Bioquímica- Biochemistry

BQ01 - Identification and functional characterization of a multi-substrate specific UDP-Sugar pyrophosphorylase from *Leishmania major*

Sebastian Damerow [1], Anne-Christin Lamerz [1], Thomas Haselhorst [2], Mark von Itzstein [2], Françoise Routier [1], Rita Gerardy-Schahn [1*]
[1]Cellular Chemistry, Hannover Medical School, Hannover, Germany
[2]Institute for Glycomics, Griffith University (Gold Coast Campus), Australia
*Gerardy-Schahn.Rita@mh-hannover.de

Although *Leishmania major* is able to take up and incorporate radioactive galactose into its glycoconjugates, the genome of this protozoan parasite lacks an obvious UDP-glucose hexose-1-phosphate uridyltransferase. Here we report the cloning, purification and characterization of a UDP-sugar pyrophosphorylase (USP) from *L. major* that constitutes a salvage pathway to UDP-galactose in this organism. The monomeric enzyme exhibits a broad substrate specificity forming both UDP-hexoses and -pentoses from the corresponding monosaccharide-1-phosphate and UTP. Using a bidirectional assay system we identified UDP-galactose (or galactose-1-phosphate in the forward reaction) as the primary substrate followed by UDP-glucose (or glucose-1-phosphate). Lower activities with xylose-1-phosphate, UDP-L-arabinose and UDP-galacturonic acid were observed, whereas mannose-1-phosphate, N-acetylglucosamine-1-phosphate and UDP-N-acetylgalactosamine were not converted. Interestingly *Leishmania* USP homologs are found in plants and display very similar substrate specificity, but are not found in other organisms suggesting a horizontal gene transfer from plants to *Leishmania*. A detailed analysis of the kinetic parameters, and the characterization of epitopes recognized in bound sugar substrates by STD-NMR will be presented. With the identification of the *L. major* USP we provide evidence for the existence of the *Isselbacher* pathway in this trypanosomatid. This enzyme catalyzing the synthesis of nucleotide sugars from sugar-1-phosphates occupies a central position in both anabolic and catabolic pathways and as such might constitute an attractive target for the development of novel therapeutic concepts.

BQ02 - Elongation of very long chain fatty acids in *L.major* and *T.cruzi*

Livore V.I* and Uttaro A.D
IBR-CONICET, Dpto. Microbiología, FCByF, UNR, Suipacha 531, 2000-Rosario. Argentina.
**livore@ibr.gov.ar*

De novo fatty acid (FA) synthesis takes place in the cytoplasm and is catalyzed by FAS (Fatty Acid Synthase) system I or II. The products of these enzymes, 16:0 – 18:0, can be further elongated up to 26:0 by an alternative group of microsomal enzymes called elongases, ELO.
Trypanosomatids utilize the ELO enzymes to produce *de novo* FA, instead of the well known FAS system. Until now, this has only been described in these protozoans (Lee et al, 2006, Cell **126:** 691).
T.brucei genome encodes four ELO. Three of them, Tb-ELO1, Tb-ELO2, Tb-ELO3, synthesize the novo fatty acids up to 18:0, while the remaining ELO, Tb-ELO5, is involved in PUFAs synthesis.
T.cruzi encodes 5 ELO enzymes. Four of them are orthologues to those found in *T.brucei* while the extra ELO, Tc-ELO4, has shown to be involved in elongation up to 26:0, (unpublished data). In *L.major* genome we have found 10 putative ORFs to ELO enzymes. Four of them are orthologues to Tc-ELO4.
It is not well established the substrate specificity of each ELO, but they are thought to function in a processive manner, the products of one ELO being substrate for the next one.
We have already proved that Tc-ELO4 elongates 18:0 to 24:0 and in a less extend to 26:0. We are also working in establishing the shortest FA capable of being elongated by Tc-ELO4 by using ΔFAS II/ΔELO1 yeast mutant.
We are currently characterizing the four ELO4 enzymes of *L.major*. We show here the FA profiles obtained by the heterologous expression of two of these enzymes in three yeast mutant strains: ΔELO2, ΔELO3, ΔELO2/ΔELO3, respectively. It allowed us to characterize Lm-ELO4.1 and Lm-ELO4.4 as responsible to elongate (al least) 20:0 to 22:0 and 24:0, respectively.
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BQ03 - STRUCTURAL ANALYSIS OF *TRICHOMONAS VAGINALIS* LIPOPHOSPHOGLYCAN

Ryan, C.M.[1], Mehlert, A.[2], Ferguson, M.A.[2], Johnson, P.J.[1*]
[1]Department of Microbiology, Immunology, and Molecular Genetics, University of California, Los Angeles, Los Angeles, CA, USA.
[2]Division of Biological Chemistry and Drug Discovery, College of Life Sciences, University of Dundee, Dundee, UK.
[*]johnsonp@ucla.edu

T. vaginalis is coated with a poorly characterized, complex structure of carbohydrates termed the glycocalyx. Due to its extracellular lifestyle, the glycocalyx is likely to play a critical role in pathogenesis of *T. vaginalis*. One major component of this structure, the lipophosphoglycan (LPG), is involved in the adherence and cytotoxicity of *T. vaginalis* toward vaginal epithelial cells. In this study, we began structural analysis of LPG from wild type adherent parasites and mutant non-adherent parasites with altered LPGs. LPG was isolated and purified using an organic extraction and hydrophobic chromatography. Purified samples were analyzed by a combination of approaches including chemical modification, GC-MS, methylation linkage analysis, MALDI, ES-MS/MS, and enzymatic digests. These analyses showed that the glycan structure is linked to the lipid anchor through an inositol phosphate bond. Composition analyses revealed that wild type LPG is composed of galactose, N-acetylglucosamine (GlcNAc), rhamnose, xylose and glucose at a ratio of 1.9: 1.7: 2.3: 1.0: 0.2. Mutant LPGs also contain galactose, GlcNAc, rhamnose, xylose, and glucose with the addition of N-Acetylgalactosamine (GalNAc), at a ratio of 0.5: 2.1: 5.7: 1.0: 1.0: 1.2. The most common monosaccharide, rhamnose, makes up the backbone of the structure and is branched at almost every residue. The branches are primarily composed of galactose-GlcNAc repeats that terminate in galactose, GlcNAc or xylose. In the mutant LPGs we propose a model with rhamnose backbone and GalNAc-GlcNAc branches that are shorter than the corresponding wildtype galactose-GlcNAc repeats. This detailed structural analysis of *T. vaginalis* LPG reveals a unique, highly branched carbohydrate structure and suggests that biological activity of this molecule is dependent on the monosaccharide composition of the side chains.
Ryan, CM was supported by NIH, ACS, and BIF travel grant.

BQ04 - PRODUCTION OF THE PLANT HORMONE INDOLE-3-ACETIC ACID (IAA) BY *PHYTOMONAS SERPENS*

Ienne, S.[1], Purgatto, E.[2], Vidotto, V. F.[1], Freschi, L.[3], Pappas Jr., G.[4], Mercier, H.[3], Zingales, B.[1*]
[1] Instituto de Química, Universidade de São Paulo, São Paulo, Brasil.
[2] Faculdade de Ciências Farmacêuticas, Universidade de São Paulo, São Paulo, Brasil.
[3] Instituto de Biociências, Universidade de São Paulo, São Paulo, Brasil.
[4] Programa de Ciências e Biotecnologia, Universidade Católica de Brasília, Distrito Federal, Brasil
*bszodnas@iq.usp.br

The plant trypanosomatid *Phytomonas* spp. is associated with devastating diseases in commercial crops, whereas in other plant species no apparent damage is observed. In the *P. serpens* genome we detected ~10^3 gene copies coding for a putative pyruvate/indolepyruvate decarboxylase, sharing high similarity with a single copy gene in *Leishmania* spp. Indolepyruvate decarboxylases (IPDC) are found in phytobacteria and participate in the conversion of Tryptophan (Trp) to the major phytohormone indole-3-acetic acid (IAA). Pyruvate decarboxylases (PDC) are ubiquitous enzymes involved in alcoholic fermentation. Both enzymes display high sequence and structural similarities, that preclude unequivocal functional assignment based solely on sequence data. Since the production of IAA could be related to the plant-*Phytomonas* association, the goal of this study was to investigate the functionality of the PDC/IPDC gene. *P. serpens* and *L. major* were cultured in three Trp concentrations (0.1g/L; 0.5g/L; 2.5g/L). Parasites' conditioned media (triplicates) were methanol extracted and submitted to HPLC analysis. At 0.1g/L Trp, *P. serpens* produced ~4µg/mL/10^8cells of IAA, whereas a ~8.2-fold lower IAA concentration was observed in *L. major* media. For *P. serpens* the increase of Trp concentration promoted a 8.4-fold (0.5g/L Trp) and 20.6-fold (2.5g/L Trp) increase in IAA production. At 2.5g/L Trp, we also observed ethanol accumulation in *P. serpens* but not in *L. major* media.The effect of Trp on PDC/IPDC transcript abundance was assessed by northern blot. Quantification of the hybridization signal showed a 2.6-fold (0.5g/L Trp) and 3.4-fold (2.5g/L Trp) increase of the specific transcript level in *P. serpens*. PDC/IPDC transcripts were not detected in *L. major*, most probably due to its single copy nature. The data suggest that the *P. serpens* PDC/IPDC gene is bifunctional determining the production of IAA and ethanol. Experiments are underway to assess IAA and ethanol production in the cell lysates of the two trypanosomatids. Support: FAPESP; CNPq.

BQ05 - MITOCHONDRIAL BIOENERGETICS IN *TRYPANOSOMA CRUZI* OVEREXPRESSING THE CYTOSOLIC AND MITOCHONDRIAL TRYPAREDOXIN PEROXIDASE

[1]Peloso, E.F.*, [1]Vitor, S.C., [2]Alves, M.J.M., [2]Colli, W., [3]Wilkinson, S., [1]Gadelha, F.R.
[1]Departamento de Bioquímica, Instituto de Biologia, Universidade Estadual de Campinas, São Paulo, Brazil.
[2]Instituto de Química, USP, São Paulo, Brazil.
[3] School of Biological and Chemical Sciences, Queen Mary University of London, London, UK
*eduardofpeloso@yahoo.com.br

Cytosolic and Mitochondrial tryparedoxin peroxidase (TcCPx and TcMPx, respectively) play a fundamental role in *Trypanosoma cruzi* antioxidant system. This pathway differs from the mammalian host offering new options for the development of a more specific therapy for Chagas' disease. Herein, we compare the growth index (GI), O_2 consumption, H_2O_2 generation, mitochondrial membrane potential ($\Delta\Psi$) and NADPH production among control, TcCPx (pTEX-TcCPx) and TcMPx overexpressing (pTEX-TcMPx) cells. Regarding the GI, no significant difference was observed between pTEX-TcMPx and pTEX-TcCPx (~15.06±1.06), however, pTEX-TcMPx GI was significantly different from pTEX (12,88±0.35). No significant differences were observed in succinate supported O_2 consumption (~6.7±0.06 nmol O_2 consumed/min.10^8cells) and respiratory control (~1.5±0.01), for pTEX and pTEX-TcCPx, respectively. On the other hand, pTEX-TcMPx had values of 9.4±0.44 nmol O_2 consumed/min.10^8 cells and 1.3±0.02 for succinate supported O_2 consumption and respiratory control, respectively. pTEX and pTEX-TcCPx cells had no differences on the maximum rates of O_2 consumption (~7.37±0.09 nmol O_2 consumed/min.10^8cells), but pTEX-TcMPx had higher respiratory rates (11.01±0.84 nmol O_2 consumed/min.10^8cells). Using malate/ pyruvate as substrate for the respiratory chain (RC), H_2O_2 production by pTEX was significantly different from tryparedoxin peroxidase overexpressing cells which showed similar levels. Significant differences among these cells were observed upon RC inhibition by antimycin A or thenoyltrifluoroacetone where pTEX-TcMPx had 43% and 28% lower levels than pTEX-TcCPx, respectively. Regarding $\Delta\Psi$, no significant difference was observed among the cells. In relation to NADPH production, pTEX-TcCPx and pTEX-TcMPx showed similar values (~23.06±1,61 nmol/min.10^8cells), however, significantly different from pTEX (21.28±0.95 nmol/min.10^8cells). Experiments are being carried out to clarify the influence of TcMPx on O_2 consumption. Also, the results indicate that TcCPx is able to contribute to the decrease in the H_2O_2 levels generated inside mitochondria. Overexpression of both tryparedoxin peroxidases led to a lower H_2O_2 generation, reinforcing the role of those enzymes in hydroperoxide detoxification.
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BQ06 - IMMUNOCYTOCHEMICAL LOCALIZATION AND EXPRESSION OF MATRIX METALLOPEPTIDASE-9 HOMOLOGUES IN *TRYPANOSOMA CRUZI*-INFECTED MOUSE EMBRYO CARDIOMYOCYTES

Nogueira de Melo, A.C.[1], Souza, E.P.[1], Velloso, A.S.[2], Pereira, M.C.[2], Meirelles, M.N.L.[2], Vermelho, A.B.[1Σ]
[1] *Departamento de Microbiologia Geral, Instituto de Microbiologia Prof. Paulo de Góes (IMPPG), Centro de Ciências da Saúde (CCS), Bloco I, Universidade Federal do Rio de Janeiro (UFRJ), Ilha do Fundão, 21941-902, Rio de Janeiro, RJ, Brazil*
[2] *Departamento de Ultraestrutura e Biologia Celular, Instituto Oswaldo Cruz, FIOCRUZ, Avenida Brasil 4365, 21045-900, Rio de Janeiro, RJ, Brazil*
[Σ] abvermelho@micro.ufrj.br; anamelo11@hotmail.com

Studies have been performed on the explanted myocardial specimens from patients with Chagas' disease, and identified matrix metallopeptidases (MMPs) as the main ECM proteolytic pathway that likely contribute to the ECM remodeling process. In the present study we investigated the presence of matrix metallopeptidases in *T. cruzi*-infected mouse embryo cardiomyocytes, by use of gelatin zymography, Western blot and imunocytochemical analyses. The analysis of the cell-associated peptidases from the systems showed two cysteine peptidases with an apparent molecular mass of 80 and 50 kDa. The spent culture media analysed displayed two distinct proteolytic classes on both systems: a band of 120 kDa, which was completely inhibited by E-64, suggesting that it belongs to the cysteine peptidase class, and a 97 kDa metallopeptidase, since it was restrained by 1,10-phenanthroline. Western blot analysis using anti-MMP-9 and anti-MMP-2 antibodies recognized the 100 and 70 kDa protein bands, indicating that metallo-type peptidases belong to the matrix metallopeptidase-9 and -2 family, respectively. The anti-cruzipain antibody reacted with several isoforms of cruzipain. Additionally, the cells were analysed by immunocytochemistry using anti-mouse MMP-9 antibody. These studies showed that gold immune complexes were distributed in the flagellar pocket and cytoplasmatic membrane of the intracellular parasite. The cytoplasm of infected cardiomyocyte cells showed few immunogold complexes. Our observations suggest that MMP-9 activity during *T. cruzi*-cardiomyocytes interaction might contribute to the ECM remodeling process, leading to the severe chagasic myocarditis during Chagas' disease. Supported by: FAPERJ, MCT/CNPq, FIOCRUZ, CEPG/UFRJ, FUJB, CAPES

BQ07 - Studies of enzymes involved in heme A biosynthesis in *Trypanosoma cruzi*

Celeste Buchensky[1], Paula Almirón[1] and Julia A. Cricco*[1,2]
[1]Instituto de Biología Molecular y Celular de Rosario (IBR) Consejo Nacional de Investigaciones Científicas y Técnicas, CONICET – Universidad Nacional de Rosario UNR, Argentina.
[2]Área Biofísica. Facultad de Ciencias Bioquímicas y Farmacéuticas UNR, Argentina.
*cricco@ibr.gov.ar

Heme A is an essential cofactor for eukaryotic cytochrome *c* oxidases. This cofactor is derived from heme B *via* two enzymatic reactions. The first one, catalyzed by heme O synthase (HOS, Cox10p), results in the conversion of the vinyl group on pyrrole ring A into a 17-hydroxyethylfarnesyl moiety. In the second transformation, heme A synthase (HAS, Cox15p) catalyzes the oxidation of the methyl group on pyrrole ring D into an aldehyde. The biosynthesis of this cofactor is carried out in the mitochondria, and HOS and HAS enzymes are encoded by nuclear genes *COX10* y *COX15* respectively. Although *T. cruzi* does not synthesize heme *de novo*, we have identified by sequence homology searches the open reading frames for putative Cox10p and Cox15p enzymes. To assign the function of above mentioned genes (Tc*COX10* and Tc*COX15*), they were cloned and expressed in *S. cerevisiae cox10 and cox15* knock out cells respectively (*cox10*□□and *cox15*□). These mutant cells are respiratory deficient and show impaired heme A biosynthesis. The expression of TcCox10 and TcCox15 in yeast mutants suppresses the phenotype of the knock out cells, restoring the standard levels of respiratory activity and mitochondrial heme A concentration. These results support that *T. cruzi* presents a heme A biosynthetic pathway. In addition, these data are in agreement with early studies showing the aa3 type of cytochrome *c* oxidase as a main oxidase in epimastigotes and the fact that recent proteomic reports revealed the presence of different subunits of cytocrome *c* oxidase in epimastigotes and trypomastigotes forms. Notwithstanding this parasite does not synthesize heme, the presence of Cox10p and Cox15p enzymes and other hemoproteins suggest that this cofactor has to be imported and transported into the mitochondria, where it is modified in order to be utilized by hemoproteins involved in key processes.
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BQ08 - Characterization of post-translation modification of eIF5A in *Trypanosoma cruzi*

Chung, J[1]., Schenkman, S.[1*]
[1]Departamento de Microbiologia, Imunologia e Parasitologia, UNIFESP.
*sschenkman@unifesp.br

In trypanosomatids the analysis of post-translational modifications (PTM) is particularly important because these organisms do not use transcription initiation as a regulatory step to control gene expression. 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 a small, highly conserved protein in eukaryotic cells and although it was identified as a translation initiator factor, the precise functional role in eukaryotic cells is not defined yet. It is the unique protein known to be hypusinated at a conserved lysine residue, which is essential for cell survival. However, little is known about how the phosphorylation affects its function. In the present work we identified the phosphorylation and hypusination sites on *T. cruzi* eIF5A after affinity purification using monospecific antibodies and ESI-Q-TOF and MALDI-Q-TOF mass spectrometry. The conserved lysine 53 was found hypusinated and serine 2 was found acetylated and phosphorylated. Three methylated residues were also found. We are currently investigating whether the serine 2 is the one that phosphorylation changes during stationary phase, or if there other phosphorylated sites.
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BQ09 - EFFECT OF MYRIOCIN ON
Leishmania (L.) amazonensis* and *Leishmania (V.) braziliensis

CASTRO, E.V.; TAKAHASHI, H.K.; STRAUS, A.H.
Department of Biochemistry,
Universidade Federal de São Paulo, São Paulo-SP, Brazil
straus.bioq@epm.br

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.* In this study it was investigated the effect of myriocin, which blocks the first step of sphingolipid synthesis, serine + palmitate forming 3 ketodihydrosphingosine (3KDS), on *L. (L.) amazonensis* and *L (V.) braziliensis* promastigotes growth, morphology and sphingolipid and phospholipid expression. We observed that *L (V.) braziliensis* promastigotes are more sensitive to myriocin than *L. (L.) amazonensis*, 1μM myriocin inhibited 50% of *L. (V.) braziliensis* growth while for *L. (L.) amazonensis* the same rate of inhibition was observed with 5μM myriocin. Besides growth rate reduction, myriocin promoted significant morphological changes, i.e. parasites presented rounded shape in presence of myriocin. For *L. (L.) amazonensis* the elongated morphology was reverted by addition of 5□M 3KDS. Analysis of *L. (L.) amazonensis* phospholipids showed a significant decrease in IPC expression from 14.5% (control parasites) to 4% and 2% in 2.5 and 5μM myriocin treated promastigotes, accompanied by increase in phosphatidylserine expression (from 6% to 17% and 25%) and lyso-phosphatidylinositol expression (from 2.6% to 5.3% and 7.7%, respectively). Analysis of *L (V.) braziliensis* phospholipids showed a decrease of IPC expression from 15% (control parasites) to 11% and 9% for 0.4 and 1μM myriocin treated promastigotes respectively, with concomitant increase of phosphatidylserine from 0.3% to 4.0% and 4.5%, respectively. Significant reduction in macrophage infectivity (43.6 % and 76.9%) by *L. (L.) amazonensis* myriocin 5μM and 12μM treated promastigotes, was observed. These results clearly indicate that myriocin inhibits parasite IPC synthesis, promotes morphological alterations, reduces parasite growth rate, and also reduces *L. (L.) amazonensis* promastigotes infectivity. On a next step infectivity of *L. (V.) braziliensis* treated with myriocin will be investigated.
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BQ10 - EFFECT OF SPHINGOLIPID SYNTHESIS INHIBITORS ON *Trypanosoma cruzi*

BRITO, T.K.; TAKAHASHI, H.K.; STRAUS, A.H.
Department of Biochemistry,
Universidade Federal de São Paulo, São Paulo-SP, Brazil
straus.bioq@epm.br

Trypanosoma cruzi presents high amounts of glycolipids as GIPLs/GIPCs as well as GPI-anchored mucin-like glycoproteins. These molecules have been implicated in host cell invasion and also in evasion from the host immune response. Variation in the lipid structures among different *T. cruzi* developmental stages, and strains are already well described. For example, *T. cruzi* G strain epimastigotes present glycolipids containing almost exclusively ceramide, whereas over 60% of the glycolipid expressed in CL strain epimastigotes contain ceramide and 40% of sn-1-alkyl-2-acylglycerol (Carreira et al 1996). In this study it was investigated the effect of myriocin, an inhibitor of serine-palmitoyltransferase, and Aureobasidin A, inhibitor of inositol phosphoryl ceramide synthase, on the growth rate, morphology and (glyco)lipid expression of epimastigotes. Myriocin (30μM) decreased in 27% the growth rate of epimastigotes G strain and altered their morphology. On the other hand, on strain CL no effect on parasite growth rate and morphology was observed. Interestingly, myriocin at 10μM promoted a drastic decrease on IPC levels on both strains, i.e., reduced about 90% IPC expression in G strain and 65% in CL strain. Aureobasidin A at 10 μM led to an inhibition on the growth rate of 58% and 74% for strains CL and G, respectively. In order to confirm the presence of glycolipids containing ceramide or alkyl-acylglycerol the lipid fraction was submitted to alkali treatment and analyzed by HPTLC stained with orcinol and immunostained with BST-1 monoclonal antibody. Reduction of glycolipids containing ceramide accompanied by increase of glycolipids containing alkyl-acylglycerol was detected in parasites treated with myriocin. No alteration of glycolipid pattern was observed after Aureobasidin A treatment. These results indicate that G strain epimastigotes in presence of myriocin start synthetize glycolipids containing sn-1-alkyl-2-acylglycerol. The (glyco)lipid biosynthetic mechanisms and the relationship among sphingolipids, morphology, growth rate are under investigation.

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BQ11 - EXPRESSION IN *E. coli* AND PARTIAL PURIFICATION OF THE THERMOSTABLE CARBOXYPEPTIDASE OF *Leishmania major*

Bernardo, G.S.*, Salmazo, P.V., Momo, P.B., Ward, R.J., de Oliveira, A.H.C.
Departamento de Química – FFCLRP/USP, Ribeirão Preto-SP, Brazil
*gisellesimoes@aluno.ffclrp.usp.br

Leishmaniasis is considered by the World Health Organization to be the second most important disease caused by a protozoan parasite. Biochemical and molecular biology studies of specific trypanosomatid proteins can help in the understanding of the biology of the *Leishmania* parasite. Metallocarboxypeptidases are carboxypeptidases containing a zinc ion bound in its active site and these proteins have been implicated in various biological functions, including degradation/recycling of proteins and processing of protein precursors. The thermostable carboxypeptidase of *L. major* (LmTCP) is classified as a peptidase family M32 domain and contains a zinc $H_{263}ESGH_{267}$. It was identified in a sub-proteome of the soluble microsomal fraction of *Leishmania major*. In this work, the region containing the LmTCP coding sequence was amplified from *L. major* genomic DNA by and cloned into the pET28a vector using *Nde*I and *BamH*I enzyme sites. The pET28aTCP construct were used for protein expression in *E. coli* BL21(DE3)pLysS during 5 hours by IPTG induction. The rLmTCP containing a His_6-tag showed high levels of expression and it was detected in the insoluble extract as inclusion bodies. The protein refolding was performed using a 6M-0M urea descending gradient plus 1mM 2-mercaptoethanol and subsequently the rLmTCP was purified by affinity chromatography. The protein pureness was observed in SDS-PAGE gel. The secondary structure of the rLmTCP was evaluated by circular dichroism, showing that the refolded protein was purified with a native-like structure.

Keywords: Leishmania, Protease, Expression, Purification.
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BQ12 - *LEISHMANIA MAJOR* CARRYING AN EXOGENOUS SPLICED LEADER GENE ARRAY DISPLAY DIFFERENTIAL ACTIVATION OF PROTEOLYSIS

Ferreira, T.R.*[1], Toledo, J.S.[1], Cloutier, S.[2] , Papadopoulou, B.[2], Cruz, A.K.[1]
[1]Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, Brasil
[2]Research Centre in Infectious Diseases of CHUL, Laval University, Québec, Canada
*tiago.rf@usp.br

Leishmania parasites exhibit complex regulatory mechanisms of gene expression, which are not yet fully understood. There is no evident control over activation of transcription due to inexistence of RNA polymerase II promoters in the parasite genome, with a few exceptions. The DNA transcription generates long primary polycistronic RNAs, which turn into mature mRNAs after a *trans*-splicing reaction, with the addition of a ~39nt sequence to their 5' extremity coupled to 3' polyadenylation of the adjacent upstream gene. This small sequence is the *spliced leader* (SL)**.** Due to these specific features the main processes ruling over gene expression in *Leishmania* spp. occur at post-transcriptional and post-translational levels, such as degradation and recycling of proteins. In a previous work we generated a *L. major* transfectant carrying a cosmid containing ~100 repeats of the SL gene. A remarkable phenotype presented by this mutant is virulence attenuation in *in vivo* infections. Based on the SL central role in RNA maturation, we believe that the surplus of SL RNA leads to abnormal changes in cell transcript and protein levels. In this work we confirmed by polysome profile analysis that protein translation in the SL overexpressor is slightly affected when compared to the transfectant control. Based on differences observed in a previous proteomic analysis we investigated the ubiquitin-proteasome system and the activity of cysteine and metalloproteases in the mentioned transfectants. By using 2D Western Blot we evaluated the pool of ubiquitin-labeled proteins, which is exacerbated in the SL RNA overexpressor. There is also an increase of cysteineprotease activity, despite the decrease in the metalloprotease, as revealed by enzymatic assays. Therefore we propose that changes observed indicate the parasite efforts to maintain cell homeostasis in face of the SL excess interfering with the amastigotes fitness within the host organism, a stressful environment for the parasite.
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BQ15 - PHOSPHOGLYERATE KINASE A (PGKA) OF *Trypanosoma cruzi*: PURIFICATION, KINETIC CHARACTERIZATION AND INIBITION STUDIES

Barros-Álvarez, X[1*]; Quiñones, W[1]; Michels, P. A. M[2]; Concepción, J. L[1].
[1] Unidad de Bioquimica de Parasitos, Facultad de Ciencias, Universidad de Los Andes, Merida, Venezuela
[2] Research Unit for Tropical Diseases, Christian de Duve Institute of Cellular Pathology and Laboratory of Biochemistry, Université Catholique de Louvain, Avenue Hippocrate 74, B-1200 Brussels, Belgium
[*]barrosximena@ula.ve

Glycolysis in *Trypanosoma cruzi* is not regulated in the classic enzymes, HK and PFK, and it is compartmentalized up to phosphoglycerate kinase (PGK) in a peroxisome-like organelle known as glycosome. PGK catalyzes the conversion of ADP and 1,3-bisphosphoglycerate (1,3BPG) to ATP and 3-phosphoglycerate (3PGA). In *T. cruzi*, PGK is present in the cytosol (PGKB), as well as in glycosomes (PGKA and PGKC). Although the role of PGKA in the intermediate metabolism of *T. cruzi* is not known with certainty, it has been proposed that PGKA should be involved in the maintenance of ATP balance inside the glycosome participating in the glycolytic flux control. It was confirmed that PGKA is located in the glycosome, associated to the membrane, and it is suggested that part of PGKA N-terminal domain is outwards, to the cytosol. The major difference between PGK isoenzymes in *T. cruzi* is the presence of an insertion of 80 aminoacids at PGKA's N-terminus. In order to determine its function, we constructed and overexpressed a recombinant truncated PGKA protein (tPGKA), without the 80 aminoacids insertion. Apparently, tPGKA wasn't capable of taking a correct conformation as seen by an increase in K_m values and a decrease in solubility. Kinetic constants of purified native PGKA (PGKA) and recombinant PGKA (rPGKA) were determined. V_{max} of PGKA for ATP and 3PGA suggest that the enzyme works in glycolytic sense. Km values for recombinant (K_mATP= 236 µM, K_m3PGA= 850 µM) and native (K_mATP= 217 µM, K_m3PGA= 174 µM) PGKAs are similar (same magnitude order) between them and to those reported for *T.brucei*. Similar inhibition effects of both PGKAs suggest that the active rPGKA -with high expression levels- could be useful in the design of drugs with specific targets looking for *T. cruzi* death.
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BQ16 - MOLECULAR STRUCTURE OF PHOSPHOINOSITOL-CONTAINING GLYCOLIPIDS FROM *Crithidia deanei*

Previato, J.O., Leitão G.C.S.[*], Dias, W.B., Romeiro, A., Mendonça-Previato, L.
Instituto de Biofísica Carlos Chagas Filho, Centro de Ciências da Saúde-Bloco G, Universidade Federal do Rio de Janeiro, Cidade Universitária, Ilha do Fundão, Rio de Janeiro, RJ, Brasil.
[*]gcleitao@biof.ufrj.br

Crithidia deanei is a monoxenic trypanosomatid that harbors a bacterium-like endosymbiont within the cytoplasm. This endosymbiont is integrated to the protozoan physiology in a way that its cell division is synchronized to the host cell cycle. *Crithidia* spp. are non-human parasites, which have the ability of easily growing in axenic cultures, sharing several metabolic pathways with higher eukaryotes. *Crithidia* spp. have been widely used as models for several studies on cell and molecular biology, ultrastructure and glycobiology. In the present study, we characterized the chemical structure of phosphoinositol-containing glycolipids (GIPL) from *C. deanei*. After hot phenolic/water extraction and alkaline hydrolysis of native *C. deanei* GIPL, the phosphoinositol (PI)-glycans were purified by gel filtration chromatography. Analytical data demonstrated that the PI-glycan contained mannose (Man), fucose (Fuc), glucose (Glc), N-acetyl glucosamine (GlcNAc) and inositol (Ins) in a molar ratio of 2:4:5:1:1, respectively. Eletron-Spray Mass Spectrum of *C. deanei* PI-glycan, in the positive ion mode, produced doubly charged ion $[M + 2H]^{2+}$ at m/z 1307. This pseudomolecular ion is compatible with a composition of 7 hexoses (Hex); 4 deoxyhexoses (deoxyHex); 1 N-acetylhexosamine (GlcNAc); 1 hexosamine (HexN); 1 inositol-phosphate (InsP) and 1 ethanolaminephosphate (EtNP). These data demonstrate for the first time the presence of Fuc and GlcNAc in the GIPL of a trypanosomatid. Recently (Turnock et al., J. Biol. Chem., 2007; Turnock and Ferguson, Euk Cell, 2007), the sugar nucleotide pool of GDP-Fuc in *Trypanosoma brucei*, *T. cruzi* and *Leishmania major* was described, suggesting the presence of Fuc-containing glycoconjugates in these parasites.
Supported by CNPq and FAPERJ.

BQ17 - DBL1&alpha DOMAIN OF *PLASMODIUM FALCIPARUM* ERYTHROCYTE MEMBRANE PROTEIN (PfEMP1) BINDS SPECIFICALLY TO BLOOD GROUP A TYPE 2: IMPLICATIONS IN THE SEVERE MALARIA

Carvalho, ST*[1]; Sousa, FJR[1]; Martins, TR[1]; Queiroz NR; Oliveira IA[1]; Mohana Borges, R[1]; Previato, JO[1]; Mendonça-Previato, L[1]; Todeschini, AR[1].
[1]Instituto de Biofísica – Universidade Federal do Rio de Janeiro - Brasil
sebagemino@yahoo.com.br

Protozoan parasite *Plasmodium falciparum* is responsible for most of the mortality and morbidity associated with malaria. Rosetting has been related with the occurrence of severe malaria in individuals infected with *P. falciparum*. The *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) expressed on the outer surface of infected red blood cells, though encoded by the parasite genome, has been identified as a rosetting promoter. PfEMP-1 binds to a variety of host molecules on the surface of uninfected red blood cells including to the ABH antigens from blood group. Several works suggest that individuals of blood group A, are more susceptible to rosette formation and severe malaria. However, there are no details on the molecular interaction between epitopes of the ABH blood group and PfEMP-1. In this work, we aimed to determine the structural requirements involved in the interaction of DBL1&alpha domain of PfEMP1 with blood group A, B and H epitopes and its subtypes A type1, A type 2 and A lac, B type 1, B type 2 and B lac, H type 1, H type 2 and H2. A recombinant DBL1&alpha□-GST was expressed and purified from *Escherichia coli* BL-21 (DE3) and its preference for the blood group A, B and H epitopes was analyzed by ELISA using biotinylated oligosaccharides. We observed that DBL1&alpha□domain showed higher affinity for the A type 2 epitope, GalNAc&alpha 1-3[Fuc&alpha 1-2]Gal□beta□1-4GlcNAc. Binding was dependent on the concentration of the oligosaccharide, being maximal at 5 μg/ml. DBL1&alpha showed low levels of biding or no binding to the other epitopes utilized. These observations demonstrate that interaction relies on the presence of GalNAc and the Gal□1-4GlcNAc moieties. These studies shed light on structural requirements for interaction of DBL1&alpha□□with ABH blood group epitopes and migth□serve as basis for the development of compounds to block roseting mediated by the PfEMP1.
Supported by CNPq, FAPERJ and CAPES

BQ18 - CHARACTERIZATION OF *TRYPANOSOMA CRUZI* PROTEINS DIFFERENTIALLY EXPRESSED IN TRYPOMASTIGOTE FORMS OF BENZONIDAZOLE RESISTANT AND SUSCEPTIBLE POPULATIONS

Pais, F.S.*, Silva-Pereira, R.A., Lopes dos Santos, S., Murta, S.M. F., Romanha, A.J.

Laboratório de Parasitologia Celular e Molecular, Centro de Pesquisas René Rachou - FIOCRUZ, Avenida Augusto de Lima 1715, 30190-002, Belo Horizonte, MG, Brasil.

*fspais@cpqrr.fiocruz.br

Previous work in our laboratory has identified several proteins by bidimensional electrophoresis and mass spectrometry as differentially expressed in trypomastigotes forms of *Trypanosoma cruzi* populations with *in vivo* selected resistance to Benznidazole (BZ). Our goal is to identify proteins potentially involved in the BZ resistance mechanisms and to indicate proteins that could be target for new drug development, since BZ presents a reasonable efficacy in the acute phase, but very low activity in the long-term chronic phase and undesirable side-effects. In the present work we have selected seven proteins identified by proteomic analysis as being differentially expressed in trypomastigotes forms of *T. cruzi* population susceptible and resistant to BZ for characterization. The expression of mRNA transcripts of genes: cofilin/actin depolymerizing factor, L-threonine 3-dehydrogenase, eukaryotic initiation factor 5a (eIF5a), malate dehydrogenase, malic enzyme, elongation factor 1α and ATpase β subunit, are being confirmed using primers designed to amplify the corresponded cDNA from *T. cruzi* CL Brener strain. In addition, Western blot results using a polyclonal antibody against eIF5a from *T. cruzi* (kindly provided by Dr. Sergio Schenkman/UNIFESP) confirmed that this protein is differentially expressed between epimastigotes forms from *T. cruzi* populations susceptible and resistant to BZ. Polyclonal antibodies against the remaining proteins will also be obtained in order to confirm their differential expression in populations with variable levels of BZ resistance. Further experiments, including functional analysis of these genes by inducing changes in the gene expression patterns in different populations of the parasite, will be performed to confirm the role of these proteins in the BZ resistance phenotype. Supported by: CNPq, PDTIS-FIOCRUZ.

BQ19 - *TRYPANOSOMA CRUZI* PEPTIDASES: MODULATION OF EXPRESSION BY GROWTH CONDITIONS AND DETECTION OF MATRIX METALLOPEPTIDASE-9- HOMOLOGUES

Nogueira de Melo, A.C.[1], Souza, E.P. [1] , Pereira, M.C. [2], Meirelles, M.N.L. [2], Vermelho, A.B.[1Σ]

[1] *Departamento de Microbiologia Geral, Instituto de Microbiologia Prof. Paulo de Góes (IMPPG), Centro de Ciências da Saúde (CCS), Bloco I, Universidade Federal do Rio de Janeiro (UFRJ), Ilha do Fundão, 21941-902, Rio de Janeiro, RJ, Brazil*
[2] *Departamento de Ultraestrutura e Biologia Celular, Instituto Oswaldo Cruz, FIOCRUZ, AvenidaBrasil 4365, 21045-900, Rio de Janeiro, RJ, Brazil*
[Σ] abvermelho@micro.ufrj.br; anamelo11@hotmail.com

Trypanosoma cruzi, the etiological agent of Chagas' disease, has been shown to contain several proteolytic activities. In the present study, the cell–associated and extracellular peptidases of *T. cruzi* grown in four different culture media were analyzed by measuring peptidase activity in gelatin-containing zymograms. Our results showed that the cell-associated peptidases as well as peptidases extracellularly released by *T. cruzi* displayed two distinct proteolytic classes: cysteine and metallopeptidase activities. Due to composition of the culture medium few qualitative changes could be detected in *T. cruzi* peptidase profiles. The major cysteine peptidase, cruzipain, synthesized by *T. cruzi* cells were detected in cellular parasite content, as a 50 kDa reactive polypeptides, after probing with anti-cruzipain antibody. In addition, metallo-type peptidases belonging to the matrix metallopeptidase-9 (MMP-9) family were revealed after western blot as a 97 kDa protein band in cellular extract, and an 85 kDa polypeptide in both cellular and secreted parasite extracts. The surface location of homologues of MMP-9 in *T. cruzi* was also evidenced by means of flow cytometry analysis. This is the first report on the presence of MMP-9-like molecules in *T. cruzi*. The presence of a matrix extracellular-degrading enzyme may play a role in *T. cruzi*-host cell interaction, making this enzyme an attractive potential target for future drug development against this pathogenic trypanosomatid.

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BQ20 - *TRYPANOSOMA CRUZI* AND NAPHTHOIMIDAZOLES: PROTEOMIC APPROACHES OF MECHANISM OF ACTION

Menna-Barreto, R.F.S. [1*], Beghini, D. G. [2], Ferreira, A. T. S. [2], Pinto, A. V. [3], Perales, J. [2]

[1] Laboratório de Biologia Celular, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz, Rio de Janeiro, Brasil.
[2] Laboratório de Toxinologia, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz, Rio de Janeiro, Brasil.
[3] Núcleo de Pesquisas em Produtos Naturais, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brasil.
*rubemb@ioc.fiocruz.br

In an effort to develop alternative drugs for Chagas disease derivatives of natural quinones were synthesized and screened on *Trypanosoma cruzi*. The most active were the naphthoimidazoles N1, N2 and N3 derived from β-lapachone. In trypanosomatids, transcription is not a regulatory step in gene expression, increasing the importance of proteomic approach for the understanding of drugs´ mechanism of action. In this work, we focused in the proteomic analysis of *T. cruzi* epimastigotes using two-dimensional electrophoresis (2D) and mass spectrometry and employed four experimental groups: control and treated with naphthoimidazoles N1, N2 and N3. The gels were tested using different conditions for protein extraction, pH range and sample buffer for protein solubilization. The optimal conditions for 2D electrophoresis were: freeze-thaw lysis as cell disruption method, 4-7 (18 cm) as pH range and 7M urea, 2M thiourea, 4% CHAPS, 60 mM DTT and 1% ampholytes as sample buffer. The gels were stained by Colloidal Coomassie Blue and automatically analyzed by Image Master® Platinum Software. Around 617 proteins were detected in each gel and the control group showed over expression in approximately 20 spots. Further experiments will be performed in order to identify differentially expressed spots. The present study could help identify the proteins implicated in the protozoan response to treatment with the three naphthoimidazoles and its implications on its biology.
Supported by CNPq, FIOCRUZ and FAPERJ.

BQ21 - KINETIC CHARACTERIZATION OF THE K30A AND H50Q MUTANTS OF THE NUCLEOSIDE DIPHOSPHATE KINASE FROM *Leishmania major*

Salmazo, P.V.*. , Leone, F. A., Masui, D. C., Ward, R.J., de Oliveira, A.H.C.
Departamento de Química, FFCLRP – USP, Ribeirão Preto – SP, Brazil
*pliniosv@aluno.ffclrp.usp.br

Leishmania is the causative agent of leishmaniasis, a disease that affects 12 million people worldwide. The clinical forms of the disease vary from skin to the visceral, which can lead to death. Unlike mammals, *Leishmania* is unable to synthesize purines de novo, and nucleoside diphosphate kinases are involved in the salvage pathway by which free purines are converted to nucleosides and subsequentely to nucleotides. The site-directed mutagenesis was performed in 3 steps of PCR reactions, the fragments obtained were purified, sequenced and cloned to the vector pET28a using *Nde*I/*BamH*I restriction sites. The expression induced by 5h using *E. coli* BL21(DE3)pLysS transformed with the pET28NDK-K30A and pET28NDK-H50Q constructions. The mutant enzymes were purified from crude extracts by affinity chromatography using Ni^{2+} resin. The eluted fractions were analyzed by 15% SDS-PAGE and the circular dichroism spectra confirmed the correct secondary structure of the mutant enzymes. The activity was assayed continuously at 30°C, in a Femto 700Plμs spectrophotometer equipped with thermostatted cell holder. The standard conditions were 50mM Tris/HCl buffer, pH 7.5, containing 10mM $MgCl_2$, 25mM KCl, 1.15mg phosphoenol-pyruvate, 0.2mg NADH, 0.4mM TDP, 1ng of proteins and different concentrations of donor substrate. Both site-directed mutants presented a reduced in about 50% of the phosphotransfer activity, showing altered values for apparent K, velocities and specific activities for different ATP donor substrates.
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BQ22 - Energy requirement for host cell invasion by *Trypanosoma cruzi* metacyclic forms

Martins, R. M.[1], Covarrubias, C.[1], Rojas, R. G.[2], Silber, A. M.[2], Yoshida, N.[1]*

1 Department of Microbiology, Immunology and Parasitology, Universidade Federal de São Paulo, R. Botucatu, 862, 6o andar, 04023-062, São Paulo, Brasil.
2 Department of Parasitology, Universidade de São Paulo, Av. Prof. Lineu Prestes, 1374, 05389-970, São Paulo, Brasil.
*nyoshida@unifesp.br

Trypanosoma cruzi requires energy to invade host cells. So far, the source of energy for that event is still unknown. To address this and other questions related to *T. cruzi* energy requirement and cell invasion, we analyzed metacyclic trypomastigote forms from *T. cruzi* group I and group II, G and CL respectively. In both strains, nutritional stress in PBS for 24, 36 or 48 h reduced the ATP content and the parasite ability to invade HeLa cells proportionally to the starvation time. Inhibition of ATP production by treating parasites with rotenone plus antimycin A also diminished the infectivity. When glucose, L-proline or glutamate, known to stimulate oxygen consumption in epimastigotes, was given to 36 h-starved metacyclic forms, L-proline restored the ATP levels to those of non-starved controls in both strains, but only CL strain recovered full infectivity towards HeLa cells. L-proline also increased parasite motility, as assessed by the ability to traverse a gastric mucin layer onto a transwell filter. Metacyclic forms of both strains transported glucose and L-proline in a similar fashion, whereas the glutamate uptake was lower. The ATP content restoration profile and infectivity by L-proline in starved Y strain parasites, a *T. cruzi* group II strain, was similar to the CL strain. In the same way, metacyclic forms from group I, Dm28 and Dm30, showed a profile comparable to that of G strain. This difference between the two groups was accompanied by alterations in the surface proteins known to be involved in the host cell invasion process. Supported by Fapesp, CAPES and CNPq.

BQ23 - Comparative Characterization of human and leishmanial UDP-Glucose Pyrophosphorylase

Jana Führing, Anne-Christin Lamerz, Rita Gerardy-Schahn*
Cellular Chemistry, Hannover Medical School, Hannover, Germany
*Gerardy-Schahn.Rita@mh-hannover.de

The enzyme UDP-glucose pyrophosphorylase catalyzes the synthesis of UDP-glucose from UTP and glucose-1-phosphate. Besides its central position in carbohydrate metabolism, UDP-activated glucose is a prerequisite for the formation of UDP-galactose, which is a major component of *Leishmania* cell surface glycoconjugates (including glycosylinositolphospholipids, GIPL; lipophosphoglycans, LPG; and proteophosphoglycans, PPG). By targeted gene replacement of the gene encoding UGP in *Leishmania major*, we generated a mutant in which LPG and PPG were drastically reduced, demonstrating that UDP-glucose is a major route to UDP-galactose in *Leishmania major*. Since the glycocalyx is essential for the parasite's survival and proliferation in the sandfly vector and the mammalian host, disturbing its formation by specific inhibition of *Leishmania* UGP might constitute an approach for the development of drugs. However, in order to solely target *Leishmania major* UGP, which presents significant homology to its human counterpart, a detailed characterization of both UGPs is indispensable. For this purpose, the parasitic enzyme and two isoforms of human UGP were cloned, recombinantly expressed, purified and characterized. Analyses highlight major differences in the quaternary organisation of the two species' pyrophosphorylases. While *L. major* UGP exists only as an enzymatically active monomer, octamerisation of the human enzyme seems to be required for activity. Furthermore, creation of single point mutations within a C-terminal beta-helix of the human enzyme revealed several conserved, mostly hydrophobic residues essential for oligomerisation. Our presentation will summarize the differences between human and leishmanial UGP that might be exploitable for specific inhibition.
Supported by Deutsche Forschungsgemeinschaft.

BQ24 - EFFECTS OF ETHANOLIC AND HEXANIC CRUDE EXTRACTS FROM *ALTERNANTHERA MARITIMA* UPON TRYPANOSOMA CRUZI BIOENERGETICS

[1]Fabretti, D. C.; [1]Silva, T. M.; [2]Salvador, M. J.; [1*]Gadelha, F. R.
[1]Departamento de Bioquímica, [2]Departamento de Biologia Vegetal, Instituto de Biologia, Universidade Estadual de Campinas, Campinas, Brazil
thiagobioms@yahoo.com.br

The search for new therapies for Chagas' disease treatment is necessary due to its high prevalence in Latin America and the lack of an efficient treatment. The great biodiversity of Brazilian flora can provide bioactive compounds for the development of new phytomedicines. *Alternanthera maritima* belongs to the Amaranthaceae family, which has many species with medical and food relevance. Some extracts from this family showed promising activity against bacteria, fungi and cellular viability in *Trypanosoma cruzi* and *Leishmania (Leishmania) amazonensis*. Hence, the objective of this work was to analyze the effects of *A. maritima* crude extracts from roots (hexanic and ethanolic extracts, AMRH and AMRE, respectively) and aerial parts (hexanic and ethanolic extracts, AMPAH and AMPAE, respectively) on *T. cruzi* epimastigote (Y strain) mitochondrial bioenergetics. Once determined the IC_{50} values (AMPAE: 3.42mg.mL^{-1}; AMPAH: 0.83mg.mL^{-1}; AMRH: 1.67mg.mL^{-1}; AMRE: 10.34mg.mL^{-1}), oxygen consumption and mitochondrial membrane potential ($\Delta\Psi$) were determined by the Safranine O method in cells incubated in the presence of sub-lethal extract concentrations, except for AMRE. No significant differences related to control was observed in succinate-supported oxygen consumption in cells treated with all extracts (~0.40 ± 0,05 nmoles O_2 consumed / min / 10^7 cells). Also, lower respiratory control (State 3/State4) in treated cells (1.56 ± 0.09, 1.62 ± 0.17 and 1.58 ± 0.14 for AMRH, AMPAE and AMPAH, respectively) related to control (2.0 ± 0.22) was observed. Treated cells also showed lower $\Delta\Psi$ compared to control, but AMRH had a stronger effect than the others. Direct addition of AMRH led to $\Delta\Psi$ collapse although the other extracts produced no alterations. Further experiments are under development to elucidate the mechanism of action of these extracts.
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BQ25 - *TRYPANOSOMA CRUZI* RESPIRATORY CHAIN BEHAVIOR UPON MALONATE AND CYANIDE INHIBITION

Silva, T.M.*; Mariano, T.S., Peloso, E. F. & Gadelha, F.R.
[1]Departamento de Bioquímica, Instituto de Biologia, Universidade Estadual de Campinas, Campinas, Brazil
*thiagobioms@yahoo.com.br

Biochemical and molecular variations confer a high degree of heterogeneity among *Trypanosoma cruzi* strains, representing a challenge for the development of an effective Chagas' disease treatment. We have previously shown that oxygen consumption rates supported by different substrates were similar in *T. cruzi* strains (Y and Tulahuen 2). No difference was observed in mitochondrial membrane potential along the growth curve. Also, hydrogen peroxide generation decreased significantly from log to late stationary phase in Y strain, while in Tulahuen 2 it was not possible to detect in our conditions. In order to continue our work we evaluated the effect of KCN (2mM) and malonate (10mM) on *T. cruzi* respiratory chain along the growth curve of the strains mentioned. The substrates used were succinate (5mM) or malate (5mM) / pyruvate (5mM). Malonate inhibition, when succinate was used as substrate, was higher than in the presence of malate/pyruvate and reached lower inhibition levels in the stationary phase (31% and 25% lower than the log phase in Tulahuen 2 and Y strain, respectively). Using malate/pyruvate as substrate, no significant differences were observed along the growth curve of both strains related to malonate inhibition (~35% and 28% for Tulahuen 2 and Y strain, respectively). KCN promoted a strong inhibition (~90%) but did not bring respiration to a complete stop in both strains, even when added after malonate and regardless of the substrate or growth phase. Similar results were obtained when cells harvested in different points of the growth curve were starved for 3 hours, although lower oxygen consumption rates were observed. Data suggest that another source for oxygen consumption besides the known respiratory chain exist, but in relation to malonate and KCN inhibition no significant differences appears to exist between strains. Further experiments are being developed to better understand *T. cruzi*´s respiratory chain.
Supported by CAPES and FAPESP.

BQ26 - EFFECT OF *CRATYLIA MOLLIS* SEED LECTIN ON *TRYPANOSOMA CRUZI* TRYPOMASTIGOTES AND EPIMASTIGOTES CELL DEATH

Fernandes, M. P.[1*]; Inada, N. M.[1]; Araújo, F. F. B.[2]; Gadelha, F. R.[3]; Alves, M. J. M.[4]; Correia, M. T. S.[2]; Coelho, L. C. B. B.[2]; and Vercesi, A. E.[1]
[*]e-mail:marianapfernandes@yahoo.com.br

[1]Dept. Patologia Clínica, FCM, UNICAMP, Campinas, SP, Brazil; [2]Dept. Bioquímica, CCB, UFPE, Recife-PE, Brazil; [3]Dept. Bioquímica, IB, UNICAMP, Campinas, SP, Brazil; [4]Dept. Bioquímica, IQ, USP, São Paulo, Brazil.

Lectins are proteins or glycoproteins that serve as tools in glycobiology research and can be employed for the detection of cell surface glycoconjugates. Herein we evaluated, whether *Cratylia mollis* seed lectin (Cramoll 1,4) has cytotoxic effects on *Trypanosoma cruzi*. Cramoll 1,4 recognized glycoconjugates present on parasite cell surface leading to agglutination of the epimastigotes and trypomastigotes in a dose-dependent manner. Trypomastigotes plasma membrane permeabilization by Cramoll 1,4 was documented by fluorescence microscopy using propidium iodide. Lectin decreased epimastigotes proliferation, reaching 93% inhibition at 50 µg/ml. Incubation of epimastigotes (1.25×10^8 /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 mitochondrial Ca^{2+} overload. As a result, reactive oxygen species (ROS) production was increased by 5 times, mitochondrial membrane potential was significantly decreased and ADP phosphorylation was impaired. Interestingly, plasma membrane permeabilization promoted by 20 µM digitonin in a Ca^{2+} containing medium led to similar results as Cramoll 1,4. Uncoupled respiration of *T. cruzi* epimastigotes was not affected by Cramoll 1,4 plus Ca^{2+} treatment, but oligomycin poisoned respiration was 60% higher than the respective control. Besides that, lectin decreased the $\Delta\Psi$ of *T. cruzi* mitochondrial fractions, but in the presence of EGTA it had no effect. In conclusion, Cramoll 1,4 toxicity to *T. cruzi* seems to result from a concerted action on parasite plasma and mitochondrial membranes, leading to mitochondrial Ca^{2+} overload and ROS production.
Supported by: CAPES, CNPq and FAPESP.

BQ27 - *TRYPANOSOMA CRUZI* EXPLORES CD8$^+$ T CELL SIALYLATION TO DAMPEN AG-SPECIFIC CD8+ T CELL RESPONSES

Adriane R. Todeschini[1], Leonardo Freire-de-Lima[1], Frederico A. Silva[1], Sebastião T. Carvalho[1], Maurício M. Rodrigues[2], Thomas Oeltmann[3], George A. DosReis[1], Lucia Mendonça-Previato[1], José O. Previato[1],

1Instituto de Biofísica Carlos Chagas Filho, Centro de Ciências da Saúde-Bloco G, Universidade Federal do Rio de Janeiro, 21949-900, Cidade Universitária, Ilha do Fundão, Rio de Janeiro, Brasil.adrianet@biof.ufrj.br. **2**Centro Interdisciplinar de Terapia Gênica e Parasitologia, Escola Paulista de Medicina, Universidade Federal de São Paulo, 04044-010, Rua Mirassol 207, São Paulo, Brasil. **3** Vanderbilt University, School of Medicine, Nashville, TN, USA.

Trypanosoma cruzi, the protozoan parasite that causes Chagas´ disease, shows a number of biological peculiarities and several interesting aspects of its glycobiology. One of the most notable is the presence of a unique cell surface *trans*-sialidase activity. This enzyme can catalyze desialylation and sialylation in glycan structure during T-cell activation on specific glycoproteins, implying that these glycans modulate precise functions in T-cell biology. For example, upon activation, cytotoxic CD8$^+$ T lymphocytes loose loss of sialic acid enhancing their effector activity and which can be monitored on the basis of increased binding of the lectin peanut agglutinin (PNA^{high}). In this study we investigated the effect of sialylation mediated by *trans*-sialidase on the CD8$^+$ T cell responses of mice infected with *T. cruzi*. Despite presenting a phenotype of activated cells (CD44^{high}) CD8$^+$ T cells from *T. cruzi*-infected mice treated with active *trans*-sialidase presented decreased binding of the peanut agglutinin (PNA^{low}) suggesting a sialylation of CD8$^+$ T cell surface by active *trans*-sialidase. Our results indicate that CD43 on CD8$^+$ T cells surface is a target of *trans*-sialidase activity during *T. cruzi* infection. Cytotoxic activity of activated CD8$^+$ T cells against an immunodominant peptide was decreased upon active *trans*-sialidase mediated sialylation *in vitro* and *in vivo.* Also, we found that the parasite's native *trans*-sialidase acts on CD8$^+$ T cells during infection, as CD8$^+$ T cells from infected mice treated with a specific competitive inhibitor of *trans*-sialidase (an enzymatically inactive analogous) had strongly decreased sialylation, i.e. they reverted to the glycosylation status expected in the absence of parasite manipulation. Our data open a possibility that sialylation of CD8$^+$ T cells, as result of *trans*-sialidase activity, might dampen CD8$^+$ T cell response in *T. cruzi*-infected host.
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BQ28 - Analysis of metallopeptidase activity during interaction of peritoneal macrophages and *Leishmania (Viannia) braziliensis* isolates associated with distinct clinical manifestations of leishmaniasis

Camila Mesquita-Rodrigues[1]; Jose Batista de Jesus[2, 3]; Patricia Cuervo[1]

[1]Laboratório de Pesquisa em Leishmaniose, Instituto Oswaldo Cruz, FIOCRUZ, Rio de Janeiro, Brasil; [2]Laboratório de Biologia Molecular e Doenças Endêmicas, Instituto Oswaldo Cruz, FIOCRUZ, Rio de Janeiro, Brasil; [3]Departamento de Ciências Naturais, UFSJ, Minas Gerais, Brasil

Leishmania (*Viannia*) *braziliensis*, a parasite autochthonous of the American continent, is the main etiological agent of American Tegumentary Leishmaniasis (ATL). The disease encompasses a broad spectrum of clinical manifestations ranging from self-healing cutaneous lesions to disseminated forms, with mucosal damage. Previous works of our group showed that *L. (V.) braziliensis* strains isolated from patients with cutaneous, disseminated and mucosal forms of ATL display different profiles of metallopeptidase activities. Our studies also showed that zymographic profiles from those isolates remain unaltered during prolonged *in vitro* culture. In the current work, throughout a substrate-gel electrophoresis approach, we performed a comparative analysis of the peptidase expression from those *L. (V.) braziliensis* isolates during *in vitro* interaction with peritoneal macrophages. Zymographic assays were carried out over 10% polyacrylamide gel copolymerized with 0.1% porcine gelatin. Peptidases were characterized according to their pH range of activity and sensitivity to specific peptidase inhibitors. Zymographic assays revealed a proteolytic profile consisting of 4-6 bands ranging from 80–200 kDa in non-infected macrophages at pH range from 7.5 to 10. Any enzymatic activity was observed at pH 3.5 and 5.5. Time course assays of non-infected macrophages performed from 24 to 96 h at pH 10 showed that both the intensity and number of proteolytic bands progressively increased over time. During interaction of macrophages with mucosal, disseminated and cutaneous isolates of *L. (V.) braziliensis* it was observed a complex profile of proteolytic activities composed of the sum of activities from macrophages and intracellular parasites. Use of 1,10-phenantroline, a specific metallopeptidase inhibitor, abrogated all enzymatic activities. When the proteolytic profile of *Leishmania*-macrophage interaction was compared with the proteolytic profile from promastigote´s extracts it was observed that intracellular amastigotes express some of the main metallopeptidase activities observed in promastigote forms. Using optical and scanning electron microscopies it was observed that *L. (V.) braziliensis* mucosal, disseminated and cutaneous isolates exhibited different abilities to infect macrophages. Finally, use of 1,10-phenantroline during interaction affected the invasion and multiplication abilities of mucosal isolate. *This work was supported by CNPq-FIOCRUZ.*

BQ29 - *LEISHMANIA CHAGASI* ECTO-3' NUCLEOTIDASE AND ECTO-PHOSPHATASE ACTIVITIES ARE MODULATED BY THE AMOUNT OF ADDED INORGANIC PHOSPHATE INTO THE CULTURE MEDIUM

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

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

In the New World, *Leishmania chagasi* is the parasite causative of visceral leishmaniasis. Some trypanosomatids, including *Leishmania* species, present a unique bifunctional externally oriented cell surface membrane enzyme 3'-nucleotidase/ nuclease, which is involved in the salvage of host-derived purines. Phosphorylation–dephosphorylation of serine, threonine, and tyrosine residues triggers conformational changes that modulate protein biological properties. In this work, we analyzed the growth and some ecto-phosphohydrolase activities of *Leishmania chagasi* modulated by the absence or the presence of added inorganic phosphate to 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. The parasites grown in the absence of added phosphate inorganic presented 50% growth reduction as compared to the cells grown in the presence of the phosphate source. On the other hand, a two-fold increase in the ecto-3' nucleotidase activity was observed 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. We also analyzed the ecto-3' nucleotidase activity of some *Leishmania* species, such as *L. major, L. amazonensis, L. tropica, L. braziliensis* and *L. chagasi.* This last one presented four-fold as much activity, as compared to the other species. Also, 92% inhibition of the phosphatase activity was observed when β-glycerol phosphate was used as a substrate, when the *L. chagasi* promastigotes were grown in the absence of added inorganic phosphate. However, using *p*-nitrophenylphosphate as substrate, only 35% increase of the phosphatase activity was observed, when *L. chagasi* was grown in the absence of added inorganic phosphate.
Supported by: CNPq, FAPERJ.

BQ30 - Exploring the Role of Molecules From The Blood-Sucking Bug and Their Influence Upon Proliferation and Differentiation Processes of *Trypanosoma cruzi*

Sultano PE[1], Saraiva FMS[1], Laranja GAT[1], Nogueira NPA[1] and Paes MC[1].
[1] Instituto de Biologia Roberto Alcântara Gomes – Departamento de Bioquímica – Universidade do Estado do Rio de Janeiro - RJ

Trypanosoma cruzi undergoes several morphogenetic modifications inside the triatomine vector. There are two most important processes: (i) the multiplication of the non infecting and replicative form, the epimastigotes, that takes place in the insect midgut, and (ii) the transformation of epimastigotes into the non-replicative and infective metacyclic trypomastigotes (metacyclogenesis, which occurs mainly in the rectum). It is known that insect factors may influence the biology of *T. cruzi*. Then, we ask if heme (found in high concentrations, about 10 mM, in the midgut), hemozoin (heme crystals present in midgut and feces) and urate (present in the vector urine), would regulate proliferation and differentiation of parasites. For that, metacyclogenesis were analyzed for several times in the presence of these molecules and at 96h, we observed a decrease in total cell number in parasites treated with heme or hemozoin, while urate showed an increment of total parasite number when compared to the control. We also investigated the effects of urate and hemozoin upon proliferation. For that, parasites were grown in BHI, 10% FCS and 30 μM heme, and then, exposed to urate or hemozoin. Thus, we observed that urate impaired the proliferative effect of heme, while, hemozoin presented no effect upon proliferation. According to the observation that urate decreased epimastigote multiplication in an appropriated nutritional medium, we searched for metacyclic forms and surprisingly, parasites exposed to urate presented an enrichment of trypomastigotes when compared to cells incubated with heme. Hence, our data suggest that hemozoin, a byproduct of the vector digestion, is not implicated in proliferation and decreases differentiation rates. Differently, urate found in the insect urine, stimulates metacyclogenesis, even in a high nutritional medium. Oppositely, heme, a molecule previously shown to increase epimastigotes proliferation, decreases differentiation, suggesting a novel range of molecules present in the vector modulating parasites multiplication and differentiation. Supported by FAPERJ

BQ31 - EFFECT OF ANGIOTENSIN PEPTIDES DURING THE INFECTION OF RED BLOOD CELLS BY *Plasmodium falciparum*

Saraiva, V.B.[1]*; Souza-Silva, L.[2]; Ferreira-Da Silva, C.T.[2]; Souza M. C.[3]; Teixeira-Ferreira, A.[4] Caruso-Neves C.[2]; Pinheiro, A.A.S.[2].
[1]Instituto Federal de Educação Ciência e Tecnologia, Instituto Federal Fluminense Campus Cabo Frio, Rio de Janeiro, Brasil.
[2]Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brasil.
[3]Fundação Oswaldo Cruz, Instituto de Tecnologia em Fármacos, Rio de Janeiro, Brasil.
[4]Fundação Oswaldo Cruz, Rio de Janeiro, Brasil
*vsaraiva@cefetcampos.br

The genus *Plasmodium* is the etiologic agent of malaria, a severe parasitic disease, and *P. falciparum* specie is responsible for the most fatal cases in humans. Recent studies have shown that signaling processes through G protein in red blood cells (RBC) is important for invasion and parasite proliferation and is considered a new target for antimalarial-drug development. The aim of this study is to evaluate the effect of angiotensin II (AngII) and angiotensin 1-7 (Ang1-7), two hormone peptides that bind to and signal through G-coupled receptors, during the invasion of RBC by *P. falciparum* merozoites. The erythrocytic forms of *P. falciparum* of the W2 strain were isolated in schizont stage through Percoll gradient and used in interaction assays with RBC maintaining 1.5% parasitemia and 5% hematocrit. The parasitemia and appearance of ring forms were recorded 24h after any treatment. Both AngII and Ang1-7 (10^{-6}–10^{-12} M) were able to decrease parasitemia in a biphasic effect with maximum inhibition observed at 10^{-8}M. Interestingly, losartan and PD123319, antagonists of AngII receptors (AT1 and AT2, respectively) did not change the inhibitory effect of AngII. However, A779, Ang1-7 receptor blocker named MAS receptor, was able to partially reverse the inhibitory effect of AngII and Ang1-7 in the same level. In addition, when AngII and Ang1-7 were added concomitantly to the coincubation assay, they showed a similar but non addictive effect. Taken together, these results suggest that AngII and Ang1-7 are able to modulate merozoite/erythrocyte interaction by inhibiting the infection of new RBC and this effect is independent of AT1 or AT2 receptors but it does depend on MAS receptor. The effect of A779 in the presence of AngII could be explained by its possible degradation to Ang1-7.
Supported by: CAPES, CNPq, FAPERJ and WHO

BQ32 - *TRYPANOSOMA CRUZI*: INHIBITION OF GROWTH AND INTERACTION WITH MAMMALIAN CELLS BY THE CALPAIN INHIBITOR MDL28170

Ennes-Vidal, V.[1], Menna-Barreto, R.F.S.[1], da Motta, F.F.[1], Santos, A.L.S.[2], Branquinha, M.H.[2], d`Avila-Levy, C. M[1].

[1] Instituto Oswaldo Cruz, Fiocruz, Rio de Janeiro, Brazil
[2] Instituto de Microbiologia Paulo de Góes, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil.

Calpains constitute a large family of calcium-dependent cytosolic cysteine peptidases. Recently, the presence of a large number of calpain-related proteins in trypanosomatids has been revealed, but there are no data on the specific functions of the calpain-like proteins in these microorganisms. In this context, we investigated the presence of these molecules in *Trypanosoma cruzi* (Y strain) by investigating the inhibitory capability of the calpain inhibitor III (MDL28170), aiming to show its effects on the growth of epimastigotes forms and on amastigotes intracellular development. Our results showed that an antibody raised against a calpain from *Drosophila melanogaster* (Anti-Dm-calpain) reacted with the cell surface of *T. cruzi* as judged by flow cytometry analysis. In addition, a polypeptide band migrating at approximately 80 kDa was detected by Western blotting with the same antibody. A search in GenBank data base revealed 4 sequences homologues to *D. melanogaster* calpains in *T. cruzi* genome, which presented a molecular mass in the range of 80 kDa. MDL28170 arrested the growth of epimastigotes forms, with a 50% lethal dose (LD50) of 34.7 µM at 48 hours. The treatment of infected murine peritoneal macrophages with 6,25 to 50 µM of MDL28170 led to a dose- and time-dependent significant decrease on the percentage of infection and on the number of interiorized parasites. The treatment of the infective tripomastigotes forms with the calpain inhibitor before the interaction with macrophages cells also resulted in a reduction in the infection. This data may contribute for the study of the functions of calpains in trypanosomatids and add new in vitro insights into the possibility of using calpain inhibitors in treating parasitic infections.
Supported by: MCT/CNPq, FAPERJ and Fiocruz.

BQ33 - Glycolytic flux control by aldolase and glyceraldehyde-3-phosphate dehydrogenase in bloodstream-form *Trypanosoma brucei*

Cáceres, A.J.[1], Haanstra, J.R[2], Bakker, B.[2], Michels, P.A.M[3], Hannaert, V[3*].
[1]Laboratorio de enzimología de parásitos, Facultad de Ciencias, Universidad de Los Andes, Mérida, Venezuela.
[2]Institute of Molecular Cell Biology, Vrije Universiteit Amsterdam, The Netherlands.
[3]Research Unit for Tropical Diseases, de Duve Institute, Université catholique de Louvain, Brussels, Belgium.
[*]veronique.hannaert@uclouvain.be

Glycolysis of *Trypanosoma brucei* is considered a promising target pathway for chemotherapeutic treatment of African sleeping sickness, because it is the single source of ATP for the parasite when living in the human bloodstream. There is a desperate need for new effective and safe drugs against sleeping sickness and other human diseases in tropical and subtropical countries caused by related protozoan parasites, because compounds currently used are largely inadequate due to inefficacy, toxicity and increasing drug resistance. A mathematical model of glycolysis in bloodstream-form *T. brucei* predicted that glucose transport would be the most effective target to inhibit glycolysis, which was experimentally confirmed. However, it also predicted that the glycolytic enzymes aldolase (ALD) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) too play a role in determining the flux. Interestingly, ALD and GAPDH appeared to possess distinct kinetic and structural properties that have already been exploited for the discovery of effective and selective inhibitors with trypanocidal activity. Here we present an experimental, quantitative assessment of the importance of these enzymes for flux control. This was achieved by decreasing the concentrations of ALD and GAPDH in bloodstream-form *T. brucei* by RNA interference (RNAi). The effects of these knockdowns on parasite growth, levels of various enzymes and transcripts, specific activities of aldolase, glyceraldehyde-3-phosphate dehydrogenase, hexokinase, phosphoglycerate kinase and pyruvate kinase, and glycolytic flux were studied and compared with model predictions. A partial depletion of ALD and GAPDH was already sufficient to rapidly kill the trypanosomes. An effect was also observed on the activity of some other glycolytic enzymes, and the overall effect on the glycolytic flux was more important than predicted by the model, in particular in the ALD-RNAi cell line. The discrepancy between model and experiment may at least partly be attributed to a decreased expression of other glycolytic enzymes.
Supported by INCO-DEV programme and CDCHT-ULA.

BQ34 - GLUCOSE KINASES FROM *Trypanosoma cruzi* AND *Leishmania spp.*

G. Gottberg[1], D. Mercado[1], A. Cáceres[1*], J. Concepción[1].
Laboratorio de Enzimología de Parásitos, Facultad de Ciencias, Universidad de Los Andes, Mérida, Venezuela
*acaceres@ula.ve

Trypanosoma cruzi and *Leishmania spp* are protozoan parasites that produce diseases of public health importance worldwide. In all Trypanosomatids, the glycolytic pathway is important for ATP generation, being its first step, the phosphorilation of glucose. Two novel glucokinases were found and characterized in *Trypanosoma cruzi* (*Tc*GlcK) and *Leishmania major* (*Lmj*GlcK). Both Trypanosomatids enzymes were only able to phosphorylate glucose, being true glucokinases. None of them were inhibited by glucose-6-phosphate or by inorganic pyrophosphate, in contrast with the inhibition reported for hexokinases of those parasites. The studies of subcellular localization determined that GlcKs are in the glycosomes. Because, GlcKs and HKs are both in the same organelle; their relative amount was measured in purified glycosomes. HKs were 9 to 16- fold higher concentrations than GlcKs. Furthermore, it was evaluated if these enzymes were expressed differentially throughout the growth curves of epimastigotes of *T. cruzi* and promastigotes of *L. mexicana*. It was shown that both enzymes are expressed constitutively. However, when the expression of GlcKs and HKs was evaluated in all morphological stages (epimastigotes, tripomastigotes and amastigotes for *Trypanosoma cruzi*; promastigotes and amastigotes for *Leishmania mexicana*), it was found that HKs were expressed in all stages, while GlcKs were present only in insect stages of both parasites, even when its transcripts were present in all forms. These results suggest a posttranscriptional regulation of the *glck* genes; it could be mediated by sequences within the 3'-untranslated regions (3'UTRs). Indeed, was found a short interspersed degenerated transposon (LmSIDER, 588 bp) in the 3'UTR of *Lmjglck*, which could be promoting RNAm destabilization in the amastigote form of *Leishmania*. However, in *T. cruzi* it remains to be determined. Finally, a phylogenetic analysis of the trypanosomatids GlcKs showed that these enzymes belong to group A of the HK family, in which they form a separate cluster. Supported by CDCHT-ULA.

BQ35 - Heme and other porphyrins may be competing for the same carrier in *Trypanosoma cruzi* epimastigotes

Cupello, M. P.[1*], Saraiva, FMS[1], Souza, C.F., Correa-Soares, J.B.R.[2], Costa-e-Silva, C.[1], Laranja, G.A.T.[1], Coelho, M.G.P.[1], Oliveira, M.M.[1] and Paes, M.C.[1]
[1]Departamento de Bioquímica, IBRAG – UERJ.
[2] Instituto de Bioquímica Médica – UFRJ.
*Mauricio.cupello@yahoo.com.br

Trypanosoma cruzi is the etiological agent of Chaga´s disease. This pathogenic protozoa take up heme (Fe-protoporphyrin IX) from the environment to supply their nutritional needs. Heme is an important molecule in several biological reactions such as oxygen transport and cell respiration. Since heme is not synthesized in *Trypanosoma cruzi* epimastigotes (EPI) and it is in high concentrations (about 10 mM) in the environment of these cells, we have been investigating its metabolism in EPI. Our results showed peaks at the same retention times of heme and billiverdin standards when analyzed by HPLC. In spectrophotometric analysis these results was confirmed. Heme oxygenase (HO) is one of the major catabolic enzymes of heme that converts heme into carbon monoxide, iron and biliverdin, which is lately converted into bilirrubin. Until now there is no HO described in *T. cruzi* genome. In order to investigating a possible HO activity involved in heme catabolism in EPI, we evaluated the effect of Sn Protoporphyrin IX (SnPPIX - an inhibitor of heme oxygenase). When SnPPIX and heme were added to cells at the same time we did not observe the peak referent to biliverdin. Surprisingly, we also did not observe the peak referent to heme in these conditions. So, the uptake of heme was evaluated and it was observed that SnPPIX decreased this uptake. Then we evaluated the effect of SnPPIX on the parasite proliferation. Our previous results showed that the addition of heme increases the proliferation in a dose-dependent manner. According to the competition between the porphyrins, we observed that in the presence of SnPPIX there was a decrease of the parasites growth. Based on these results others porphyrins as PdPPIX and ZnPPIX were tested and the same effects were observed, indicating the possible existence of a carrier of porphyrins in *T. cruzi*.
Supported by FAPERJ

BQ36 - PROTEASOME DEPENDENT PROTEOLYSIS IN T. CRUZI DRUG RESISTENT STRAINS

Barboza, N.R.*[1] Leal, T.F.[1] Oliveira C.B. Lana, M.[1] Bahia, M.T.[1] Murta, S.[2] Romanha, A.J.[2] Guerra-Sá, R.[1]
[1]Departamento de Ciências Biológicas Universidade Federal de Ouro Preto
[2]Instituto Oswaldo Cruz, Centro de Pesquisa René Rachou
nrbarboza@gmail.com

Differences in susceptibility to Benzonidazole (BZ) and Nifurtimox (NFX) between T. cruzi strains may explain, at least in part, differences in the efficacy of these treatments in vertebrate hosts infected with T. cruzi. strains that are naturally resistant or susceptible to BZ and NFX have been described. Proteasome has an important role in degradation of normal, damaged, mutant, or misfolded proteins. This includes the degradation of normal and regulatory proteins in the cellular metabolism and additionally the removal of damaged proteins as a stress response. The objective of our work was understand the contribution of the proteasome in phenotype of resistant and susceptibleto benznidazole using the following strains: 17WTS,17LER, BZS, BZR, CL and Colombian. Our results showed that the RNA levels from beta subunit 1, 2 and 5 were similar, but the reactivity against the anti-alpha subunit showed a dramatic decrease in resistant when compared to susceptible strains. No significant changes in the chymotrypsin-, trypsin-, and caspase like activities of the proteasome were measured. The rate of protein degradation was measured by the accumulation of free tyrosine in crude extracts. Our results show similar levels of tyrosine between the strains after 90 min of incubation in presence of ATP plus ubiquitin. At this moment it is not clear that the bulk of all intracellular protein is degraded by the Ub-proteasome pathway in all strains investigated in this work. Finally, these findings suggest that protein breakdown during epimastigote proliferation can be modulated by activation of the ubiquitin and post translation modification in the 20S proteasome. Now this hypothesis will be investigated.
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BQ37 - Metalloproteinase inhibitors reduce *Blastocrithidia culicis* adhesion to the insect midgut

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 antibodies 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. Studies with the metalloproteinase inhibitors EDTA, EGTA and 1,10 phenanthroline revealed that the pre-treatment of *B. culicis* with these inhibitors reduced parasite adhesion to *Aedes aegypti midgut*. These results suggest an important role of gp63 molecules and metalloproteinases in the interaction between this trypanosomatid and the invertebrate host.

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BQ38 - HIV Aspartyl Peptidase Inhibitors Interfere with Cellular Proliferation, Ultrastructure and Macrophage Infection of *Leishmania amazonensis*

Santos, LO[1], Marinho, FA[2], Altoé, EF[1], Vitório, BS[1], Alves, CR[1], Britto, C.[1], Motta MC[3], Branquinha, MH[2], Santos, ALS[2], d'Avila-Levy, CM[1]

[1] Laboratorio de Biologia Molecular e Doenças Endêmicas (FIOCRUZ); [2] Instituto de Microbiologia Prof. Paulo de Góes (UFRJ); [3] Instituto de Biofísica Carlos Chagas Filho (UFRJ).

Leishmania is a protozoan etiologic agent of leishmaniasis. Current therapy for leishmaniasis is suboptimal due to toxicity of the available therapeutic agents and the emergence of drug resistance. Compounding these problems is the increase in the number of cases of *Leishmania*-HIV coinfection, due to the overlap between the AIDS epidemic and leishmaniasis. In the present report, we have investigated the effect of HIV aspartyl peptidase inhibitors (PIs) on the *Leishmania amazonensis* proliferation, ultrastructure, interaction with macrophage cells and expression of classical peptidases which are directly involved in the *Leishmania* pathogenesis. All the HIV PIs impaired parasite growth in a dose-dependent fashion. PIs treatment caused profound changes in the *Leishmania* ultrastructure, including cytoplasm shrinking, increase in the number of lipid inclusions and some cells presenting the nucleus closely wrapped by endoplasmic reticulum, as well as chromatin condensation, which is suggestive of apoptotic death. The treatment of promastigote forms with PIs drastically reduced the association indexes during the interaction with murine macrophage cells and intracellular development of *Leishmania*. Despite all these beneficial effects, the PIs induced an increase in the expression of cpb and gp63, two well-known virulence factors expressed by *Leishmania* spp. The results presented herein add new in vitro insight into the wide spectrum efficacy of HIV PIs and suggest additional studies about the synergistic effects of classical antileishmanial compounds and HIV PIs in *Leishmania*-HIV-1 macrophages coinfection.

Key-words: HIV Aspartyl Peptidase Inhibitors, *Leishmania amazonensis*
Suppoted by: CNPq, Faperj and Fiocruz

BQ39 - Proteomic characterization of *Trypanosoma cruzi* membrane proteins

Oliveira, F.L.[1]*, Ferrão, P.M.[1], Waghabi, M.C.[1] and Mendonça-Lima, L.[1]
[1]Laboratório de Genômica Funcional e Bioinformática, Instituto Oswaldo Cruz, Fiocruz, Rio de Janeiro, Brasil
*fabiane@ioc.fiocruz.br

Trypanosoma cruzi (T. cruzi) is the etiological agent of Chagas's disease. The completion of the *T. cruzi* genome project has opened many questions that could be investigated by protein expression studies using a proteomic approach. Several *T. cruzi* proteomic studies have been reported and recent analysis of an enriched organellar fraction demonstrated that sample fractionation could potentialize the identification of proteins expressed in low levels, such as the membrane proteins. In this context, the aim of the present work is to characterize and identify *T. cruzi* membrane proteins. Protein extracts from axenic epimastigotes (Y strain, exponential growth phase) were prepared for two-dimensional electrophoresis (2-DE) using the *Subcellular Protein Extraction Kit* (Calbiochem) for membrane fraction. 2-DE gels were performed using both epimastigotes total protein extract and membrane fraction to characterize and compare both maps. Excised spots from gels were tripsinized and sent to MALDI-TOF/TOF and identified using the *Mascot online* program. Comparative proteome analysis were carried out, revealing that the protocol used for subcellular protein extraction was able to enrich the membrane fraction, presenting higher levels of surface proteins (around 120%) as compared to total extract proteomic maps. We could observe the presence of typical surface proteins such as trans-sialidase and mucine associated surface protein (MASP) and proteins from organellar membranes such as the vesicle- associated membrane protein. Both proteomic maps presented a large number of metabolic proteins suggestting traffic of these proteins into cellular compartments. The present study could help understand *T. cruzi* complex biology investigating membrane proteins that could be involved in: a) adesion and invasion process; b) intracellular proliferation and differentiation steps and c) pathogenesis. Financial Support: IOC – Fiocruz, CNPQ.

BQ40 - PHOSPHOPROTEOMIC APPROACH TO UNDERSTAND TGF-β RESPONSE IN *T. cruzi* BIOLOGY

Ferrão, P.M.[1]*, Oliveira, F.L.[1], Da Cunha, M.R.[1], Waghabi, M.C.[1], Mendonça-Lima, L.[1]
[1]Laboratório de Genômica Funcional e Bioinformática, Instituto Oswaldo Cruz, FIOCRUZ, Rio de Janeiro, Brasil
* pmf@ioc.fiocruz.br

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 on *T. cruzi* biology, 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 times and dose were assayed for differential protein induction. Comparative proteomic 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, being these differences more evident in short incubation times (until 5 minutes). This result shows that the phosphorylation events related to TGF-β pathway happen relatively fast, which is expected for signal transduction events. At longer times of incubation (30- 60 minutes), we observed the loss of phosphorylation sign which could indicate the deactivation of this pathway.
Mass spectrometry identification of differential expressed phosphoproteins is being carried out and we have already identified many of our proteins of interest.
Considering the crucial role of TGF-β in *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, indicating some potential candidates to the development of blockers of the TGF-β pathway in *T. cruzi*.
Supported by CNPq and Fiocruz.

BQ41 - Cysteine peptidase relevance on the interaction of *Trypanosoma cruzi* with an invertebrate host model

Altoé, E.C.F.[1], Vitório, B.S.[1], Santos, L.O.[1], Branquinha, M.H.[2], Santos, A.L.S.[2], Lima, A.P.C.[3], & d'Avila-Levy, C.M.[1*]

[1] *Instituto 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.*
[3] *Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil.*

The main lysosomal cysteine peptidase of *Trypanosoma cruzi*, the causative agent of Chagas' disease is called gp57/51 or cruzipain. This molecule has been involved in the differentiation of the parasite, multiplication of the parasite and host cell invasion. Despite cruzipain being abundantly expressed in epimastigotes of *T. cruzi*, found in the insect vector, its possible relevance in the interaction with the invertebrate host has not been explored. Recent work from our group, showed that a synthetic inhibitor of cysteine peptidases, E-64, promotes a significant reduction in the rate of adhesion between the parasite and the intestinal epithelium of *Aedes aegypti*, an insect routinely used as a model for analyzing the interaction of trypanosomatids and vectors. Here, we analyzed the rate of *T. cruzi* adhesion after treatment of the parasite with anti-cruzipain, and another synthetic inhibitor, leupeptin. Our data showed that anti-cruzipain, at concentrations of 1:1000 and 1:2500, reduced the rate of adhesion in approximately 58% and 35% respectively and leupeptin at 10μM promoted a reduction of approximately 47% when compared with the control. In addition, the adhesion rate to insect midguts of *T. cruzi* overexpression chagasin, an endogenous cruzipain inhibitor, was virtually abolished. Therefore, our results suggest that there may be an involvement of cysteine peptidases in the interaction between *T. cruzi* and the invertebrate host, possibly the cruzipain.
SUPPORTED BY: MCT/CNPq,FAPERJ, FUJB and FIOCRUZ.

BQ42 - PARTIAL CHARACTERIZATION OF METALLOPROTEINASES IN THE BLOODSTREAM FORMS OF *TRYPANOSOMA BRUCEI*

Karina P. de Sousa, Jorge M. Atouguia and Marcelo S. Silva[#].

Centre for Malaria and Tropical Diseases – Institute of Hygiene and Tropical Medicine, Lisbon - Portugal.

[#]Corresponding author: mssilva@ihmt.unl.pt

Metalloproteases are vital in the physiology and pathogenesis of parasites, swaying the infection with their ability to degrade proteins, to ease dissemination through host tissues, and by evading from host immune response. In its life cycle, *Trypanosoma brucei* – agent of African Trypanosomiasis (AT) – might produce metalloproteases (MP) that may be assist its migration through host tissues by hydrolyzing matrix proteins. In the encephalitic stage of AT, trypanosomes invade the brain, crossing the blood-brain barrier with no obvious disruption of tight junctions. We put forward the hypothesis that the proteolytic activity of *Trypanosoma* products may play a role in its migration through host tissues by degrading matrix components, disassembling the organization of the extracellular matrix. In this study we assess this activity in *T. brucei* (Tb) bloodstream forms extract, and report the identification and partial characterization of a neutral TbMP displaying marked pH-dependant proteolytic activities on gelatin and casein, and its inhibitors. To this end, *T. brucei* extract was subjected to electrophoresis in a 0.15% gelatin-and casein-SDS-PAGE gel, followed by renaturation, washing and incubation for 12h at 37ºC in reaction buffer, with or without inhibitors, adjusted for the desired pH, and then stained. This allowed direct results on the substrates and molecular weight of TbMP. The results seem to indicate that bloodstream forms of *T. brucei* contain TbMP that a) can degrade gelatine and casein proteins; b) are inhibited by EDTA, tetracycline and derived; and c) display marked pH-dependent activity. These results might be important to grasp the host/parasite interaction, considering the significant role of TbMP as mediators of vital parasite processes. Further studies may unlock new opportunities for better understanding AT and also create new pathways for researching targets for new drugs.

BQ43 - A PROTEOMIC APPROACH TO INVESTIGATE DIFFERENT INFECTION PROFILES BETWEEN TWO *Trypanosoma cruzi* ZYMODEME 3 STRAINS

Kikuchi S.A. [a], Sodré C.L[a], Santos A[b], Guarany C[b], Soeiro M.N[c], Meuser M[c] , Kalum D[a] ,Chapeaurouge A[d], Perales J[d], and Fernandes O[a]*

[a] Laboratório Interdisciplinar de Pesquisas Médicas, Instituto Oswaldo Cruz , FIOCRUZ, Rio de Janeiro, Brasil.
[b] Laboratório de Estudos Integrados em Bioquímica Microbiana, Universidade Federal do Rio de Janeiro , Brasil
[c] Laboratório de Biologia Celular, Instituto Oswaldo Cruz, FIOCRUZ, Rio de Janeiro, Brasil.
[d] Laboratório de Toxicologia, Instituto Oswaldo Cruz, FIOCRUZ, Rio de Janeiro, Brasil.
*octaviofernandes@fiocruz.br

Two *Trypanosoma cruzi* Z3 strains were isolated from insect vectors captured in the Brazilian Amazon region: 3663 (Z3A) and 4167 (Z3B). These Z3 strains were cultivated at 28 ºC for a period of 20 days. Both strains presented the log, stationary and decline phases in distinct days: 4167 reached the log phase on day 4, while 3663 strain on day 10. In addition, 4167 revealed a poor growth pattern compared to the abundant proliferation of 3663. The strains exhibited different infection patterns in Vero, C6/36, Raw and Hep-2 cells. Interestingly, the epimastigote forms of these Z3 strains presented distinct morphological characteristics. We also present a proteomic approach to investigate the differences in the global patterns of gene expression between the two strains, especially aiming the identification of protein targets involved in their distinct virulence profile. Spots were identified by MS. Proteins related to *T. cruzi* infectivity such as glutathione peroxidase-like, S-adenosylmethionine synthetase, tryparedoxin peroxidase, arginine kinase, and cruzipain were detected. Cruzipain is the major cysteine proteinase involved in peptidase dependent mechanisms of induced Ca^{+2} signaling in mammalian cells, an important key regulator of *T.cruzi* infectivity. This might be significant to *T. cruzi* biology considering that 3663 trypomastigotes was much less infective than 4167, Dm28c or CL Brener.Two-DE gel showed low expression profile of cruzipain in 3663 and up regulation in 4167, that was corroborated by the FACS. The difference in cruzipain expression profile among the strains could be elucidated in view of the remarkable genetic and biological diversity that exist in *T. cruzi* groups. The discrepancy in infectivity between the two Z3 strains could accounted for the different protein profiles.This was the first study to describe the proteomic map of Z3 strain, in which we found potential targets with infectivity implications and for drug development.
Supported by PDTIS (FIOCRUZ), CNPq

BQ44 - HEME INDUCES MITOCHONDRIAL SUPEROXIDE PRODUCTION IN *TRYPANOSOMA CRUZI* EPIMASTIGOTES

Saraiva FMS[1], Nogueira NPA[1], Silva, TM[2], Mariano, TS[2], Laranja GAT[1], Oliveira MF[3], Gadelha, FR[2] and Paes MC[1]

[1] Instituto de Biologia Roberto Alcântara Gomes - Departamento de Bioquímica – Universidade do Estado do Rio de Janeiro – RJ - Brasil.
[2] Instituto de Biologia- Departamento de Bioquímica - Universidade Estadual de Campinas – SP – Brasil.
[3] Instituto de Bioquímica Médica – Universidade Federal do Rio de Janeiro – RJ
natty_nogueira@yahoo.com.br

Trypanosoma cruzi epimastigotes proliferate inside the hematophagous insects midgut in the presence of high concentrations of heme (about 10 mM), an important molecule serving as the prostetic group of hemeproteins involved in respiration and oxygen transport. Additionally, previous results of our group showed that heme significantly increased parasite proliferation in a dose-response manner (Lara *et al*, 2007). Despite of that, free heme potentially induces the formation of reactive oxygen species (ROS), such as superoxide ($O_2^{\bullet-}$). In this regard, here, we evaluated the mitochondrial $O_2^{\bullet-}$ formation induced by heme in *T. cruzi* epimastigotes. Parasites were maintained in BHI supplemented with 10% FCS and 30 μM heme for 7 days at 28ºC. Then, superoxide production by epimastigotes was analyzed by flow citometry and epifluorescence microscopy using the $O_2^{\bullet-}$ sensitive-hydroethidine-analogue MitoSOX. This probe localizes to the mitochondrion due to its hydrophobic nature and its positively charged moiety. Parasites were pre-loaded with MitoSox and then, incubated in the absence or in the presence of heme, FCCP, a mitochondrial uncoupler or antymicin A (AA), an inhibitor of the mitochondrial electron transport. Our results showed that parasites treated with increasing concentrations of heme presented an augmentation in mitochondrial $O_2^{\bullet-}$ production compared with the control. Moreover, addition of the uncoupler FCCP partially inhibited MitoSOX oxidation in heme-treated cells, indicating that superoxide production is dependent on the mitochondrial membrane potential. On the other hand, the treatment of epimastigotes with AA (2□g□ml□ induced the formation of large amounts of $O_2^{\bullet-}$. This phenomenon was observed in the absence as well as in the presence of heme showing a cummulative effect. Taken together, our results suggest that heme, found in high concentrations in epimastigotes environment, virtually targets to the mitochondrion, thus, modulating mitochondrial superoxide production in the parasite. Supported by FAPERJ

BQ45 - *PNEUMOCYSTIS CARINII ERG6* GENE EXPRESSED IN *TETRAHYMENA THERMOPHILA:* PURIFICATION AND ACTIVITY OF THE RECOMBINANT *S*-ADENOSYL-L-METHIONINE:STEROL C-24 METHYLTRANSFERASE (SAM:SMT)

Johnston, L.Q.[1*], Cassidy-Hanley, D.,[2] Clark, T.[2], Kaneshiro, E.S.[1]
[1]Department of Biological Sciences, University of Cincinnati, Cincinnati, Ohio, USA
[2] Department of Microbiology and Immunology, Cornell University, Ithaca, New York, USA.
* johnstlq@email.uc.edu

Pneumocystis is an opportunistic fungal pathogen that causes a type of pneumonia in immunocompromised individuals. The organism lacks the major fungal sterol ergosterol but synthesizes its own unique sterols with a double bond at C-7 of the sterol nucleus and an alkyl group at C-24 of the sterol side chain. The *S*-adenosyl-*L*-methionine:sterol C-24-methyltransferase (SAM:SMT) enzyme, encoded by the *erg6* gene is an attractive drug target as mammals does not have this enzyme. The recombinant enzyme expressed in *E. coli* was previously characterized in homogenates of transformed bacteria. In the present study, we are examining *Tetrahymena thermophila* as an expression system, as its GC content is low like that of *Pneumocystis* and *Tetrahymena* does not synthesize sterols. Two vector constructs were used to transform the rat-derived *P. carinii erg6* gene into *T. thermophila*. The constructs utilized tandem affinity purification (Tap) tags, one fused at the N-terminus and the other fused at the C-terminus of the target sequence. The construct containing the *erg6* gene with Tap tags was transformed into the MTT1 locus of *Tetrahymena*. We obtained ten clones carrying the *P. carinii erg6* gene, which we verified by PCR and these were screened by Western Blot analysis for the recombinant SAM:SMT protein. Two of the clones found to express the recombinant *P. carinii* SAM:SMT protein under the initial culture conditions tested were chosen for subsequent purification and enzyme analysis; one containing the N-terminal construct and one containing the C-terminal construct. Tandem affinity protocols purified the target protein to homogeneity as indicated by SDS-PAGE. Preliminary enzyme assays using ^{14}C-labeled SAM and lanosterol as substrates showed that the preparation has enzyme activity by the detection of radiolabeled lanosterol derivative product(s). Enzyme kinetic assays and substrate preference analyses on the purified SAM:SMT are in progress. Supported by a grant from the NIAD RO1 AI064084.

BQ46 - IDENTIFICATION, CLONING AND CHARACTERIZATION OF AN ALDO-KETO REDUCTASE FROM *TRYPANOSOMA CRUZI* WITH QUINONE OXIDOREDUCTASE ACTIVITY

Garavaglia PA[1], Cannata JJB[2], Ruiz AM[1], Maugeri D[3], Bua J[1], Galleano M[4], Duran R[5] and García GA[1]*.
[1] INP "Dr. Mario Fatala Chabén"-ANLIS "Dr. Carlos G. Malbrán", Buenos Aires, Argentina. [2] CEFYBO, Universidad de Buenos Aires, Buenos Aires, Argentina. [3] IIB–INTECH Universidad Nacional de General San Martín-CONICET, Buenos Aires, Argentina. [4] Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Buenos Aires, Argentina. [5] Instituto de Investigaciones Biológicas "Clemente Estable", Montevideo, Uruguay.
*E-mail: gaandgarcia@yahoo.com.

Drugs currently used for treatment of *Trypanosoma cruzi* infection, the ethiological agent of Chagas' disease, have shown inadequate side effects and variable efficiency. With the aim to describe parasitic enzymes involved in the action mechanisms of trypanocidal drugs, we attempted to identify novel NADPH-dependent oxido-reductases from *T. cruzi*, since it has been reported that reductases are crucial in this metabolism. The passage of soluble fraction of epimastigote lysates through Cibacron Blue-Sepharose followed by NADPH elution yielded a predominant protein with an apparent molecular weight of 32 kDa identified by MALDI-TOF as an aldo-keto redutase superfamily member (*Tc*AKR). The release profile of *Tc*AKR from intact epimastigotes by titration with digitonin suggested that *Tc*AKR is mainly localized in the cytosol. We confirmed by western blot that *Tc*AKR is also present in trypomastigote/amastigote lysates. The *TcAKR* gene was cloned in *E. coli* and the recombinant protein (rec*Tc*AKR) showed NADPH-dependent reductase activity with most common AKR substrates, 4-NBA and 2-DHA, with a substrate saturation curve consistent with the Michaelis-Menten model. No activity was detected with NADH as cofactor. We also tested whether rec*Tc*AKR may reduce naphtoquinones (NQ), since many of these compounds have displayed important trypanocidal activity. rec*Tc*AKR showed reductase activity with o-NQ (1,2 NQ, 9,10 PQ and β-lapachone) with concomitant generation of free radicals, but did not exhibit affinity for ρ-NQ (5H-1,4-NQ, 2H-1,4-NQ, α-lapachone and menadione). The substrate saturation curve with o-NQ fitted to a sigmoidal curve, suggesting that rec*Tc*AKR presents a cooperative behavior. In agreement with this hypothesis, three peaks assigned to monomers, dimers and tetramers were obtained when rec*Tc*AKR was submitted to a Superose 12 gel chromatography column. Our results indicate that *Tc*AKR, the first member of the AKR family described in *T. cruzi*, may participate in the action mechanisms of trypanocidal drugs.
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BQ47 - INFLUENCES OF HEME OXYGENASE -1 AND NADPH OXIDASE DURING INFECTION WITH *TRYPANOSOMA CRUZI* METACICLIC INFECTED MACROPHAGES

Breder L.O.[1*], Nogueira N.P.A.[1], Souza C.F.[1], Laranja G.A.T.[1], Arruda M.A.[2] and Paes, M.C.[1]

[1]Departamento de Bioquímica - IBRAG – UERJ – Rio de Janeiro, Brasil

[2] Departamento de Farmacologia e Psicobiologia – IBRAG – UERJ – Rio de Janeiro, Brasil
[*]luiza.breder@gmail.com

Heme Oxygenase is an important enzyme involved in inflammatory process and its expression can be increased by reactive species of oxygen (ROS). Another enzymatic system involved in the same process is NADPH oxidase, a multimeric complex specialized in superoxide anion generation which was first characterized in phagocytes. Then, we are investigating the correlation between these two enzymes during *T.cruzi* infection since the presence of parasite leads a pathophysiological condition. First, we analyzed the expression levels of HO-1 during *T. cruzi* infection and/or NADPH oxidase inhibitor treatments. Thus, RAW 264.7 macrophage in the exponential growth phase, were infected with *T. cruzi* trypomastigotes at a 1:10 rate and afterwards incubated with heme and/ or diphenyleneiodonium (DPI). After that, western blotting assays were performed. We observed an increase in HO-1 expression in cells infected with trypomastigotes as well as treated with heme. This augmentation was even more evident when cells were infected and incubated with heme concomitantly. On the other hand, the use of the DPI decreased HO-1 expression. NADPH oxidase inhibition by DPI was also able to diminish macrophage infection by *T. cruzi*, as analyzed by light microscopy. We have also observed that *T. cruzi* infection increases ROS production by macrophages in a NADPH oxidase-dependent manner, once it was totally abolished by pre-treatment of macrophages with DPI, as assessed by dihydrorhodamine assay. Our results show, for the first time, the modulation of HO-1 expression by *T. cruzi* infection, which seems to be a NADPH oxidase-dependent phenomenon. The impact of those enzymatic systems on macrophage infection rates are now under investigation. Supported by CNPq, CAPES and FAPERJ.

BQ48 - *Trypanosoma cruzi*: A mitochondrial permeability transition pore could be involved in the parasite programmed cell death.

Bustos, P.L.[1], Perrone, A.E.[1,2], Fuchs, A.G.[1,2], García, G.A.[1], Postan, M.[1] Búa, J.[1,2,*]
[1]Instituto Nacional de Parasitología “Dr. Mario Fatala Chaben”, ANLIS Malbrán; [2]Universidad Abierta Interamericana; Buenos Aires, Argentina.
[*]jacbua@yahoo.com

Cyclophilins (CyPs) are enzymes involved in protein folding and target of the immunosuppressive drug Cyclosporin A (CsA). CyPD is a mitochondrial cyclophilin, involved in the mitochondrial permeability transition pore (mPTP) opening in mammals. We have previously described the cyclophilin gene family in *Trypanosoma cruzi* and some of their isoforms were isolated by CsA affinity chromatography. *T. cruzi* has a CyPD homologue, a cyclophilin of 21 kDa, *Tc*CyP21, expressed in the parasite (SAP, Rosario, Argentina 2008). We aimed to establish if *Tc*Cyp21 could be involved in the parasite mPTP opening. Bioinformatic analysis of the mammalian components of the mPTP pore such as adenine-nucleotide translocase, voltage-dependant anion channel, hexokinase, among others, were found in the *T. cruzi* genome. *T. cruzi* cell death was induced in CL Brener epimastigotes by oxidative stress with 5 mM H_2O_2, and some apoptosis-like features such as DNA degradation, a PARP-like protein cleavage and a cytochrome *c* translocation from mitochondria to cytosol were observed. These apoptotic signs were inhibited by pre-treatment of parasites with CsA suggesting a *T. cruzi* cyclophilin involved in the mPTP. Enhanced Hexokinase enzymatic activity was observed when *Tc*CyP21 recombinant protein was added to an enriched mitochondrial parasite fraction, but pre-incubations with CsA or polyclonal antibodies anti-*Tc*CyP21 synthetic peptide, dramatically reduced the mitochondrial hexokinase activity. As these inhibitors affect *Tc*CyP21 Peptidyl Prolyl *cis-trans* isomerase (PPIase) activity, it is possible they interfere with the initial velocity of the hexokinase. This has been previously reported for the mammalian mPTP, in which CyPD PPIase activity stabilized mitochondrial hexokinase binding, indicating a close relationship between both proteins. This is the first time a mPTP pore opening is described for a protozoan parasite, encouraging further studies on protein complex involved, and events leading to the parasite programmed cell death.
Supported by ANLIS Malbrán; NIH D43TW007888 and Universidad Abierta Interamericana.

BQ49 - IDENTIFICATION FROM *LEISHMANIA* RIBOSOMAL PROTEINS EXPRESSED IN DIFFERENT SPECIES TO USE IN THE DIAGNOSIS OF CANINE VISCERAL LEISHMANIASIS

Coelho, V.T.S. [1], Costa, M.A.F. [1], Ramírez, L. [2], Oliveira, J.S. [1]; Farias, L.M. [3], Carvalho, M.A.R. [3], Veloso, L.C. [3], Santos, K.V. [3], Soto, M. [2] Santoro, M. M[1], Coelho, E.A.F. [1,4]*

[1]Departamento de Bioquímica e Imunologia, Instituto de Ciências Biológicas e [4]Departamento de Patologia Clínica, Coltec, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brasil.
[2]Departamento de Biologia Molecular, Centro de Biologia Molecular Severo Ochoa, Universidade Autónoma de Madrid, Madrid, Espanha.
[3]Departamento de Microbiologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brasil.

* viniciotadeu@yahoo.com.br / dudafercoelho@pq.cnpq.br

Leishmaniasis comprises a disease caused by intracellular parasites that infect fagocitic cells in human and dogs. A model for *Leishmania* virulence involving two different groups of parasite molecules has been proposed. The first one is formed by surface and secreted proteins necessary for the infection. A second group is formed by conserved intracellular molecules referred as ‘‘pathoantigens’’ since that an immune response against them is thought to result in pathology. Several lines of evidence suggest that *Leishmania* ribosomal proteins (LRPs) are immunologically relevant molecules during infection and also are conserved in different *Leishmania* species. In this work, we identified some proteins in the LRPs complex that are expressed in the three different *Leishmania* species and which were reactive with sera of dogs with active visceral leishmaniasis (VL). Ribosomal proteins from *Leishmania chagasi*, *L. amazonensis* and *L. infantum* were fractionated by 2-D electrophoresis and submitted to a Western blot analysis with sera from dogs with VL. The stained gels were used to computational analysis and the more reactive spots were identified by mass spectrometry. In conclusion, we observed that some proteins in the LRPs complex were specifically recognized by antibodies from dogs with VL and will be individually analyzed for serological diagnosis of canine visceral leishmaniasis.
Supported by FAPEMIG and CNPq.

BQ50 - Biochemical characterization of enzymes involved in cysteine biosynthesis in *Leishmania* sp and *Trypanosoma cruzi*.

Marciano Daniela, Santana Marianela and Nowicki Cristina. Facultad de Farmacia y Bioquímica UBA, Buenos Aires, Argentina. cnowicki@criba.edu.ar.

In TriTryps, the metabolism of sulfur-containing amino acid is complex and poorly understood yet. *T. cruzi* and *Leishmania spp,* but not *T. brucei,* might be able to assimilate *in vivo* inorganic sulfur to synthesize *de novo* cysteine. Different research groups reported that in both parasites two alternative routes might be operative involving, as in plants and bacteria, a serine acetyltransferase (SAT) and a cysteine synthase (CS), in addition to a broad specificity cystathionine beta synthase (CBS). The latter *in vitro* is capable to form cysteine by condensation of serine or O-acetylserine (OAS) with inorganic sulfide (IS). Nevertheless, in *T. cruzi* CS was not functionally characterized, and the operability of the putative SAT in *Leishmania* sp is still not well documented. Leishmanial and *T. cruzi* CSs closely resemble plant and bacterial enzymes; by contrast, SATs exhibit a non-conserved extension in their N-terminus yet the residues involved in catalysis are highly conserved in the C-terminal region. In this work we report the biochemical characterization of *T. cruzi* CS and leishmanial SAT. Both enzymes were cloned and functionally expressed in *E. coli*. The recombinant CS was highly specific towards OAS and IS whereas the leishmanial recombinant SAT catalyzed the O-acetylation of serine, and its specific activity highly resembled the values exhibited by plant SATs not complexed with CS. Moreover, our results showed that *T. cruzi* and leishmanial CSs are cytosolic enzymes. The biological relevance of *T. cruzi* CS is strengthened since when compared with epimastigotes, the expression of CS is notably up-regulated in trypomastigotes and amastigotes.

BQ51 - EVOLUTION AND ORIGIN OF EUKARYOTIC PYRUVATE FORMATE LYASE – AN ANAEROBIC SOLUTION FOR PYRUVATE CATABOLISM

Hampl, V.[1*], Roger, A. J.[2]
[1]Department of Parasitology, Faculty of Science, Charles University in Prague, Vinicna 7, Prague 2, 128 44, Czech Republic
[2]Department of Biochemistry and Molecular Biology, Faculty of Medicine, Dalhousie University, Halifax, Nova Scotia, B3H 1X5, Canada
*vlada@natur.cuni.cz

Although anaerobic eukaryotes that contain anaerobic derivates of mitochondria like hydrogenosomes and mitosomes represent a number of independent lineages in the tree of eukaryotes, their energy metabolisms exhibit considerable similarities. Many of them use pyruvate:ferredoxin oxidoreductase (PFO), or the related pyruvate:NADP+ oxidoreductase (PNO) enzyme, instead of pyruvate dehydrogenase (PDH) to convert pyruvate to acetylCoA. In this pathway, [FeFe] hydrogenase is often used to transfer electrons from ferredoxin to protons to generate hydrogen gas. Whereas the origin and evolutionary history of eukaryotic hydrogenases is unresolved, phylogenetic analyses of the PFO/PNO family suggest a monophyletic origin for eukaryotic homologues suggesting that this enzyme may have been present in the last common ancestor of eukaryotes and secondarily lost in predominantly aerobic lineages. Curiously, several unrelated anaerobes (chytrids, several green algae, *Mastigamoeba*, diatom *Thallasiosira*, *Amoebidium*, *Cyanophora*) use another unrelated enzyme for catabolism of pyruvate – pyruvate formate lyase (PFL). In order to elucidate the evolutionary history of the latter enzyme we performed phylogenetic analyses using available sequences of PFL and PFL activating enzymes. Like PFO, eukaryotic PFL homologues form a weakly supported clade suggesting a single-source origin of this enzyme in eukaryotes.
Supported by The Czech Ministry of Education Project 0021620828 and Canadian Institutes for Health Research Grant MOP-62809.

BQ52 - GLUCOSYLCERAMIDE SYNTHASE IN *TRYPANOSOMA CRUZI*, A KEY ENZYME OF THE GLYCOSPHINGOLIPID PATHWAY

Piñero, T.A.[1], Landoni, M.[1],Soprano[2], L.L., Esteva, M.I.[2], Duschak, V.G.[2],and Couto, A.S.[1]
[1]CIHIDECAR-Dpto.Qca.Orgánica, FCEN-UBA, [2]Inst. Nac. Parasitología, ANLIS-Malbrán, Min de Salud de La Nación, Buenos. Aires. Argentina. tpinyero@qo.fcen.uba.ar

Sphingolipids are important components of eukaryotic cells, many of which function as bioactive signaling molecules. Although in mammalian cells, metabolic products of sphingolipid pathway have been largely studied and several enzymes involved have been characterized, very little is known about this pathway in parasitic protozoa. The core structure of the glycosphingolipids, glucosylceramide, is synthesized by a UDP-glucose: ceramide glucosyltransferase (GCS). Recently, we have shown the presence of this active enzyme in the intraerythrocytic stages of *P. falciparum*. In this work we report the characterization of an active GCS in different strains of epimastigote forms of *T. cruzi.* Purification of GCS was achieved *via* a 5 step protocol involving a Green-dye agarose affinity column. This procedure led to a protein with an apparent MW of 70 kDa whose specific activity was proven using NBD-Ceramide and UDP-Glucose as substrates. Alternatively, active GCS was purified in a single step by immunoaffinity chromatography using a specific polyclonal anti-*T. cruzi* GCS mouse antibody. In order to get some insight on the subcellular location of this enzyme, western blot developed with antihuman GCS showed that the enzyme was present in the enriched membrane fraction obtained by subcelullar fractionation after parasite grinding with silicon carbide. In addition, immunoelectron microscopy techniques showed label not only in the Golgi apparatus as expected, but also in microdomains all around the parasite membrane. The effect of PPMP (D,L-*threo*-phenyl-2-palmitoylamino-3-morpholine-1-propanol) on culture epimastigote forms of *T. cruzi* showed a significant reduction on parasite growth. Similar results were obtained on blood trypomastigote forms. In addition, glycosphingolipids obtained from PPMP-treated parasites, showed a significant decrease of the synthesized glucosylceramide (60%) indicating a possible association between both events. Differences between this key enzyme of the glycosphingolipid pathway and the mammalian enzyme would allow to consider *T. cruzi* GCS as a new chemotherapeutic target. Supported by UBA-CONICET-ANPCYT

BQ53 - Oxygen and *Entamoeba*: Similarities to *Giardia*

T. Paget[1*], D Lloyd[2] , B. Goldberg[3], D. Eichinger[4],
K. M.Harris[5], E. Jarroll[6,7]

[1*] School of Pharmacy, University of Kent at Medway, Medway, UK, [2] Cardiff School of Biosciences, Cardiff, Wales, UK 3AT [3]Dept. of Chemistry, NYU, NY, NY; [4] Medical Parasitology, NYU Medical School, NY, NY, [5] Barnard College, Columbia University, NY, NY [6] Department of Biology, Northeastern University, Boston, MA, USA, [7]Division of Natural and Social Sciences, Lehman College-CUNY, Bronx, NY, USA (address as of July 1, 2009)
*t.a.paget@kent.ac.uk

Its is now well established that the gut environment is not anaerobic since oxygen is found near the mucosa and may be present after eating. The impact of this on protozoan parasites that are found in this environment is significant and previous work by our group has shown that the gut parasite *Giardia* is an aerotolerant anaerobe and that oxygen uptake (OU) increases with increasing levels of oxygen up to 60µM (approx 28% air saturation at 37^{o}C). We have also shown that these low levels of oxygen can also modify the metabolism of *Giardia*. We have studied the response of *Entamoeba histolytica* and *E. invadens* to varying concentrations of oxygen and have observed a effect similar to that seen in *Giardia*. For *E. histolytica* maximum OU occurred at an oxygen concentration of 53 µM; for *E. invadens* this level was significantly higher (90 µM).Interestingly, OU rates were comparable. We have also shown that exogenous glucose supports OU. OU was also detected in the cysts of *E. invadens* suggesting that these forms are not truly cryptobiotic. OU was monitored during nutrient depravation-induced encystment in *E. invadens.* Interestingly, OU was significantly higher during the early stages of this process (5-10h) but then decreased, and by 24h it was significantly reduced. These data suggest that like *Giardia*, *Entamoeba* is well adapted to its environment but also highlight differences in oxygen response between *E. invadens* and *E histolytica*; *E. invadens* would seem to be able to tolerate higher levels of oxygen. It is clear that organisms that colonise the gut have to adapt to the presence of oxygen and some like *Giardia* may use this for some aspect of redox balancing.

BQ54 - THE SEARCH FOR HIGH AFFINITY INHIBITORS OF *Leishmania (L.)amazonensis* ARGINASE

Leme, C.W.[1*], da Silva, M.F.L.[1], dos Santos, M.G[1], Floeter-Winter, L.M.[1]
[1]Universidade de São Paulo, São Paulo, Brasil.
*camila.leme@usp.br

Leishmania is an intracellular parasite of mammals that, to survive inside the host macrophage, escapes from several microbicidal mechanisms, such as the production of NO and superoxide radicals (Bogdan et al., 1996). The NO synthesis by inducible nitric oxide synthase (iNOS) requires L-arginine as substrate, the same aminoacid used by arginase to produce urea and L-ornithine. The synthesis of L-ornithine is fundamental for the production of polyamines which assists the proliferation of *Leishmania* (Camargo, 1979). Moreover, the arginase from the parasite can compete with iNOS for the same substrate, modulating L-arginine levels, decreasing the production of NO and reducing the host microbicidal response (Boucher, Moali et al. 1999). The use of inhibitors of arginase, as NOHA, reduced the infection of *L. infantum in vitro* (Iniesta, Gomez-Nieto et al., 2001). However, NOHA is not a good chemotherapic candidate because it also inhibits the host arginase, an essential enzyme of the mammal's urea cycle. After the characterization of Leishmania's arginase we are searching for arginase inhibitors looking at a higher affinity for parasite arginase than host arginase. For this purpose, the recombinant enzyme was expressed and purified in two stages: the first with a column of Ni^{2+}, the second with a DEAE-Sepharose column. With the purified arginase, enzymatic assays were performed and the previously determined K_m was confirmed (da Silva et al., 2008). Then, we started to test inhibitors already described, such as ABH, L-norvaline and nor-NOHA (Baggio et al., 1997; Wheatley et al., 2003; Moali et al., 2000). The inhibitor L-norvaline, showed a significant inhibition of the recombinant enzyme. At a concentration of 80mM of L-norvaline and a L-arginine concentration near the K_m, we observed 70% inhibition of reaction. The infection in the presence of this inhibitor will be evaluated to determine the effects of L-norvaline *in vivo*.

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BQ55 - RELEVANCE OF SULFATED OLIGOSACCHARIDES AS MAIN COMPONENTS OF CRUZIPAIN, THE MAJOR CYSTEINE PROTEINASE OF *TRYPANOSOMA CRUZI*

Acosta D.M [1],Soprano L.L.[1],Landoni M.[3], Pourcelot M [2],Esteva M.I.[1],Kovensky J.[2], Couto A.S.[3] and. Duschak V.G.[1]
[1]Inst Nac Parasitol Dr Mario Fatala Chaben, ANLIS-Malbrán, Ministerio de Salud de la Nación Argentina; [2]Laboratoire des glucides, Université Jules Verne, Amiens- France; [3]CIHIDECAR-Dpto.Q.Org., FCEN, UBA, Argentina.

Trypanosoma cruzi contains a major cysteine proteinase, cruzipain (Cz). This lysosomal enzyme bears an unusual C-terminal domain (C-T) that contains a number of post-translational modifications and is responsible for most antibodies in natural and experimental infections. UV-MALDI-TOF MS analysis, allowed us to identify the presence of sulfated high-mannose type oligosaccharides in the C-T as a new striking feature of this molecule. In order to evaluate the immune responses to sulfated moieties on Cz, the involvement of anionic charged structures in the immune recognition of sulfated glycoproteins, and /or in the crossreactivity between myosin and cruzipain, BALB/c mice were immunized with purified Cz and C-T prior and after desulfation treatment. The humoral immune response to sulfates on Cz or C-T was mainly IgG2b. This reactivity was abolished when desulfated antigens were used as immunogens showing that sulfates are absolutely required for eliciting IgG2b response to Cz. A significant reduction of C-T-specific delayed-type hypersensitivity reaction in C-T-immunized mice was observed when desulfated C-T was challenged, suggesting the involvement of sulfate groups in the generation of memory T-cell responses. Moreover, immunization with C-T elicited ultrastructural abnormalities in heart tissue. Surprisingly, pathological alterations were not observed in hearts from sulfate depleted-C-T-immunized mice and a lower labelling was observed by immunoelectron microscopy using myosin-adsorbed specific policlonal anti-Cz serum. In order to study the involvement of sulfates in immunerecognition, different sulfated/desulfated molecules were coupled to BSA and preliminar dot assays showed a growing recognition to sulfated structures with specific anti-Cz serum: SO_4^{2-}/COO^- = 1(+++), 0.67 (++), 0.4 (+), 0 (-). Our results highlight the relevance of sulfated groups as main components of this molecule. Ongoing assays using specific anti-sulfate antibodies will help to elucidate the involvement of sulfates in the antigenicity and crossreactivity of the molecule and/or in the immunopathology of Chagas disease. Supported by CONICET-ANPCYT-UBA-Min.CyT-ECOS.

BQ56 - Is IL-6 a nexus between the inmune-neuroendocrine response and the effectors mechanisms of the inflammation in the infection with *Trypanosoma cruzi*?

Hernandez, S.[1*], Roggero, E.[2], Villalonga X[1] Arnaiz, M. [1] , Schwarcz, M. [1]
[1] Centro de Altos Estudios en Ciencias de la Salud, Universidad Abierta Interamericana, Buenos Aires
[2] Universidad Abierta Interamericana, Rosario, Buenos Aires, Argentina
susana.hernandez@vaneduc.edu.ar

Relationship between interleukine 6 (IL-6) as a mediator of the immune response with a) the regulation of the neuro-endocrine response, b) oxidative stress and c) metaloproteinase activity (MMPs) was investigated.
C57Bl/6 (B6) and IL-6 knock-out (KO6) mice were infected sc with 100 *Trypanosoma cruzi* trypomastigotes (Tulahuen strain). On day 18 post infection: survival, weight, parasitemia, plasmatic levels of corticosterone (RIA), INF-γ, TNF-α and IL-6 (ELISA), xantine oxidase and superoxide dismutase activities, liver lipoperoxidation and MMP 2 and 9 activities in skeletal muscle, heart and spleen were determined. The basal levels of the assayed cytokines (except IL-6) and corticosterone were similar in both B6 and KO6. The infected KO6 died earlier and presented higher parasitemia. In KO6, there was an increase in INF-γ levels (3516,5 ± 320,5 vs 1766,1 ± 399,6) and MMP-9 in spleen (1.29 ± 0.169 vs 0.98 ± 0.013) and myocardium (1.145 ± 0.17 vs 0.98 ± 0.013) when compared with infected B6, while TNF-α levels did not show any difference (1763, 3 ± 843,2 vs 1512,2 ± 310,5). IL-6 increased only in B6 (46,4±11,8 vs 421±252,9). To 18 dpi a similar 10 fold increase was observed in both B6 and KO6 when compared to the basal levels. In B6 the infection increased serum XO (162 ± 25 vs 64 ± 12) and did not modify liver SOD, whereas in KO6 the XO activity remained the same and SOD decreased (3,35 ± 1,02 vs 1,12 ± 0,24). Liver lipoperoxidation was not changed.
These results suggest that the IL-6 is not the main mediator in the activation of the HPA axis during the infection with *T. cruzi*, nevertheless it would stimulate oxidative stress and the activity of the metaloproteases.

BQ57 - INTRA- AND INTER-SPECIES STRUCTURAL SPECIFICITIES OF *EUPLOTES* PHEROMONE FAMILIES

Alimenti C.[1], Vallesi A[1*]., Pedrini B.[3], Wüthrich K.[2,3] and Luporini P.[1]
[1]Dipartimento di Biologia Molecolare Cellulare Animale, University of Camerino, Camerino MC, Italy;
[2]Department of Molecular Biology and Skaggs Institute for Chemical Biology, The Scripps Research Institute, La Jolla, CA, USA;

[3]Institut für Molekularbiologie und Biophysik, ETH Zürich, Zürich, Switzerland.
* adriana.vallesi@unicam.it

The experimental availability of a variety of strains of *Euplotes nobilii* and *E. raikovi*, which are species phylogenetically closely related yet ecologically separated (the former inhabiting Antarctic and Arctic waters, and the latter inhabiting temperate waters), permitted us to carry out a comparative study of the NMR solution structures that distinguish the psychrophilic (cold-loving) and the mesophilic pheromone families of these two species. The molecular structures of available members of the two families show a tight conservation of a three-helix bundle core, which ensures long-lasting structural integrity and biological activity of these water-borne signal proteins in their natural marine environment. In addition to this conserved scaffold, each pheromone shows individual structural traits primarily committed to confer specificity on its autocrine (mitogenic) and paracrine (sexual) signaling activities. These traits mainly involve variations in the length and regularity of the three helices, as well as in the shape and orientation of the carboxy-terminal polypeptide segment. At inter-species level, the *E. nobilii* pheromone family is clearly distinguished by structural modifications that appear to have evolved in functional correlation with cold-adaptation. Most relevant are extensions of polypeptide segments devoid of regular secondary structure, a unique distribution of polar and hydrophobic amino acids, the presence of solvent-exposed clusters of negatively charged amino acid side chains, and a special role of aromatic residues in anchoring regions of the molecular architecture. Overall, these cold-adaptive modifications make the psychrophilic pheromone family of *E. nobilii* an elegant example of how signal molecules combine high global stability of their three-dimensional structures with sufficient levels of local structural plasticity for efficient functioning at physiologically low temperatures.
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BQ58 - AFRICAN TRYPANOSOME TRAVERSAL OF THE HUMAN BLOOD-BRAIN BARRIER: A ROLE FOR PROTEASE ACTIVATED RECEPTOR SIGNALING?

Grab, D.J.[1,2*], Garcia-Garcia, J.C.[1], Nikolskaia, O.V.[1], Brown, A.[3], Pardo, C.A.[3], Kim, Y.V.[4], Zhang, Y.[5], Becker, K.G.[5], Wilson, B.A.[6], Lima, A.P.C.A.[7], Scharfstein, J.[7], Stephen Dumler, J.[1]
[1]Department of Pathology, The Johns Hopkins University School of Medicine, Baltimore, Maryland, USA
[2]Department of Pediatrics, The Johns Hopkins University School of Medicine, Baltimore, Maryland, USA
[3]Department of Neurology, The Johns Hopkins University School of Medicine, Baltimore, Maryland, USA
[4]Institute of Human Virology, School of Medicine University of Maryland, Baltimore, Maryland, USA.
[5]Gene Expression and Genomics Unit, National Institute on Aging, NIH, Baltimore, Maryland, USA
[6]Department of Microbiology, University of Illinois at Urbana-Champaign, Urbana, Illinois, USA
[7]Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil
*dgrab@jhmi.edu

Human African trypanosomiasis (HAT) is a vector-borne disease for which death is inevitable if the patient is untreated. During the course of infection, African trypanosomes spread from the blood to the brain over the blood-brain barrier (BBB). Using our human brain microvascular endothelial cell (HBMEC) *in vitro* model for how African trypanosomes cross the human BBB, we previously found that a parasite cysteine protease (i.e. brucipain), induced calcium activation signals that allowed this barrier to open-up to parasite crossing. Because HBMEC express G protein coupled receptors (GPCR) known as Protease-Activated Receptors (PARs) that trigger calcium signals in HBMEC, we hypothesized a functional link between parasite brucipain and HBMEC PARs. Using RNA interference we found that *in vitro* PAR-2 gene (*F2RL1*) expression in HBMEC monolayers could be reduced by over 95%. We also found that the ability of *T. b. rhodesiense* to cross *F2RL1*-silenced HBMEC monolayers was reduced (39-49%) and that HBMECs silenced for *F2RL1* maintained control levels of barrier function in the presence of the parasite. Consistent with a role for PAR-2, we found that HBMEC barrier function was also maintained after blockade of Gαq with *Pasteurella multocida* toxin (PMT). PAR-2 signaling has been shown in other systems to have neuroinflammatory and neuroprotective roles and our data implicate a role for proteases (i.e. brucipain) and PAR-2 in African trypanosome/HBMEC interactions. Using gene-profiling methods to interrogate candidate HBMEC pathways specifically triggered by brucipain, we identified several pathways that potentially link brucipain activity with pathophysiologic processes associated with CNS HAT. Together, the data support a role, in part, for GPCRs as molecular targets for the parasite leading to the activation of Gαq-mediated calcium signaling. The consequence of these events is predicted to be increased permeability of the BBB to parasite transmigration and the initiation of neuroinflammation, events precursory to CNS disease.
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