Bioquimica - Biochemistry

BQ01 - EFFECT OF MYRIOCIN ON *Leishmania (L.) amazonensis*

CASTRO, E.V.; TANAKA, A. K.; TAKAHASHI, H.K.; STRAUS, A.H.

Department of Biochemistry, Universidade Federal de São Paulo, São Paulo-SP, Brazil

Sphingolipids have been related with several biological processes, such as intracellular transport, modulation of signal transductions and apoptosis. Inositol phosphorylceramide (IPC) is the major sphingolipid expressed in promastigote forms of *Leishmania (Leishmania) amazonensis*, and recently it was demonstrated that the IPC synthase inhibitor, Aureobasidin A, blocked *L. (L.) amazonensis* amastigote and promastigote growth (Tanaka et al 2007 J. Antimicrobial Chemotheraphy. 59:487). In this study it was investigated the effect of myriocin, which blocks the first step of sphingolipid synthesis (serine + palmitate forming 3 ketodihydrosphingosine), on *L. (L.) amazonensis* promastigote growth, morphology and on sphingolipid and phospholipid expression. Myriocyn at 12 μM inhibited completely promastigote growth, independently of the supplemention with exogenous ethanolamine, which together with ethanolamine-phosphate (a product of sphingoid base) can be used to synthesize phosphatidylethanolamine. Examination of myriocin-treated promastigote cells stained with HEMA-3 showed more rounded appearance when compared to control parasites. Cells staining for DNA with DAPI showed a small decrease in the proportion of interphase cells (1k1n, 82.5% for control versus 72.5% for myroicin treated) with a concomitant increase of 2k2n and not defined cells. Parasites incubated for 3 days with myriocin at 2.5 μM and 5 μM presented a reduction of growth rate of 31% and 50%, respectively. Analysis of parasite phospholipids showed a significant decrease of IPC expression from 14.5% (control parasites) to 4.0% and 2.0% (myriocin 2.5 and 5 μM treated parasites), with a concomitant increase of phosphatidylserine from 6% to 17% and 25% and lyso-phosphatidylinositol from 2.6% to 5.3% and 7.7%, respectively. Besides, myriocin growth inhibition of the parasites, the role of sphingolipids on parasite lipid raft formation and infectivity is under investigation.
Supported by FAPESP, CAPES, CNPq and FADA.

BQ02 - Heme and not its analogs is essential to *Trypanosoma cruzi* epimastigotes proliferation

Saraiva FMS [1], Nogueira NPA[1], Souza, CF [1], Laranja, GAT [1], Coelho, MGP [1], Paes MC [1].

1- *Universidade do Estado do Rio de Janeiro – Departamento de Bioquímica*

Trypanosoma cruzi, the ethiologic agent of Chagas disease, is transmitted through triatomine vectors during their blood-meal on the vertebrate host. *T. cruzi* epimastigotes (EPI) proliferate inside of the hematophagous insects that usually ingest in a single meal about 10 mM heme bound to hemoglobin. We have recently shown that the addition of heme increases significantly the parasite proliferation in a dose-dependent manner (Lara *et al*, 2007). In order to investigate whether their nutritional needs is heme or other porphyrins analogs we tested the effects of porphyrin rings without iron or with metal substitutions to induce epimastigotes proliferation. *T. cruzi* epimastigotes Dm28c strain, were maintained in BHI supplemented with 10% FCS at 28ºC for 7 days. Afterwards cells were incubated in the absence or in the presence of, the protoporphyrin ring (PPIX), the immediate precursor of heme differing from it by the absence of the atom of iron, zinc protoporphyrin (ZnPPIX), the mesoporphyrin ring (MPIX) a protoporphyrin analog that has two ethyl groups substituting its vinyl groups as well as palladium mesoporphyrin IX (PdmPIX) and the malaria pigment, hemozoin (Hz) witch consists of heme molecules crystallized into dimmers. Thus, growth of epimastigotes in the presence of these porphyrins was evaluated. PPIX, ZnPPIX, MPIX, PdmPIX and Hz were unable to increase epimastigotes proliferation when compared to heme. Therefore, our data suggests that both the coordinated iron and the vinyl groups in the porphyrin ring of the heme molecule are necessary for the increment of epimastigotes proliferation.
Supported by FAPERJ and CAPES.

BQ03 - KINETIC AND SPECTROSCOPIC STUDIES WITH RECOMBINANT *Leishmania major* MACROPHAGE MIGRATION INHIBITORY FACTOR

Momo P.B.[1], Pinto M.R.[2], Tosi L.R.O.[2]; Cruz A.K.[2]; Ward R.J.[1] and de Oliveira A.H.C.[1]

[1]Departamento de Química, FFCLRP-USP; [2]Departamento de Biologia Celular e Molecular e Bioagentes Patogênicos, FMRP-USP.

Migration Inhibitory Factor (MIF) was the first cytokine to be identified from the human T cells and participates in innate and adaptive immune response. MIF has been considered an important factor in the control of parasites infections, presenting a beneficial or a detrimental role, depending on the pathogen and MIF deficient mice were more susceptible to infections caused by *L. major* and *T. cruzi*. Interestingly, homologues of mammalian MIF, which act as a modulator factor of the immune response from infected host, have been isolated from parasites species, including *Leishmania* ortholog Lm1740MIF. Here we have characterized the Lm1750MIF, which was described two times more expressed than Lm1740 in all *L. major* forms. The recombinant Lm1750MIF was purified by affinity chromatography from *E. coli* extract and showed inhibitory macrophage migration activity *in vitro*. The purification yield showed that 51% of total activity (31.1U/mg) was retained by the Ni-NTA column, yielding a final activity purification of 79 fold. The phenylpyruvate tautomerization activity of the rLmMIF1750 was assayed by enol-borate complex method and kinetics parameters were obtained at 30°C and pH6.2. The far-UV circular dichroism spectra revealed a well-defined secondary structure of the purified rLmMIF and that this secondary structure is maintained even in pH3.0. Further, intrinsic tryptophan fluorescence spectroscopy showed an increased emission coupled with a λmax red-shift suggesting an non-native tertiary structure of the protein in pH3,0. The results may be useful for understanding of the structural arrangements involved in the LmMIF oligomerization and its interaction with the membrane receptor of the host cell during the modulation of the immune response. Supported by FAPESP, CNPq and CAPES.

BQ04 - IDENTIFICATION OF MOLECULES OF INTEREST IN THE IMMUNODIAGNOSTIC OF CANINE VISCERAL LEISHMANIASIS

CAMPOS, A.C.F.A.[1], MARTINS, V.Tamietti[1], MACHADO, C.M.[2], ALVES, D.C.R.[1], MOREIRA, R.S.M.[1], OLIVEIRA, D.M.[1], LIMA, M.P.[1], COSTA, L.E.[1], PFEIFFER, V.N.[1], COELHO, V.T.S.[1], FUMAGALLI, M.A.C.[2], TAVARES, C.A.P.[1], CHÁVEZ-OLORTEGI, C.[1], MOTA-SANTOS, T.A.[1], COELHO, E.A.F.[1,3]

1. *Departamento de Bioquímica e Imunologia, Instituto de Ciências Biológicas, UFMG, Minas Gerais, Brazil.*
2. *Departamento de Farmacologia Bioquímica e Molecular, Instituto de Ciências Biológicas, UFMG, Minas Gerais, Brazil.*
3. *Setor de Patologia Clínica, Coltec, UFMG, Minas Gerais, Brazil.*

Canine visceral leishmaniasis (CVL) is a serious health public problem. Serological diagnosis of CVL appears to be hampered by various factors, such as low specificity of tests used in endemic areas common to other diseases. Phage display technology was used to select clones of bacteriophages expressing peptides able to react with IgG antibodies purified from serum samples from dogs with visceral leishmaniasis (VL). After several bio-pannings cycles, five highly reactive phages clones were tested in the ELISA experiments. As positive control, the same serum samples were tested against the protein extract of *Leishmania chagasi*. Results showed that five phage clones reacted with serum samples from dogs with VL with high sensitivity and specificity. More importantly, no cross-reaction with serum samples from dogs with Chagas´disease was observed. In conclusion, the five selected clones or a pool of them can be used in the composition of a kit for the immunodiagnostic of CVL.

SUPPORT: FAPEMIG, CNPq, PRPq/UFMG

BQ05 - EFFECT OF METHYL-β-CYCLODEXTRIN ON *LEISHMANIA AMAZONENSIS* DETERGENTE-RESISTANT MEMBRANE (DRM) FUNCTION

**DE CICCO, N. and *ATELLA, G.C.*

**Instituto de Bioquímica Médica, UFRJ, Rio de Janeiro, Brazil*
cicco@bioqmed.ufrj.br

Recent studies showing that detergent-resistant membrane (DRM) fragments can be isolated from

different cell types suggest that they're not always in a liquid crystalline phase. DRMs or lipids rafts are lipid-enriched microdomains being cholesterol their principal component. Cyclodextrins are cyclic oligosaccharides consisting of six, seven or eight glucopyranose units, usually referred to as α-, β- or γ-cyclodextrins, respectively. It has a hydrophobic inner cavity, that can encapsulate hydrophobic molecules, like cholesterol, rendering the molecules water soluble. Methyl-β-cyclodextrin (MBCD) is used in cell culture applications to incorporate and remove cholesterol. The objective of this work is to study the influence of MBCD treatment on DRM composition and function in *Leishmania amazonensis*. In order to verify the effect of MBCD on *L. amazonensis*'s DRM, the parasites were pre-treated with MBCD for 30min, washed, lysed and incubated at 4°C in Triton X-100 1% for 20min. After incubation the homogenate were subjected to sucrose density gradient ultracentrifugation for 20hs. Ganglioside GM1 and Flotillin were descript as a DRM components and can be used for detection of DRM containing fractions. In order to investigate the presence of GM1 and Flotillin after MBCD treatment, the fractions were subjected to a dot-blot using antibody Cholera toxin B subunit (CTB) and anti-Flotillin. The MBCD treatment did not alter the amount of GM1 and Flotilin in *L. amazonensis* DRM but decreased its density (22% to 15% after treatment). To examine the involvement of DRM on LDL endocytosis, the parasites were pre-treated with MBCD for 30 min and incubated in the presence of LDL-I^{125}. After different times, cells were collected and the radioactivity determined by gamma counting. It was observed that LDL endocytosis was significantly inhibited in cells pre-treated, suggesting that this process is dependent on the presence of DRMs. Supported by CNPq, FAPERJ, IFS

BQ06 - Indolepyruvate decarboxylase gene and production of indole-3-acetic acid (IAA) phytohormone in *Phytomonas* and *Leishmania*

IENNE, S.[1], *PURGATTO, E.*[2], *FRESCHI, L.*[3], *PAPPAS Jr., G.*[4], *MERCIER, H.*[3], *ZINGALES, B.*[1]

[1] Departamento de Bioquímica, Instituto de Química, Universidade de São Paulo, São Paulo, Brazil; [2] Departamento de Alimentos e Nutrição Experimental, Faculdade de Ciências Farmacêuticas, Universidade de São Paulo, São Paulo, Brazil; [3] Departamento de Botânica, Instituto de Biociências, Universidade de São Paulo, São Paulo, Brazil; [4] Genomic Sciences and Biotechnology Program, Universidade Católica de Brasília, Distrito Federal, Brazil.

Introduction: The consensus sequence of one EST of *Phytomonas serpens* shows high similarity with indolepyruvate decarboxylases (IPDC) of phytobacteria and putative IPDCs of *Leishmania* species. IPDCs are involved in the pathway for the conversion of tryptophan to indole-3-acetic acid (IAA), the major plant growth hormone. **Objectives:** The goals of this study were: (i) to verify the presence of putative *IPDC* genes in additional trypanosomatids and (ii) to attest the production of IAA by *P. serpens* and *L. major*. **Results and conclusions:** Degenerated primers were designed based on the conserved regions of IPDC sequences *of P. serpens* and *Leishmania* spp. PCR assays indicated the presence of IPDC orthologs in *Crithidia*, *Herpetomonas* and *Leptomonas* genera. PCR products will be sequenced. Southern blot hybridizations are in progress to investigate the genome organization and copy number of these putative genes. To verify the production of IAA, *P. serpens* and *L. major* were cultured (initial inoculum 1.5 x 10^7 cell·mL^{-1}), respectively, in Grace's and 199 media, supplemented with 10% FCS, at 28°C (*P. serpens*) and 25°C (*L. major*), for 4 days. The control culture media and parasites' conditioned media (~10 mL) (triplicates obtained on different occasions) were methanol extracted and submitted to HPLC analysis. As standards IAA, and its precursors Indole-3-pyruvic acid (IPyA) and Tryptophol (Tol) were analyzed. We observed a 3-fold higher concentration of IAA (4.19±1.3 μg·mL^{-1}·10^8 $cells^{-1}$) and Tol (3.0±1.2 μg·mL^{-1}·10^8 $cells^{-1}$) in *P. serpens* medium as compared to *L. major*. On the other hand, *L. major* conditioned medium contained a high concentration of IPyA (6.2±3.4 μg·mL^{-1}·10^8 $cells^{-1}$), which was absent in *P. serpens.* These data suggest that IPDC activity is higher in *P. serpens*, as the reflection of the high copy number of *IPDC* genes (~10^3 copies), or kinetic parameters. These will be measured in parasite cell lysates. **Support:** FAPESP; CNPq.

BQ07 - NEW ANTIGENS IN VISCERAL LEISHMANIASIS: A PROTEOMIC APPROACHES

COSTA, M. M. S.[1], *ANDRADE, H. M.* [1], *CHAPEAUROUGE, D. A.* [2], *MELO, M. N.* [1], *GAZZINELLI, R.T.* [1]

[1]*Universidade Federal de Minas Gerais, Minas Gerais, Brazil,* [2]*Fundação Oswaldo Cruz, Rio de Janeiro, Brazil*

Leishmania chagasi, a causative agent of visceral leishmaniasis in Americas, during its digenetic life cycle, alternates between the alimentary tract of the sandfly vector as an extra cellular promastigote and the acidic phagolysosomes of macrophage cells as an intracellular amastigote. We used difference gel electrophoresis (DIGE), and mass spectrometry methodologies to highlight and identify proteins that are differentially expressed in both stages of the parasite. The amastigotes forms were purified from spleen hamster infected, and promastigotes forms were cultivated in Schneider medium. Proteins differentially expressed in the intracellular form of the parasite are thought to be important for intracellular survival and pathogenesis. We used narrow pH range strips for isoelectric focusing to resolve soluble proteins of both developmental stages of *L. chagasi.* More than 70 spots differentially expressed in amastigotas were detected among almost 1000 protein spots resolved by DIGE, meanwhile in promastigotes about 40 spots differentially expressed were detected. A MALDI time-of-flight (MS/MS) analysis of all differentially expressed proteins in both stage, permitted the identification of proteins over expressed in these forms. The proteins identified were bioinformatic analyzed to identify immunological domains. These findings bring a new insight in our understanding of interaction between intracellular form of *Leishmania* and immunological response in vertebrate host. Comparative analysis of the proteome of both developmental stages of the protozoan parasite *Leishmania* should permit the identification of protein candidates for the development of vaccines and new drugs. Financial support: CNPq and FAPEMIG.

BQ08 - COMPARATIVE ANALYSIS OF METALLOPROTEASE EXPRESSION IN *Leishmania* sp.

CABRAL B.[1]; *CUPOLILLO E.*[1]; *DE JESUS J.B*[2,3]; *CUERVO P.*[1]

[1]*Laboratório de Pesquisas em Leishmanioses, IOC, FIOCRUZ, Rio de Janeiro, Brasil;* [2]*Laboratório de Biologia Molecular e Doenças Endêmicas, IOC, FIOCRUZ;* [3]*Departamento de Ciências Naturais, UFSJ, Minas Gerais, Brasil*

Leishmania parasites are responsible for a broad spectrum of clinical manifestations ranging from self-healing cutaneous lesions to a systemic form of the disease, visceral leishmaniasis, which is fatal if left untreated. Previous works of our group showed that (i) *L. (V.) braziliensis* strains isolated from patients with distinct clinical manifestations display different pattern of metalloprotease activities and (ii) zymographic profiles remain unaltered during prolonged *in vitro* culture, suggesting that the proteolytic activity pattern is a stable phenotypic characteristic of *Leishmania* parasites. In the present work, in order to detect variability in the protease expression of *Leishmania* species, a comparative analysis of proteolytic activities was performed. Zymographic assays were carried out over SDS- polyacrilamide gels (10%) copolymerized with 0.2% gelatin. Enzymes were characterized according to their pH range of activity and sensitivity to distinct protease inhibitors. Zymographic assays revealed that all analyzed species exhibit different activity profiles ranging from 50 to 200 kDa. Biochemical characterization showed that these enzymes belong to the metalloprotease class, presenting optimal activity in the pH range between 5.5 and 10.0. These results showed that different *Leishmania* species, including species in the New and Old world, can be distinguished by their proteolytic profile, indicating that zymographic analysis might be used as an additional tool for the characterization of parasites of this genus, as suggested for other trypanosomatids.

BQ09 - PHOSPHOPROTEOME APPROACH TO UNDERSTAND TGF-BETA RESPONSE IN *T. CRUZI* BIOLOGY

FERRÃO, P.M.[1], OLIVEIRA, F.L.[1], DA CUNHA-REIS, M.[1], WAGHABI, M.C.[1], MENDONÇA-LIMA, L.[1]
[1]Laboratório de Genômica Funcional e Bioinformática, Instituto Oswaldo Cruz, FIOCRUZ, Rio de Janeiro, Brasil.

Trypanosoma cruzi, the protozoan parasite that causes Chagas disease, possesses a complex life cycle involving different intracellular steps. The anti-inflammatory cytokine transforming growth factor beta (TGF-β) plays an important role, being involved on invasion, proliferation and differentiation steps during parasite cell cycle. *T. cruzi* gene expression is regulated primarily at the post-transcriptional level, making proteomics a promissing tool for the study of adaptative changes. The aim of the present work is to study differential expression of *T. cruzi* phosphoproteins in response to TGF-β addition to clarify its role on parasite biological events. Axenic epimastigotes from the Y strain, cultivated or not in the presence of TGF-β were prepared for two-dimensional electrophoresis (2-DE). Optimal TGF-β incubation time were assayed for differential protein induction. Comparative proteome analysis were carried out, revealing that few proteins present in 2-DE maps displayed significant differential expression due to the presence of TGF-β in cultures. A comparative method for phosphoprotein analysis was generated using 2-DE electrophoresis followed by ProQ diamond staining coupled to PhosphoProbe detection and Western-blot analysis. Mass spectrometry identification of differential protein spots is being carried out. Considering the crucial role of TGF-β on *T. cruzi* infection, the present study could help identify the molecules implicated in the parasite's response to this important cytokine and its implications on parasite biology. Financial Support: IOC-Fiocruz and CNPq

BQ10 - *T. CRUZI* SUBCELLULAR FRACTIONATION: A PROTEOMIC APPROACH

OLIVEIRA, F.L.[1], FERRÃO, P.M.[1], DA CUNHA-REIS, M.[1], WAGHABI, M.C[1]., MENDONÇA-LIMA, L.[1]
[1]Laboratório de Genômica Funcional e Bioinformática, Instituto Oswaldo Cruz, FIOCRUZ, Rio de Janeiro, Brasil.

Chagas disease is a widely distributed, debilitating disease process affecting 16-18 million people in Latin America, caused by the intracellular protozoan *Trypanosoma cruzi*. The completion of the *T. cruzi* genome project opened the possibility to address questions that could be investigated by protein expression studies using a proteomic approach. Several *T. cruzi* proteomic studies have been reported and recently the analysis of an enriched organellar fraction demonstrated that sample fractionation could potentialize the identification of proteins expressed in low levels. In this context, the aim of the present work is to characterize *T. cruzi* subcellular fractionation procedure by proteomic analysis. Protein extraction from axenic epimastigotes (Y strain) in the exponential phase were prepared for one and two-dimensional electrophoresis (2-DE) using the *Subcellular Protein Extraction Kit* from Calbiochem developed for mamalian cells. Briefly, the characterization of the fractionation procedure for trypanosomatidae is based on differential extraction in buffers specific for each fraction. The observation of protein patterns from the obtained fractions (soluble, membrane, nucleus and cytoskeleton proteins) was done by silver-stained 12% SDS/PAGE. Comparative proteomic analysis were carried out, revealing that proteins present in 2-DE maps displayed significant differential expression, confirming the one- dimensional data. To better characterize these fractions and confirm the subcellular fractionation procedure, Western-blot analysis is being carried out using specific antibodies for each fraction. In parallel, representative proteins spots from each subcellular fraction are being identified by mass spectrometry. The present study could help understand *T. cruzi* biology investigating the compartimentalization of differentially expressed proteins in a given studied moment, correlating to its cellular function. Financial support: IOC - Fiocruz

BQ11 - COMPARATIVE EXPRESSION AND POST-TRANSLATIONAL MODIFICATIONS OF EIF5A DURING *TRYPANOSOMA CRUZI* GROWTH

CHUNG, J, TONELLI, R.R., CASTILHO, B.A., SCHENKMAN, S.

Universidade Federal de São Paulo, São Paulo, Brazil.

Chagas' disease is caused by the protozoan parasite *Trypanosoma cruzi*, which is transmitted

by blood-sucking bugs of the subfamily Triatominae. In the gut of the insect vector, proliferating extracellular epimastigotes replicate and differentiate into the non-dividing infective form of metacyclic trypomastigotes. Post-translational modifications (PTM) of proteins are key events in intracellular signaling. One such modification is phosphorylation. In our laboratory, proteomic 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 highly conserved from Archaea to mammals, and although it was identified as a translation initiator factor, no evidence supported it is involvement in this process. The protein is also subjected to hypusination a modification dependent on the polyamine spermidine. In the present work we compared the presence of these PTMs and the expression of eIF5A between epimastigotes at different growth phases. The large difference found in growing versus arrested cells suggests that phosphorylation of eIF5A may be a key factor for growth control in this parasite.

Supported by FAPESP and FINEP

BQ12 - IDENTIFICATION OF POST-TRANSLATIONAL MODIFICATIONS IN H49-CALPAIN LIKE PROTEINS OF *Trypanosoma cruzi*

GALETOVIĆ A[1], SANTOS, M.R[2], CORDERO, E.M[1], DA SILVEIRA, J.F[1]
1. Depto de Microbiologia, Imunologia e Parasitologia, Universidade Federal de São Paulo
2. Universidade Bandeirante de São Paulo

The H49-calpain like cysteine peptidases are part of a novel protein family associated to cytoskeleton of *T. cruzi*. Previous results from our laboratory suggested that H49-calpain like proteins are involved in the attachment of the flagellum to the cell body. Sequence analysis showed that they contain a catalytic domain (Cys Pc) and blocks of tandemly arranged repeats of 68 amino acids (aa). In this work, we studied the presence of post-translational modifications in H49-like proteins by 2-dimensional gel electrophoresis and western blotting with monoespecific antibodies against the Cys Pc catalytic domain and 68-aa repeats. Protein extracts from epimastigotes and metacyclic trypomastigotes of G and CL strains were firstly submitted to isoelectric focusing electrophoresis (pH gradient 4-7) and to SDS-PAGE (6%) gel electrophoresis (second dimension). Polyclonal rabbit antibodies raised against the Cys Pc domain reacted with several 50 kDa proteins (pH 4.5-5) and 60 kDa proteins (pH ~5.5), respectively, in metacyclic trypomastigotes and epimastigotes of both strains. In contrast, polyclonal rabbit antibodies raised against 68-aa repeats reacted with high molecular weight proteins (>200 KDa) focused on over wide pH range in metacyclic forms. These results indicate that only the high molecular calpain like proteins carry the 68-aa repeats. We showed that H49-calpain like proteins presented self-proteolysis on pH 5 in both forms of the two strains which could be due to catalytic activity of the molecule. These results indicate that H49-calpain like cysteine peptidases undergo post-translational modifications in epimastigote e metacyclic forms and suggest that H49-calpain like cysteine peptidases are modified in a stage-specific manner. The role of post-translational modification will be investigated in our laboratory.

Support: Beca Presidente de la República-Chile, FAPESP, CNPq, CAPES.

BQ13 - CELLULAR LOCALIZATION AND EXPRESSION OF GP63 HOMOLOGOUS METALLOPROTEASES IN *Leishmania (Viannia) braziliensis* STRAINS

MESQUITA-RODRIGUES C.[1], CUERVO P,[1,2]; SANTOS A.L.S[3]; ALVES C.[4]; MENEZES G.C.[5]; SILVA B.A[3]; BRITTO C.[4]; FERNANDES O.[2]; CUPOLILLO E.[1]; DE JESUS J.B.[4,6]

[1]Laboratório de Pesquisas em Leishmanioses, Instituto Oswaldo Cruz, FIOCRUZ, Rio de Janeiro; [2]Laboratório de Epidemiologia Molecular de Doenças Infecciosas, Instituto Oswaldo Cruz, FIOCRUZ, Rio de Janeiro; [3]Departamento de Microbiologia Geral, Instituto de Microbiologia Prof. Paulo de Góes, UFRJ, Rio de Janeiro; [4]Laboratório de Biologia Molecular e Doenças Endêmicas, Instituto Oswaldo Cruz, FIOCRUZ, Rio de Janeiro; [5]Laboratório de Biologia da Superfície Celular, Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Rio de Janeiro; [6]Departamento de Ciências Naturais, Universidade Federal de São João del Rei, São João del Rei, MG, Brasil.

Introduction: *Leishmania* (*Viannia*) *braziliensis* is the major causative agent of American tegumentary leishmaniasis, a disease that

encompasses a broad spectrum of clinical manifestations. In a previous study, we showed that Brazilian and Colombian *L. braziliensis* strains, isolated from patients with distinct clinical manifestations, display different pattern of metalloprotease activities. Following these results, we investigated the cellular localization of these molecules and their relation to the major surface protease (gp63) of *Leishmania.* Comparative analyses of metalloprotease expression among different clinical isolates as well as an evaluation of the effect of long-term *in vitro* passage on the expression pattern of these metalloproteases were also performed. **Methods and Results**: Western blot analysis, using an anti-gp63 antibody, revealed polypeptide patterns with a similar profile to that observed in zymographic analysis. Flow cytometry and fluorescence microscopy analyses corroborated the presence of metalloproteases with homologous domains to gp63 in the parasites and revealed differences in the expression level of such molecules among the isolates. The cellular distribution of metalloproteases, assessed by confocal analysis, showed the existence of intracellular metalloproteases with homologous domains to gp63, predominantly located near the flagellar pocket. **Conclusions**: It was observed that differential zymographic profiles of metalloproteases exhibited by *L.* (*V.*) *braziliensis* isolates remain unaltered during prolonged *in vitro* culture, suggesting that the proteolytic activity pattern is a stable phenotypic characteristic of these parasites.

BQ14 - Biochemical characterization of the serine transport in Leishmania (Leishmania) amazonensis

dos Santos, MG [1]; Paes, LS [2]; Zampieri, RA [1]; da Silva, MFL [1]; Silber, AM [2]; Floeter-Winter, LM [1]

[1]Instituto de Biociências, Universidade de São Paulo, São Paulo, Brasil.
[2]Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, Brasil.

The role of amino acids in trypanossomatids goes beyond protein synthesis, involving processes such as differentiation, osmoregulation, energy metabolism and precursors of other metabolites. The availability of the amino acids involved in those functions depends, among other things, on their transport into the cell. As serine is a precursor for the synthesis of phosphatidylserine, which is involved in the invasion and inactivation of the host macrophage, we characterized the serine transport in *Leishmania (Leishmania) amazonensis* by measuring the uptake of labeled serine by promastigotes and amastigotes. Kinetic data show a single saturable system with Km of 0.82598 +/- 0.18325 mM and maximum velocity of 355.37 +/- 19.41 pmol/min x 10^7 cells for promastigotes. These values are greater then the values described for the arginine transport in *Leishmania donovani* (Km=0,01428mM and Vmax= 33,2 pmol/min x 2 x 10^7cells), but are of the same magnitude of the proline transport described for *Trypanosoma cruzi* (Km=0,3 and Vmax=98,34pmol/min x 2 x 10^7cells). The serine transport increased linearly with temperature in a range from 20 to 45 ºC, allowing the calculation of the activation energy 7.09 kJ/mol. Serine uptake was dependent on pH, with an optimum activity at pH 7.5. Alanine, cysteine, glycine, threonine, valine and ethanolamine competed with the substrate in a ten-fold excess concentration, suggesting that the serine uptake is driven by a broad specificity transport system. The characterization of the serine transport process in amastigotes revealed a transport system with a similar Km, energy of activation and pH response to that found in promastigotes, suggesting that the same transport system is active in both insect vector and mammalian host *Leishmania* stages.
Supported by FAPESP and CNPq.

BQ15 - THE MAJOR NEUTRAL LEUCYL AMINOPEPTIDASE OF TRYPANOSOMA CRUZI (LAPTC) BELONGS TO THE PEPTIDASE FAMILY M17

Thiago Santana Gastardelo[1], Gloria Restrepo-Cadavid[1], Izabela Bastos[1], Teresa Assumpção[1], Carlos R. Félix[2], Bergmann M. Ribeiro[3], and Jaime M. Santana[1].

[1]Laboratório de Interação Parasito-Hospedeiro, Universidade de Brasília; [2]Laboratório de Enzimologia, Universidade de Brasília; [3]Laboratório de Microscopia Eletrônica, Universidade de Brasília. jsantana@unb.br

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 *Trypanosoma cruzi* using the fluorogenic substrate leucine-7-amido-methylcoumarin. This activity was

isolated from cell-free extract from epimastigote forms of the parasite by a two-step chromatographic procedure and associated with a single ~220 kDa homo-tetrameric protein. Molecular and enzymatic analyses indicated that this leucyl aminopeptidase of *T. cruzi* belongs to the peptidase family M17 or leucyl aminopeptidase (LAP) family and was named LAP-Tc to indicate its activity. Its sequence shares 43 to 47% 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 recombinant LAPTc also assembles into homo-oligomer, has neutral/alkaline pH and displays maximal activity at 60 °C. Immunofluorescence assay revealed that LAPTc is located inside vesicles in the cytoplasm of *T. cruzi* developmental forms. Gel zymographic experiments indicated that the enzyme retains its oligomeric structure up to 80 °C. The activity of this metalloaminopeptidase 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 biossinthetic 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. Supported by Finep, CNPq, CAPES and FAP-DF.

BQ16 - MITOCHONDRIAL BIOENERGETICS IN *TRYPANOSOMA CRUZI* OVEREXPRESSING THE CYTOSOLIC TRYPAREDOXIN PEROXIDASE

PELOSO, E. F[1]., VITOR, S. C. [1], ALVES, M. J. M.[2], COLLI, W.[2], GADELHA, F. R[1].

[1]Instituto de Biologia, Departamento de Bioquímica, UNICAMP, São Paulo, Brazil.
[2]Instituto de Química, USP, São Paulo, Brazil.

Cytosolic tryparedoxin peroxidase (TcCPx) plays a fundamental role in *Trypanosoma cruzi* antioxidant system where it participates in hydrogen peroxide (H_2O_2) detoxification. This pathway differs from the mammalian host offering new options for the development of a more specific therapy for Chagas' disease. The purpose of this work was to compare oxygen (O_2) consumption, H_2O_2 and superoxide ($O_2^{\cdot-}$) generation and mitochondrial membrane potential ($\Delta\Psi$) between TcCPx overexpressing (pTEX-TcCPx) and control (pTEX) cells. No significant differences were observed in succinate supported O_2 consumption (9.1 ± 0.5 and 8.4 ± 1.0 nmol O_2 consumed/min.10^8 cells for pTEX and pTEX-TcCPx, respectively) and respiratory control (1.5 ± 0.1 and 1.5 ± 0.2 for pTEX and pTEX-TcCPx, respectively). Using malate and pyruvate as substrate for the respiratory chain (RC), a decrease in H_2O_2 production was observed in pTEX-TcCPx cells (47%) when compared to pTEX cells, even upon RC inhibition by antimycin A (AA) (35% lower). A 48% increase in $O_2^{\cdot-}$ generation upon RC inhibition by AA was observed for both groups. Regarding $\Delta\Psi$, both groups showed no significant differences even in the presence of the inhibitor thenoyltrifluoroacetone (TTFA). TcCPx overexpression did not alter mitochondrial bioenergetics but led to a lower H_2O_2 generation, reinforcing the role of that enzyme in hydroperoxide detoxification.

Supported by: CAPES, SAE-UNICAMP and FAPESP.

BQ17 - MITOCHONDRIAL BIOENERGETICS AND ROS GENERATION ALONG THE GROWTH CURVE OF TWO *TRYPANOSOMA CRUZI* STRAINS

HULSHOF, L.; SILVA. T. M.; ARANTES, S. C.; PELOSO; E. F.; GADELHA, F. R.

Universidade Estadual de Campinas, São Paulo, Brazil.

Biochemical and molecular variations confer a high degree of heterogeneity among *Trypanosoma cruzi* strains, which represents a challenge for the development of an effective therapy for Chagas' disease. Due to the central role of mitochondria on parasite survival, mitochondrial bioenergetics studies are fundamental for a better understanding of the characteristics of the parasite. The objective of this work was to compare mitochondrial bioenergetics in two different strains of *T. cruzi*, Tulahuen 2 and Y. The parameters analyzed along the growth curve (3, 5 and 7 days (log, early stationary and late stationary phase, respectively)) were oxygen consumption, mitochondrial membrane potential ($\Delta\Psi$) and reactive oxygen species (ROS) generation. Results showed no significant difference on oxygen consumption using different respiratory chain substrates in each growth phase for both strains. A 100% increase on oxygen consumption was observed in Y strain on the late stationary phase in comparison to the

other phases. Also, along the growth curve, Y had lower oxygen consumption when compared to the other strain (58, 71 and 30% on 3, 5 and 7 days, respectively). In both strains it was observed a significant decrease in the respiratory control rate when cells reached the late stationary phase. No difference was observed in $\Delta\Psi$ determined by flow cytometry between strains along the growth curve. Superoxide (O_2^-) generation was significantly higher in Y strain (228%) when compared to Tulahuen 2 in the late stationary phase. Thenoyltrifluorocetone did not increase O_2^- generation compared to the control in both strains. Hydrogen peroxide (H_2O_2) generation decreased significantly from log to late stationary phase in Y strain, while in Tulahuen 2 it was not possible to detect H_2O_2 production under the same conditions. The data obtained reinforce the heterogeneity among *T. cruzi* strains.

Supported by: CAPES and PIBIC-CNPq.

BQ18 - PRELIMINARY CHARACTERIZATION OF THE Δ^1-PYRROLINE-5-CARBOXYLATE DEHYDROGENASE OF *TRYPANOSOMA CRUZI*

SUÁREZ, B.A; PAES, L.S; SILBER, A.M[1].

[1]*Departamento de Parasitologia – Instituto de Ciências Biomédicas – Universidade de São Paulo, São Paulo, Brazil.*

Δ^1- pyrroline-5-carboxylate dehydrogenase (P5CDh) (E.C. 1.5.1.12) is the second enzyme of the proline catabolic pathway. It catalyzes the conversion of pyrroline-5-carboxylate (P5C) into glutamate with $NAD(P)^+$ dependence. Previous studies demonstrated that proline induces metacyclogenesis as well as its involvement in the intracellular differentiation from epimastigote-like form to trypomastigote stage. Besides, it was shown for other organisms that this amino acid is involved in the resistance to several stress conditions. We identified on the *T. cruzi* genome databases the gene *TcP5CDh*, encoding for P5CDh. When amplified by PCR, a 1.7 kb fragment was obtained, which was cloned and sequenced. The activity of its product was initially evaluated by a complementation assay on a knock-out strain of *Saccharomyces cerevisiae* for the orthologue gene *PUT2*. The expression analysis performed by real time PCR in the five means *T. cruzi* stages revealed that the highest expression levels of *TcP5CDh* gene corresponded to the intracellular epimastigote-like form, the stage showing the lowest intracellular free proline content and the highest proline uptake activity as previously shown. The measure of the P5CDh activity in epimastigote extracts using glutamate and NAD(P)H as the substrates adjusts to a classical model of exponential decay. Those experiments will be used as a tool to correlate the *TcP5CDh* enzymatic activity with other physiological functions using the complemented yeast model.

-Financial support: CNPq, FAPESP.

BQ19 - DETERMINATION OF OXYGEN CONSUMPTION, MITOCHONDRIAL MEMBRANE POTENTIAL AND NADPH PRODUCTION IN *TRYPANOSOMA CRUZI* STRAINS ISOLATED FROM CARDIAC AND ASYMPTOMATIC PATIENTS

MENDONÇA, M. A.[1], *OLIVEIRA T. C.*[1], *KOWALTOWSKI, A. J.*[2] & *GADELHA F.R*[1]

[1]*Universidade Estadual de Campinas, São Paulo, Brazil;*
[2] *Departamento de Bioquímica, Instituto de Química, Universidade de São Paulo, S. Paulo, Brazil.*

We have previously reported that some *T. cruzi* strains present a 455-bp deletion in the center of the maxicircle gene that code for NADH dehydrogenase subunit 7 (ND7) (Baptista et al., 2006). The aim of this work was to analyze mitochondrial bioenergetics and NADPH production by the pentose phosphate pathway (PPP) in three strains with the wild type gene (wt) (CL Brener, Esmeraldo and 115) and two strains with the *ND7* deletion (Δ) (FAMEMA and VL10) The parameters studied were (i) oxygen consumption using a Hansantech oxygraph; (ii) mitochondrial membrane potential using safranin O as a probe, and (II) NADPH production by the PPP, as described by Mielniczki-Pereira et al, 2007. No over differences in respiratory rates were observed between the strains tested. Significant differences were observed in safranin uptake, among the isolates when succinate was used as substrate. Upon addition of ADP, significant differences were observed between CL Brener and 115, with all substrates used (succinate, malate/pyruvate and malate/pyruvate in the presence of malonate). Regarding NADPH production no correlation could be established either, since FAMEMA (Δ) and Esmeraldo (wt) had a high activity (31±1.7 and 29±1.7 nmoles NADPH

produced/min.10^8cells, respectively), whereas VL10 (Δ) and 115 (wt), a low activity (9±0.8 and 16±0.6 nmoles NADPH produced/min.10^8cells, respectively). These findings indicate that the deletion in *ND7* does not affect the mitochondrial parameters under study and the NADPH production by the PPP.

Supported by: PIBIC and MCT/CNPq (Edital Estudo de Doenças Negligenciadas)

BQ20 - Proteases analyses of congenital Chagas causing *Trypanosoma cruzi* strains reveal a high molecular weight cysteine-protease activity.

Patrícia Fampa[1], Anderson A. Moura[1], Fernando Sánchez Valdez[2], Cintia Fernández[2], Olga Sánchez Negrette[3], Miguel Ángel Basombrío[2], Maria Celia Mora[2] & Marcel I. Ramirez[1].*

1- Laboratório de Biologia Molecular de Tripanosomatídeos e Flebotomíneos, Instituto Oswaldo Cruz – FIOCRUZ. Av. Brasil, 4365, 21045-900; Rio de Janeiro, Brasil.
2- Instituto de Patología Experimental, Facultad de Ciencias de la Salud, Universidad Nacional de Salta, Av. Bolivia, 5150, Salta, Argentina.
3- Primer Nivel de Atencion en Salud, Ministerio de Salud Publica de Salta, Argentina.

**Corresponding author: marcelr@fiocruz.br*

Due to the increasing control of the transmission of *Trypanosoma cruzi* mediated by vector, blood transfusion and organ transplant, congenital Chagas disease (CCD) has emerged as a public health problem. The transmission rates of CCD vary in different geographical areas, ranging from 0.1% in regions of Brazil and Argentina to 9% or more in some areas of Bolivia, Chile and Paraguay.
Little is known about the mechanisms of transplacental transmission, however, Congenital Chagas disease may result from a complex equilibrium between maternal immune response, placental factors and features of the parasitic strains.
In order to understand the parasite factors that could be involved with the ability to cross the transplacental barrier, we have started a biochemical and biology characterization of 20 congenital Chagas causing *T. cruzi* strains, collected from new-born or children patients from Salta, Argentina. Interestingly, Triatomine vectors were eradicated from Salta city and no reinfestations have been registered, being congenital transmission the most important way of infection.

These parasite strains, previously typed as TCIId, have been assayed by proteases gelatinolytic activity gels (zymography), followed by incubation with digestion buffers of pH 5.5 and 10.0 for 24 or 48h, respectively. Active proteases at acid pH are cysteine-proteases and those active at alkaline pH are metalloproteases, as demonstrated by specific inhibitors utilization. We have found that:

I)All of the isolates showed higher protease activities when compared to other wild isolates studied in our lab before and,

II) All of them present a high weight molecular cysteine-protease band (around 100 KDa), besides the ubiquitous cruzipain 50 KDa one.

We are purifying and identifying this high-weight molecular band (in progress) and we intend to characterize its putative role in transplacental barrier crossing by the parasite trough *in vitro* and *in vivo* assays.

Supported by: CNPq, FIOCRUZ & FAPERJ.

BQ21 - PROBING MITOCHONDRIAL COMPLEX I OF *TRYPANOSOMA CRUZI*

CARRANZA, J.C.[1,2], SILVA, MN.[1], KOWALTOWSKI, A.J.[1], ZINGALES, B.[1]

[1]Departamento de Bioquímica, Instituto de Química, Universidade de São Paulo, S. Paulo, Brasil; [2]Universidad del Tolima, Ibagué, Colombia.

Introduction: Complex I is a multimeric enzyme located in the mitochondrial inner membrane that catalyzes electron transfer from NADH to coenzyme Q. Complex I is also an important site of superoxide production within mitochondria. The functionality of complex I in trypanosomatids is debated. We showed that maxicircle genes coding for two subunits of complex I (ND4 and ND7) present deletions in some *T. cruzi* strains. **Objectives**: The goal of this study was to characterize the respiratory/bioenergetic activity and generation of mitochondrial hydrogen peroxide in parasites with wild type and deletion-bearing *ND* genes. **Results and conclusions:** Digitonin-permeabilized epimastigotes of CL Brener and 115 (wild type genes), Esmeraldo (deleted *ND4*), VL10

and Famema (deleted *ND7*) were probed for mitochondrial respiration. Measurements of oxygen consumption were made with a Clark-type electrode fitted to a HansaTech oxygraph. The respiratory control ratio (RCR) (state III/ state IV) was determined. With complex I substrates (5mM piruvate; 5mM malate), no significant differences in the RCR values (~2.2) of the strains were observed. The same results were obtained for RCR (~2.1) with complex II substrate (5mM succinate). The data indicate that either the deletions in the *ND* genes do not affect complex I activity, or that complex I is non-functional and NADH may be oxidized by fumarate reductase (FR). To test this hypothesis, RCR was determined in the presence of the complex II inhibitor malonate (10mM). The data confirm that NADH is mostly oxidized by FR. Mitochondrial production of H_2O_2 was also measured. We observed no correlation between the amount of peroxide produced and *ND* deletions. The lowest H_2O_2 level was detected in CL Brener (~12 $pmoles.min^{-1}.mg^{-1}$) and the highest, in 115 (~21 $pmoles.min^{-1}.mg^{-1}$). It is concluded that complex I displays very low activity in *T. cruzi* epimastigotes. **Support**: FAPESP; Edital MCT/CNPq/MS-SCTIE-DECIT 25/2006-Estudo de Doenças Negligenciadas.

BQ22 - Identification of immunodominant proteins complex of 30-34 KDA of *Trypanosoma cruzi* (Y strain) by two-dimensional electrophoresis and mass spectrometry

da costa, g. c. v.[1], *von kruger, w. m. a.*[2], *lery, l*[2], *peralta, j. m.*[1]

[1]*Instituto de Microbiologia Prof.Paulo de Góes-UFRJ, Rio de Janeiro, Brazil.* [2]*Instituto de Biofísica Carlos Chagas Filho - UFRJ, Rio de Janeiro, Brazil*.

Chagas' disease is a serious problem of public health in Latin America. Detection of antibodies anti-*T. cruzi* by serological techniques is the best choice for diagnosis of infection in the chronic fase of the disease. However, with the absence of a gold standard test and problems with specificity when total extracts are used as antigen, the production of new recombinant and synthetic peptides are stimulated to be develop. Parasite proteins of 30 to 34 kDa have demonstrated an excellent specificity when immunoblot analysis were done. Then further characterization studies with such proteins are needed. Proteomic approaches have been successfully used as tools for discovery new antigenic molecules in some parasite agents. In this work, we first compare different caotropic substances and detergents for solubilization of epimastigote *Trypanosoma cruzi* (Y strain) protein extracts for application in two-dimensional electrophoresis gels. Following, immunoblotting assay was used to identify immunoreactive spots and to compare the results with stained gels. Subsequently, spots were excised and peptides analysed by mass spectrometry (MALDI-TOF/TOF). The results showed that addition of ASB-14 (1%) detergent in combination with CHAPS (3%) in the solubilization buffer increase the number of spots and the resolution in the total epimastigote protein extract analysis. Using these conditions, four parasite proteins between 30 to 34 kDa were identified: methylthioadenosine phosphorylase, peptidase M20/M25/M40, arginase and cathepsin B, with isoelectric point between 5.1 and 6.6. These proteins were not demonstrated by early studies. Immunoproteomic mapping with serum samples from *T. cruzi* infected patients showed a significantly heterogeneity of reactivity profiles with 30 to 34 kDa proteins, although one spot was recognized by all serum samples.

FINANCIAL SUPPORT: CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) e FAPERJ (Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro)

BQ23 - EFFECT OF SPHINGOLIPIDS SYNTHESIS INHIBITORS ON *Trypanosoma cruzi*

BRITO, T.K.; BERTINI, S.; TAKAHASHI, H.K.; STRAUS, A.H.

Department of Biochemistry, Universidade Federal de São Paulo, São Paulo-SP, Brazil

Sphingolipids have been related with several biological processes, such as intracellular transport, modulator of signal transductions, and apoptosis mediator. Glycosyinositolphosphorylceramides (GIPCs) are the main glycolipid expressed in *T. cruzi* (strain CL) epimastigotes. In the present study it was analyzed the effect of myriocyn (inhibitor of serine palmitoyltransferase), fumonisin B (inhibitor of ceramide synthase) and Aureobasidin A (inhibitor

of inositolphosphorylceramide synthase) on epimastogotes growth, morphology, and sphingolipids expression. Aureobasidin A (10 µM) inhibited completely epimastigotes growth and myriocyn (10 µM) inhibited about 30% of parasite growth. Conversely no effect on promastigote growth was observed when fumonisin 50 µM was added to the cultures. Parasites treated for three days with Aureobasidin A 10µM became smaller and presented a rounded body when compared to control parasites. In order to understand the effect of Aureobasidin A on epimastigotes, parasites were cultivated in presence of Aureobasidin A 0.1 µM (concentration that inhibits 75% of epimastgotes growth) and no significant reduction of inositolphosphorylceramide and glycolipid expression was detected by thin layer chromatography. Since GIPCs represent the major sphingolipids in *T. cruzi* epimastigotes (strain CL), it will be analyzed if glycolipids synthesized in presence of Aureobasidin A or other sphingolipids inhibitors are glycosylinositolphosphoglycerolipids or glycosylinositolphosphoceramides. It was also tested Aureobasidin A (20 µM) and Fumonisin (20 µM) in Vero cell infected with trypomastigotes, and a significant reduction of number of parasites per infected cell (90% and 65%, respectively) was observed.
Supported by FAPESP. CAPES, CNPq, and FADA.

BQ24 - MILTEFOSINE AS A TOOL TO STUDY PHOSPHOLIPID BIOSYNTHESIS IN ENDOSYMBIONT-BEARING TRYPANOSOMATIDS

FREITAS-JUNIOR, P.R.G.[1]; AZEVEDO-MARTINS, A.C.A.[1]; CAVALCANTI, D.P.[2]; CATTA-PRETA, C.M.C.[1]; PROBST, C.[3], KRIEGER, M.A.[3], DE SOUZA, W.[1,2]; EINICKER-LAMAS,M.[1]; MOTTA, M.C.M.[1]

[1]Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil
[2]Instituto Nacional de Metrologia, Normalização e Qualidade Industrial, Rio de Janeiro, Brazil
[3]Instituto de Biologia Molecular do Paraná, Curitiba, Brazil

Crithidia deanei is a trypanosomatid that maintains a mutualistic relationship with an intracellular bacterium. The endosymbiont envelope lacks sterols; the phospholipid composition is characterized by a major quantity of cardiolipin (CL), followed by phosphatidylcholine (PC) and phosphatidylethanolamine (PE). In prokaryotes, PC is present only in species closely associated with eukaryotes. Miltefosine impairs the PC biosynthesis in mammalian cells by inhibiting the translocation of CTP phosphocholine citidyltransferase (CCT), a key enzyme in the Kennedy pathway, from its inactive cytosolic form to its active membrane-bound form. In trypanosomatids, this drug affects the choline transport and the activity of the phosphatidylethanolamine N-methyl-transferase, an enzyme which composes the Greenberg pathway, for PC biosynthesis in prokaryotes and lower eukaryotes. In this work, we used the miltefosine as a tool to study phospholipid biosynthesis in *C. deanei*. Our results showed a low effect of this drug on protozoan proliferation after treatment for 24h with different drug concentrations. However, when higher concentrations were used, cell proliferation was strongly inhibited. Assays with aposymbiotic cells were also performed in order to verify if endosymbiont influences phospholipid biosynthesis. Data obtained by transmission electron microscopy, showed ultrastructural effects of miltefosine on protozoa, as plasma membrane shedding and blebbing, mitochondrial swelling and convolutions of endosymbiont envelope. Biochemical analysis were performed to check the phospholipid composition of the protozoa and its symbiontic bacterium, revealing that miltefosine affected the phospholipid biosynthesis on *C. deanei*. Search on the endosymbiont genome annotation database, did not identify sequences that share homology with enzymes involved in PC biosynthesis, but a sequence of a putative phospholipase D was found and may participates in change of polar head between phospholipids. Insights about mechanisms of phospholipid biosynthesis in *C. deanei* might help us to understand the establishment of symbiosis in trypanosomatids.
Supported by: CNPq, FAPERJ, CAPES.

BQ25 - AVALIATION *IN VITRO* OF L-THIAZOLIDINE-4-CARBOXYLIC ACID ON THREE SPECIES OF TRIPANOSOMATIDS

SUÁREZ, B.A[1]*; GODOY, P.D.M* [2]*; TAUNAY-RODRIGUES. A*[1]

[1] *Departamento de Parasitologia – Instituto de Ciências Biomédicas – Universidade de São Paulo, São Paulo, Brasil.*
[2] *Laboratório de Parasitología – Instituo Butantã, São Paulo, Brasil.*

The pathogenic species of genus *Leishmania* and *Trypanosoma* currently affects millions people worldwide. Several metabolic pathways had been suggested as targets for new drugs. Particularly, the L-proline is metabolized by many species of the Kinetoplastidia order. It is also involved in many biological processes such as metacylogenesis, intracellular differentiation, osmoregulation, the feeding of Kreb´s cycle with intermediary metabolites. The use of amino acid analogues may interfere with different metabolic pathways and synthesis of proteins in several prokariotic and eukariotic organisms. The aim of this work was to evaluate the activity of the L-proline analogue L-thiazolidine-4-carboxylic acid (T4C) on three pathogenic trypanosomatids species. We used the replicatives forms of *Trypanosoma cruzi*, *Trypanosoma. brucei* and *Leishmania amazonensis* cultured in media LIT, 199 and ME-99, respectively. The effect of the analog by itself and combined with two stress conditions (temperature 37 ºC and pH 5,5) was analyzed. The T4C concentrations are ranged between 0.1, and 10 mM using a rotenone (200 μM) and antymicin (0,5 μM) mixture as positives controls. The data were obtained daily, monitoring the absorbance change to 620 nm in 96 well-plates during seven days. The dose–response on growing showed IC_{50} of 3.5 ± 0.2 mM, 1,08 ± 0,07 mM, 1.5 ± 0.2 mM for *T. cruzi*, *T. brucei* and *L. amazonensis*, respectively. The T4C showed a synergistic effect with pH and temperature stresses on the three species, showing a highest sensitivity for *L. amazonensis*. When comparing the cell growing with both stress conditions, statistical differences were found ($p \leq 0{,}007$). Our *in vitro* results showed that T4C affects the cell growing of the species tested, and the combined effect under stress conditions increases this activity. These data show that T4C is promising as a trypanocid compound, and further experiments will be done to confirm its potential as a therapeutic drug.

Financial support: FAPESP, USP.

BQ26 - Enhancement of 3’ nucleotidase activity in *Leishmania chagasi* grown in culture medium in the absence of added inorganic phosphate

Vieira, D. P.[1]*, Lopes, A. H. C. S.*[1] *and Meyer-Fernandes, J. R.*[2]

[1]*Instituto de Microbiologia Prof. Paulo de Góes, UFRJ;* [2]*Instituto de Bioquímica Médica, UFRJ; Cidade Universitária, Ilha do Fundão, Rio de Janeiro, R.J. 21941-590, Brasil.*
E-mail: meyer@bioqmed.ufrj.br

Visceral leishmaniasis (VL) is a systemic protozoan disease that is transmitted by phlebotomine sandflies. In the New World, this form of leishmaniasis is caused by *Leishmania chagasi.* Some tripanosomatids, including *Leishmania* species, possesses a unique bifunctional externally oriented cell surface membrane enzyme 3’-nucleotidase/ nuclease which is involved in the salvage of host-derived purines. Purine salvage is critical for these parasites survival because they are incapable of *de novo* purine synthesis. In this work, we analysed the growth and enzymatic activity of *Leishmania chagasi* modulated by the absence or the presence of added inorganic phosphate in the culture medium. The parasites were grown in the absence (2 mM Pi) or in the presence of added inorganic phosphate (83 mM) and the cell growth was daily determined by counting the number of parasites in a Neubauer chamber. The parasites grown in the absence of added phosphate inorganic presented a reduction of growth (50 %) as compared to the cells grown in the presence of this phosphate source. Also, several enzymes activities were assayed, including ecto-ATPase, ecto-ADPase and ecto-5’nucleotidase, whose did not presented significative differences in the activity assays between the cells grown in the absence and in the presence of added inorganic phosphate. In the other hand, the ecto-3’nucleotidase activity assay showed a two-fold increase in the enzyme activity when *L. chagasi* was grown in the absence of added inorganic phosphate as compared to the cells grown in the presence of this phosphate source. These results are suggestive that absence of inorganic phosphate addition in the culture medium modulates some signaling pathways leading to an increase in ecto-3’nucleotidase activity and culminating in reduction of cellular proliferation in *Leishmania chagasi*.

Supported by: CNPq, FAPERJ.

BQ27 - *LEISHMANIA INFANTUM* DISPLAYS LOW LEVEL OF POLYMORPHISM IN ITS MAJOR SURFACE MOLECULE LIPOPHOSPHOGLYCAN (LPG)

Junara Miranda Coelho[1],Vanessa Cabreira de Freitas[1], Rafael Ramiro de Assis[2], Natalia Novozhilova[3] , Maria Norma Melo[2], Lenea Campino[4], Paulo Pimenta[1], Salvatore Joseph Turco[3], Rodrigo Soares[1]

1Centro de Pesquisas René Rachou; 2 Universidade Federal de Minas Gerais; 3 University of Kentucky; 4 Universidade Nova de Lisboa

The LPG of *Leishmania* is a multivirulent factor that enables the parasites to survive and develop both in vertebrate and invertebrate hosts. Modifications of the basic LPG phosphoglycan (Gal-Man–P repeat units) backbone by side chain sugars play important roles in parasite survival and sand fly specificity. However, it is still unknown the level of polymorphism in this moiety in a given species. In this project, the level of variability in 20 *L. infantum* strains (16 from Brazil and 4 from Portugal) was evaluated. New World *L. infantum* strains were first named *L. chagasi*. However, molecular studies have defined them as being the same species. In a previous work, *L. chagasi* (PP75 strain from Brazil), the side chains consisted primarily of one β-1,3 glucose residues, being very similar to *L. mexicana* LPG. Here, it was characterized the repeat units of Brazilian strains from different regions (BACV, JP15, 211, 268, 269, 241, 291, 640, BA262, BH46, PP75, LTA110, 2507, 2566, 2504, 0957) e Portuguese strains (LIPA 116, IPT1, Pharoah and IMT254). LPGs were purified and subjected to western-blot, fluorophore-assisted carbohydrate electrophoresis (FACE) and capillary electrophoresis (CE). To determine side chain substitutions, enzymatic treatment and high performance liquid chromatography (HPLC) were performed. It was observed three profiles on *L. infantum* LPG: (1) no side-chain substitution (90% of the strains); (2) with one side-chain (5%, strain PP75) and (3) with more than one side-chain (5%, BH46). Our results showed that the level of polymorphism in *L. infantum* is low and most of Brazilian strains and all Portuguese strains were devoid of side-chains. HPLC analysis revealed the presence of glucosylation in PP75 and BH-46 strain and background levels in the HP-EMO strain. The presence of glucoses in the repeat units of *Leishmania* seems to be a constant feature only in the LPG of New World strains.

BQ28 - RESISTANCE INDUCED BY KETOCONAZOLE PRESSURE IN *Leishmania amazonensis* INVOLVES CHANGES IN LIPID COMPOSITION AND OVER-EXPRESSION OF C-14 DEMETHYLASE

ANDRADE-NETO, V.V[1], PACIENZA-LIMA, W[2], GUEDES, H.L[1,2] CUNHA-JÚNIOR, E.F.[1], ATELLA, G.[2], CANTO-CAVALHEIRO, M.M.[1] TORRES-SANTOS, E.C[1]
[1]Instituto Oswaldo Cruz, Rio de Janeiro, Brazil; [2]Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil

Ergosterol biosynthesis route is a valuable target to the treatment of leishmaniasis. The antifungical azoles, which inhibit the C14α-demethylase, have shown strong *in vitro* activity on *L. braziliensis* and *L. amazonensis*. Despite the mechanism of action of these agents to be very well studied, the leishmanial resistance has not been aimed yet. Here, we investigated the *in vitro* acquired resistance mechanisms of leishmania to azoles. *L. amazonensis* promastigotes were grown in Schneider plus 10 % fetal calf serum (FCS) with increasing concentrations of ketoconazole. The drug pressure begun with the IC50 (1 μM) and the concentration was increased step by step, each other passage, until 12 μM. In order to investigate the differences in lipid composition between the wild and resistant strains, both parasites were cultured in Schneider plus 10 % FCS for 96 h. After extensive washing in saline, the cells number was adjusted. Lipids were extracted with chloroform/methanol (2:1), dried with N_2 and then submitted to thin-layer chromatography (TLC) in hexane:diethilether:acetic acid (60:40:1) followed by hexane:chloroform:acetic acid (80:20:1), and visualized with Charring reagent. The TLC bands were then analyzed by densitometry. An overall change in lipid composition of resistant strain was observed, with reduction of endogenous sterols (63%), di- and triglycerides (66% and 14%) and fatty acids (62%) and increase of phospholipids (11%) and monoglycerides (54%). In addition, mRNA expression of C-14 demethylase gene was studied in both wild and resistant strains by RT-PCR. Significantly higher C-14 demethylase gene expression was observed in ketoconazole-resistant leishmania in relation to wild type, and no difference was observed using actin gene

expression as control. These results suggest that the ketoconazole induced resistance in leishmania could be multifactorial, involving adaptation in membrane lipid composition and over-expression of the target enzyme as adaptative mechanisms to overcome the drug ergosterol biosynthesis inhibition. FAPERJ

BQ29 - PHOSPHOLIPASE A2 (PLA2) IN THE SALIVA OF TRIATOMA INFESTANS

Lima S.M; Cardoso,L.S; Silva-Neto, M.A.C. and Atella, G.C.

Instituto de Bioquímica Medica - CCS – UFRJ

Triatoma infestans (T. infestans), is a blood sucking bug from subfamily Triatominae. It is widespread in the Southern Cone countries of South America and it is a vector of Chagas' disease. An adult Triatoma will ingest from two to three times its own weight of blood at a single meal. Blood-sucking insects possess a variety of anti-hemostatic factors in their salivary glands to maintain blood fluidity during feeding. In our lab we previously demonstrated the anti-hemostatic properties of a lipid, lysophosphatidylcholine (LPC) isolated from the salivary glands of another kissing-bug, Rhodnius prolixus. Phospholipase A2 (PLA2) is an enzyme responsible for the hydrolysis of phospholipids at sn-2 position which results in the release of lysophospholipids and fatty acids. The aim of the present study was to verify the activity of PLA2 in the saliva of the T. infestans. Thus, the saliva of 200 adult insects was collected in the presence of saline and the total protein content was determined (1,1 ug/ul). Enzyme activity was assayed using fluorescent phosphatildylcholine as substrate and product formation was measured with the aid of a fluorimeter. PLA2 activity was time- and concentration-dependent. Our results confirm the presence of PLA2 activity in the saliva of T. infestans. In addition to being involved in the production of salivary LPC, PLA2 may also display direct anti-hemostatic effects itself. This possibility, together with the PLA2 activity profile during salivary gland development are currently under investigation.

Supported by CNPq, Faperj, IFS

BQ30 - DETECTION OF CALPAIN-LIKE ACTIVITIES IN TRYPANOSOMATIDS THROUGH ZYMOGRAMS

OLIVEIRA, S.S.C.[1], SANGENITO, L.S.[1], CHAGAS, M.G.[1], MARINHO, F.A.[1], D'AVILA-LEVY, C.M.[2], SANTOS, A.L.S.[1], BRANQUINHA, M.H.[1]

[1]Departamento de Microbiologia, Instituto de Microbiologia Prof. Paulo de Góes, UFRJ, Rio de Janeiro, Brazil;
[2]Laboratório de Biologia Molecular e Doenças Endêmicas, Instituto Oswaldo Cruz, FIOCRUZ, Rio de Janeiro, Brazil.

Polyacrylamide gels containing a protein as a proteolytic substrate, usually gelatin, has been widely used as a simple standard technique to assess the complexity of peptidases in cell systems, including protozoa. However, this assay might not be sensitive enough to detect minor proteolytic activities, as it is likely that gelatin might not be an adequate substrate for all peptidases. In this sense, we started to search for calpain-like activities in trypanosomatids. Calpains are calcium-regulated cysteine peptidases that exists in many cell systems, including humans, and calpain homologues have recently been detected in trypanosomatid human pathogens. However, it is difficult to detect calpain activity in gelatin-SDS-PAGE due to its sensitivity to alkaline pH and SDS and the preferential use of casein as substrate. In this study, we show that casein zymography can be used to detect calpain activity in trypanosomatids. Samples were run into the polyacrylamide gels under nondenaturing conditions using two distinct systems: a Tris-glycine alkaline buffer or a continuous neutral pH buffer system containing Hepes and imidazole. Gels were then incubated in calpain activation buffers containing calcium and dithiothreitol. Using these techniques, calpain-like activities were detected in cell extracts from *Trypanosoma cruzi* clone Dm28c and from *Leishmania amazonensis*, as well as in the culture supernatant from *Blastocrithidia culicis* and *Crithidia deanei*. Calpain activity was found to decrease with E-64 and EGTA treatment. Our data showed that these modifications in the zymography procedure markedly enhance detection of calpain-like enzymes in trypanosomatids.

Supported by: CNPq, FAPERJ, FIOCRUZ and CEPG/UFRJ.

BQ31 - *T. CRUZI* CELL DEATH INDUCED BY *CRATYLIA MOLLIS* SEED LECTIN IS MEDIATED BY PLASMA MEMBRANE PERMEABILIZATION FOLLOWED BY MITOCHONDRIAL Ca^{2+} LOAD AND ROS PRODUCTION

Fernandes, M. P.[1]; Inada, N. M.[1]; Araújo, F. F. B.[2]; Gadelha, F. R.[3]; Correia, M. T. S.[2]; Coelho, L. C. B. B.[2] and Vercesi, A. E.[1]

[1]Dept. Patologia Clínica, FCM, UNICAMP, Campinas, SP, Brazil; [2]Dept. Bioquímica, CCB, UFPE, Recife-PE, Brazil; [3] Dept. Bioquímica, IB, UNICAMP, Brazil.

Plant lectins are proteins or glycoproteins that serve as valuable tools in glycobiology research and can be employed for the detection and preliminary characterization of cell surface glycoconjugates. This work was aimed at evaluating whether *Cratylia mollis* seed lectin (Cramoll 1,4) have toxic effects on *Trypanosoma cruzi* epimastigotes (Tulahuen 2 strain). Mitochondrial membrane potential and changes in medium Ca^{2+} were measured by the safranine and arsenazo techniques, respectively. It was observed that Cramoll 1,4 decreased the parasite proliferation in a dose-dependent manner, with a 93% inhibition at a concentration of 50 µg/ml. Incubation of cells (1.25 x 10^8 /ml) in the presence of Cramoll 1,4 (50 µg/ml) and 10 µM Ca^{2+}, during 1 h, induced plasma membrane permeabilization followed by an increase in intracellular Ca^{2+} concentration and by mitochondrial Ca^{2+} overload. This increased the production of reactive oxygen species (ROS) by 3 times and significantly decreased the mitochondrial membrane potential and impaired ADP phosphorylation. Basal and uncoupled respiration (FCCP) were not affected by Cramoll 1,4 plus Ca^{2+} treatment, but oligomycin poisoned respiration was increased by 60%, probably at the expense of Ca^{2+} influx. Interestingly, digitonin permeabilization of the plasma membrane, in medium containing Ca^{2+}, led to similar results. In conclusion, Cramoll 1,4 toxicity to *T. cruzi* epimastigotes may result from a concerted action on parasite plasma membrane, mitochondrial Ca^{2+} uptake and ROS production.

Supported by: CAPES, CNPq and FAPESP.

BQ32 - PROTEOLYTIC ACTIVITIES IN *TRYPANOSOMA CRUZI* SYLVATIC ISOLATES FROM STATE OF RIO DE JANEIRO, BRAZIL

Misael D.[1]; Gomes S.A.O.[1]; Stahl C.V. [1]; Silva B.A.[2], Gonçalves T.C.M.[1]; Santos A.L.S.[2] , Feder D.[3] and Santos-Mallet, J.R.[1].

[1]Setor de Morfologia e Ultraestrutura e Bioquímica de Artrópodes e Parasitos. Laboratório de Transmissores de Leishmanioses, Instituto Oswaldo Cruz – FIOCRUZ, Rio de Janeiro, Brasil. [2]Laboratório de Estudos Integrados em Bioquímica Microbiana, Instituto de Microbiologia Paulo de Góes (IMPPG), Bloco I, CCS, UFRJ, Rio de Janeiro, Brasil.
[3] Laboratório de Biologia de Insetos, Departamento de Biologia Geral, Universidade Federal Fluminense – UFF, Niterói – Rio de Janeiro, Brasil.

Trypanosoma cruzi, Chagas (1909), ethiologic agent of Chagas' disease, is represented by a set of wild parasites which circulate between men, vectors, reservoirs and domestic animals. Depending of vector capacity, the triatomines is able to participate actively on the parasite transmission inside of its geographic distribution area. *Triatoma vitticeps* (Stal 1859) has been found in the southern states of Brazil, including Bahia, Espírito Santo, Minas Gerais and Rio de Janeiro. Using epimastigote forms of seven wild samples of *T. cruzi*, isolated from *T. vitticeps,* from Triunfo locality, Santa Maria Madalena, State of Rio de Janeiro, we investigated the polypeptides profile and the proteolytic activity using polyacrylamide gel electrophoresis. One of the proteolytic activities demonstrated, a major band 45 kDa, is the main cysteine peptidase of *T. cruzi*, the cruzipain molecule. This data was confirmed by western blotting assay using anti-cruzipain antibody. Other cell-associated proteolytic activities were detected with apparent molecular masses of 113 and 80 kDa. We are currently characterizing these enzymes with proteolytic inhibitors.

Financial support: FUJB, CNPq and FAPERJ/FIOCRUZ.

BQ33 - The influence of 2-difluoromethyl-4-nitrophenyl N-acetyl-α-neuraminic acid on the inhibition mechanism of *Trypanosoma cruzi trans*-sialidase (TcTS)

Sebastião Teixeira de Carvalho[1], Mauro Sola-Penna[2], Shin-Ichiro Nishimura[3], Hiroshi Hinou[3], Lucia Mendonça-Previato[1], Adriane Regina Todeschini[1], Jose Osvaldo Previato[1].

[1]Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro.
[2] Hokkaido University, Sapporo, Japan.
3 Faculdade de Farmácia, Universidade Federal do Rio de Janeiro.

Carbohydrates present on the surface of cells are recognized as playing important roles in the host-parasite interactions. *Trypanosoma cruzi* has undergone a process of incorporation of sialic acid from exogenous sialyl-containing molecules into sialylated glycoprotein on the parasite surface by a glycosidase hydrolase *trans*-sialidase (TcTS), rather than by the well known route in which CMP-sialic acid is an intermediate. The TcTS is a set of proteins with enzymatic or lectin-like properties that modulate or interact with the host T cells, and is a possible target for drug design, combining structural biology and crick chemistry. Recently we reported that 2-difluoromethyl-4-nitrophenyl N-acetyl-α-neuraminic acid (TSinhibitor) inactivated the TcTS through a covalent bond formation between TS amino acids with the reactive aglycon generated by the hydrolysis of inhibitor. Here we report the kinetic parameters for TcTS inhibition by this compound assaying sialidase and *trans*-sialidase activities. Both activities present similar patterns of activation by the pseudo-substrate 4-methylumbeliferyl N-acetylneuraminic acid, presenting a $K_{0.5}$ of 0.21 ± 0.02 mM and 0.19 ± 0.02 mM for sialidase and *trans*-sialidase activities, respectively. The inhibition constant for the compound ($I_{0.5}$) was evaluated for both activities. The $I_{0.5}$ calculated for sialidase activity, when the TcTS was pre-incubated with the inhibitor compound was 0.58 ± 0.06. However, without the pre-incubation, the $I_{0.5}$ was 0.68 ± 0.07 mM. Similar results were obtained when TcTS *trans*-sialidase activity was assayed using N-acetyllactosamine or lactose as substrate acceptor. In these experiments, when the TSinhibitor concentration was higher than 1 mM, the *trans*-sialidase activity was less inhibited when compared to sialidase activity. This difference might be due to the fact that, since the TSinhibitor is a substrate for TcTS when it is in the presence of the acceptor substrate, the hydrolysis rate of TSinhibitor is faster, giving rise to more reactive species (aglycon), augmenting the inhibitory effect of the TSinhibitor.

Supported by CNPq, FAPERJ, The Millennium Institute for Vaccine Development and Technology (420067/2005-1)

BQ34 - The antioxidant enzyme network of *Trypanosoma cruzi*: an emerging virulence factor?

Piacenza, L[1]; Zago, M.P[2]; Peluffo, G[1]; Alvarez, MN[1]; Wilkinson, S.R.[3]; Basombrio, M.A[2]; Radi, R[1]

***1 -** Departamento de Bioquímica and Center for Free Radical and Biomedical Research, Facultad de Medicina, Universidad de la República, Montevideo-Uruguay, Uruguay **2 -** Instituto de Patología Experimental, Facultad de Cs. de la Salud, UNSa-CONICET, Salta-Argentina **3 –** School of Biological and Chemical Sciences, Queen Mary University of London.*

Virulence of *Trypanosoma cruzi* strains towards the host depends on different genetic and biochemical factors known to participate in the severity of Chagas disease. The suggested increased expression of molecules of the antioxidant network including trypanothione synthase (TcTS), tryparedoxin, tryparedoxin peroxidases, and iron-containing superoxide dismutases during metacyclogenesis, constitutes a remarkable event that allows to hypothesize that parasites are preconditioned in an effort to overcome the challenge raised by macrophage-derived reactive oxygen and nitrogen species upon infection. In this line, and using several isolates (10 strains) that belongs to the major phylogenetic lineages TCI and TCII, we investigated for an association between virulence (ranging from highly aggressive to extremely attenuated isolates at the parasitemia and histopathology level) and the antioxidant enzyme content. Antibodies rised against TcTS, ascorbate peroxidase (TcAPX), mitochondrial and cytosolic tryparedoxin peroxidases (TcMPX, TcCPX) and trypanothione reductase (TcTR) were used to evaluate the antioxidant enzyme levels in epimastigotes and metacyclic trypomastigotes of the different *T. cruzi* strains. A direct correlation was found between levels of TcTS, TcMPX and TcCPX with the parasitemia elicited by the different isolates studied (r=0.908, 0.953, 0.738 respectively, P<0.05). The

strongest association was found with the TcTS which was not only increased on the more virulent strains but also rised during the differentiation to the infective form, whereas TcAPX showed the weakest correlation. Moreover, independently of its virulence, levels of antioxidant enzymes were higher in the trypomastigote stage. At the cellular level, this was accompanied by an elevated resistance of highly virulent *T. cruzi* populations to H_2O_2 and peroxynitrite challenge. Finally, *T. cruzi* transformed for the overexpression of TcCPX were more infective in macrophages activated for the production of peroxynitrite suggesting that the parasite antioxidant armamentarium at the onset of infection represents a new virulence factor involved in the establishment of the disease.

BQ35 - BIOCHEMICAL EVIDENCES INVOLVE DNA POLYMERASE BETA OF *Trypanosoma cruzi* IN REPAIR OF OXIDATIVE LESIONS IN DNA

SCHAMBER, B.L.F.[1], RÉGIS-DA-SILVA, C.G.[1], NARDELLI, S.C.[2], MACEDO, A.M.[1]; FRANCO, G.R.[1]; SCHENKMAN, S.[2] , PENA, S.D.J.[1], TEIXEIRA, S.M.R.[1], MACHADO, C.R.[1]

[1]Departamento de Bioquímica e Imunologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Minas Gerais, Brazil; [2]Departamento de Microbiologia, Imunobiologia e Parasitologia, Universidade Federal de São Paulo, São Paulo, Brazil.

During its life cycle *Trypanosoma cruzi* deals with the deleterious action of reactive oxygen species (ROS). ROS can oxidize DNA to generate 7,8-dihydro-8-oxoguanine (8-oxoG) that can mispair with adenine, generating mutations. Specific biochemical pathways to minimize the mutagenic consequences of 8-oxoG were identified in *T. cruzi*. It is believed that base excision repair (BER) is essential to maintain equilibrium between generation of genetic variability and genome protection against oxidative genotoxic stress. DNA polymerase beta (polβ) is involved in BER, and *T.cruzi* possesses two mitochondrial polβ (Tcpolβ and TcpolβPAK). We showed that the *in vitro* DNA synthesis capability for both enzymes is differentially modulated by NaCl and pH. Different from human polβ, Tcpolβ was unable to synthesize DNA across an oligonucleotide containing 8-oxoG or a 3´ mismatch. To explore Tcpolβ function *in vivo*, we isolated clones overexpressing Tcpolβ. One of the 12 clones obtained was tested for survival in presence of zidovudin (AZT), a potent DNA replicating blocking agent. The overexpressing clone was more sensible to this treatment compared to control, suggesting that Tcpolβ can incorporate AZT in DNA. This was also seen in *in vitro* experiments. Furthermore, Tcpolβ was immunolocalized in the two kinetoplast antipodal sites just only in replicative cellular forms of the parasite, suggesting involvement in mitochondrial DNA replication. In addition, the overexpressing clone was treated with hydrogen peroxide (H_2O_2) and methylmethanesulphonate (MMS). H_2O_2 treatment increase survival rate, while this effect was not seen after MMS treatment. Curiously, a third focus of Tcpolβ was identified near the kinetoplast of *wild type* CL-Brener cells after H_2O_2 treatment. We are investigating the participation of this focus in DNA repair. This focus was not observed after MMS treatment. The experimental data obtained suggest participation of Tcpolβ in replication and repair of oxidative damage in mitochondrial DNA of *T. cruzi.*

Financial Support: CNPq, CAPES and Howard Huges Medical Institute

BQ36 - ROLE OF L- PROLINE ACCUMULATION IN THE RESISTANCE TO OXIDATIVE STRESS IN *TRYPANOSOMA CRUZI*

Paes, L.S.; Suárez,B.A.; Magdaleno, A. ; Silber, A.M.

Depto de Parasitologia - ICBII - Universidade de São Paulo – Brasil

The flagellated parasite *Trypanosoma cruzi* uses L-proline as energy source. In some eukaryotic organisms, L-proline is involved in stress resistance, being remarkable its role in the oxidative stress. Biochemical evidences support the hypothesis that *T. cruzi* oxidizes L-proline. We showed the participation of a proline dehydrogenase (TcPDH) (EC 1.5.1.2) in this process. In the present work we determined the activity of enzyme TcPDH and role of their products in the tolerance to oxidative stress. Stress tolerance assays were performed by using early-log-phase in *T. cruzi* epimastigotes cultures challenged with H_2O_2 at different times and concentrations. Cell viability was determined by MTT assay. The enzymatic activity assays and quantification of the intracellular free proline levels were evaluated. The highest activity TcPDH gene showed lower free intracellular proline levels and enhanced sensitivity

to oxidative stress in epimastigotes of *T. cruzi*. The obtained data suggest that the L-proline oxidation by TcPDH increases the sensitivity to oxidative stress in *T. cruzi* epimastigotes, involving the regulation of this gene and free proline content in oxidative stress resistance.
Supported by: FAPESP and CNPq

BQ37 - Resveratrol as a probing molecule to interfere with signaling pathways controlling *Aedes aegypti* lifespan.

Nunes, R.D.[1]; Gazos-Lopes F.[1]; Melo-Oliveira, R.[1]; Bouts, D.[1]; Gusmão, D.[3]; Alviano, D.S.[2]; Lemos, F.J.[3]; Silva-Neto, M.A.C[1].

1-Instituto de Bioquímica Médica, UFRJ
2-Instituto de Microbiologia, UFRJ
3-Laboratório de Biotecnologia, UENF

Decrease in mosquito lifespan have significant epidemiological implications to arthropod-borne diseases. Knowledge of central metabolic and signaling pathways may reveal new targets for *Aedes aegypti* longevity regulation, the major vector of Dengue. As polyphenols are known to enhance longevity in all animals studied and mosquitoes also uptake resveratrol when fed on sugar we are currently identifying the mecanism of action of these substances on mosquito diet in order to block these mecanisms and make *A. aegypti* live less. The survival of two resveratrol-fed strains of *A. Aegypti* (Liverpool and Red Eye) increased up to 50% in males and 35% in females. The survival of *Anopheles aquas*alis was also improved but with a different profile. The most important bacterial population isolated from A. Aegypti midgut wasn't affected by resveratrol *in vitro*. Other important pathogenic microorganism didn't have their population size changed, excluding an effect based on microorganism control. The insulin signaling pathway was affected as we observed an increase on the activity of a tyrosine phosphatase homologous to mammalian PTP1B. This effect is organ-specific and suggests a hormonal-mediated signaling. Corroborating the xenormesis hypothesis, the PFK activity increased when resveratrol was previously fed on mosquitoes which were next exposed to a calorie restriction diet. Total protein kinase activity assayed as AMPK was increased in male heads, although weight differences weren't detected. The phosphorylation profile of heads from old males was reverted to a profile close to that found in younger insects. This finding is in agreement with the hypothesis of repressed development.

Supported by FAPERJ/CNPq/WHO.

Keywords:
Resveratrol/Longevity/*Aedes*/Metabolism

BQ38 - Reconstruction of the parasite synapse: pivotal role of the *T. cruzi* trans-sialidase.

Butler CE, Wheeler G, Tyler KM

School of Medicine, Health Policy and Practice, University of East Anglia, Norwich, UK

A variety of active and inactive Trans-sialidase family molecules are expressed on the surface of *T. cruzi* trypomastigotes which have been demonstrated to be important in successful cell invasion. The active enzyme transfers host cell sialic acids to the surface of the parasite which cannot synthesise its own, and as such is vital for parasite survival. Inactive enzymes retain the ability to interact with host glycopeptides in a lectin-like manner and have also been implicated in cell invasion.
We utilized active recombinant trans-sialidase (Tcts) and inactive trans-sialidase (Tcts2V0) immobilized on 3 micron beads and introduced them to Madin-Darby Canine Kidney cells (MDCKs). Both Tcts and Tcts2V0 can attach, with significantly greater attachment observed with the active enzyme. This activity, when quenched by competitive inhibitor lactitol, reduces the ability of the beads to attach but has no effect on the inactive trans-sialidase. An increase in GFP-tubulin intensity was observed at the bead-cell synapse as was the recruitment of vesicles which could be stained with early endosome antibody. Using two methods (acridine orange cytoplasmic staining and biotinlyation) we found that incubation for an hour was sufficient for endocytosis of beads coated with TcTs internalisation being significantly more effective. To determine the mechanism of entry we considered the three potential endocytic pathways and utilized live-imaging of transformed cell lines together with a fixed cell marker-based analysis. Our systems describe a caveolin-associated, microtubule-mediated uptake mechanism for trans-sialidase coated particles into a vacuole surrounded by intermediate filaments.

This research is sponsored by: UEA

BQ39 - Signaling pathways triggered by platelet-activating factor (PAF) during *Trypanosoma cruzi* metacyclogenesis

Marta T. Gomes[1,], Luciana T. Zimmermann[1], Antônio Ferreira-Pereira[1], Mário A. C. Silva-Neto[2] & Angela H. Lopes[1]*

*[1]Instituto de Microbiologia Prof. Paulo de Góes (IMPPG)/ UFRJ; [2]Instituto de Bioquímica Médica (IBqM)/ UFRJ; *mtgomes@micro.ufrj.br*

Differentiation is one the most important events in the development of eukaryotic cells. In trypanosomatids, differentiation enables non-infective parasites to transform into infective forms, which are then competent to complete their life cycles. The signals involved in triggering these morphological and physiological changes are implicated in the activation of protein kinase cascades and a variety of complex physiological signals. 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. PAF also stimulates cell differentiation in *Trypanosoma cruzi* and *Herpetomonas muscarum muscarum*, through a signal transduction pathway that activates protein kinase CK2. Here we further investigated the signaling pathways triggered during metacyclogenesis of *T. cruzi*, clone Dm28c. Parasites were maintained in TAUP medium in the absence or in the presence of PAF (10^{-6} M) and/or PKC inhibitor (10 nM BIS I) and/or CK2 inhibitor (20nM TBB and 100 μM DRB). These inhibitors were able to reduce PAF-induced differentiation by 57% (PAF + BIS), 51% (PAF + TBB) and 54% (PAF + DRB). Also, BIS I and DRB promoted a reduction in PAF-stimulated protein phosphorylation. Interesting, parasites incubated with PAF presented a 40% increase in calcium released by intracellular compartments, when the parasites were permeabilized with digitonin. These results suggest that signal transduction induced by PAF during *T. cruzi* differentiation occurs via PKC and CK2.

Supported by: CNPq, FAPERJ and CAPES.

BQ40 - EFFECT OF ANGIOTENSIN II DURING THE INFECTION OF RED BLOOD CELLS BY *Plasmodium falciparum*

Saraiva, V.B.; Pinheiro, A.A.S.; Kelli XX; Mendonça-Previato L.; Previato J.O. and Caruso-Neves C.

Malaria is a severe parasitic disease and remains a serious public health problem in the world. The etiologic agent of malaria is the parasitic protozoa of the genus *Plasmodium*, and the specie *P. falciparum* responsible for the most fatal cases in human. Recent studies have shown that signaling processes via G protein in red blood cells (RBC) is important not only for invasion but also to parasite proliferation, and is considered a new target for antimalarial-drugs development. The objective of this study is to check the effect of angiotensin II (Ang II), a hormone peptide which has pro-inflammatory action during the invasion of RBC by *P. falciparum* merozoite. Ang II signals via AT1 receptors, sensitive to losartan, and AT2, sensitive to PD123319 (PD), both coupled to protein G. The erythrocytic forms of *P. falciparum* of the W2 strain were isolated in schizont stage through Percoll gradient. Schizonts purified were incubated at 2.5% parasitemia in the absence or presence of Ang II (10^{-6} - 10^{-12}M), losartan (10^{-6}M), PD (10^{-7}M), Ang II + losartan or Ang II + PD. The parasitemia and appearance of ring forms were recorded after 24 and 48 h. In this experiment we observed a decrease of parasitemia in the presence of Ang II, in a biphasic effect with maximum inhibition observed at 10^{-8}M. In 24 hours, at the same concentration, there is a decrease in the number of ring forms accompanied by a reduction in the number of schizonts, after 48 hours of interaction. Furthermore, we found that losartan and PD did not modulate the inhibitory effect of Ang II. Taken together, these results suggest that Ang II modulates merozoite/erythrocyte interaction but this effect is independent of AT1 or AT2 receptors.

BQ41 - BINDING OF *LEISHMANIA AMAZONENSIS* LEISHPORIN TO TARGET MEMBRANES IS INDEPENDENT FROM CYTOLYSIN ACTIVATION

CASTRO-GOMES, T.[1], *FORTUNATO-TELES, D.*[1], *MONTEIRO-VIEIRA, T. H.*[1], *FRÉZARD, F.*[2], *HORTA, M.F.*[1]

[1]*Departamento de Bioquímica e Imunologia,* [2]*Departamento de Fisiologia e Biofísica, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil*

Leishporin is a pore-forming protein found in the Genus *Leishmania*. Because it is is optimally active at 5.5 pH and 37°C, it may act inside the mammalian host in important steps of leishmania infection, such as rupture of host cells, leading to infection amplification. In previous works, we found that leishporin binds to target cells lipids before pore-formation. We also found that leishporin must be activated to lyse cells. This activation is accomplished after the removal of a non-covalently linked oligopeptide inhibitor by proteolysis or dissociating conditions. Recently, we have shown that liposomes can remove active leishporin from promastigote extracts. However, hemolytically inactive extratcts can be further activated by proteases or dissociating agents, demonstrating the presence of inactive forms of the cytolysin. In the present work, we have studied the binding of inactive and active forms or leishporin to target membranes in order to study the mechanism of pore formation/cytolysis. We show that inactive leishporin can also bind to the target membranes although active forms bind better. However, lysis only occurs after leishporin activation by removal of the inhibitor. These results suggest that lipid-binding domains are present in leishporin molecule, regardless its activation state, but removal of inhibitor increase their affinity for lipids. Taken together our findings indicate that lysis occurs in at least three steps (the first two not necessarily in this order): 1) binding of leishporin to target membrane lipids (independent from but optimized by activation); 2) activation by removal of oligopeptide inhibitor; 3) pore-formation/membrane permeabilization, step under activation control. **Support: CAPES, CNPq, FAPEMIG, PRONEX, WHO.**

BQ42 - INFLUENCE OF PEPTIDASES IN THE INTERACTION PROCESS *LEISHMANIA BRAZILIENSIS* – HOST CELL: COMPARATIVE STUDY BETWEEN VIRULENT AND AVIRULENT STRAINS

Lima, A.K.C.[1], *Souza, J.E.O.*[1], *Guerra, R.J.A.*[1], *Azevedo, E.S.*[1], *Oliveira, V.F.*[1], *Santos, A.L.S.*[2], *Dutra, P.M.L.*[1]

[1]*Disciplina de Parasitologia, DPL, FCM, UERJ.* [2]*Instituto de Microbiologia Prof. Paulo de Góes, UFRJ. E-mail: pdutra@uerj.br*

Leishmaniasis is a group of diseases with a wide spectrum of clinical manifestations, which range from self-limited skin lesions to the severe visceral involvement that can lead to death. The study of the surface molecules related to the process of host-parasite interaction is of fundamental importance, and among them, peptidases are clearly involved in the growth, differentiation and virulence of several protozoa. In this paper, we have analyzed the cellular and extracellular proteolytic profiles in two distinct *Leishmania braziliensis* isolates, virulent and avirulent strains, of. Both quantitative and qualitative differences were observed in the *L. braziliensis* strains. For instance, in acidic pH value, cysteine peptidases of low molecular masses, sensitive to E-64, were detected exclusively in virulent strain. Curiously, both metallo and cysteine peptidases were drastically reduced during several in vitro passages of the virulent strain. In order to detect homologous of the major metallopeptidase (gp63) as well as the major cysteine peptidase (cpb) in both virulent and avirulent strains of *L. braziliensis*, we performed experiments of western blotting, flow cytometry and fluorescence microscopy. Our results demonstrated that the virulent strain produced a higher amount of gp63 and cpb molecules, detected on both surface and cytoplasm region, when compared to avirulent ones. Metal-based drugs and metallopeptidase inhibitors {EGTA, 1,10-phenanthroline, phendio, [Cu(phendio)$_2$] and [Ag(phendio)$_2$]}, as well as E-64 (a potent cysteine peptidase inhibitor) were able to inhibit only the growth only of virulent strain. Similarly, these compounds also inhibited the association index betweenvirulent strain and macrophage. To evidence more specifically the role of both gp63- and cpb-like molecules during the parasite interaction, the pre-treatment of the virulent strain with both antibodies promoted a drastic reduction in the interaction with macrophages, while the

association index of the avirulent strain to macrophage was only slightly reduced.
Supported by: CNPq and FAPERJ.

BQ43 - INFLUENCE OF ECTO-NUCLEOSIDE TRIPHOSHATE DIPHOSPHOHYDROLASE ACTIVITY IN TRYPANOSOMA CRUZI INFECTIVITY

SANTOS, R.F.[1]; MOREIRA, B.P.[1]; PÔSSA, M.A.S.[2]; BASTOS, M.S.[1]; GUEDES, P.M.M.[3]; LAMÊGO, M.R.A.[1]; DeMARCO, R.[4]; VERJOVSKI-ALMEIDA, S.[5]; BAHIA, M.T.[2]; FIETTO, J.L.R.[1]

[1]Universidade Federal de Viçosa, Minas Gerais, Brazil, [2]Universidade Federal de Ouro Preto, Minas Gerais, Brazil, [3]Universidade de São Paulo, Ribeirão Preto, Brazil, [4]Universidade Federal de São Carlos, São Carlos, Brazil, [5]Universidade de São Paulo, São Paulo, Brazil

In this study we evaluated the influence of Ecto-Nucleoside-Tri-Phosphate-Diphosphohydrolase (E-NTPDase) activity in infectivity of *T. cruzi* using in vitro model of infection. To that effect, we followed ecto-NTPDase activities of Y strain infective forms (trypomastigotes) obtained during sequential sub-cultivation in mammalian cell. Our results showed that ATPase/ADPase activities ratio and infectivity of cell derivate trypomastigotes decrease during sequential sub-cultivation. Surprisingly, after third to fourth passages cell derivate trypomastigotes could not penetrate mammalian cells and have differentiated into amastigote-like parasites that exhibited low levels of ecto-NTPDase activities. To evidence the participation of *T. cruzi* Ecto-NTPDase1 in the infective process, we evaluated the effect of known ecto-ATPDase inhibitors (ARL 67156, Gadolinium and Suramin), or anti-NTPDase-1 polyclonal antiserum on ATPase and ADPase hydrolytic activities in recombinant *T. cruzi* NTPDase-1 and in live trypomastigotes. All tests showed a partial inhibition of ecto-ATPDase activities and a marked inhibition of trypomastigotes infectivity. Our results suggest that E-ATPDases act as facilitators of infection in vitro and emerge as targets in chemotherapy of Chagas disease.

SUPPORTED BY
CNPq
FAPEMIG
CAPES

BQ44 - INFLUENCE ON HOST CELL INVASION BY THE SERINE PEPTIDASE INHIBITOR (ISP2) OF *Trypanosoma cruzi*.

Mayra Fonseca Abreu[1], Flavia Coelho Garcia dos Reis[1], Roberta Eitler Bruno[1], Graham H. Coombs, Jeremy C. Mottram[2], Ana Paula C. A. Lima[1]

[1]Instituto de Biofísica Carlos Chagas Filho, Federal University of Rio de Janeiro, Brazil; [2]Wellcome Centre for Molecular Parasitology, University of Glasgow, UK.

Ecotins are bacterial high affinity inhibitors of family S1 serine peptidases that are found in the periplasm of several bacteria species. They present a broad range of inhibition, strongly inactivating trypsin, chymotrypsin, cathepsin G, neutrophil elastase (NE), proteinase 3 and also inhibiting members of the coagulation cascade with moderate to high potency. In *E. coli*, ecotin was found to exert a protective role against the deleterious effect of NE. Genes similar to ecotins were identified in the genomes of *T. cruzi, T. brucei* and *Leishmania*, and were designated Inhibitors of Serine Peptidases (ISPs). Since there is no gene encoding S1 peptidases in the *T. cruzi* genome, we raised the possibility that ISPs could modulate host enzymes. In order to evaluate the biochemical properties of *T. cruzi* Dm28c ISP, we cloned and expressed the only ISP gene in the *T.cruzi* genome - ISP2. Our results showed that is a thermo resistant, high affinity slow binding inhibitor of trypsin, chymotrypsin, cathepsin G and NE, exhibiting *K*i's in the nanomolar range. We transfected *T. cruzi* Dm28c epimastigotes with pTEX containing the *ISP*2 gene and after drug selection and plate cloning we successfully obtained parasites overexpressing functional ISP2. Western blot analysis of parasite lysates revealed that ISP2 expression is higher in epimastigotes than in trypomastigotes and amastigotes. A possible role of ISP2 in host-parasite interaction was analyzed by performing invasion assays of human smooth muscle cell lines with tissue culture trypomastigotes. We observed that the addition or the pretreatment of the cells with rec-ISP2 during the interaction significantly reduced parasite entry. The same result was observed in invasion assays of muscle cells with trypomastigotes that overexpress ISP2, suggesting that host serine peptidases might play a role in parasite invasion.

BQ45 - SELENIUM DETERMINATION BY ET AAS: EVALUATION IN MICE WHOLE BLOOD DURING ACUTE PHASE OF *TRYPANOSOMA CRUZI* INFECTION

MEDEIROS, M.M. [1], *FÁTIMA RAMOS MOREIRA* [2], *SOUZA, A.P.* [1], *RAMOS, T.D.* [2], *DUN, J.A.* [1], *ARAÚJO-JORGE, T.C.* [1]

[1] *Laboratório de Biologia Celular/Instituto Oswaldo Cruz/FIOCRUZ, Rio de Janeiro, Brasil;* [2] *Laboratório de Metais/CESTEH/ENSP/FIOCRUZ, Rio de Janeiro, Brasil*

Chagas' disease is caused by infection with the protozoan *Trypanosoma cruzi* and it affects 13 million people in Latin American. Selenium (Se), an essential micronutrient, has presented important role in resistance in experimental infection, and chagasic patients with severe cardiopathy presented low Se serum levels. Electrothermal atomic absorption spectrometry (ET AAS), has been widely used for the trace metals determination, since it provides the following advantages: requires low sample volume; provides adequate limit of detection; reduces the contamination risk; allows direct determination without any previous treatment. The main purposes of this work were: (1) Adapt the ET AAS technique using the best conditions of analysis (time and temperature program, dilution samples, concentrations of diluents, chemical modifier mass); (2) test ET AAS efficacy to determine Se in mice whole blood; (3) monitor Se blood levels in adult mice infected with 10^4 Y strain of *T. cruzi*. Herein, infected (Tc) and non-infected (N) Swiss mice whole blood was collected in heparinized tubes decontaminated with 10% nitric acid. Before infection, no difference was observed between the control and infected groups, which presented similar Se blood levels (N= 184.4 ± 37.4 g/L; Tc= 188.0 ± 43.4 g/L). At 7 days post-infection, group Tc showed a significant (p<0.05) increase in Se levels (274.3 ± 31.0 g/L) in comparison with control group (200.9 ± 30.7 g/L). Interestingly, at 14 and 21 days post-infection (dpi), group Tc presented a significant decrease in Se levels (dpi 14= 240.5 ± 57.3; dpi 21= 252.4 ± 53.0 g/L), when compared to the group N (d14= 303.0 ± 58.6; d21= 300.0 ± 56.9 g/L). Our findings demonstrated that ET AAS is an adequate technique which has a good sensitivity to determine Se in mice whole blood, which was altered during *T cruzi* infection.

This work was supported with grants from IOC-Fiocruz/CNPq/Faperj.

BQ46 - Molecules from the triatomine environment as Heme, Urate and Hemozoin and their involvement in the metacyclogenesis.

Sultano PE[1], *Nogueira NPA*[1], *Breder LO* [1], *Laranja GAT*[1], *Coelho MGP*[1], *Paes MC*[1].

1- Universidade do Estado do Rio de Janeiro – Departamento de Bioquímica

Metacyclogenesis is the complex differentiation process from *T. cruzi* epimastigotes (EPI), proliferative and non-infectious forms, to metacyclic trypomastigotes (MT), nonproliferative and infective forms. Heme is an important molecule in the metabolism of all living organisms. EPI proliferate inside the hematophagous insects that usually ingest about 10 mM heme bound to hemoglobin. We showed recently that EPI acquires extracellular heme from the medium and the addition of heme increases significantly the parasite proliferation in a dose-response manner (Lara *et al*, 2007). However, until now, the role of this molecule in the metacyclogenesis process has not been ruled out. For *in vitro* differentiation, EPI incubated TAU medium and then diluted 1:100 in TAU3AAG medium in the presence of: (i) heme, (ii) urate (that is found in the urine of the triatomine) and (iii) hemozoin (Hz), witch consists of heme molecules crystallized into dimers. Parasites were permeabilized in PBS containing 0.1% Triton X-100 and then incubated with 10 μg/mL DAPI for 15 min. Therefore, cells were observed for different times and analyzed by fluorescence microscopy. The total cells number decreased when parasites were treated with heme when compared to other treatments, mainly at 96 hours. Parasites treated with urate presented a slight increase in MT that remained alive up to 14 days in TAU3AAG. We also investigated the effects of urate upon EPI proliferation. Parasites were incubated in the presence of urate and/or heme during 12 days. Thus, we observed that urate impaired the proliferative effect of heme. So, taken together, our data suggests that heme is a molecule involved in parasites proliferation and not in the differentiation process. On the other hand, urate seems to be involved in the metacyclogenesis process instead of epimastigotes proliferation. Supported by FAPERJ

BQ47 - EVIDENCE FOR AN ATP-SENSITIVE K^+ CHANNEL IN MITOPLASTS ISOLATED FROM *TRYPANOSOMA CRUZI* AND *CRITHIDIA FASCICULATA*

COSTA, A.D.T. , *GOLDENBERG, S. and KRIEGER, M.A.*
Instituto Carlos Chagas (IBMP/Fiocruz), Rua Prof. Algacyr Munhoz Mader, 3775, Curitiba, PR, Brazil CEP 81350-050

In past years, an ATP-sensitive K^+ channel ($mitoK_{ATP}$) was identified and pharmacologically characterized in mammalian mitochondria. Opening of $mitoK_{ATP}$ and the subsequent K^+ entry into the matrix was shown to have three effects on mitochondrial physiology: (i) increase in mitochondrial volume (swelling), (ii) acceleration of respiration, and (iii) increase in ROS production. Although its activity has been shown to be related to several pathophysiological processes in mammalian tissues, the channel or its activity has not yet been described in trypanosomatids. In the present study, we show pharmacological evidence for the presence of a $mitoK_{ATP}$ in trypanosomatids. *Trypanosoma cruzi* or *Crithidia fasciculata* cells were incubated in hypotonic medium followed by mild detergent exposure to isolate mitoplasts (i.e. mitochondria devoid the outer membrane). Measurement of mitoplasts volume showed that they swelled when incubated in KCl medium due to respiration-driven K^+ entry into the matrix. Swelling was inhibited in the presence of ATP when the mitoplast suspension was incubated in a K^+-containing but not in a K^+-free medium. This result suggested the presence of $mitoK_{ATP}$ in the mitoplasts of these trypanosomatids. Indeed, we observed that the ATP-inhibition of swelling in KCl medium could be reversed by the $mitoK_{ATP}$ agonist diazoxide. Furthermore, the diazoxide-induced swelling could be inhibited by the $mitoK_{ATP}$ blockers 5-hydroxydecanoate or glibenclamide. Valinomycin, a potassium ionophore, could also reverse the ATP-inhibited state, but it was not sensitive to 5-hydroxydecanoate or glibenclamide. Although it is necessary to demonstrate its existence *in vivo*, the results shown herein provide strong evidence for the presence of an ATP-sensitive K^+ channel in trypanosomatid mitochondria.

Supported by CNPq, CAPES and Fiocruz.

BQ48 - gp63 MOLECULES IN *BLASTOCRITHIDIA CULICIS* MEDIATE ADHESION TO INSECT GUT EPITHELIUM

Vitório, B.S.[1], *Santos, L.O.*[1], *Altoé, E.C.F.*[1], *Britto, C.*[1], *Santos, A.L.S.*[2], *Branquinha, M.H.*[2], *& d'Avila-Levy, C.M.*[1]

[1] *Instituto de Oswaldo Cruz, FIOCRUZ, Rio de Janeiro, Brazil.*
davila.levy@ioc.fiocruz.br
[2] *Instituto de Microbiologia Prof. Paulo de Góes, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil.*

Blastocrithidia culicis is a parasite 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 and cured strains of *B. culicis* was compared by western blotting and flow cytometry analysis using anti-gp63 antibodies. It was shown that the wild strain expresses less gp63 in comparison to the cured strain. We also analyzed the effect of the pre-treatment of *B. culicis* with anti-gp63 on the parasite adhesion to *Aedes aegypti* midgut. The interaction rate of anti-gp63 treated cells of the wild strain of *B. culicis* was reduced in at least 60% in relation to the cells treated with pre immune serum. These results suggest an important role of gp63 molecules in this trypanosomatid.

Supported by: MCT/CNPq, FAPERJ and FIOCRUZ.