mechanism, we generated a mutation at the active site of TXNI, by replacing the resolving cysteine residue (Cys43). The mutant TXNC43S gene was transfected and expressed in *T. cruzi* with an additional 6xHis tag. Protein extracts from these parasites were obtained, and purified with magnetic beads that bind His-tagged proteins. The eluted proteins were separated through one and two-dimensional electrophoresis, and analysed by mass spectrometry. Our *in vivo* approach led to the discovery of several putative redox-regulated proteins belonging to unexpected pathways, indicating that tryparedoxin plays a relevant role as a "redox hub" in *T. cruzi*.

BQ.06 – MDL28170, A POTENT CALPAIN INHIBITOR, AFFECTS *TRYPANOSOMA CRUZI* METACYCLOGENESIS, ULTRASTRUCTURE AND ATTACHMENT TO THE LUMINAL MIDGUT SURFACE OF *RHODNIUS PROLIXUS*

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Trypanosoma cruzi is the etiological agent of Chagas' disease. During the parasite life cycle, several molecules are involved in the differentiation process and infectivity, but less is known about which of them are key molecules. Peptidases are relevant for crucial steps of *T. cruzi* life cycle, as such, it is conceivable that they may participate in the metacyclogenesis and interaction with the invertebrate host. In this paper, we have investigated the effect of the calpain inhibitor MDL28170 on the attachment of *T. cruzi* epimastigotes to the luminal midgut surface of *Rhodnius prolixus*, as well as on the metacyclogenesis process and ultrastructure. In addition, we have analyzed the effect of anti-calpain antibodies on the interaction of epimastigotes to the midgut surface of the insect. MDL28170 treatment was capable of significantly reducing the number of bound epimastigotes to the luminal surface midgut of the insect. Once the cross-reactivity of the anti-*Dm*-calpain was assessed, it was possible to block calpain molecules by the antibody, leading to a significant reduction in the capacity of adhesion to the insect guts by *T. cruzi*. Moreover, the *in vitro* metacyclogenesis process was impaired by the calpain inhibitor presenting a significant reduction in the number of metacyclic trypomastigotes. The calpain inhibitor also demonstrated a direct effect against bloodstream trypomastigotes. Ultrastructural analysis of epimastigotes treated with the calpain inhibitor revealed disorganization in the reservosomes, Golgi and plasma membrane disruption. The presence of calpain and calpain-like molecules in a wide range of organisms suggests that these proteins could be necessary for basic cellular functions. Herein, we demonstrated the effects of MDL28170 in crucial steps of the *T. cruzi* life cycle, such as attachment to the insect midgut and metacyclogenesis, as well as in parasite viability and morphology. These results help to shed some light on the functions of *T. cruzi* calpains. Considering the potential roles of these molecules on the interaction with both invertebrate and vertebrate hosts, it is conceivable to explore them as an alternative target to treat Chagas' disease.
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BQ.07 – HETEROLOGOUS EXPRESSION AND PURIFICATION OF THE PROTEIN ECTO-NUCLEOSIDE TRIPHOSPHATE DIPHOSPHOHYDROLASE (E-NTPDASE 2) OF *LEISHMANIA MAJOR*

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The Leishmaniasis is a parasitic disease caused by protozoaries of the kinestoplatida order, Trypanosomatidae family and Leishmania gender. Nowadays, these diseases represent a great problem concerning the Brazilian Public Health System and also in some parts of the world. The E-NTPDases or apyrases are proteins that can be found in these parasites and they also have the major function of extracellular nucleotide degradation, which in turn allows the parasite to modulate purinergic signling and "dribble" the immunological system of its host. The aim of the present work is to obtain the heterologous expression of *Leishmania major* E-NTPDase-2 protein for future studies. Initially, a specific pair of primers was designed in order to remove the amino-terminal transmembrane coding region. A PCR reaction was conducted and subsequently, the cloning procedures in the amplification vector (pJET-blunt) and in the expression vector (pET-21b) were obtained. The NTPDase-2 of recombinant *L. major* was expressed through a heterologous bacterial system and then purified through affinity chromatography in nickel agarose (Ni-NTA). The samples were analyzed by SDS-PAGE 10% and quantified through the Bradford method. The confirmation of the correct expression of an e-NTPDase protein was obtained by a Western Blot. The results show that the *primers* were able to amplify the coding region of the protein without the N-terminal transmembrane coding region. The events of cloning were successfully conducted and in addition, it was possible to obtain the heterologous expression for the E-NTPDase-2 protein of *L.major* which was properly confirmed through the Western Blot method.
Supported by CNPq and FAPEMIG.

BQ.08 – HETEROLOGOUS EXPRESSION AND BIOCHEMICAL CHARACTERIZATION OF *Leishmania chagasi* NUCLEOSIDE TRIPHOSPHATASE (NTPDase) AS A GENUÍNE APYRASE

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Visceral Leishmaniasis is a serious tropical neglected disease, responsible for around 57,000 deaths per year worldwide. The protozoa *Leishmania chagasi* is the mainly pathogenic agent of this disease in the New World.This parasite is classified in the Tripanosomatidae family, which is unable to synthesize purines. It is believed that the Nucleoside Triphosphate Diphosphohydrolases (NTPDases) participate in the process of uptake of purines in "purine salvage pathway" and in the modulation of host immune responses induced by extra cellular nucleotides in the purinergic signaling pathway, acting as facilitators of parasites infections. In this work we cloned the putative nucleoside phosphatase gene predicted in *L. chagasi* genome and expressed it in *Escherichia coli* pET21-b system. The recombinant active NTPDase was purified from inclusion bodies, renaturated and was biochemically characterized according to nucleotidase activity. The recombinant enzyme (LcNTPDase) showed preference for GTP, UDP and ADP as substrates and was active only in presence of magnesium as cofactor. The use of calcium as cofactor completely abolished nucleotidase acitivty. The pH dependent activity showed that the optimum pH range between 7.0 and 8.0. The known apyrase partial inhibitors: ARL 67156, Suramin, sodium azide and Gadolinium were tested. The presence of Gadolinium 300μM showed 97% enzyme inhibition. These results showed that LcNTPDase is a genuine apyrase and presented for the first time an active *Leishmania* recombinant apyrase. LcNTPDase ADPase and UDPase activities could be related with the host immune system modulation. This enzyme can be used in biological assays to study its participation in *L. chagasi* infection and can be applied in biotechnological applications such as rational drug design to be used in Leishmaniasis chemotherapy. Supported by UFV, CNPq, FAPESP and CAPES

BQ.09 – BIOCHEMICAL AND BIOLOGICAL STUDIES WITH A RECOMBINANT E-NTPDase from *Leishmania infantum chagasi*

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An putative GDPase, from E-NTPDase family, was identified in genome of *Leishmania infantum chagasi*. This enzyme family seems to be related with trypanosomatids virulence molecules, In this work the full-length coding region of this putative E-NTPDase, with 2043 bp was cloned into pJET vector (Fermentas). To further characterize the GDPase, we performed the heterologous expression of the active recombinant enzyme using pET21b system (Novagen). In silico analyses predicted a possible aminoterminal signal peptide cleavage sitebetween amino acid positiosn 28-29, immediately following an amino-terminal predicted transmembrane segment, thus suggesting that GDPase could be produced as a soluble exported protein. Using this information we design a strategy to express the soluble GDPase. Full-lenght coding regioncloned in pJET vector was used as template to amplify a 1953 bp DNA fragment that was transferred to pET21b vector (that codes for Hexa-HIS at the carboxy terminal of the recombinant fusion protein). This construction was used to transform *E. coli* BL21-codonplus RIL (Estratagene) cells. Recombinant protein was expressed after 2 hours of induction. Insoluble recombinant GDPase was solubilized, renaturated and purified by Ni-NTA affinity chromatography in the AKTA PURIFIER UPC10 system (GE Healthcare). Nucleotidase activity assays showed preference for GTP hydrolysis (0,186 nmol Pi/ug $xmin^{-1}$) than GDP 0,083 nmol Pi/ug x min^{-1}. The GTPase activity was higher in pH 7,4 than pH 8. Now we are performing another biochemical characterizations and producing polyclonal antibodies anti-GDPase in rabbit to do immunolocalization assays in *L. infantum chagasi*
Supported by: UFV and FAPEMIG.

BQ.10 – Quercetin induces death in *Leishmania amazonensis* by mitochondrial dysfunction and reactive oxygen species production

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Leishmaniasis, a parasitic disease caused by protozoa of the genus *Leishmania*, affects more than 12 million people worldwide. Quercetin has generated considerable interest as a pharmaceutical compound with a wide range of therapeutic activities. One such activity is exhibited against the bloodstream parasite *Trypanosoma brucei* and amastigotes of *Leishmania donovani*. However, the mechanism of protozoan action of quercetin has not been studied. In the present study, we report here the mechanism for the antileishmanial activity of quercetin against *Leishmania amazonensis* promastigotes. Quercetin inhibited *L. amazonensis* promastigote growth in a dose- and time-dependent manner beginning at 48 hours of treatment and with maximum growth inhibition observed at 96 hours. The IC_{50} for quercetin at 48 hours was 31.4 µM. Quercetin caused mitochondrial dysfunction due to collapse of mitochondrial membrane potential. In addition, mitochondrial reactive oxygen species (ROS) production was increased in *L. amazonensis* treated with quercetin. Pre-incubation of promastigote forms of *L. amazonensis* with reduced glutathione (GSH) or N-Acetyl-cysteine (NAC) inhibited quercetin activity. Recently, the effects of several drugs that interfere directly with mitochondrial physiology in parasites such as *Leishmania* have been described. The unique mitochondrial features of *Leishmania* make this organelle an ideal drug target while minimizing toxicity. Taken together, our results demonstrate that quercetin exerts its antileishmanial effect on *L. amazonensis* promastigotes by generating ROS and affecting parasite mitochondrial function.
Supported: FAPERJ, IOC/FIOCRUZ

BQ.11 – A MITOCHONDRIAL PROLINE DEHYDROGENASE OF *TRYPANOSOMA CRUZI* (*Tc*PRODH) PARTICIPATES OF RESPIRATION CHAIN AND IS REGULATED DURING LIFE STAGE

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Trypanosoma cruzi is capable of using carbohydrates and amino acids as carbon and energy sources. Preferentially, epimastigotes catabolize glucose and, after carbohydrate exhaustion, a shift to amino acid metabolism occurs. The amino acid L-proline participates in *T. cruzi*'s bioenergetic metabolism and also supports the differentiation processes, resistance to oxidative metabolic and osmotic stresses. Besides, it was recently shown that proline is a relevant metabolite to supply the energy that powers host cell invasion. Biochemical evidences supports the hypothesis that *T. cruzi* oxidizes L-proline through a proline dehydrogenase (*Tc*PRODH) (EC 1.5.1.2) and a Δ^1pyrroline-5-carboxylate dehydrogenase (*Tc*P5CDH) (EC 1.5.1.12). Here, we describe the biochemical activity, mitochondrial localization and the role of *Tc*PRODH in the mitochondrial respiratory chain. The mRNA analysis, profile protein expression and specific activity showed that this enzyme is up-regulated in the intracellular epimastigote, a stage that requires external supply of proline. It was also showed by two methods indirect immunofluorescence and partial permeabilization that *Tc*PRODH is located in the mitochondrial membrane. These data, together with the fact that this enzyme uses FAD as cofactor indicates that *Tc*PRODH can also contribute with reduced equivalents to the respiratory chain. No significant differences between succinate and proline supported oxygen consumption rates were observed. Experiments are being carried out in order to see how *Tc*PRODH interacts with the other components of the mitochondrial respiratory chain.

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BQ.12 – INOSITOL PHOSPHORYLCERAMIDE EXPRESSION KINETIC DURING *L. (L.) amazonensis* DIFFERENTIATION

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In eukaryotes, sphingolipids (SLs) are important membrane components and powerful signaling molecules. In *Leishmania*, the major group of SLs is inositol phosphorylceramide (IPC), which is common in yeast and Trypanosomatid but absent in mammals and, thus, the IPC metabolical pathway could be considered a good target for new therapy drugs. In order, to analyze the *L. (L.) amazonensis* sphingolipid expression in amastigotes and promastigotes, indirect immunofluorescence assay were carried on with mAb LST-1, directed to IPC. Amastigote forms were isolated from hamster footpad lesions (0h) and cultivated in LIT medium at 23°C for 6h, 24h, 48h and 72h. Parasites were fixed and the reactivity with LST-1 was analyzed by indirect immunofluorescence using of confocal microscopy. 0h and 6h Amastigotes are not recognized by LST-1. On the other hand, after 24h, LST-1 reactivity was observed in all plasmatic membrane. After 48h and 72h the LST-1 reactivity was also identified in parasites vesicles structures. Quantitative RT-PCR for sphingolipids metabolic enzymes are under investigation during the differentiation of amastigote to promastigote.

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BQ.13 – LYSOPHOPHATIDYLCHOLINE (LPC) EFFECT ON THE PROLIFERATION AND DIFFERENTIATION OF *TRYPANOSSOMA CRUZI*

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Trypanosoma cruzi, etiological agent of Chagas disease, is an heteroxenous species that is submitted to morphological and physiological changes during its life cycle. Parasite epimastigote forms replicate and differentiate into infective metacyclic trypomastigotes at the Triatominae insect vector midgut. They are released with kissing bug feces during blood feeding, leading to vertebrate infection. Our experimental model is *Rhodnius prolixus*, Chagas disease vector at Central and South Americas. *R. prolixus* saliva contains lysophosphatidylcholine (LPC), a lysophosphatidyl lipid resulted of phosphatidylcholine hydrolysis by phospholipase A_2 enzyme. Once this bioactive multisignaling ubiquitous lipid is found in human plasma ingested by the insect during blood feeding, the goal of the work is determining the role of LPC in the proliferation and differentiation of *T. cruzi*. We analyzed the effect of LPC (1nM, 1µM, 10µM, 50µM) on parasite proliferation (Y and Dm28c strains) grown in LIT medium supplemented with 10% fetal bovine serum for 7 days. We observed a discrete increasing in LPC-treated-parasite growth, mainly in Y strain. In the presence of 10% delipidated fetal bovine serum and of 10% purified human low-density-lipoprotein (LDL), the effect was more evident with the growth rate of 10µM LPC/ LDL-treated group 3 fold higher at the 6^{th} day post-treatment than control. LPC effect on parasite differentiation was performed by assaying Dm28c strain metacyclogenesis *in vitro*. Parasites were incubated in a nutritional poor medium TAU, that mimetizes the composition of kissing bugs urine, in the absence or presence of LPC (1nM, 1µM, 10µM), and the percentage of trypomastigotes was obtained at each day post-treatment for 7 days. We observed no difference between untreated and treated groups. From now on, we intend to identify the signaling pathways that are activated by LPC in *T. cruzi* and that lead to parasite increased proliferantion. Supported by: CNPq, FAPERJ& IFS.

BQ.14 – CHARACTERIZATION OF A SIR2-RELATED PROTEIN 1 FROM *LEISHMANIA (L.) AMAZONENSIS* (LaSir2) AS A CYTOPLASMIC, GLYCOSYLATED NAD^+-DEPENDENT DEACETYLASE.

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LaSir2 was obtained by a PCR-based cloning using primers designed from the *LmSir2* sequence. *LaSir2* nucleotide sequence analysis revealed an 1122pb gene that encodes for a 373 amino acid protein with a predicted MW of ~40kDa. Amino acid sequence analysis showed that LaSir2 shares identity with other Kinetoplastid Sir2 orthologues (92% for *L.infantum* and 49% for *T.brucei)* and similarities of 53% and 59% with the catalytic site of yeast Sir2 and human Sirt1, respectively. The catalytic site of LaSir2 presents all of the elements essential for the transcriptional silencing, deacetylase and ribosyltransferase activities and, additionally, LaSir2 presents an N-terminal signal anchor, a putative C-terminal NES and it is predicted to be O-glycosylated and phosphorylated. The recombinant 6xHisLaSir2 was purified to homogeneity and was monomeric (41kDa±1%) as investigated by SEC-MALS. Spectroscopic studies agreed with tridimensional models from orthologues as 6xHisLaSir2 presented a characteristic alpha-helix rich Circular Dichroism spectrum, with minima at 208 and 222 nm, and fluorescence emission spectrum with λ_{max} at 335nm suggesting that the tryptophan residue is buried. Also, the 6xHisLaSir2 displayed NAD^+-dependent deacetylase activity, being able to accept as acetylated substrates, a p53-derived peptide and a ~50kDa protein from promastigote extract. A serum raised against 6xHisLaSir2 detected a major ~50kDa protein in extracts of both promastigotes and lesion-derived amastigotes. Analysis of subcellular fractioning of promastigotes revealed that LaSir2 is excluded from cytosol and mitochondria. Indirect immunofluorescence of promastigotes showed LaSir2 distributed into cytoplasmic granules and in secretory-like vesicles. Furthermore, by Western blot probed with the anti-6xHisLaSir2 serum, LaSir2 was detected in both promastigotes and amastigotes secreted material. A concanavalin A-affinity purification of either promastigotes or amastigotes extracts, followed by LaSir2 immunodetection, revealed a major protein of ~66kDa indicating that, according to *in silico* predictions, LaSir2 could be highly glycosylated *in vivo*.
Supported by FAPESP and CNPq.

BQ.15 – EFFECT OF PLATELET-ACTIVATING FACTOR (PAF) ON THE INFECTION OF PERITONEAL MOUSE MACROPHAGES BY *LEISHMANIA CHAGASI*

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In the New World, the visceral form of leishmaniasis is caused by *Leishmania chagasi*. Platelet-activating factor (PAF) is a potent phospholipid mediator of several cellular functions in diverse biological and pathophysiological processes, such as cell differentiation, inflammation and allergy. Previous study from our group showed that PAF modulates the interaction of peritoneal mouse macrophages with *Leishmania amazonensis*. PAF also stimulates cell differentiation of *Trypanosoma cruzi* and *Herpetomonas muscarum muscarum*, triggering a signal transduction pathway that activates a protein kinase CK2 in *H. m. muscarum*. PAF effects in trypanosomatids seem to occur through membrane receptor and intracellular signaling, via protein kinase C (PKC). In this work, we describe the effects of PAF on the interaction of *L. chagasi* with peritoneal mouse macrophages. Prior to the infection, *L. chagasi* promastigotes and/or the macrophages were treated for four hours with PAF and/or one of the following modulators: WEB 2086 (antagonist of PAF receptor), BIS I (PKC inhibitor), TBB (CK2 inhibitor), KT 57 and H89 (PKA inhibitors). The interaction was inhibited when the macrophages or both the promastigotes and the macrophages were treated with PAF. On the other hand, when only the promastigotes were treated with PAF, a two-fold increase in the association indices was observed. The antagonist of PAF receptor, WEB 2086, as well as the protein kinase inhibitors abrogated all PAF effects. We also showed that PAF stimulated nitric oxide production when the macrophages or both the parasites and the macrophages were treated with this phospholipid. Corroborating these results, we demonstrated that PAF can modulate some protein kinases of *L. chagasi*. When the promastigotes were treated with PAF, the activity of PKC, PKA and CK2 increased 57%, 60% and 50%, respectively. This set of results suggests that PAF triggers key intracellular signaling pathways in *L. chagasi* that may lead to an increase in parasite infectivity. Supported by: CNPq, FAPERJ, CAPES and INCT-EM.

BQ.16 – COMPARATIVE ANALYSIS OF METALLOPROTEASE SECRETED BY *L. (V.) braziliensis* IN TWO-DIMENSIONAL AND THREE-DIMENSIONAL COLLAGEN MATRICES

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Leishmania (V.) braziliensis is the main causative agent of American Tegumentary Leishmaniasis (ATL) in Brazil and it is responsible for heterogeneous clinical manifestations ranging from cutaneous to mucosal lesions. Previous work of our group showed that proteases secreted by *L. (V.) braziliensis* strains isolated from patients with distinct clinical manifestations of ATL display different pattern of metalloproteases. In addition, we showed that the proteolytic profile is a stable phenotypic character. Currently, studies are being developed to better explore the interaction of the cells with the extracellular environments using collagen matrices. Previous works showed the important role of the extracellular matrix in the signaling and activation of immune cells during *Leishmania* infection. So, in this work, we aimed to determine the profile of secreted proteases of *L. (V.) braziliensis* strains, isolated from patients with mucosal, cutaneous and disseminated clinical manifestations, during interaction with both two-dimensional (2D) and three-dimensional (3D) collagen matrices in co-culture with macrophages host cells. Zymographic assays were performed using SDS-polyacrilamide gels (10%) copolymerized with 0.2% porcine gelatin. Enzymes were characterized according to their pH range of activity, and sensitivity to distinct protease inhibitors. We observed that proteolytic activities were due to enzymes belonging to the metalloprotease class and present optimal activity between the pH range 5.5 and 10.0. Zymographic assays also revealed that proteolytic profile is similar in 2D and 3D matrices and remains unchanged in co-culture with macrophages. However, when we compare the interaction of *L. (V.) braziliensis* strains (mucosal, cutaneous and disseminated) with the collagen, we observed significant differences regarding the affinity, interaction and invasion of the collagen matrices, as revealed by scanning electron microscopy. Supported by CAPES

BQ.17 – METALLOPEPTIDASE ZYMOGRAPHIC PROFILES TO DISTINGUISH *LEISHMANIA (VIANNIA) BRAZILIENSIS* AND *LEISHMANIA* (*VIANNIA*) *PERUVIANA* ISOLATES FROM PERU

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American Tegumentary Leishmaniasis (ATL), a disease caused by protozoa of the *Leishmania* genus, comprises a broad range of clinical manifestations ranging from mild skin ulcers that may spontaneously heal to disfiguring mucosal lesions that imply parasite dissemination from the primary cutaneous lesion. *L.* (*V.*) *peruviana* and *L.* (*V.*) *braziliensis* from Peru, are species genetically so related that their characterization as distinct species have been intricate. Despite such genetic similarity, these species are associated to different clinical manifestations of ATL: *L.* (*V.*) *peruviana* only causes cutaneous leishmaniasis, whereas *L.* (*V.*) *braziliensis* can cause both cutaneous and mucocutaneous leishmaniasis. Since the primary cutaneous lesions caused by the infection with these species are indistinguishable, it is necessary to develop a suitable method to differentiate the infecting *Leishmania* species in order to prevent possible metastasis to oropharingeal mucosa. A different electrophoretic pattern in the mannose phosphate isomerase isoenzyme was the first approach that allowed distinguishing these two species. However, such isoenzymatic distinction depends on the electrophoretic system used for the characterization, is time-consuming and requires high-tech facilities that are not always available in the regions where the infection is endemic. In the present study, we investigate the proteolytic profile from *L.* (*V.*) *peruviana* and *L.* (*V.*) *braziliensis* by zymographic analysis. Enzymes were characterized according to their pH range of activity, and sensitivity to distinct protease inhibitors. We observed that *L. (V.) peruviana* isolates displayed three proteolytic bands with molecular mass ranging from 55 to 80 kDa whereas *L. (V.) braziliensis* isolates showed five proteolytic activity between 55 and 130 kDa. Using specific inhibitors we determined that proteolytic activities are due to metallopeptidases enzymes and present optimal activity between the pH range 5.5 and 10.0. Finally, we propose the metallopeptidase profile as a potential phenotypic marker to distinguish between these species.
Supported by FIOCRUZ CNPq

BQ.18 – TRANSLATION FACTOR eIF5A OF *TRYPANOSOMA CRUZI*

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In trypanosomatids the post translational modifications is particularly important because these organisms do not use transcription initiation as a regulatory step to control gene expression. In our laboratory, phosphoproteomic studies revealed that a protein known as eukaryotic translation initiation factor A (eIF5A) is highly phosphorylated in exponentially growing cells and that phosphorylation is decreased in stationary *T. cruzi* cells. The protein eIF5A is a small, acidic and highly conserved protein in eukaryotic cells. It's unique as it contains a hypusinated lysine residue. eiF5A is involved in multiple cellular functions including translation initiation, mRNA decay, cell cycle progression, cell survival, and in translation elongation. Therefore, we identified that the serine 2 is the phosphorylated modified residue by using mass spectrometry analysis. By overexpressing wild type and serine 2 eIF5A we found that this serine is possible related to the hypusination, which is probably required for protein in the translation elongation.
Supported by FAPESP and FINEP

BQ.19 – ANALYSES OF INHIBITORS CANDIDATES FOR THE *Leishmania (Leishmania) amazonensis* ARGINASE

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To survive inside the host macrophage organisms from *Leishmania* genus escape from several microbicidal mechanisms such as the production of NO and superoxide radicals (Bogdan et al., 1996). The NO synthesis by inducible nitric oxide synthase (iNOS) requires L-arginine as substrate, the same aminoacid used by arginase to produce urea and L-ornithine. The synthesis of L-ornithine is fundamental for the production of polyamines essencial for the proliferation of *Leishmania* (Camargo, 1979). Moreover, the arginase from the parasite can compete with iNOS for the same substrate, modulating L-arginine levels, decreasing the production of NO and reducing the host microbicidal response (Boucher, Moali et al. 1999). Previously in the laboratory the *Leishmania*'s arginase was characterized and a purification method was described for a recombinant enzyme produced by *E.coli*. The molecular model based on the rat arginase crystal also enable an *"in silico"* search for arginase inhibitors looking for a compound that could present a higher affinity for the parasite arginase than for the mammal enzyme. We selected three of the screened compounds - L-norvaline, L-citrulline and L-lysine - that showed the inhibition rates of 83,6%, 52,8% and 97,5%, respectively, in assays using 80 mM of each and the same concentration of the substrate L-arginine. After this we tested the inhibitors effect on the growth of cultured *L. (L.) amazonensis* promastigotes and observed that L-norvaline and L-lysine were capable of inhibit the parasite growth by 75% and 89%, respectively. Finally we realized "*in vitro*" infections of murine macrophages treated with those compounds and found out that with 40 mM of L-lysine, 20% of the cells were infected, while using 150 mM of L-norvaline only 10% of the macrophages were infected but when we used the same concentration tested for L-lysine no significant inhibition of the infection was detected. So the analyzes of the results allowed to conclude that L-lysine would be the best inhibitor among the selected ones in respect of potency and effect on the parasite growth and infection capacity. Supported by FAPESP and CNPq

BQ.20 –BIOCHEMICAL ANALYSYS OF THE PROLINE DEHYDROGENASE IN *TRYPANOSOMA CRUZI*

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In the flagellated parasite *Trypanosoma cruzi,* the amino acid L-proline is involved in energy metabolism, differentiation processes and resistance to oxidative, metabolic and osmotic stresses. Biochemical evidences supports the hypothesis that *T. cruzi* oxidizes L-proline through a proline dehydrogenase (*Tc*PRODH) (EC 1.5.1.2) and a Δ^1pyrroline-5-carboxylate dehydrogenase (*Tc*P5CDH) (EC 1.5.1.12) producing glutamate. In the present work, we describe the experimental conditions for the expression and purification of the active enzyme, which was used to determine its biochemical characteristics. The recombinant *Tc*PRODH was expressed in *Escherichia coli* BL21 (codon plus) strain using pAE vector. The apparent molecular weight for *Tc*PRODH fused with a 6-histidine tag (N-termus), was of 66 kDa as verified by SDS-PAGE. The recombinant protein was purified by affinity chromatography using NTA-Ni^{2+} resin and eluted with 250 mM of imidazole. A policlonal serum anti- *Tc*PRODH was produced by mice immunization and used for protein detection in western blot assays. Specificity parameters were obtained from the recombinant *Tc*PRODH showed a highest specific activity for L-proline than 4-hydroxyl-L-proline or D-proline. In addition, the activity of recombinant *Tc*PRODH resulted in a reduction of FAD^+ cofactor instead of NAD^+ and $NADP^+$. *In silico* protein analyses showed the presence of a mitochondrial address signal, the active site recognition signature and intriguingly an EF-hand domain, which was not previously described for any PRODH. Since EF-hands are putative Ca^{2+} or Mg^{2+} binding sites, we evaluated its functionality. Our results showed that *Tc*PRODH has a functional EF-hand domain which confers to the enzyme the ability of having its activity regulated by Ca^{2+} or Mg^{2+}, an unique feature for these enzymes.
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BQ.21 – AN ECTO-PYROPHOSPHATASE ACTIVITY FROM PROCYCLIC PROMASTIGOTE FORMS OF *LEISHMANIA AMAZONENSIS.*

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Leishmania amazonensis parasites are intracellular protozoa and the etiological agent of cutaneous and diffuse cutaneous leishmaniasis. The flagellated metacyclic promastigote forms are transmitted to vertebrate hosts by sandfly bites. The promastigotes develop into aflagellated amastigotes in the vertebrate host and multiply by binary division, inside macrophages. Membrane interactions between parasites and hosts are crucial for its survival, from both physiological and immunological viewpoints. Cytoplasmic membranes contain enzymes whose active sites face the external medium rather than the cytoplasm. The activities of these enzymes, referred to as ecto-enzymes, can be measured using intact living cells. During its life cycle, the protozoa pass through different compartments of insect, presenting different pH values. So, it is of great importance the study of proton pumps, like the H^+-PPases, the integral membrane protein that utilizes the energy released by hydrolysis of pyrophosphate (PPi) to transport protons across the membrane, against the electrochemical potential gradient. In this context, we have characterized a pyrophosphatase activity on the external cell surface of procyclic promastigote forms of *L. amazonensis*. This activity is stimulated by $MgCl_2$ in a dose-dependent manner, with $S_{0.5}$ value of 2,0 mM $MgCl_2$, while the *p*-nitrophenylphosphatase and β-glycerophosphatase activities described in these parasites are not stimulated by $MgCl_2$. Furthermore, sodium orthovanadate, an acid phosphatase inhibitor, and levamisole, an alkaline phosphatase inhibitor, did not inhibit the ecto-pyrophosphatase activity. However, sodium fluoride, an inhibitor of pyrophosphatases, inhibited the ecto-pyrophosphatase activity in a dose-dependent manner with IC_{50} value of 2,5 μM reaching maximal inhibition of 15% in 30 μM NaF. The pyrophosphatase activity of *L. amazonensis* was stimulated in pH 7.5-8.5, presenting optimum pH at alkaline range. These data suggest that this enzyme probably is a pyrophosphatase of family I, specially because its activity is stimulated by $MgCl_2$, but not by $MnCl_2$. Supported by CNPq, FAPERJ and UFRJ/PIBIC.

BQ.22 – ECTO-PHOSPHATASES ACTIVITIES IN *BLASTOCRITHIDIA CULICIS* AND *AEDES AEGYPTI* SALIVARY GLAND

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Blastocrithidia culicis is a monoxenous trypanosomatid that inhabits mosquitoes. Using the mosquito *Aedes aegypti* as an experimental model to exploit monoxenous life cycle we demonstrated that *B. culicis* colonizes mosquito midgut and reaches its hemocoel. Indeed, we show that *B. culicis* interacts and invade mosquito salivary glands *in vitro* and *in vivo*. Ecto-phosphatases are enzymes capable to hydrolyze phosphorylated substrates in the external side of cells. Phosphorylation/dephosphorilation events have several biological roles and ecto-phosphatase activity has been implicated in microorganism differentiation, proliferation and parasite-host interaction. In order to investigate if mosquito or protozoa ecto-phosphatases are involved in protozoa-insect interaction, we first characterize these enzymes present in both, protozoa and *A. aegypti* organs. In order to characterize the hydrolytic activity of ecto-phosphatases, *B. culicis* or mosquito salivary glands were incubated with *p*-nitrophenylphosphate (*p*-NPP) for 1h in different pHs or in the presence of phosphatase inhibitors. The variation on pH range shows that *B. culicis* ecto-phosphatases present an acid activity while mosquito salivary gland has a neutral activity. Vanadate (1mM), molybdate (1mM) and sodium fluoride (1mM) inhibited both activities, as well as, inorganic phosphate (1mM). The samples were either incubated in the presence of divalent cations to evaluate its involvement on ecto-phosphatases activity. Our results show that $MgCl_2$ (5mM) enhanced significantly the *B. culicis* ecto-phosphatase activity, but inhibited the salivary gland activity. Interestingly, the aposymbiotic strain of *B. culicis*, that is unable to colonize *A. aegypti* midgut,,has a ecto-phosphatases activity 64% lower than the symbiont-containing cells and $MgCl_2$ have no had effect on its activity. Collectively, these results will be useful to elucidate the participation of ecto-phosphatases in *B. culicis* mosquito colonization. Supported by CAPES, CNPq and FAPERJ.

BQ.23 – IDENTIFICATION AND CHARACTERIZATION OF AN ECTO-PYROPHOSPHATASE ON EXTERNAL SURFACE OF PLASMA MEMBRANE OF *TRYPANOSOMA RANGELI* AND ITS POSSIBLE ROLE IN INTERACTION WITHIN THE VECTOR *RHODNIUS PROLIXUS*

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We have identified by PCR a putative H^+-PPase in epimastigotes of *T. rangeli* that is recognized by antibodies raised against the enzyme of *A. thaliana*. Its location in the plasma membrane was confirmed by immunofluorescence microscopy technique. The optimum pH for the ecto-H^+-PPase activity is 7.5. The activity is inhibited by about 75%, 80% and 90% by NaF, Pi and AMDP, respectively. There are two sites of hydrolysis of PPi, one of high affinity with Km of 0.32 mM PPi, and another of low affinity, with Km of 1.69 mM PPi. The ecto-H^+-PPase of *T. rangeli* is stimulated by $MgCl_2$, and Mg^{2+}-dependent ecto-H^+-PPase activity is inhibited by $CaCl_2$. The ecto-pyrophosphatase activity is modulated by the extracellular concentration of Pi, reaching about twice as much activity when the cells are maintained in culture medium depleted of Pi. Cells grown with PPi have their ecto-H^+-PPase activity increased by about three times compared to that of cells grown in culture medium control, cells grown in the presence of NaF for 2 days, about twice, and cells grown for 6 days in the presence of the inhibitor showed the same activity of cells grown in control medium. Oddly enough by feeding insects *Rhodnius prolixus* 5th instar with such parasites, cell density of parasites found in crops of these insects three days after blood meal obeys exactly the same descending order of activity: grown with PPi > grown with NaF for 2 days > grown with NaF for 6 days = control. We have also found ecto-pyrophosphatase activities in other protozoa of Trypanosomatidae family, such as *T. cruzi*, *Leishmania chagasi* and *L. amazonensis*, and in *Euglena gracilis*. These enzymes presented different biochemical characteristics suggesting that this protein could be a differential marker for these protozoa.
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BQ.24 – ECTO-ATP DIPHOSPHOHYDROLASE FROM *Leishmania (Leishmania) amazonensis*: A POSSIBLE NEW BIOCHEMICAL TARGET FOR THE DEVELOPMENT OF THERAPIES

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Chemotherapy remains the mainstay for the control of leishmaniasis. We characterize a novel ATP diphosphohydrolase (apyrase) in *L. (L.) amazonensis* promastigote forms. By Western blots, rabbit polyclonal antibodies against potato apyrase reacted with bands de 50-63 kDa from enriched plasma membrane, flagellum and microssomal fractions. By ultrastructural cytochemical, ATP diphosphohydrolase activity was found distributed as an electron-dense lead phosphate deposit at the plasma membrane surface, flagellar pocket, and flagellar membrane of promastigote forms when they were incubated in the cytochemical medium containing ATPases, nucleotidase and phosphatase inhibitors plus ADP or ATP as substrate. The *in vitro* effect of cisplatine, 6-mercaptopurine, thionicotinamide, N-27, T-27 or allopurinol, which has antileishmanicidal, antitumoral, antibacterial and/or antifungal activity, was evaluated. The measurement in duplicate (n= 5) was performed in reaction medium containing 50 mM MOPS buffer, pH 7.4, 1 mM CaCl2, 1 mM MgCl2, 100 µM ortovanadate, 1 mM NaN3, and plasma membrane fraction (0.03 mg protein/ml), plus 100 µM of the compound. After preincubation for 30 min at room temperature, the hydrolytic assay was initiated by addition of 3 mM ATP or ADP, and allowed to proceed for 30 min at 37°C. Inorganic phosphate liberated was determined spectrophotometrically according to Taussky&Shorr. Control samples, preincubated in the presence of 1% (v/v) dimethyl sulfoxide, hydrolyzed ATP or ADP in the range (mean±SD) of 112±55 and 102±10 nmol Pi.mg-1.min-1, respectively. Cisplatine significantly inhibited 90% ATPase and 80% ADPase activities; mercaptopurine inhibited approximately 35% of these activities, whereas thionicotinamide has lower effects. N-27 and T-27, two nicotinamide derivatives, inhibited significantly ADPase activity, 50% and 30% respectively, whereas allopurinol inhibited 70% of the ATPase activity. These results appointed the *L. (L.) amazonensis* ATP diphosphohydrolase as a new target for therapies development. In addition, these inhibitors may contribute for further functional assays that may elucidate the role(s) of this enzyme. Supported by FAPEMIG, CNPq, CAPES, IOC/FIOCRUZ and UFJF

BQ.25 – Δ^1-PYRROLINE-5-CARBOXILIC ACID (P5C) METABOLISM IN *TRYPANOSOMA CRUZI*

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P5C is a metabolic node for proline, ornithine and glutamate pathways, linking the Kreb´s and urea cycles in eukaryotes. Regulation of P5C metabolism and the relative sizes of P5C pools in the cytosol and mitochondria are important in controlling flux between these pathways, and may prevent futile cycles among these metabolites. In *T. cruzi,* P5C is generated from proline and might be generated also from ornithine. The proline-glutamate pathway in *T. cruzi* (being characterized by our group), is mediated by two enzymatic steps: the proline oxidation by the proline dehydrogenase (*Tc*ProDH), rendering P5C, and the subsequent oxidation of P5C into glutamate by P5C dehydrogenase (*Tc*P5CDH). Previously we reported the functionality of both genes by yeasts complementation assays. In this work, the expression during the life cycle of *T. cruzi* is analyzed. The mRNA and protein levels were similar in most of stages, with the only exception of intracellular epimastigote stage, in which both duplicated those in other stages. The subcellular location was analyzed by immunolocalization and digitonin permeabilization assays in epimastigote forms, being predominantly mitochondrial. However, a relevant P5CDH activity was also detected in the cell cytoplasm. For biochemical analysis, the recombinant *Tc*P5CDh was expressed in the active form fused to a his-tag in the C-termus in *Escherichia coli* (64 kDa), and P5C was synthesized, isolated, and characterized. The enzymatic activities for P5CR, OAT and P5CDH detected in crude extracts from *T. cruzi* showed the ability of *T. cruzi* to synthesize proline and glutamate from α-ketoglutarate (Krebs cycle) or ornithine. The fact that *Tc*ProDH localizes only in the mitochondria and *Tc*P5CDH in the mitochondria and cytosol also suggest that P5C could be a H^+ acceptor in an electron shuttle system operating between the mitochondria and the cytoplasm, with a physiological role in the regulation of the cellular redox status. Supported by FAPESP.

BQ.26 – *PHYTOMONAS SERPENS*: PYRUVATE/INDOLEPYRUVATE DECARBOXYLASE GENE AND PRODUCTION AND FUNCTIONALITY OF INDOLE-3-ACETIC ACID PHYTOHORMONE

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A gene encoding a putative pyruvate/indolepyruvate decarboxylase (PDC/IPDC) is present in the plant trypanosomatid *Phytomonas serpens*. This gene shares high similarity with a *Leishmania* spp. gene. Pyruvate decarboxylases (PDCs) convert pyruvate to acetaldehyde, a key step in the alcoholic fermentation, whereas indolepyruvate decarboxylases (IPDCs) participate in the conversion of indolepyruvate into the phytohormone indole-3-acetic acid (IAA). Both enzymes display high sequence and structural similarities, which preclude unequivocal functional assignment based solely on sequence data. The goal of this study was to investigate the functionality of *P. serpens PDC/IPDC* gene. HPLC and GC-FID analyses of *P. serpens* conditioned medium indicates the production of IAA (4 µg/10^8 cells) and ethanol (40 mg/10^8 cells). PDC activity was evaluated in semi-purified parasite lysates. The enzyme shows a typical Michaelis-Menten behavior with a K_M of 1.1 mM for the substrate pyruvate. Addition of 2 mM indolepyruvate, the substrate of IPDC, promoted a ~10-fold increase of the K_M to pyruvate, without alteration of V_{max}. Such behavior indicates that indolepyruvate is a competitive inhibitor of PDC activity, suggesting that PDC/IPDC is a bifunctional enzyme determining the production of IAA or ethanol, according to the substrate availability. Work is in progress to clone the *PDC/IPDC* gene to assay both enzymatic activities in the recombinant protein. The phytohormone produced by *P. serpens* is biologically active since it promoted typical curvature responses and the elongation of tomato hypocotyls (~15% size increase compared with control), with an effect analogous to that obtained with the synthetic auxin. In addition, we observed an increase in the amount of auxin conjugated with amino acids and sugars in tomato fruits infected with *P. serpens.* Taken together the data indicate that the *PDC/IPDC* gene is active *in vivo* and may play an important role in the association plant-trypanosomatid. Support: FAPESP; CNPq.

BQ.27 – IDENTIFICATION OF A CATALYTICALLY ACTIVE ATP DIPHOSPHOHYDROLASE ISOFORM FROM *Leishmania (Leishmania) chagasi* PROMASTIGOTES

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A putative protein identified as NDPase, homologous to the members of the ATP diphosphohydrolase (or apyrase) family, was found in the genome of *L. infantum* (sin. *L. chagasi*). In order to search for an active ATP diphosphohydrolase, phosphohydrolytic activity was assayed in promastigote preparations from *L.* (*L.*) *chagasi* (MHOM/BR/1972/BH46 strain) using standard reaction medium. These preparations presented a phosphohydrolytic activity that was equally effective at hydrolysis of either ATP or ADP, in the range of 65 $\pm$ 38 or 59 $\pm$ 37 nmol Pi.mg-1.min-1, respectively. CTP, UDP and GDP were also hydrolyzed. Ca^{2+}, Mg^{2+} or Mn^{2+} was activating metal ion for both ATPase and ADPase activities. No significant hydrolysis of AMP, inorganic pyrophosphate or *p*-NPP could be observed discarding 5'-nucleotidase, pyrophosphatase or phosphatase activity, respectively. Several inhibitors of classical ATPases were tested. Sodium orthovanadate (100 μM; P-type ATPase inhibitor) showed a slight effect, and inhibited 27% of the ATPase activity. No significant interference was observed when DCCD (100 μM; mitochondrial ATPase inhibitor), bafilomicyn A (1 μM; vacuolar ATPase inhibitor) or Ap5A (100 μM; adenylate kinase inhibitor) was tested. On the other hand, sodium azide (1 mM), an known inhibitor of either *L. amazonensis* or *L. braziliensis* ATP diphosphohydrolase isoforms, inhibited partially (24%) and almost totally (85%) the ATPase and ADPase activities, respectively. Additionally, rabbit polyclonal anti-potato apyrase antibodies recognized a polypeptide of approximately 50 kDa in Western blots and, when immobilized on Protein A-Sepharose it was capable to immunoprecipitate 97% and 75% of the ATPase and ADPase activities, respectively, from a C12E9-solubilized promastigotes preparation. Ultrastructural cytochemical microscopy showed ATP diphosphohydrolase activity at plasma membrane, flagellar pocket and flagellum of the promastigote. All together, these results confirmed the presence of an active ATP diphosphohydrolase isoform in *L. (L.) chagasi* promastigotes that shares conserved epitopes with potato apyrase. Supported by FAPEMIG, CNPq, CAPES, IOC/FIOCRUZ and UFJF

BQ.28 – CYTOCHEMICAL LOCALIZATION OF ATP DIPHOSPHOHYDROLASE FROM *Leishmania (Viannia) braziliensis* PROMASTIGOTES

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We identified an ATP diphosphohydrolase activity (EC 3.6.1.5) in the preparation of *L. (V.) braziliensis* promastigotes. Localization of the ATP diphosphohydrolase activity in *L. (V.) braziliensis* promastigote forms was obtained by ultrastructural cytochemical techniques. The promastigote forms were fixed in 1% paraformaldehyde and 1% glutaraldehyde in 0.1 M Na-cacodylate buffer, pH 7.4, for 30 min. Subsequently, the cells were washed with the cacodylate buffer and incubated in the cytochemical medium, which contained 50 mM MOPS, pH 7.4, 1 mM $CaCl_2$, 100 µM orthovanadate (P-type ATPase inhibitor), 100 µM DCCD or 1 mM sodium azide (mitochondrial ATPase inhibitors), 100 µM ammonium molybdate (nucleotidase inhibitor), 1 mM levamisole (phosphatase inhibitor), 2 mM $CeCl_3$ and 3 mM of either ATP, ADP or GDP for 1 h at 37^o C. Post-fixation was done in 1% OsO_4 in Na-cacodylate buffer for 1 h at 4^o C. Dehydration was done in acetone and inclusion in Epon. Stained and unstained thin sections were observed in a JEM-1011 electron microscope. Enzyme activity was found distributed as an electron dense cerium phosphate deposit at the surface of the plasma membrane, and at flagellar pocket and flagellum of *L. (V.) braziliensis* promastigote forms when they were incubated in the cytochemical complete medium containing ATP, ADP or GDP as substrate. Besides its ecto-localization, the hydrolytic activity appeared to be identically distributed at outer surface of the mitochondria. In the absence of nucleotides, controls showed no electron dense deposits. This enzyme activity is possibly associated with purine recuperation and/or as a protective mechanism against the host organism under conditions that involve nucleotides. Furthermore, this subcellular location suggests the participation of this enzyme in metabolic pathways still not investigated, possibly essential for parasite survival. Effects of leishmanicidal compounds on the ATP diphosphohydrolase activity is the present subject of study of our laboratory. Supported by FAPEMIG, CNPq, CAPES, IOC/FIOCRUZ and UFJF.

BQ.29 – ISOLATION OF AN ACTIVE ATP DIPHOSPHOHYDROLASE ISOFORM FROM *Leishmania (Viannia) braziliensis* PROMASTIGOTE FORMS

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Isolation of an active ATP diphosphohydrolase (EC 3.6.1.5) isoform from *L. (V.) braziliensis* (Lb) was obtained by cross-immunoreactivity with polyclonal anti-potato apyrase antibodies. Rabbit polyclonal anti-potato apyrase antibodies recognized bands of approximately 48 and 43 kDa in Western blots of the promastigotes preparation (50 μg of total protein). Anti-potato apyrase antibodies (serum diluted 1:500) were tested for their ability to immunoprecipitate ATP diphosphohydrolase from a detergent-homogenized *L. (V.) braziliensis* promastigotes preparation. After homogenization with non ionic detergent $C_{12}E_9$ and centrifugation, significant parasite enzyme activity was maintained in the high-speed supernatant (ADPase activity, 249 nmol Pi.mg-1.h-1; ATPase activity, 206 nmol Pi.mg-1.h-1). No significant difference was observed in control assays with either pre-immune serum (ADPase activity, 242 nmol Pi.mg-1.h-1; ATPase activity, 230 nmol Pi.mg-1.h-1) or Protein A-Sepharose (ADPase activity, 312 nmol Pi.mg-1.h-1; ATPase activity, 252 nmol Pi.mg-1.h-1) added alone in the absence of serum. On the other hand, rabbit polyclonal anti-potato apyrase antibodies immobilized on Protein A-Sepharose immunoprecipitated approximately 83% of the ATPase (30 nmol Pi.mg-1.h-1) and 87% of the ADPase activities (42 nmol Pi.mg-1.h-1) corresponding to depletion of an ATPase/ADPase activity ratio of approximately 1. The immunoprecipitated resin-rabbit antibody-antigen complex was washed and subjected to electrophoresis and Western blots. The rabbit polyclonal antibodies against potato apyrase immobilized on Protein A-Sepharose immunoprecipitated the same bands of approximately 48 kDa and, in lower amount, the band of 43 kDa, which were recognized by mouse polyclonal anti-potato apyrase antibodies (serum diluted 1:500). This band of approximately 43 kDa could be result of proteolysis of the band of 48 kDa, as a consequence of experimental conditions or even a natural occurrence *in vivo*. These results confirmed the identity of an active ATP diphosphohydrolase isoform in *L.* (*V.*) *braziliensis* promastigote forms, which shares conserved epitopes with potato apyrase. Supported by FAPEMIG, CNPq, CAPES, UFJF

BQ.30 – 5261 NELFINAVIR, AN HIV ASPARTYL PEPTIDASE INHIBITOR, IS EFFECTIVE IN INHIBITING THE MULTIPLICATION AND ASPARTYL PEPTIDASE ACTIVITY OF SEVERAL *LEISHMANIA* SPECIES

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There is a general lack of effective and non-toxic chemotherapeutic agents for leishmaniasis and there is yet no study about the effect of HIV peptidase inhibitors on *Leishmania*-HIV co-infected patients. In the present work, we performed a comparative analysis of the spectrum of action of HIV peptidase inhibitors on different *Leishmania* species, including strains obtained from HIV^+ patients under antiretroviral treatment or not. The effect of HIV peptidase inhibitors, nelfinavir and saquinavir, on *Leishmania* promastigotes proliferation was assessed by means of a colorimetric assay (MTT). Subsequently, the effect of nelfinavir on aspartyl peptidase activity of *Leishmania* species was assessed by a cathepsin D fluorogenic substrate MCA-Gly-Lys-Pro-ILe-Leu-Phe-Phe-Arg-Leu-Lys(DNP)-Arg. Nelfinavir was capable of significantly reducing the multiplication of *Leishmania amazonensis*, *Leishmania braziliensis*, *Leishmania donovani*, *Leishmania major*, *Leishmania chagasi*, *Leishmania chagasi* from HIV^+ patient under antiretroviral treatment or not, with a strong growth inhibition of at least 50% to *Leishmania major* and 94% to other *Leishmania* species including strains isolated from HIV^+ patients. In addition, Nelfinavir was also capable of inhibiting aspartyl peptidase activity of the *Leishmania* strains tested at either 1μM or 10 μM. Present data may contribute to the study of the effect of HIV peptidase inhibitors on *Leishmania* infection and add new in vitro insights into the possibility of exploiting aspartyl peptidases as promising targets to treat leishmaniasis. Supported by Fiocruz, FAPERJ and CNPq.

BQ.31 – MOLECULAR CHARACTERIZATION OF THE MEVALONATE KINASE FROM *TRYPANOSOMA CRUZI*

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In eukaryotic cells, sterols are important determinants of membrane fluidity and permeability and serve as precursors for bioactive molecules, which function as regulators of cell cycle and development. The importance of sterols for trypanosomatids has been proved and there are some inhibitors that act at different points of the pathway. Mevalonate Kinase (MK) plays a central role in the cholesterol biosynthesis pathway, catalyzing the phosphorylation of mevalonic acid to form mevalonate 5-phosphate. Recently,a MK study showed that when trypanosomatids invasion occurs in the host, this enzyme is overexpressed. This suggests the importance of this enzyme as a target for the development of future drugs. Although structural characterization of some MK from trypanosomatids is known, but there have been no studies that characterize the MK from *Trypanosoma cruzi* (TcMK). Herein, we report biochemical and structural preliminary studies of the TcMK. TcMK was cloned into pet28a(+) vector, overexpressed in *E. coli* at 22 °C in auto-induction medium (ZYM 5052) and purified by affinity chromatography. Size exclusion chromatography (SEC) was performed in order to determine the oligomeric state. The SEC result revealed that the protein has two different oligomeric states. These oligomers of TcMK protein were used for activity assays suggesting that the enzyme is active mainly in the dimeric form. Crystallization experiments are been realized aiming to obtain suitable crystals for diffraction measurements.
Supported by CNPq.

BQ.32 – EXPRESSION ANALYSES OF *T. CRUZI'*S ANTIOXIDANT ENZYMES ALONG THE GROWTH CURVE

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One of the difficults to find therapeutics targets to Chagas's disease is the huge biochemistry and molecular variation in *T. cruzi* different strains. We evaluated the expression of enzymes that act in parasite's antioxidant system, i.e., cytosolic and mitochondrial tryparedoxin peroxidases and superoxide dismutase A and B (TcCPx, TcMPx, SODA and SODB, respectively) on two different strains of *T. cruzi* (Tulahuen 2 and Y) along the growth curve; logarithmic, early and late stationary phase (3, 5 and 7 days, respectively). TcCPx, in Tulahuen 2, had an increase of expression in approximately 50% after 5 days and 105% after 7 days, in relation to the log phase. For Y, the TcCPx showed a similar expression pattern, except by a decrease of 16% after 5 days. TcMPx had its expression increased after fifth day (~633% in Tulahuen 2 and ~202% in Y) and decreased to lower levels than the previous one in the late stationary phase for both strains. In SODA, its expression increased in 12 and 15% in early and the late stationary phase (comparing with the logarithmic phase) for Tulahuen 2, whereas for Y reduced in 16 and 49%, in this same phases. The results of SODB expression for Y were approximately 97% higher than Tulahuen 2, in the logarithmic phase. However, its expression decreased 6 and 16% after 5 and 7 days for Y, whereas for Tulahuen 2 this expression enhanced in ~85% in the stationary phase and reduced 29% in the late one comparing to 5 days. This results suggests that TcCPx increases its expression toward the late stationary phase and TcMPx presents high expression in the stationary phase, whereas a low one in the late phases.
Supported by FAPESP, CAPES and SAE-UNICAMP.

BQ.33 – *TRYPANOSOMA CRUZI*'S ANTIOXIDANT ENZYMES PROFILE UNDER OXIDATIVE STRESS

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The enzymes involved in *T. cruzi's* antioxidant system of play a fundamental role in parasite survival against oxidative stress. We evaluated cytosolic and mitochondrial tryparedoxin peroxidases and superoxide dismutase A and B (TcCPx, TcMPx, SODA and SODB, respectively) expression on control (pTEX), cytosolic and mitochondrial tryparedoxin peroxidases (pTEX-TcCPx and pTEX-TcMPx, respectively) overexpressing cells treated or not with 200μM H_2O_2 (lethal dose). TcMPx expression in non treated cells increased in pTEX-TcMPx (138%) and interestingly in pTEX-TcCPx (46%) in relation to pTEX. TcCPx had an increase of 42 and 24% on pTEX-TcCPx and pTEX-TcMPx, respectively, in relation to pTEX under no treatment. Similar levels were observed when those cells were treated with H_2O_2, leading to an increase of 43 and 16% in pTEX-TcCPx and pTEX-TcMPx, respectively in relation to pTEX H_2O_2-treated. The treatment with H_2O_2 led to a higher protein expression in all cells in relation to their respective control. SODA expression decreased in overexpressing cells under both conditions compared to their respective controls. On the other hand, SODB levels in non-treated overexpressing cells increased whereas upon H_2O_2 treatment a decrease was observed. These results suggest a collaboration between cytosolic and mitochondrial tryparedoxin peroxidase, since the overexpression of one increased the expression of the other. Experiments are being carried out to clarify SOD expression under oxidative stress. Supported by FAPESP, CAPES and SAE-UNICAMP.

BQ.34 – BIOCHEMICAL CHARACTERIZATION OF THE BIOSYNTHESIS OF VITAMIN E IN INTRA-ERYTHROCYTIC STAGES OF *P. falciparum*.

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The study of isoprenoid biosynthesis in *Plasmodium falciparum* by 2C-methyl-D-erythritol-4-phosphate pathway (MEP) is presented as a therapeutic target once that it is absent in humans. Our group found in intra-erythrocytic stages of *P. falciparum* the biosynthesis of isoprenoids by the MEP pathway.This pathway was described exclusively in plants, fungi or bacteria. The shikimate and MEP pathways are the precursors of biosynthesis of vitamin E and both pathways have already been described in *P. falciparum*. It is suggested that the biosynthesis of vitamin E might occur in the parasite, representing a possible target for developing new antimalarial drugs. Using metabolic labeling with [^{3}H]farnesil-PP or [^{3}H]geranilgeranil-PP, three different methods of RP-HPLC and mass spectrometry analyses confirmed the biosynthesis of vitamin E in the three stages of parasites (ring, trophozoites and schizonts). The treatment with usnic acid, an inhibitor of hydroxyphenylpyruvate dioxigenase - enzyme responsible by the biosynthesis of vitamin E - showed an inhibition of this biosynthesis (53,5 ± 7%) and of the growth of parasite (IC_{50} 24,6 ± 4μM). We are trying to demonstrate by means of a fluorescent probe, the acid Parinaric, that vitamin E acts as a lipophilic antioxidant protecting the membrane of lipoperoxidation. These findings not only contribute to the current understanding of **P. falciparum** biology but shed light on a pathway that could serve as a chemotherapeutic target. Supported by FAPESP and CNPq.

BQ.35 – ASCORBATE PEROXIDASE PROTEIN EXPRESSION IN *Trypanosoma cruzi* IS MODULATED BY STRESS GENERATED BY HYDROGEN PEROXIDE

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Ascorbate peroxidases (APXs) are Class I heme-containing peroxidase enzymes. They catalyze the reduction of hydrogen peroxide to water. They are not present in mammals what make them potential target for chemotherapy of Chagas disease. Literature data have shown the regulation of ascorbate peroxidase gene by H_2O_2. Here we have investigated the levels of TcAPX protein in *T. cruzi* populations susceptible and resistant to benznidazole (BZ) after H_2O_2 exposure. *T. cruzi* populations with *in vitro*-induced (17LER) and *in vivo*-selected (BZR) resistance to BZ and their susceptible counterparts 17WTS and BZS were used in this study. Epimastigote forms of these populations were incubated in the absence or presence of different concentrations of H_2O_2 at room temperature for 1 h. Following exposure, cells were harvested, washed and the pellet submitted to protein extraction for Western blot analysis using rabbit anti-TcAPX polyclonal antibody. The results showed that the levels of TcAPX protein increased in a dose-dependent manner only when the resistant strains were treated with H_2O_2. Such modulation does not happen with the susceptible strains. We also observed that H_2O_2 concentration higher than 400 µM and longer incubation period are lethal for *T. cruzi* parasites. The results suggest that TcAPX protein expression was up-regulated in response to cellular H_2O_2 levels and that parasites use APX to overcome the oxidative stress. Interestingly, *Leishmania major* and soybean cells also overexpress APX in the presence of H_2O_2. Supported by CNPq, FAPEMIG, CPqRR/ FIOCRUZ

BQ.36 – INVASION OF HOST CELLS BY *TRYPANOSOMA CRUZI*: A NEW PUTATIVE RECEPTOR

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Trypomastigotes of *Trypanosoma cruzi*, express at the cell surface glycoproteins known as Tc85 belonging to the gp85/trans-sialidase superfamily. Several members of the superfamily have been implicated in the invasion of host cells by *T. cruzi* and components of the extracellular matrix, as fibronectin and laminin, were described as their ligands. Using the phage display technique, a sequence (pp7) was identified that specifically binds in a dose-dependent manner to H3.3p, a recombinant protein corresponding to an internal fragment of a cloned member of Tc-85. Alignment analysis identified the prokineticin receptor 2 (PKR2), as a putative candidate. Prokineticin receptors (PKR1, PKR2) are expressed in many tissues, structurally are members of the rhodopsin family, with seven transmembrane domains and putative post-translational modifications. The ligands, prokineticins 1 and 2, are peptides involved in a variety of biological processes. In order to verify whether PKRs may be a Tc85 receptor, MCF10A, a human mammary epithelial cell line, which expresses PKR2, as assessed by immunofluorescence and RT-PCR, was employed as host cell. We showed that: 1. H3.3p binds to the surface of MCF10A, detected by immunofluorescence, employing anti-His antibody or G1/G8 monoclonal antibody (raised against H3.3p); 2. The synthetic peptide pp7 inhibits the binding of H3.3p to a ~45 kDa band in a nitrocellulose blot of MCF10A extract and the same region is recognized by anti-PKR2 antibody; 3. The antigens recognized by anti-PKR2 antibody and by anti-pp7 antibody colocalize at the cell surface of MCF10A, as evaluated by confocal microscopy; 4. Anti-PKR2 antibody inhibits by ~60% the infection of MCF10A by *T. cruzi*; 5. The peptide pp7 (0.2 mM) inhibits by ~40% the infection of MCF10A by *T. cruzi*. Altogether, the data indicate a possible role of PKR2 as a ligand for *T. cruzi* infection, in particular the amino acid sequence pp7. Suported by FAPESP and CNPq.

BQ.37 – THE INTERACTION OF *TRYPANOSOMA CRUZI* MEVALONATE KINASE PROTEIN WITH HOST CELL MEMBRANE MAY MODULATE THE INVASION OF EXTRACELLULAR AMASTIGOTE (EA) FORMS

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Trypanosoma cruzi extracellular amastigotes (EA) forms, which are generated by the extracellular differentiation of trypomastigotes are able to invade cultured cells. EAs of the G strain are more infective than CL strain parasites. EA microarray analysis has demonstrated that mevalonate kinase (TcMVK) has higher expression in G than in CL parasites, suggesting an important role of the MVK pathway in EA infectivity. Interestingly, bioinformatics tools have shown that *Trypanosoma cruzi* is the only trypanosomatid studied that has two MVK isotypes, one of which contains a signal peptide, indicating that MVK is secreted into the extracellular medium. This was confirmed by Western blot assay. Furthermore imunofluorescence assays showed that MVK colocalizes with aldolase, a glycosomal protein which is also present in other trypanosomatids. We cloned and obtained a purified form of TcMVK protein (MVKR). A kinase activity assay demonstrated that MVKR is functional in both monomeric and dimeric forms. The secreted isoform of MVKR dose-dependently binds to HeLa cells membrane, suggesting an interaction between MVKR and cholesterol at the host cell. MVKR added on the invasion assay led to an increased rate of EA invasion**.** Preliminary studies showed that MVKR increases the phagocytic ability of host cells. Taken together, these data suggest that TcMVK may be an important modulator in EA invasion and could become an important chemotherapeutic target.
Support: FAPESP

BQ.38 – MECHANISM OF TUNICAMYCIN RESISTANCE AND EVALUATION OF N-GLYCOSYLATION INHIBITION OVER VIRULENCE IN *LEISHMANIA* PARASITES

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Leishmaniasis is spectral disease caused by 20 species of *Leishmania* and the clinical manifestation profile is specie-dependent. The molecular mechanism of virulence remains largely unknown, but it is known that glycoproteins on the surface of the parasite are often implicated in evasion of host immunity and invasion of host cells to establish parasitism. Glycosylation of proteins usually lead to changes in their biological function and the regulation of this event may be essential to determine the parasite's virulence pattern. Inhibition of protein glycosylation of several parasitic protozoans by drugs, such as tunicamycin (TM), as well as parasites made resistant to them are useful for host-parasite interaction studies.Herein we report our success to generate and evaluate TM resistant *L. braziliensis* variants. Tunicamycin-resistant variants were produced by gradual acclimatization of cells to increasing concentrations of the drug, up to 40 μg/mL (IC_{50}= 0.3 μg/mL). Four clones were recovered from TM-resistant population by limiting dilution but only one of them showed phenotype stability after one cycle of promastigote-amastigote-promastigote differentiation (the amastigote stage occurring in BALB/c mice under drug-free conditions). Transmission and scanning electronic microscopy revealed morphological modifications in the mutant and *in vitro* immune response survey indicates remarkable changes in cytokine and nitric oxide production in comparison with the parental wild type line. *In vivo* assays have been conducted to evaluate whether virulence and cytokine production pattern is altered in murine model infected with this mutant. To further delineate the mechanism of tunicamycin resistance and elucidate how this affects immune response and host-parasite interaction we have used a proteomic approach to identify proteins that may be involved in this phenomenon. These results highlight the relevance of glycosylation and shed light on host-parasite interaction issues revealing possible targets to pharmacological intervention. Supported by: FAPESP and CNPq.

BQ.39 – SITE-DIRECTED MUTATIONS OF MACROPHAGE MIGRATION INHIBITORY FACTOR (MIF) OF LEISHMANIA MAJOR

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The macrophage migration inhibitory factor (MIF) is considered an important factor in the control of infection by parasites. Studying the structure and function of proteins of *Leishmania* allows the understanding of the molecular host-parasite interaction and the presentation of new targets to use in the development of therapies against the parasite, which is responsible for several different forms of Leishmaniasis. The aim of this study is to investigate the effect of site-directed mutations of the coding sequence of MIF *L. major* residues, involved in the tautomerase enzymatic activity and the maintenance of its quaternary structure. The site-directed mutagenesis was performed in 3 steps of PCR reactions, the fragments obtained were purified, sequenced and cloned to the pET21b vector using NdeI/HindIII restriction sites. The recombinant MIF and the mutants, all of them containing a His6-tag, were expressed as soluble form in E. coli and subsequently highly purified from the cell lysate by affinity chromatography using a Ni-NTA resin. With the purified proteins had been performed experiments gel filtration to check the status of oligomerization of MIF and its mutants in solution. The preliminary results and next experiments may contribute to the understanding of the state of oligomerization and the structural mechanism involved in *L. major* MIF protein interactions during the modulation of the host immune response.
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BQ.40 – THE ROLE OF SELENIUM IN INTESTINAL MOTILITY DISTURBANCE CAUSED by *Typanosoma cruzi* INFECTION

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Infection with *Trypanosoma cruzi* causes megasyndromes of the gastrointestinal (GI) tract in humans and animals. In the present study, we employed magnetic resonance imaging to non-invasively monitor the effect of selenium supplementation on alterations in the GI tract of *T. cruzi*-infected mice. CD1 mice infected with *T. cruzi* (Brazil strain) exhibited dilatation of the intestines similar to that we recently reported in infected C57Bl/6 mice. The average intestine lumen diameter increased by 65% and the increase was reduced to 29% in mice supplemented with 2 ppm selenium in the drinking water. When supplemented with 3 ppm selenium in chow the lumen diameter was also significantly reduced although the difference between the infected and infected supplemented mice was smaller. Intestinal motility in infected mice fed with selenium-enriched chow was increased compared with infected mice fed with normal unsupplemented chow and was not significantly different from intestinal motility in uninfected mice. We suggest that Se may be used to modulate the inflammatory, immunological, and/ or antioxidant responses involved in intestinal disturbances caused by *T. cruzi* infection.
Supported by CNPq (Decit Nº25/2006), CAPES, FAPERJ, FIOCRUZ, NIH grants CA123334, AI062730, and AI076248.

BQ.41 – INHIBITORY ACTIVITY OF RISEDRONATE AGAINST PLASMODIUM PARASITES *IN VITRO* AND *IN VIVO*

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The biosynthesis of several end products of isoprenoid pathway in *Plasmodium falciparum* was recently described. Interestingly, the intermediates and final products biosynthesized by this pathway in mammals differ from those biosynthesized in *P. falciparum.* Thus, the intermediates and some end products of the isoprenoids pathway, could be a potential drug target in *P. falciparum*. Recent studies have shown that bisphosphonates containing nitrogen blocked the biosynthesis of isoprenoid and inhibit protein isoprenylation in humans. Risedronate showed an IC_{50} of 20 µM on cultures of the intraerythrocytic stages of *P. falciparum.* We have also demonstrated that risedronate treated or untreated parasites, labeled with either [H^3] farnesyl pyrophosphate or [H^3] geranylgeranyl pyrophosphate showed a decrease of intensity of the band corresponding to farnesylated protein and, on the other hand, an increase in intensity in geranylgeranylated protein. Using Ras and Rap-specific monoclonal antibodies, putative Rap and Ras proteins of **P. falciparum** schizont stages were immunoprecipitated upon treatment with risedronate. Another time, we confirmed the results shown by SDS-PAGE. Risedronate showed strong inhibitory activity on the biosynthesis of the menaquinone and phylloquinone in the schizont stage. By thin-layer chromatography, we showed that isoprenoids attached to the proteins are partially modified confirming the activity of risedronate on the isoprenoid metabolism in *P.falciparum*. Additionally, we have shown that treatment of BALB/c mice infected with *P.berghei* at 25 mg/Kg i.p. for 7 days with risedronate decreased the parasitemia in approximately 90%.Supported By: FAPESP e CNPQ (Brazil)

BQ.42 – EFFECT OF MYRIOCIN IN *Leishmania (V.) braziliensis* GROWTH, SPHINGOLIPID SYNHESIS AND MORPHOLOGY

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Sphingolipids have been related with several biological processes, such as intracellular transport, modulation of signal transduction and apoptosis. Inositol phosphorylceramide (IPC) is the major sphingolipid expressed in promastigote forms of *Leishmania.* In this study it was investigated the effect of myriocin, inhibitor of serino palmitoyltransferase, the first step of sphingolipid synthesis, in promastigote forms of *L. (V.) braziliensis*. Parasite growth, morphology and sphingolipid/phospholipid expression were analyzed in cultures treated with myriocin and supplemented or not with ethanolamine (EtN) and 3-ketodihidrosphingosine (3-KDS). We observed that *L (V.) braziliensis* treated with 1µM of myriocin showed a 52% inhibition of growth, the promastigotes presented a more round form when compared to control parasites. Aberrant cell phenotypes were observed after parasite incubation with 1 µM and 5 µM of myriocin. After 72 hours, a defective cytokinesis was observed by optic microscopy and confirmed by transmission electron microscopy, resulting in giant cells with multiples nucleus and flagella. Addition of exogenous EtN and 3-KDS on myriocin-treated promastigotes did not revert myriocin growth inhibition effect, and also addition of EtN did not revert morphologic alterations, suggesting that the myriocin effect is not due to blockage of synthesis of ethanolamine phosphate necessary for Kennedy pathway as verified in *L.major* (Zhang *et al.*, 2007). Addition of exogenous 3-KDS on 1 µM and 5 µM myriocin-treated parasite reduced the number aberrant cell phenotypes, and partially reverted the expression of parasite IPC. These results suggest that sphingolipids are important for completion of cytokinesis and may also act as signals for *L (V.) braziliensis* proliferation.
Supported by FAPESP, CAPES, CNPq.and FADA.

BQ.43 – INHIBITION OF STEROL BIOSYNTHESIS IN *LEISHMANIA* IS COUNTERACTED BY A SHIFT ON INTRACELLULAR CHOLESTEROL ENDOCYTOSIS AND METABOLISM

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The development of more effective and selective drugs is a priority for the treatment of leishmaniasis and the identification of exclusive metabolic pathways of the parasite that can be used as a target could be an interesting starting point. In spite of leishmania parasites synthesize their own sterols, they accumulate significant percentage of exogenous cholesterol, reaching in some cases to be the major sterol at all, indicating a biological role for this molecule. This work aims to study the importance for *L. amazonensis* of the use of cholesterol from the serum in various situations, assessing the potential of this system as a possible drug target. The activity of the ergosterol biosynthesis inhibitors (ketoconazole, miconazole, terbinafine and simvastatin) was evaluated in the presence of normal or delipidated serum. It was observed that the deprivation of serum lipoprotein potentiates the effect of inhibitors of ergosterol. The promastigotes treated with these inhibitors showed differences in their lipid composition, with accumulation of cholesterol in treated cells, mainly with ketoconazole and miconazole, suggesting a compensation mechanism in leishmania, which may overcome ergosterol inhibition. Experiments with LDL-I^{125} indicated that leishmania promastigotes increase uptake of LDL upon pressure with either ketoconazole or simvastatin, two sterol biosynthesis inhibitors with different mechanism of action. Suramine, an inhibitor of the uptake of LDL, showed a decrease in cholesterol content of the leishmania and in combination with simvastatin, had a synergistic effect, showing that content of LDL, especially cholesterol, could be involved in maintaining the cell membrane integrity. Taken together, these results suggest that cholesterol plays an important role in the activity of ergosterol inhibitors biosynthesis and that the blocking of its use by leishmania may be a possible drug target. Support by FAPERJ and CNPq/PAPES.

BQ.44 – INVOLVEMENT OF GP63 MOLECULES IN *BLASTROCRITHIDIA CULICIS* ADHESION TO THE INSECT MIDGUT

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Blastocrithidia culicis is a insect trypanosomatid that harbors an endosymbiotic bacterium in its cytoplasm. The major surface peptidase of *Leishmania* spp. is the best characterized metallopeptidase in the Trypanosomatidae family and homologues of these enzymes have been described in several monoxenic trypanosomatids. Here, the gp63 expression of endosymbiont-harboring was assessed by flow cytometry analysis using a panel of anti-gp63 antibodies. It was shown that the wild strain expresses gp63 with homology to *Leishmania* and *T. cruzi* gp63 molecules. We also analyzed the effect of the pre-treatment of *B. culicis* with these anti-gp63 on the parasite adhesion to *Aedes aegypti* midgut, and showed that anti-Tc-gp63 promoted the strongest inhibition of the parasite adhesion (75%). Finally, the pre-treatment of *A. aegypti* midgut with purified metalloproteinase also promoted a significant adhesion on parasite binding, suggesting the involvement of insect receptors to this molecule in the binding process. Taken together, these results suggest a role for gp63 molecules in *B. culicis* adhesion to the insect host midgut.
Supported by CNPq, Faperj and Fiocruz.

BQ.45 – CRUZIPAIN PROMOTES *TRYPANOSOMA CRUZI* ADHESION TO *RHODNIUS PROLIXUS* EXPLANTED MIDGUTS

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Cruzipain is the major lysosomal cysteine peptidase of *Trypanosoma cruzi*, which is the causative agent of Chagas' disease. This enzyme is expressed at variable levels in all developmental forms and strains of the parasite. Cruzipain is required for parasite infectivity and intracellular growth in mammalian cells, however, its role in parasite interaction with the vector has been overlooked. Here, we have analyzed the effects of the treatment of *T. cruzi* with anti-cruzipain antibodies and a panel of different cysteine peptidase inhibitors on the parasite adhesion to *Rhodnius prolixus* posterior midgut. In parallel, we have analyzed the adhesion rate using genetically manipulated *T. cruzi,* which superexpresses chagasin, an endogenous cruzipain inhibitor (pCHAG). Brielfy, the parasites were treated for 1 hour with iodoacetamide, leupeptin, antipain, Ca074me or E-64 at 10 μM or cystatin at 1 μg/ml and allowed to bind to *R. prolixus* explanted guts for 15 minutes. The interaction rate of the parasites treated with the cysteine peptidase inhibitors was on average 70% lower in comparison to the untreated parasites, except for Ca074me (a cathepsin B inhibitor), which showed no significant alteration. In addition, anti-cruzipain antibodies (1:1000) reduced the adhesion to the insect posterior midgut in 64%, corroborating, pCHAG parasites adhered to the insect gut 73% less than control. In vivo infection assays, performed with pCHAG or control parasites revealed that pCHAG parasites were virtually unable to colonize *R. prolixus* rectum. Furthermore, the expression of surface cruzipain in *T. cruzi* cells was drastically enhanced after passage in *R. prolixus*. Collectively, these results suggest that cruzipain somehow mediates actively the interaction between *T. cruzi* and epithelial cells from the invertebrate host.
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BQ.46 – IDENTIFICATION OF PROTEINS MODIFIED BY PHOSPHORYLATION IN *TRYPANOSOMA CRUZI* DURING THE INVASION OF HOST CELLS

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We aimed to identify proteins from the infective stage of *T. cruzi* (trypomastigotes) that were modified as a consequence of parasite adhesion to components of the extracellular matrix (ECM), as it is well established that these are involved in host cell invasion. Furthermore, members of the gp85/trans-sialidase superfamily of the parasite bind to ECM elements (laminin, fibronectin, collagen and heparin sulphate). Additionaly, approximately 190 kinases and 86 phosphatases have been identified in the genome of *Trypanosoma cruzi* (CL Brener strain). For this reason, phosphoproteomes from different stages of *T. cruzi* have been published recently. In order to address this question, trypomastigotes (Y strain) were incubated with laminin-, fibronectin- or BSA-coated surfaces for 2h, followed by 2D-PAGE analysis pattern after staining with Pro-Q Diamond Phosphoprotein Gel Stain (Invitrogen). Proteins with significant differences in stained intensity when compared to the control (BSA) (p<0.05) were selected for further identification by LC-MS/MS. From the 64 spots presenting modification to the degree of phosphorylation on incubation with laminin, 16 corresponded to cases of novel phosphorylations, 26 corresponded to cases of dephosphorylation and 22 presented alteration to the level of phosphorylation. Only 9 of the latter showed variation at a level greater than 2.0-2.5 fold. **After incubation with fibronectin:** from the 78 spots selected, 8 corresponded to novel phosphorylation, 44 to dephosphorylation events and 26 to variation in the level of phosphorylation. Of the latter, 5 spots showed variation greater than 2.0 to 2.5 fold when compared to the control. Currently the full identification of the proteins is under way. For example, tubulin (alpha and beta) showed an increase in the phosphorylation when trypomastigotes were incubated with laminin. So the identification of proteins modified by phosphorylation during the interaction of trypomastigotes with ECM may help in understanding the signaling events within the parasite during the invasion. Supported by FAPESP and CNPq.

BQ.47 – INTRAERYTHROCYTIC *Plasmodium falciparum* STAGES SYNTHESIZE MENAQUINONE AND PHYLLOQUINONE AND MODIFIES ITS UBIQUINONE/MENAQUINONE POOL DEPENDING ON OXYGEN AVAILABILITY

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During its intraerythrocytic development in the human host, the malaria parasite *P. falciparum* is submitted to considerable changes in the oxygen concentration due to intermittent cytoadherence in the deep vasculature, with consequences for the energy metabolism of the parasite. Facultative anaerobic organisms such as *E. coli* employ two types of electron carriers, ubiquinone and menaquinone which are tightly regulated depending on the oxygen supply in the environment. Herein, we show that intraerythrocytic stages of *Plasmodium falciparum* have an active pathway for biosynthesis of menaquinone (vitamin K2) and phylloquinone (vitamin K1), as well as, ubiquinone. Kinetic assays confirmed that plasmodial menaquinone acts at least in the electron transport. Similarly to *E. coli*, we observed increased levels of menaquinone in parasites kept under very low oxygen pressures. Additionally, parasite growth is strongly inhibited when menaquinone synthesis was blocked by Ro 48-8071, an inhibitor of 1,4-dihydroxy-2-naphthoate prenyltransferase. Due to its absence in humans, the menaquinone biosynthesis can be considered an important drug target for malaria. Supported by FAPESP and CNPq.

BQ.48 – PEPTIDASE ACTIVITY CHARACTERIZATION OF LYSATE AND LIVE CELLS OF *LEISHMANIA (L.) AMAZONENSIS* AMASTIGOTES AND PROMASTIGOTES

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Proteases (proteinases, peptidases or proteolytic enzymes) are enzymes present in all organisms, from viruses to vertebrates. Peptidases in parasites are involved in invasion of host cells and tissues, parasite nutrition, modification of host proteins and also in immuno evasion. The aim of this study was to identify the proteinase activity of *Leishmania amazonensis* amastigotes in lysates and live cells in of amastigote and promastigote forms. Fluorescence Resonance Energy Transfer (FRET) peptides and MCA substrates (Z-Phe-Arg-MCA, Z-Arg-Arg-MCA, D-Pro-Phe-Phe-MCA, ε-NH_2-caproyl-Cys(SBzl)-Cys(SBzl)-MCA) were used to perform inventories of the peptidase activities. The pH and temperature profiles of peptidase activities of the lysates were done in the presence of inhibitors (E-64, TLCK, PMSF, Pepstatin and EDTA). The peptidase activity in live cells were observed and quantified with confocal microscopy. The lysates presented peptidase activity in wide range of pH, with higher activity around pH 5.0 and pH 8.0. The confocal microscopy confirmed intracellular peptidase activity in pH 5.0, in agreement with the cellular environment of amastigotes, that was also inhibited for E-64 and TLCK, similar with the lysates. Proteolytic activities of live cells were not observed with hydrophobic substrates ε-NH_2-caproyl-Cys(SBzl)-Cys(SBzl)-MCA and D-Pro-Phe-Phe-MCA, indicating selectivity of the parasite. All together we observed a large peptidase activity linked to cysteine proteases but we also observed a significant peptidase activity similar to oligopeptidase B that has particular preferences for basic sequences of amino acids.
Supported by CNPq and FAPESP.

BQ.49 – LEISHMANICIDAL ACTIVITY OF SPECIFIC CERAMIDASE AND GLUCOSYLCERAMIDE SYNTHASE INHIBITORS AND IMPLICATIONS FOR *LEISHMANIA AMAZONENSIS* CERAMIDE METABOLISM

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Introduction. Sphingolipids (SLs) are essential components of biological structures and biological recognition processes between pathogen and host cells. The biosynthesis of SLs in Trypanosomatidae has been only partially characterized. In *Leishmania,* serine-palmitoyil transferase and inositol-phosphoryl ceramide synthase have been identified and glucosylsphingolipids were detected in *L. amazonensis* amastigotes. These molecules appear to be involved in signaling events that mediate metacyclogenesis and host cell-parasite interactions. (1S,2R)-D-erythro-2-(N-myristoylamino)-1-phenyl-1-propanol (MAPP) and 1-phenyl-2-decanoylamino-3-morpholino-1-propanol (PDMP), are inhibitors of eukaryotic ceramidase and glucosylceramide synthase, respectively and their activities against trypanosomatids have not been described. Objectives. We aimed to evaluate the activity of MAPP and PDMP against *L. amazonensis* and investigate the interference of these inhibitors in the incorporation of ceramide by promastigotes. Methodology. MAPP and PDMP effects were observed after incubating *L. amazonensis* promastigotes with increasing concentrations of the inhibitors. After 24h, the viability was assessed by the MTT test. Morphology and ultrastructural aspects were evaluated in treated promastigotes using optical and electron microscopy. C6-NBD-ceramide incorporation and metabolism in the presence of the inhibitors were investigated by HPTLC. Results. The EC50% values for MAPP and PDMP were 112.6 and 62.5 microM, respectively. Parasites treated with MAPP did not present significant alterations on cellular morphology. In contrast, parasites treated with PDMP became rounded with elongated flagella and, by ultrastructural analysis, showed unusual cytoplasmic multilamellar bodies. The labeling of MAPP or PDMP treated-parasites with C6-NBD-ceramide followed by the analysis of lipid extracts showed that drug treatment induced an accumulation of ceramide accompanied by changes in the biosynthesis of other SL. Conclusions. Inhibitors of eukaryotic ceramidase and glucosylceramide synthase are active against *L. amazonensis* promastigotes and induce modifications in ceramide metabolism, suggesting that these enzymes are also present in *Leishmania.* These results open new perspectives for the study of these metabolic pathways in *Leishmania*. Support: FAPESP, CNPq.

BQ.50 – LIPID METABOLISM in *Herpetomonas megaseliae*

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Herpetomonas megaseliae is a monoxenic trypanosomatid isolated from the phorid fly *Megaselia scalaris*. During its lifecycle, this parasite remains in the insect gut lumen. Despite this is not pathogenic to humans, such protozoa represents a safe models for initial studies on lipid metabolism. Lipids are hydrophobic molecules that play a variety of cellular functions of great importance to all organisms. In this work we are investigating the ability of *H. megaseliae* to incorporate and synthesize lipids. ^{3}H-palmitic acid was added to the culture medium and after 24 h of incubation, the radioactivity incorporated by the parasites was determined by scintillation counting. We observed that *H. megaseliae* were able to incorporate ^{3}H-palmitic acid added to the culture medium and to use it for *de novo* lipid synthesis. Triacylglycerol and phospholipids were the major lipids found. Also we tested for the ability of this parasite to incorporate the main lipoprotein present on vector hemocel, lipophorin (Lp). Parasites were incubated in the presence of radioactively labelled lipophorin either on the phospholipid (^{32}P-Lp) or in the protein moiety (^{125}I-Lp). We observed that parasites were able to incorporate Lp in both experiments. In order to localize the incorporated lipids parasites were incubated with fluorescent tagged lipids such as BODIPY-fatty acid (BODIPY-FA) and phosphatidylethanolamine-Texas Red (Texas Red-PE) for 24 h. The results again showed the capacity of incorporation of lipids free in cell medium and their intracellular fate. This work was supported by CNPq, FAPERJ and IFS

BQ.51 – RECEPTOR-MEDIATED ENDOCYTOSIS OF LDL BY *Leishmania amazonensis* AND LOCATION OF THIS RECEPTOR IN PARASITES LIPID MICRODOMAINS

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DRM (detergent-resistant membranes) or *lipid rafts* are lipid-enriched microdomains in which cholesterol is the principal component. Recent studies showed that it can be isolated from different cell types and are involved in cholesterol transport, endocytosis and signal transduction. Other studies showed the existence of a scavenger receptor for LDL oxidized, CD36, located in lipid rafts of Chinese Hamster Ovary cells. The aim of this work is to study the LDL endocytosis by *Leishmania amazonensis* and localize the LDL-receptor in parasites membranes microdomains. In order to verify the receptor-mediated endocytosis of LDL, the parasites were incubated in the presence of LDL-I^{125} at 28ºC and 4ºC. After 24h, cells were collected, washed and the radioactivity determined by gamma counting. The LDL was found associated with cells and endocytosis was significantly inhibited in assay with fold-excess of LDL or Transferrin. To observe the existence of a LDL-receptor like protein, we isolate *L.amazonensis* membranes. Cells were lysed with glassbeads (1:4 w/w) and separeted in a ultracentrifugation gradient. The membranes were subjected to eletrophoresis gel with a poliacrylamide gradient, using calf hepatocytes membranes as positive control. We found a 38 kDa protein that corresponds to hepatocytes lipoprotein receptor. To examine the LDL-receptor localization in DRM, we first isolated *L. amazonensis* DRMs. The parasites were lysed, homogenated, incubated at 4°C in Triton X-100 1% for 20 minutes and subjected to sucrose density gradient ultracentrifugation. The gradient fractions were subjected to a dot-blot using different agents for investigate the presence of DRM components (GM1 and Flotillin-1). We also used the antibody against the human LDL-receptor. We identified a large Flotillin, GM1 and LDL-receptor content in the fractions from 4 to 7 of sucrose gradient. These results could provide better tools for understand the interactions between parasites and their hosts. Supported by CNPq, FAPERJ, IFS.

BQ.52 – PURIFICATION OF PROTEINS LIBERATED FROM *TRYPANOSOMA CRUZI* IN RESPONSE TO CALCIUM STIMULATION

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Cell invasion by *Trypanosoma cruzi* is a multi-step process that includes host cell attachment, cell signaling events, host cell lysosome recruitment to the site of parasite entry, host cell microtubule reorganization, lysosome survival, lysosome escape and differentiation into the replicating amastigotes. The first measurable change observed in *T. cruzi* after the parasite binds to the surface of a potential mammalian host cell is an increase in intracellular calcium. Calcium is an important secondary messenger for controlling cellular responses to cell signaling events, including exocytosis in other cell types. Considering the importance of cell invasion to the life cycle of *T. cruzi*, we predict that this initial rise in calcium controls the release of proteins involved with cell invasion from internal sources. To identify these hypothetical proteins, a differential labeling protocol was designed using reversible and irreversible biotin in conjunction with a calcium stimulation using a calcium ionophore. After the labeling with biotin, marked proteins were purified using streptavidin-agarose for analysis by SDS-PAGE and western blots. Analysis consisted of identifying proteins present in the samples stimulated with calcium compared to control samples without calcium stimulation. In addition, western blots were performed using streptavidin and sera from patients diagnosed with Chagas disease. A major objective of this project is to identify potential biomarkers for diagnosis of *T. cruzi* infections and prognosis for the progression to Chagas disease. Proteins that demonstrate a differential with regards to calcium stimulation and are recognized by antibodies in the sera of patients are of the greatest interest.
Supported by FAPERJ, CNPq and Fiocruz

BQ.53 – SODIUM-DEPENDENT UPTAKE OF INORGANIC PHOSPHATE IN *TRYPANOSOMA RANGELI*

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Trypanosoma rangeli is a protozoan that infects a great number of mammals and triatomine vectors. In this work we demonstrated, for the first time, that living cells of *T. rangeli* are able to transport the extracellular inorganic phosphate (Pi) to cytosol through a carrier-mediated process. The dependence on Na^+ concentration revealed a normal Michaelis-Menden kinetics for the uptake of Pi and the values of apparent K_m and V_{max} were 1.2 ± 0,3 mM and 22.0 ± 1.2 *p*mol × min^{-1} ×10^7 $cells^{-1}$, respectively. Addition of Na^+ ionophore, monensin, reduced the Pi accumulation into the cells in the presence of NaCl to the level observed in absence of Na^+. Pi-influx showed a sigmoidal dependence on the Pi concentration with a Hill coefficient of 2.2 ± 0.3, and the values of $K_{0.5}$ and V_{max} were 0.05 ± 0.004 mM and 23.6 ± 1.0 *p*mol × min^{-1} ×10^7 $cells^{-1}$, respectively. Treatment with FCCP or by v(H^+)-ATPase inhibitor bafilomycin A1 resulted in a significant decrease in Pi influx, consistent with the predicted electrogenicity of transport. Parasites maintained at Pi-supplemented culture medium (50 mM Pi) presented a decreased Pi-influx, suggesting that this transport is stimulated by Pi-starvation in medium culture. Furthermore, these parasites showed a decrease in *TrPho89*, a Na^+, Pi symporter homologue, mRNA levels when compared to parasites maintained at Pi-depleted culture medium (2 mM Pi). Altogether, these results suggests the presence of a Na^+/Pi cotransporter present in *T. rangeli*, contribute to inorganic phosphate acquisition by epimastigotes development. Supported by CNPq, CAPES and FAPERJ.

BQ.54 – HEME UPTAKE IN *TRYPANOSOMA CRUZI* EPIMASTIGOTES INVOLVES ATP-BINDING CASSETTE (ABC) TRANSPORTERS.

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Trypanosoma cruzi, the etiological agent of Chaga's disease, takes up heme from the environment to supply their nutritional needs, since it is not synthesized in epimastigotes. Heme is a porphyrin (Fe-protoporphyrin IX) that plays a critical role in several biological reactions such as oxygen transport and cell respiration. However, the mechanisms involved in its uptake across biological membranes are poorly understood. Indeed, in these parasites, no heme transporter has yet been characterized. Thus, here we evaluated the heme import by epimastigotes. Our results showed that when parasites were pre-loaded with other porphyrins such as Sn-protoporphyrin IX, Pd-mesoporphyrin IX and Zn-mesoporphyrin IX the heme uptake decreased drastically. The same effects were observed when these parasites were treated with ATP- binding cassette (ABC) transporters blockers, verapamil, cyclosporin and indomethacin. Furthermore, we evaluated the effect of these drugs in epimastigotes growth kinetics and observed that, in contrast with heme, in the presence of heme plus these drugs the parasites growth decreased. These results suggest the existence of a mutual porphyrin carrier in *T. cruzi* epimastigotes, possibly an ABC-like transporter. Supported by CAPES, CNPQ and FAPERJ

BQ.55 – THE MAJOR NEUTRAL HEXAMERIC LEUCYL AMINOPEPTIDASE OF *Trypanosoma cruzi* (LAPTc) BELONGS TO THE PEPTIDASE FAMILY M17

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Pathogens depend on peptidase activities to accomplish many physiological processes, as well as to interact with their hosts, highlighting parasitic peptidases as virulence factors and, thus, potential drug targets. In this study, a major leucyl aminopeptidolytic activity was identified in the kinetoplastid *Trypanosoma cruzi*, the aetiological agent of Chagas' disease. It was isolated from epimastigote forms of the parasite by a two-step chromatographic procedure and associated with a single 320-kDa homo-hexameric protein as determined by sedimentation velocity. Interchain disulfide bonds do not take part in the oligomeric assembling of the active peptidase. Molecular identity of the enzyme was revealed by peptide mass fingerprinting as the predicted *T. cruzi* aminopetidase EAN97960. Molecular and enzymatic analyses indicated that this leucyl aminopeptidase of *T. cruzi* (LAPTc) belongs to the peptidase family M17 or leucyl aminopeptidase family. Its sequence shares identity to sequences of other M17 family members, including assigned and unassigned leucyl aminopeptidases of kinetoplastid parasites such as *Leishmania* ssp. and African trypanosome. The enzyme is expressed by all *T. cruzi* forms and localizes within vesicles in the cytoplasm of the parasite. LAP-Tc has a strong dependence on neutral pH, is mesophilic and retains its oligomeric form up to 80 °C. Conversely, its recombinant form, like other LAPs, is thermophilic and requires alkaline pH. The activity of this metalloaminopetidase is inhibited by bestatin and metal chelants such as 1,10-phenanthroline, restored by Zn^{+2}, and potentiated by Mn^{2+} or Ca^{2+}. Since biosynthetic pathways for essential amino acids, including leucine, are lacking in *T. cruzi* and other kinetoplastid parasites, LAPTc could have a function in nutritional supply. Furthermore, the peptidase activity could also play a role in peptide and protein processing.
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BQ.56 – MODULATION OF CELL CYCLE AND PROTEIN EXPRESSION OF *LEISHMANIA (VIANNIA) BRAZILIENSIS* BY DEPLETION OF IRON

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Iron is an essential element for *in vitro* and *in vivo* survival of microorganisms, acting as a cofactor of several enzymes and playing a critical role in host-parasite relationship. Only the more virulent strains, endowed with effective mechanisms to acquire iron from healthy hosts can invade, colonize, multiply and establish infection. Endosomes and lysosomes of macrophages are gradually depleted in iron by transporters such as Nramp1 that pump the ion into the cytosol reducing the amount accessible to pathogenic microorganisms. In *Leishmania amazonensis* iron transporter LIT1 is essential for the growth of intracellular amastigotes and development of lesions in mice. *L. (V.) braziliensis* is a parasite widespread in the new world and considered to be the major etiological agent of American Tegumentary Leishmaniasis (ATL). Despite this fact, the role of iron on the growth and virulence of this species is still unclear. In this scenario, the identification of proteins regulated by iron in *L.(V.) braziliensis* can provide important information about their mechanisms of pathogenicity contributing to the development of new therapies for the ATL. In the current work, we aimed to analyze the effect of iron depletion on the growth and protein expression of *L. (V.) braziliensis*. Promastigotes from cultures which had reached the logarithmic phase of growth were inoculated into iron-supplemented and iron-depleted medium. Iron depletion was carried out by the addition of different concentrations of 2,2-dipyridyl ranging from 25 µM to 300 µM. It was observed that parasite growth is affected by both concentration of 2,2-dipyridyl and time of culture in depleted medium. Whole extracts of promastigotes obtained from parasites growth in iron-rich and iron-depleted medium were submitted to fractionation by two-dimensional electrophoresis (2DE). Preliminary data suggest that there are several proteins involved in proteolysis and signaling suffering up- or down-regulation by iron. Supported by: CAPES

BQ.57 – OPTIMIZATION OF EXPRESSION OF A 21 KDA PROTEIN FROM *TRYPANOSOMA CRUZI*

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Chagas disease is caused by the intracellular protozoan *Trypanosoma cruzi.* It is estimated that 13 million people are directly affected in Central and South American. The current available drugs are limited and ineffective. To overcome these limitations, the identification of new macromolecular targets is very important. This work aim to conduct crystallography studies with a 21 kDa protein (P21) from *T. cruzi*, P21 shows high probability of being secreted and interacts in the mammalian cell invasion process. The recombinant P21 was expressed in *Escherichia coli* BL-21 containing pET-28a (+) vector. P21 was expressed mostly in insoluble fraction and in order to obtain soluble fraction we tested two culture media (LB end LB 2x), three temperatures to expression (37, 20 and 16°C**)**, three concentrations of isopropyl b-D-thiogalactopyranoside-IPTG (1mM, 0,5mM, 0,1mM) and three methods of bacterial lysis (French press, sonication and ***Freeze***-Thawing), the fractions soluble and insoluble were analyzed by Coomassie blue staining SDS-polyacrylamide gels. P21 showed more soluble in the following conditions: LB 2x media, 16**°**C of expression temperature**,** 0,1mM of IPTG and sonication lysis method.
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BQ.58 – E-NTPdases: PREDICTION OF FAMILIES AND CLASS DESCRIPTORS

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Enzymes of E-NTPDase family hydrolyze ATP and other nucleosides tri and diphosphates. Extracellular nucleotides act as signaling molecules in immune response of mammalian hosts. The ATPe hydrolysis plays a role on infectivity and virulence of parasites (*Trypanosoma*, *Leishmania*, *Trichomonas*, *Toxoplasma* and *Entamoeba*). All the enzymes belonging to this family have five highly conserved regions named Apyrase Conserved Regions. The mechanism of nucleotides hydrolysis is clear, but little is known about what determines the distinct nucleotide preferences. In this study we analyzed all complete E-NTPDase sequences, showing that it's possible to divide them in sub-families based on sequence similarities and aminoacid positions conservations that could be related with nucleotide preferences. For this, 566 UniProt sequences were filtered, leaving 366 threads. All reviewed proteins of this family, deposited in the SwissProt (42) were detailed analyzed through alignment and literature annotations. Statistical analysis of conserved and correlated mutations was applied to this final alignment. A matrix was constructed containing the correlation value of each correlation between the statistically significant perturbations with other alignment positions. This matrix has undergone a procedure of hierarchical clustering and removal of low signals, resulting in small groups of highly correlated positions. We showed that there is a trend in substrate preference between subfamilies. There are four positions that appear to be strongly correlated and which seem to be good descriptors of the distinct subfamilies. Based on known structural information's, we can suggest why some of these positions could act as keys to substrate specificity. Furthermore we can predict the substrate preference of tripanosomatides E-NTPDases (*T. cruzi* and *Leishmania infantum*) and confirmed many of then using enzymatic activities of recombinant expressed proteins. These data could be explaining the influence of pathogen´s E-NTPDases on hosts purinergic signaling and may be applied in new approaches to rational drug design. Supported by: INBEQMeDI, CNPq, Capes, FAPESP, UFV.