### BC001 - **METACYCLIC FORMS OF A NEW LINEAGE OF TRYPANOSOMA CRUZI ASSOCIATED WITH BATS INVADE HOST CELLS BY ENGAGING THE SURFACE MOLECULE GP82**

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The mechanisms of host cell invasion by T. cruzi isolate from bat (designated BAT), belonging to a new genetic group, were investigated. Metacyclic trypomastigotes (MT) of BAT isolate displayed a surface profile similar to that of highly infective CL strain, i.e., they expressed gp82 and mucin-like molecules recognized by monoclonal antibodies 3F6 and 2B10, respectively. BAT isolate MT efficiently invaded cultured HeLa cells, in a manner similar to CL strain, by engaging gp82. Down regulation of HeLa cell signaling pathways involving mammalian target for rapamycin (mTOR), phosphatidylinositol 3-kinase (PI3K) and protein kinase C (PKC) impaired BAT isolate internalization. This mode of target cell invasion, implicating MT-specific gp82 molecule and the referred kinases, is similar to that of CL strain. To determine the degree of similarity between gp82 molecules of BAT isolate and other T. cruzi strains, a cDNA clone coding for the C-terminal domain of gp82 was generated from BAT isolate MT RNA. The deduced amino acid sequence of the referred cDNA displayed 91% and 93% identity, respectively, with the sequences previously deduced from cDNA clones coding for G and CL strain gp82, confirming the high conservation of gp82 molecules. Analysis of BAT isolate gp82 in chromosomal size fragments showed a profile distinct from that of G or CL strain. Genomic organization of BAT isolate gp82 genes also differed from that of G and CL strains. In addition to the ability to invade host cells in gp82-mediated manner, BAT isolate MT exhibited the ability to migrate through a gastric mucin layer, a property relevant for the establishment of infection by the oral route. However, oral infection by BAT isolate MT was inefficient, in contrast to CL strain. Factors implicated in these differences between BAT isolate and CL strain in vivo are currently under investigation.
Supported by:CAPES, FAPESP e CNPQ

### BC002 - **THE ENDOSYMBIONT OF BLASTOCRITHIDIA CULICIS DEPENDS ON THE HOST CELL TO DIVIDE**

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*Blastocrithidia culicis* is a monoxenic trypanosomatid that presents single-copy structures, as the nucleus, the kinetoplast and a bacterium, which maintains an obligatory relationship with the host protozoan. Investigations of the cell cycle in symbiont-harboring trypanosomatids suggest that the bacterium divides in coordination with other host cell structures. In this work we used optical and electron microscopy approaches in order to study the symbiont division during the *B. culicis* cell cycle. During this process, the symbiotic bacterium presents different formats and different positions in relation to other cell structures. Thus, at the beginning of the protozoan cell cycle, the endosymbiont presents a constricted form that becomes more elongated until the bacterium division that occurs before the kinetoplast and nucleus segregation. During cytokinesis, symbionts are positioned close to nuclei to ensure that each daughter cell will inherit a single copy bacterium. After cytokinesis, the symbiont migrates to the posterior end of the host and a new cell cycle begins. Protozoa treatment with aphidicolin, a DNA polymerase inhibitor that blocks the nuclear DNA synthesis, promotes cell proliferation arrest in Blastocrithidia culicis. In aphidicolin treated cells the symbiont segregation is inhibited, resulting in filamentous forms. These results indicate that somehow the host protozoan controls the number of bacteria per cell in order to maintain the close symbiotic relationship.
Supported by:CNPq, FAPERJ and FAPESP

BC003 - **PROTEOMIC PROFILES OF TWO LEISHMANIA INFANTUM STRAINS WITH VIRULENCE VARIATION**

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Leishmania (Leishmania) infantum is a protozoan belonging Trypanosomatidae Family agent of Canine Visceral Leishmaniasis (CVL) in the Americas. The CVL is a severe disease characterized by chronic and progressive cachexia, hepatosplenomegaly, lymphadenopathy, onychogryphosis and pancytopenia. The symptoms are present in less than 50% infected dogs. Clinical variability suggests that host and parasite factors are involved on virulence. Here we had used proteomic approach to identify proteins that may be involved in L. chagasi virulence. The L. infantum strains named BH400 (MCAN/BR/2000/BH400) and BH46 (MHOM/BR/1972/BH46) had shown distinct virulence in hamster infections, and were used to comparative proteomic analysis. Three independent promastigotes cultures were obtained for both strains. BH400 was cultivated in α-MEM and Schneider medium while BH46 in LIT and α-MEM medium. The parasites on exponential growth phase were washed in DMEM medium and protein extract was obtained with lysis buffer. The proteins were fractionated by 2-DE (two-dimensional electrophoresis) using IPG strips (7 cm, pH 4-7 NL; GE Helthcare), SDS-PAGE 12% and stained with colloidal comassie. The gels were analyzed by ImageMaster 2D Platinum ® software. It was detected approximately 300 spots on gels. The comparative analysis of the 3 independent cultures gels had shown 85% similarity inside the same strain. The BH46 and BH400 profiles, both growing in α-MEM medium, were compared and showed 82% of correlation. This analysis points 3 and 5 over-expressed proteins in BH46 and BH400, respectively. These proteins had shown expression level twice higher than their corresponding ($p < 0.05$). The eight selected proteins are being identified by mass spectrometry (MALDI ToF/ToF). The proteomics analysis association with the genome sequencing information may to contribute for understanding of host-parasite interactions, the parasite virulence factors, the escape mechanisms and the discovery of new therapeutic potential target.
Supported by:FAPEMIG; CNPq

BC004 - **PROTEOLYTIC CLEAVAGE OF MACROPHAGE MEMBRANE FUSION REGULATORS BY LEISHMANIA PROMASTIGOTES**

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Leishmania donovani promastigotes inhibit phagolysosome biogenesis during the establishment of infection within macrophages. This inhibition is mediated by the surface glycolipid lipophosphoglycan, which causes exclusion of Synaptotagmin (Syt) V from the phagosome membrane. As a consequence, promatigotes establish infection in phagosomes from which the vesicular proton-ATPase is excluded and which fails to acidify. Syts constitute a large family of transmembrane proteins that modulate membrane fusion events by regulating the activity of soluble N-ethylmaleimide-sensitive-factor attachment protein receptor (SNARE) proteins. SNARE-mediated membrane fusion events are involved in intracellular trafficking, phagocytosis, and phagosome maturation. In the present study, we sought to further investigate the impact of Leishmania promastigotes on SNARE-mediated membrane fusion events in macrophages. We first assessed the fate of various macrophage SNAREs following infection with Leishmania promastigotes. We found that a subset of SNAREs was cleaved upon infection, and that this cleavage was mediated by the Leishmania surface zinc-metalloprotease Gp63. Next, using macrophages and dendritic cells from a knockout mouse, we found that one of those SNAREs cleaved by Gp63 is required for antigen crosspresentation. Consistently, we observed that Leishmania promastigotes inhibit antigen crosspresentation in a Gp63-dependent manner. Taken together, our results suggest the existence of a novel mechanism used by Leishmania promastigotes to evade recognition and destruction by the immune system, whereby the parasite proteolytically degrades key regulators of intracellular trafficking.
Supported by:CIHR

## BC005 - **MAXADILAN INCREASES PLASMA LEAKAGE IN POSTCAPILLARY VENULES VIA STIMULATION OF PAC1 AND CXCR1/2 RECEPTORS ON LEUKOCYTES**

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Experiments were designed to determine if the vasodilatory peptides maxadilan and pituitary adenylate cyclase-activating peptide (PACAP-38) may cause plasma leakage through activation of leukocytes and to what extent these effects could be due to PAC1 and CXCR1/2 receptor stimulation. Intravital microscopy of hamster cheek pouches utilizing FITC-dextran and rhodamine, respectively, as plasma and leukocyte markers was used to measure arteriolar diameter, plasma leakage and leukocyte accumulation in a selected area (5 mm2) representative of the hamster cheek pouch microcirculation. Our studies showed that maxadilan and PACAP-38 induced arteriolar dilation, leukocyte accumulation and plasma leakage in postcapillary venules. The recombinant mutant of maxadilan M65 and an antagonist of CXCR1/2 receptors, reparixin, and an inhibitor of CD11b/CD18 up-regulation, ropivacaine, inhibited all these effects as induced by maxadilan. Dextran sulfate, a complement inhibitor with heparin-like anti-inflammatory effects, inhibited plasma leakage and leukocyte accumulation but not arteriolar dilation as induced by maxadilan and PACAP-38. In vitro studies with isolated human neutrophils showed that maxadilan is a potent stimulator of neutrophil migration comparable with fMLP and leukotriene B4 and that M65 and reparixin inhibited such migration. The data suggest that leukocyte accumulation and plasma leakage induced by maxadilan involves a mechanism related to PAC1- and CXCR1/2-receptors on leukocytes and endothelial cells.

Supported by:CNPq, FAPERJ

## BC006 - **METACYCLOGENESIS CONTROL BY ADENOSINE IN LEISHMANIA PARASITES**

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Metacyclogenesis is important in the life cycle of trypanosomatids as the stage in which infective forms are generated. However, little is known about this fact. This work investigated the effects of adenosine in the in vitro and in vivo metacyclogenesis of *Leishmania* parasites. Parasites were cultured with CGS15943 (CGS), a potent adenosine receptor antagonist. CGS treated cultures showed a significant increase in the number of metacyclics (10X), as assessed by morphology, motility and isolation in density gradient. Furthermore, a significant increase in survival to complement-mediated lysis (15X) and a low adherence to sand fly gut was observed in treated parasites. Additionally, higher levels of infectivity of peritoneal macrophages was observed when these cells were incubated with CGS treated parasites. C57BL/6 mice were inoculated on the ear with a low dose (1x103) of control or treated promastigotes. Both lesion size and tissue parasitism were significant increased in the group inoculated with parasites from the treated culture. Interestingly, metacyclogenesis induction in vitro was completely reversed in L. *amazonensis* cultured in the presence of CGS plus adenosine. We also found that CGS can partially block adenosine uptake by these parasites. Besides, females of *Lutzomyia longipalpis* were allowed to feed in a dog naturally infected with *Leishmania infantum* and fed afterwards with sucrose or sucrose plus adenosine. A large reduction in number of metacyclic forms per gut of insects fed with sucrose plus adenosine was observed. Furthermore, ours results indicate that the effects observed for L. *amazonensis* and L. *infantum* were conserved for other species of trypanosomatids like L. *braziliensis*, L. *major* and *Trypanosoma cruzi.* In conclusion, our data propose that presence/absence of adenosine is important to control the differentiation of *Leishmania promastigotes* into infective forms.

Supported by:CNPq, CAPES, FAPEMIG and Rede Mineira de Bioterismo

BC007 - **EARLY RESONSE OF ADIPOSE TISSUE TO TRYPANOSOMA CRUZI INFECTION**

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Brown (BAT) and white adipose tissue (WAT) and adipocytes are targets of Trypanosoma cruzi infection and the parasite persists in adipose tissue into the chronic phase. Here, CD-1 mice were infected with the Brazil strain of T. cruzi. At 15 days post infection, there was no parasitemia and mice were clinically well. We found that the parasite load in adipose tissue as determined by qPCR was elevated compared to other organs. There was a significant increase in macrophages in infected adipose tissue as well as a reduction in lipid accumulation. The adipocyte size and fat mass were also reduced. There was an increase in the expression of lipolytic enzymes in infected adipose tissue. Infection resulted in increased levels of TLR-4 and TLR-9 and the expression of components of the mitogen-activated protein kinase pathway in adipose tissue. Protein and mRNA levels of PPAR-γ were increased in WAT, while protein and mRNA levels of adiponectin were significantly reduced in BAT and WAT. The mRNA levels of several cytokines, chemokines and their receptors were increased. NFkB levels were increased in BAT while Iκκ-γ levels increased in WAT. These data indicate that adipose tissue is an early target and early sensor of T. cruzi infiltration as well as a reservoir of infection. In addition, T. cruzi-infected adipose tissue creates an inflammatory phenotype which is deleterious to the host.

Supported by:NIH

BC008 - **CHOLESTEROL DEPLETION INDUCES LYSOSOMAL DEPLETION AND IMPAIRS T. CRUZI ENTRY INTO NON-PROFESSIONAL PHAGOCYTIC CELLS**

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It is well established that T. cruzi entry into non-phagocytic cells relies heavily on lysosomal fusion with the plasma membrane for parasite internalization and later for the formation of a viable parasitophorous vacuole. Since the interaction between host plasma membrane and T. cruzi play a significant role in parasite infection it is important to understand the mechanisms by which the parasite gains the intracellular milieu through interaction and signaling with this membrane. In previous works we have shown, for primary murine neonatal cardiomyocytes, that cholesterol-enriched microdomains are important for TCTs entry in these cells. In addition to that we have also shown that cholesterol depletion impairs both T. cruzi entry and lysosomal association with internalized parasites. In the present work we show that cholesterol depletion leads to a diminishment in T. cruzi entry due to a decrease in pre-docked lysosomal reservoir near the cell cortex. Cholesterol depletion by methyl-beta-cyclodextrin (MβCD) provokes lysosomal exocytosis, altering lysosomal distribution inside host cells. Depletion of pre-docked active lysosomes, at cell periphery, is confirmed by impaired lysosomal exocytosis stimulation after cholesterol depletion. On the other hand, cholesterol replenishment of MβCD treated cells reverts this effect. In conclusion, cholesterol depletion leads to massive lysosomal exocytosis events diminishing available lysosomes, which could be subverted by T. cruzi to enter host cells.

Supported by:CAPES-INCT Fluidos Complexos, FAPEMIG

BC009 - **MURINE LEISHMANIASIS: A POTENTIAL MODEL FOR CHRONIC HYPOXIA**

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Low oxygen tension is a common feature of inflammatory/infected tissues and hypoxia inducible factor (HIF)1α and HIF2α are heterodimeric transcription factors mediating the cellular response to hypoxia. The pathogenic mechanisms of HIF1α and HIF2α are still unclear and object of debate. We previously observed HIF-1α expression in the cutaneous lesions of BALB/c mice during *Leishmania amazonensis* infection. Based on this observation, we examined HIF1α, HIF2α and vascular endothelial growth factor (VEGF), a angiogenic factor directly activated by HIF-1α, in lesions of susceptible BALB/c mice and resistant C57BL/6 mice. The HIFs expressions were observed in lesions of susceptible and resistant mice during the experimental period. The BALB/c mice lesions showed HIF1α and HIF2α expression into cytoplasm of macrophages and failed to promote VEGF expression. C57BL/6 lesions showed HIF2α nuclear accumulation and VEGF expression during the chronic period. The animal models of leishmaniasis showed diversity pattern of HIFs expression, cell localization and activity of HIFs and may be a useful model to study the pathogenic mechanism of hypoxia during chronic leishmaniasis.
Supported by:CAPES, CNPQ E FAPESP

BC010 - **CROSSTALK BETWEEN LEISHMANIA AMAZONENSIS-INFECTED HUMAN NEUTROPHILS AND DENDRITIC CELLS ALTERS THEIR SURFACE MOLECULES EXPRESSION.**

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Introduction. Neutrophils are the first cell line of defense against pathogens. These cells are programmed to undergo apoptosis and are phagocytosed by macrophages, releasing anti-inflammatory mediators, inhibiting the main functions of these cells. Reports in the literature have shown that interactions between neutrophils and dendritic cells (DCs) influence the immune response mediated by T cells. This interaction seems to be highly specific between DC-SIGN expressed on DCs and, mainly, Mac-1 on neutrophils. Activated neutrophils are able to induce DC maturation through TNF-α and these cells produce IL-12, which induces a Th1 polarization. In this study, our goal is to evaluate the interactions between human neutrophils and DCs in the infection by L. amazonensis and their influence in the adaptive immune response.
Methods and Results. Human blood was obtained from healthy donors. Viable neutrophils were infected with L. amazonensis promastigotes in the ratio of 10:1 (parasite:cell) for 3 hours. We observed an increase of CD11b and CD18 expression by FACS. In contrast, a decrease in CD62L and CD16 expression, characterizing an activated profile of these cells. We also evaluated the production of chemokines in the supernatant of infected neutrophils by CBA and we detected the production of CCL2, IL-8 and CCL5. A chemotactic assay showed that migration of DCs is increased with the infection rate. After, infected neutrophils were co-cultured with dendritic cells for 18 hours and we evaluated DCs surface molecules expression by FACS. We observed a reduced expression CD1a, DC-SIGN, CD80 and MHC-II.
Conclusions. The interactions between human neutrophils and DCs seem to alter the functions of DCs. These preliminary data show the importance of initial events in the pathogenesis of Leishmania and induction of adaptive immune response.
Supported by:CNPq

BC011 - **TRYPANOSOMA CRUZI OF DIFFERENT STRAIN AND EVOLUTIONARY STAGE CAN INDUCE PLASMA MEMBRANE DERIVED MICROVESICLES PRODUCTION DURING HOST CELL INTERACTION**

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Immune evasion by pathogenic intracellular microbes involves subverting host recognition and clearance. Trypanosoma cruzi, the causative agent of Chagas disease, has evolved several mechanisms to survive the hostile environments encountered during its life cycle. We have showed previously that interaction of metacyclic trypomastigotes and monocytes from THP-1 lineage induces release of plasma membrane derived microvesicles (PMVs), in a Ca2+ dependent manner. PMVs give parasite protection against complement-mediated lysis, enhance T. cruzi invasion of eukaryotic cells and promote a high infection in mice. In this work we show that blood trypomastigote forms of T. cruzi also induce PMVs formation during interaction of parasite and THP-1 cells. This feature is shared by T. cruzi type I and type II strains. We have compared PMVs induction by metacyclic, blood trypomastigote and epimastigote forms from Y strains. Although all forms of parasite induce PMVs formation, THP-1 interaction with infective forms: metacyclic and blood trypomastigotes showed higher number of PMVs produced. Purified PMVs showed that only microvesicles from metacyclic and blood trypomastigotes interactions can increase T. cruzi infectivity in eukaryotic Vero cells. The components differentially present in PMVs from these stages are under study by proteomic analysis. Taken together our results show that PMVs induction during host cell interaction is a mechanism shared by different T. cruzi strains. Our results also suggest that evolutionary stage of parasite that interact directly with host blood may use this mechanism to facilitate host cell infection, enabling the evasion of the host innate immune system, parasite survival and a successful infection.
Supported by:Cnpq

BC012 - **CHARACTERIZATION OF PARAFLAGELLAR RODS IN GIARDIA DUODENALIS**

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Giardia duodenalis (syn. intestinalis, lamblia) is the etiological agent of giardiasis, an intestinal disease that has a widespread distribution in the world, bringing to infected individuals symptoms like diarrhea, anemia, weight loss, among others. Giardia is a flagellated protozoan that adheres to small intestine epithelium, and it has already been shown that flagellar movements are important for parasite survival. Specifically, motility is required for the initiation and maintenance of giardial infection. In other flagellates, like kinetoplastids, a structure called paraflagellar rod (PFR) is necessary for full flagellar motility and provides support for metabolic regulators that may influence flagellar beating. G. duodenalis trophozoites present several flagella. In some of them morphological studies have indicated the presence of not yet characterized structure associated to the flagellar axoneme, especially in the portion of the flagella still in contact with the cell body. In the current work we used previously well characterized antibodies associated with confocal laser scanning microscopy and transmission electron microscopy, high resolution scanning electron microscopy and atomic force microscopy (AFM) to characterize the structure associated with the flagella of G. duodenalis. Electron microscopy showed the presence of a continuous structure laterally located in relation to the anterior and ventral pairs of flagella. It starts at the fagellar basis and runs up to the point where the flagella leaves the cell body and become free. This structure was intensely labeled with antibodies recognizing PFR proteins 1 and 2 of trypanosomatids. Further biochemical and ultrastructural studies are being performed to a better characterization of the PFR of G. duodenalis.
Supported by:Capes, cnpq, faperj

BC013 - **A RECOMBINANT PROTEIN BASED ON TRYPANOSOMA CRUZI P21 UPREGULATES UNSPECIFC PHAGOCYTOSIS**

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*Trypanosoma cruzi* P21 protein was recently characterized and its possible role in the parasite internalization into host cell was examined. Continuing the characterization of functional *T. cruzi* P21 through the use of its recombinant form (P21-His6) is of great importance as it may eventually be among the potential therapeutic targets for Chagas disease treatment. To study the activity of P21-His6, we performed phagocytosis assays in vitro, in which peritoneal macrophages from C57BL / 6 mice were incubated with zymosan particles or infected with extracellular amastigotes of *T. cruzi,* promastigotes of *Leishmania amazonensis* or tachyzoites of *Toxoplasma gondii* and P21-His6 was added or not at the same time. Our results showed that P21-His6 treatment upregulated cell invasion by the different pathogens and also zymosan internalization. In order to verify the mechanism beneath this pro-phagocytic activity we observed the influence of the recombinant protein on actin filaments polymerization. We verified that treatment with P21-His6 augmented macrophages actin polymerization comparing to the non treated control. Subsequently we aimed to identify the receptor for P21-His6 on macrophage surface and also the signaling pathway activated upon interaction. Our results showed that P21-His6 binds to fusin and triggers a signaling pathway dependent on PI3 kinase, AKT, MEK-1, MEK-2 and ERK 1-2. As long as, HIV binds to fascin in order to promote host cell invasion, we are now studying the effect P21-His6 treatment on HIV propagation in vitro.
Supported by:CNPq, FAPEMIG, FAPESP, CAPES

BC014 - **STRUCTURAL ORGANIZATION OF PLASMODIUM CHABAUDI INFECTED ERYTHROCYTE: A THREE-DIMENSIONAL VISUALIZATION USING DIFFERENT MICROSCOPY APPROACHES**

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*Plasmodium* infects erythrocytes causing a series of modifications in its internal and surface organization. These alterations comprise formation of micro and small vesicles that are spread throughout the cytoplasm and a complex group of adjacent membranes named Maurer's clefts and tubovesicular network (TVN). Parasite also modifies the surface of host erythrocyte by inducing the generation of knobs. Few data are available on structural organization of *Plasmodium chabaudi*-infected erythrocytes, especially in a high-resolution 3D level. *Plasmodium chabaudi* is an attractive model for structural studies because a synchronized life cycle, controlled by circadian period, can be obtained producing bleeds enriched in specific developmental forms of the parasite. Electron microscopy observation of ultrathin sections of *Plasmodium chabaudi*-infected erythrocytes showed a membrane network in the host cell cytoplasm originated from the parasitophorus vacuole membrane. Dynamic of the interaction and formation of pores within this membrane network were revealed by electron tomography. Three-dimensional reconstruction of tubovesicular network showed that these structures are numerous and vary in shape and size. Using transmission electron microscopy, atomic force microscopy, field-emission scanning electron microscopy and dual beam microscopy we observed striking deformations, invaginations and knob formation on the surface of the erythrocyte. Alterations of the intracellular organization, such as formation of vesicles as a consequence of the presence of parasite, were observed. These results provide insights of the structural modifications that involve membrane trafficking upon *Plasmodium* infection and exemplify how front-end microscopy techniques can provide a better understanding of the mechanisms underlying the interaction of *Plasmodium chabaudi* with the host cell.
Supported by:CAPES e FAPERJ

## BC015 - **ROLE OF LIPID BODIES FROM LEISHMANIA CHAGASI IN THE PROSTAGLANDIN PRODUCTION**

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Introduction and Objective: Lipid bodies (LB) are cytoplasmic organelles involved in eicosanoid production in leukocytes. Eicosanoids as prostaglandins (PG) have been implicated in the immune response control. Parasites such as Leishmania (L.) are also capable of producing PGs, but the role of parasite LBs in biosynthesis of PGs has not yet been investigated. In this work, we studied the dynamics of LB formation and PG release from L. chagasi.
Methods and Results: Logarithmic (Log, 3rd day) and stationary (Stat, 7th day) stages of L. chagasi cultures were stained with osmium and LBs were counted by light microscopy. The number of LBs increased from Log to Stat phase (Log: $0.38 \pm 0.08$; Stat: $3.24 \pm 0.71$) and were maximal in the latter stage, suggesting a role for these organelles during differentiation. We measured PGE2, PGF2alpha and PGD2 released into extracellular medium by enzyme immunoassay (EIA), and found there were no differences in PG levels in the supernatants. Because arachidonic acid (AA), the precursor of prostaglandin cascade, enhances LB formation and activity in mammalian cells, we tested the effects of incubating Log L. chagasi with different doses of AA (3.25, 7.5, 15 and 30 uM). Treatment with AA increased LB numbers per parasite and the extracellular levels of PGE2 and PGF2alpha in dose-dependent manner (One-way ANOVA, linear trend's post-test). PGD2 were also increased. In addition, AA treatment increased the level of PGF synthase in parasites according to immunoblotting. This suggests that AA induced de novo synthesis of PGF synthase in the parasite, and this resulted in enhanced prostaglandin release.
Conclusion: Overall these results suggest that LBs are enhanced under the same conditions as increased synthesis and release of prostaglandins by L. chagasi. The data suggest novel functions for this organelle in the cellular metabolism of Leishmania, with possible implications for interactions with the surrounding host microenvironment.
Supported by:CNPq and iii - INCT

## BC016 - **NEW INSIGHTS TO UNDERSTAND THE COORDINATED CELL CYCLE OF CRITHIDIA DEANEI WITH ITS SYMBIONT BACTERIUM**

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Symbiont bearing trypanosomatids, cell division requires coordinated replication and segregation of single copy structures as the nucleus, the kinetoplast, the flagellum and the bacterium, which maintains a mutualistic relationship with the host protozoan. *Crithidia deanei* generation time is 6 hours and during this period the symbiont presents a dividing pattern for 3 hours before its segregation. Then it remains as double bacteria in rod shape until the cytokinesis, when each daughter cell receives a single symbiont. Taken together, these data indicate that the symbiont division is somehow controlled by the host protozoan. In order to better understand the mechanisms that are involved in the symbiont coordinated division, we treated *C. deanei* with several inhibitors which target essential host protozoan events as DNA replication (aphidicolin), protein synthesis (cicloheximida), as well as basal body and kinetoplast segregation (ansamitocin and acriflavine). Protozoa were cultivated in the presence of each inhibitor and samples were used in optical and electron microscopy, as well as in proliferation assays. Our data showed that the treatment of *C. deanei* with cicloheximide, induced cell proliferation arrest, by blocking nucleus and kinetoplast division and the symbiont segregation, suggesting that the bacterium depends on protein factors produced by the host to divide. In the other hand, treatment with aphidicolin arrested protozoan proliferation but did not block the symbiont segregation, resulting in an unusual *C. deanei* cell pattern which presents two symbionts. Interestingly, aphidicolin treatment of *Blastocrithidia culicis*, another symbiont harbouring trypanossomatid, resulted in the formation of filamentous bacterium that duplicates its DNA, but is unable to segregate. These results indicate that distinct mechanisms control the bacterium division in both species, suggesting different co-evolution processes between the symbiont with their respective hosts.
Supported by:CNPq, FAPERJ, FAPESP

BC017 - **HAS LEISHMANIA TURNED THE BLIND EYE TO DNA DAMAGE?**

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The proposed models for gene amplification in the protozoan parasite Leishmania imply the formation of single-stranded DNA (ssDNA). Several types of DNA damage also lead ssDNA that can be recognized and bound by the RPA protein. In superior eukaryotes, RPA-coated ssDNA is essential for the activation of the DNA repair checkpoint. One of the first players recruited to these sites is the heterotrimeric complex 9-1-1 (Rad9, Rad1 and Hus1), which functions as a docking site for proteins involved in DNA damage signaling and repair. We have found that the Hus1 homolog of L. major is a nuclear protein that binds to chromatin in response to DNA damage and improves the cell capability to cope with replicative stress. To study the limits of conservation of this pathway in the ancient eukaryote we investigated the presence of the other components of the 9-1-1 complex. In silico analysis did not identify Rad1 and Rad9 candidate genes within the parasite genome. Instead, the parasite expresses a Rad9 domain-containing protein with a predicted molecular mass that is twice as large as those of Rad1 or Rad9. We found that LmRad9-1 is a nuclear protein and that its overexpression confers resistance to MMS and Hydroxyurea. Furthermore, we have demonstrated that LmRad9-1 interacts with LmHus1, which is an indication that LmRad9-1 plays both Rad9 and Rad1 function. Next we studied the possible interaction between LmHus1 with LmRPA. Attempts to co-immunoprecipitate RPA using $\alpha$-LmHus1 antibodies were not successful suggesting that these proteins do not interact as described in other organisms. Immunofluorescence experiments revealed a partial co-localization between LmRPA-1 and LmHus1 or LmRad9-1. Surprisingly, our data indicated that binding of Hus1 is not dependent on RPA, another feature that differs from the described interaction mechanism of 9-1-1 complex and RPA. It is noteworthy that the predicted Leishmania RPA lacks the domain involved in both ATR kinase and 9-1-1 interactions. These data suggest that Leishmania LmHus1 and LmRad9-1 might be partners in the formation of a functional 9-1-1-like complex that despite of participating in DNA damage sensing does not interact with RPA. Altogether, our data suggest that Leishmania parasites operate a peculiar DNA damage sensing machinery that is compatible with their extensive genetic plasticity.

Supported by:FAPESP and CNPq

BC018 - **DIFFERENTIAL INFECTIVITY OF TRYPANOSOMA CRUZI “Y” STRAIN POPULATIONS BY THE ORAL ROUTE**

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Recently, two *T.cruzi* samples identified as “Y” strain were found to differentially express gp82 and gp30, the metacyclic trypomastigote (MT)-specific surface molecules associated respectively with high and low efficiencies in establishing infection by the oral route.Here we performed a comparative analysis of these *T.cruzi* populations,which were designated Y82 and Y30 strains. For the first time, the amino acid sequence of a putative gp30 molecule was deduced from a Y30 strain cDNA clone and the corresponding recombinant protein (D21) was generated. D21 exhibited 57% sequence identity with J18,the recombinant protein containing a gp82 sequence highly conserved between genetically divergent *T.cruzi* strains. It displayed properties similar to those of J18, namely, the ability to bind to host cells and to induce lysosomal exocytosis. Host cell invasion by Y82 and Y30 strains was inhibited by D21 or J18, as well as by mAb 3F6 that reacts with gp82 and gp30 expressed on MT surface. MT internalization of both strains required the activations of host cell signaling cascades involving mammalian target of rapamycin (mTOR), phosphatidylinositol 3 kinase (PI3K) and protein kinase C (PKC), which lead to lysosomal exocytosis. As compared to J18, the ability of D21 to bind to gastric mucin was lower. Consistent with this finding, lower number of Y30 strain MT migrated through a gastric mucin layer in vitro, as compared to Y82 strain.In the presence of gastric mucin, the host cell invasion by MT of Y30 strain, but not of Y82 strain, was inhibited. More relevant, 4 days after oral administration into mice, much fewer Y30 strain amastigote nests were found in histological sections of the stomach, as compared to Y82 strain.Taken together,our results reinforce the notion that the differential expression of gp82 and gp30 molecules determines the ability of MT to bind to and translocate through the gastric mucin layer,which is essential for reaching the target cells in oral infection.

Supported by:FAPESP, CNPq

BC019 - **THE EFFECTS OF CAMPTOTHECIN ON THE COORDINATED DIVISION OF BLASTOCRITHIDIA CULICIS AND ITS SYMBIOTIC BACTERIUM**

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*Blastocrithidia culicis* is a monoxenic trypanosomatid that presents single copy structures, as the nucleus, the kinetoplast and the endosymbiotic bacterium, which maintains a mutualistic relationship with the host protozoan. During *B. culicis* cell cycle, the symbiont divides in coordination with host structures, so that each daughter cell harbours only one bacterium. Camptothecin is a DNA topoisomerase I inhibitor that promotes cell cycle arrest and ultrastructural alterations in trypanosomatids. In this work, we investigated the effects of camptothecin on *B. culicis* proliferation, ultrastrucuture and on symbiont division. For this purpose, cells were maintained in culture medium and treated with different drug concentrations (1, 5, 10 and 50 µM). After each 12 hours, samples were collected for counting on Neubauer's chamber and for processing to fluorescence optical microscopy and to transmission electron microscopy. Our results showed that camptothecin inhibited cell proliferation and promoted protozoa ultraestructural changes, as nuclear chromatin unpacking and mitochondrion swelling. Furthermore, immunofluorescence assays showed that camptothecin induced cell cycle arrest, since the number of trypanosomatids presenting an interphasic pattern was higher when compared to non-treated cells. Interestingly, the symbiont of drug treated protozoa was able to duplicate its DNA, but not to segregate, thus presenting unusual filamentous forms. These data reinforces the idea that the bacterium division is coordinated by the host protozoa in order to maintain the close symbiotic relationship.
Supported by CNPq and FAPERJ.

BC020 - **HOST CELL LYSOSOME EXOCYTOSIS REQUIRED FOR INVASION OF TRYPANOSOMA CRUZI METACYCLIC FORMS IS ASSOCIATED WITH SIGNALING CASCADES INVOLVING MTOR, PKC AND PI3K**

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To further elucidate the molecular mechanisms of host cell invasion by T. cruzi metacyclic trypomastigotes (MT), we attempted to identify components of target cell signaling pathways leading to lysosome exocytosis required for MT internalization. Treatment of human epithelial HeLa cells with rapamycin, inhibitor of mammalian target of rapamycin (mTOR), diminished lysosomal exocoytosis and MT invasion. Downregulation of phosphatidylinositol 3-kinase (PI3K) and protein kinase C (PKC) also impaired exocytosis and MT internalization. The recombinant protein based on gp82, the MT surface molecule that mediates cell adhesion/invasion, induced exocytosis in HeLa cells. Such a property has been previously attributed to a soluble factor secreted by tissue culture trypomastigotes (TCT), but not to any T. cruzi surface molecule. Rapamycin treatment of HeLa cells diminished gp82 binding as well. Cell invasion assays under conditions that promoted lysosome exocytosis, such as 1 h incubation in starvation medium PBS++, increased MT internalization, whereas prestarvation of cells for 1-2 h had an opposite effect. In contrast to MT, invasion by TCT increased upon HeLa cell prestarvation or treatment with rapamycin. This is a novel finding that reveals another distinctive feature of the two infective forms in a key process for infection. To enter host cells, MT engage the surface molecule gp82, triggering the signaling pathways involving mTOR and/or PI3K/PKC, actin cytoskeleton disruption and lysosome mobilization to the cell periphery that culminates in exocytosis, whereas TCT rely on a mTOR-independent pathway, which may be upregulated by autophagic process.
Supported by:FAPESP e CNPQ

BC021 - **VIRULENCE CHARACTERIZATION IN LEISHMANIA INFANTUM STRAINS**

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*Leishmania infantum* (=*L. chagasi*) is the causative agent of visceral leishmaniasis (VL) in Brazil. A wide spectrum of clinical variability observed suggests that parasite factors may be involved on virulence. It was observed that animals infected with BH400 strain (MCAN/BR/2000/BH400) exhibited a more severe pattern of disease when compared to those infected with BH46 (MHOM/BR/1972/BH46) strain. In order to investigate in vitro infectivity, peritoneal macrophages from BALB/c mice were infected with BH46 or BH400 promastigotes at multiplicity of infection (MOI) of 10. The percentage of infected cells was approximately 12% and 5% for BH400 and BH46 strain ($p = 0.0042$), whereas the number of intracellular amastigotes/cell was 2.5 and 1.8, respectively. In vivo infection was performed using $10^5$ amastigotes. After 90 days, animal, liver and spleen weights, ascite and parasite quantification were evaluated. The BH400 infected animals showed higher parasite densities in the spleen ($10^7$ parasites) than those infected with BH46 ($10^2$ parasites) ($p < 0.0001$) and exhibited higher spleen weight ($p = 0.0013$). The liver samples were submitted to histophatological changes and to immunohistochemical assay. An increase in the granulome, portal inflammation and capsular thickening was more evident in BH400 than in BH46 ($p < 0.02$). In the immunohistochemical evaluation, BH400 infected animals exhibited higher parasitism (11.4-fold, $p = 0.0001$) than BH46. Those results suggested virulence variability between the strains, and will be explored to identify differentially expressed proteins in DIGE and mass-spectrometry. The virulence factors studies may contribute to the discovery of novel therapeutic targets against leishmaniasis.
Supported by:FAPEMIG, CAPES, CNPq

BC022 - **CRUZIPAIN PROMOTES TRYPANOSOMA CRUZI ADHESION TO RHODNIUS PROLIXUS MIDGUT**

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Cruzipain is the lysosomal cysteine peptidase most abundant of Trypanosoma cruzi, which is the causative agent of Chagas' disease. This enzyme is expressed at variable levels in all developmental forms and strains of the parasite. Cruzipain is required for parasite infectivity and intracellular growth in mammalian cells, however, its role in parasite interaction with the vector has been overlooked. Here, we have analyzed the effects of the treatment of T. cruzi with anti-cruzipain antibodies and a panel of different cysteine peptidase inhibitors on the parasite adhesion to Rhodnius prolixus posterior midgut. In parallel, we have compared the adhesion rate between wild T. cruzi, and a genetically manipulated parasite, which superexpresses chagasin, an endogenous cruzipain inhibitor (pCHAG). Brielfy, the parasites were treated for 1 hour with iodoacetamide, leupeptin, antipain, Ca074me or E-64 at 10 µM or cystatin at 1 µg/ml and allowed to bind to R. prolixus explanted guts for 15 minutes. The interaction rate of the parasites treated with the cysteine peptidase inhibitors was on average 70% lower in comparison to the untreated parasites, except for Ca074me (a cathepsin B inhibitor), which showed no significant alteration. In addition, anti-cruzipain antibodies (1:1000) reduced the adhesion to the insect posterior midgut in 64%, corroborating, pCHAG parasites adhered to the insect gut 73% less than control. Assessment of insect colonization 20 days after blood feeding, revealed parasites only in the rectum by direct microscopic counts. This finding led us to highlight, for the first time, the development of a methodology using quantitative real-time PCR (qPCR) with SYBR-green targeting T. cruzi satellite-DNA to quantify T. cruzi infection in R. prolixus. qPCR infection assays, performed with pCHAG or control parasites revealed that pCHAG parasites were virtually unable to colonize R. prolixus midgut or rectum, thereafter unable to complete its life cycle within their insect host, while control parasites were detected both in the midgut and rectum, being more abundant in the latter. Furthermore, the expression of surface cruzipain in T. cruzi cells was drastically enhanced after passage in R. prolixus. Collectively, these results suggest that cruzipain somehow mediates actively the interaction between T. cruzi and epithelial cells from the invertebrate host.
Supported by:FAPERJ, MCT/CNPq, FIOCRUZ

## BC023 - **LYSOSOMES ARE NOT INVOLVED IN THE DEGRADATION PATHWAY OF THE INDUCIBLE NITRIC OXIDE SYNTHASE OF MACROPHAGES CAUSED BY TOXOPLASMA GONDII INFECTION**

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Toxoplasmosis is a zoonosis widely spread throughout the world caused by Toxoplasma gondii. Cellular and humoral responses control the infection of this parasite, and macrophages are important cells in the immunological responses against T. gondii. When classically activated, macrophages become more microbicidal and control the replication of T. gondii through nitric oxide (NO) production by a reaction catalyzed by the inducible Nitric Oxide Synthase (iNOS). However, when T. gondii infects activated macrophages, NO production is inhibited because iNOS is degraded. It has been demonstrated that lysosome may be involved in iNOS degradation. Thus, we investigated whether this organelle participates in iNOS degradation after T. gondii infection of activated macrophages. For this, J774-A1 macrophage cell line was cultured with Dulbecco's Modified Eagle's Medium supplemented with 5% fetal bovine serum. The T. gondii (RH strain) was maintained in the peritoneal cavity of Swiss mice. Macrophages were activated with lipopolysaccharide and interferon-γ for 24h, treated with concanamicin A, a lysosome inhibitor, for 1h and infected with the parasite. Acidic compartments and viability of macrophages was assayed by acridine orange and ethidium bromide staining. Cells attached to coverslips and supernatant were collected after 2, 3 and 6h of infection and iNOS expression was assayed by immunofluorescence and NO production determined by the Griess reagent. Macrophages treated with concanamicin A had no acidic compartments, but were viable. Macrophages with inhibited lysosomes infected with T. gondii showed no expression of iNOS and NO was not produced, indicating that this enzyme was still degraded by the infection. These results show that lysosomes are not involved in iNOS degradation caused by T. gondii infection.
Supported by:CAPES, CNPq, FAPERJ, UENF

## BC024 - **LOCALIZATION OF ACTIN IN PHYTOMONAS SERPENS AND CRITHIDIA SPP.**

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Actin filaments play a major role in diverse eukaryotic cell processes, including cytokinesis and motility. Although abundant and well-characterized in higher eukaryotic cells, up to few years ago little data was available on the expression and distribution of this protein in trypanosomatid protozoa. Presence of this protein in the cytoplasm of some pathogenic trypanosomatids (*Trypanosoma brucei*, *T. cruzi* and *Leishmania major*) has been recently demonstrated by immunofluorescence. However no data is available on other trypanosomatids, including those that infect plants and insects, mainly due to the lack of genome information. Therefore, in this work we have screened some trypanosomatids for the presence of actin, by using a mouse polyclonal antibody produced against the whole sequence of the *Trypanosoma cruzi* actin gene, encoding for a 41.9 kDa protein. By Western blot, this antibody reacted with a polypeptide of about 42 kDa in whole cell lysates of insect (*Crithidia deanei* with symbiont and *C. fasciculata*) and plant (*Phytomonas serpens*) parasites. Analysis by confocal microscopy of cells incubated with the anti-actin antibody followed by incubation with a secondary goat anti-mouse antibody coupled to AlexaFluor 488 demonstrated a diffuse actin expression dispersed throughout the cytoplasm in all examined trypanosomatids. Interestingly, in *Crithidia deanei* labeling was intense around the bacterial endosymbionts, which are located at the posterior cell region. Our data indicate that actin is widespread in trypanosomatids and that in *C. deanei* some kind of actin-based cytoskeleton might be related to positioning the endosymbionts at the posterior cell region. Further studies are underway to immunolocalize actin in trypanosomatids by transmission electron microscopy.
Supported by:CNPq

BC025 - **ANALYSIS OF CD100 MODULATION OF MACROPHAGE ACTIVATION AND INFECTIVITY BY LEISHMANIA (LEISHMANIA) AMAZONENSIS.**

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Leishmaniasis is caused by trypanosomatids of the genus Leishmania, which infect mainly macrophages. The disease affects 12 million people worldwide and can have cutaneous, mucocutaneous and visceral forms. The main factors that influence form and severity of the disease are species of Leishmania and host immune response. Considering the importance of macrophages in Leishmania´s infection and the potential role of CD100 in the modulation of macrophage activation, we postulated that this molecule may affect the macrophage infectivity by the parasite. CD100 is known to reduce migration and expression of inflammatory cytokines in human monocytes and dendritic cells, and is induced in macrophages by inflammatory stimulus. Based on this information, our objectives are to analyze and compare the effects of CD100 on macrophages from BALB/c and C57BL/6 mice lineages in terms of their profile of activation and infectivity by L. amazonensis.
We observed that peritoneal macrophages from BALB/c mice express 29 times more CD100 than C57BL/6 cells. Macrophages from BALB/c incubated with LPS increased the expression of iNOS and IL1β, and co-incubation with LPS and CD100 minimized the increase of iNOS and increased TNFα. Soluble CD100 had no modulation in any of the genes analyzed. Macrophages from C57BL/6 stimulated with LPS showed increase only in IL1β. In infections with 2,5 L. amazonensis per macrophage for 4, 24, 48 and 72h, BALB/c showed a statistically non-significant decrease in CD100 expression. Macrophages from C57BL/6 showed a statistically non-significant decrease in expression of CD100 after 48h, and an increase afterwards. In infections with 5 parasites per macrophage for 24h CD100 expression decreased significantly in BALB/c mice (p = 0.01- C57BL/6 data not analyzed). Further experiments will analyze whether CD100 affects the infectivity of macrophages by L. amazonensis.
Supported by:FAPESP

BC026 - **ELECTRON TOMOGRAPHY ANALYSIS OF THE CONTRACTILE VACUOLE COMPLEX OF TRYPANOSOMA CRUZI SUBMITTED TO HYPOSMOTIC TREATMENTS**

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During the course of the infection, *Trypanosoma cruzi* faces environments where extreme variations in the concentration of ions and osmolytes in the extracellular milieu are found. To cope with these fluctuations, the parasite has developed adaptation mechanisms that involve signaling pathways and remodeling of parasite organelles, including the contractile vacuole complex (CVC) and acidocalcisomes. The CVC of some trypanosomatids is formed by several tubules and vesicles forming a multi-tubular structure named spongiome, connected to a central vacuole located near the flagellar pocket. This organelle has a mechanism for fluid secretion that is achieved by cooperation with acidocalcisomes, which are electron-dense acidic organelles rich in calcium, polyphosphate and other cations, and shown to be involved in several functions as calcium homeostasis, phosphate metabolism and osmoregulation. The structural modifications that take place in the CVC during hyposmotic treatment have not been yet fully characterized in *T. cruzi*. In this work we used cryotechniques and 3D electron tomography reconstruction to study the CVC of *T. cruzi* submitted to hyposmotic treatment. Results revealed a CVC in *T. cruzi* with the central vacuole positioned docked to a specific domain of the flagellar pocket, presenting an electron dense aspect, and associated with a spongiome. The tubules of the spongiome were connected to the contractile vacuole preferentially in a region opposite to the kinetoplast and parallel to the flagellum, suggesting that this structure has a polarized organization. Serial electron tomography revealed fusion of acidocalcisome with the central vacuole. Local modulation of the acidocalcisome volume after hyposmotic stress could be observed. Altogether, the results suggest that the spatial organization of CVC is defined by specific domains of the spongiome and the contractile vacuole, which may be modulated during events of regulatory volume decrease in *T. cruzi* .
Supported by:CNPq, FAPERJ, FINEP and CAPES

BC027 - **AN ULTRASTRUCTURAL STUDY OF STRIATED FIBRES OF TRITRICHOMONAS FOETUS**

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Tritrichomonas foetus is the causative agent of bovine trichomonosis disease which a major cause of infertility and abortion leading to considerable economic losses in beef-producing areas of the world. Furthermore, T. foetus presents a particular cell structure such as the costa, a striated fiber that have been implicated to serve an anchoring function for the recurrent flagella and to help absorb the mechanical stress generated by flagellar beating. Nevertheless, little is known about this structure. Thus, the aim of the present study was obtain a better understanding of the ultrastructure and morphometry of the costa, because this structure could be used as a parameter for nano measurements. For this, the isolation of costa of T. foetus was performed and the enriched fraction was analyzed by transmission electron microscopy (TEM), field emission scanning electron microscopy (FESEM) and atomic force microscopy (AFM). The costa, flagella, axostyle and basal body-associated filaments were observed by FESEM after the extraction of cytoskeleton using 2% triton X-100. In addition, a new costa filament-associated was observed. The costa-enriched fraction was also observed by negative stain and other structures such as the plasma membrane and parabasal fibrils were also found. Measurements of costa were performed, demonstranting that this structure are cross-striated with a constant periodicity of 50 nm (n = 10), consisting of a dark gap of 20 nm and a light zone of 30nm with a complex interbanding pattern. Together, these results demonstrated a new view of the costa and these methodologies were efficient to perform the measurements.
Supported by:AUSU, CAPES, CNPq, FAPERJ and PRONEX

BC028 - **TRYPANOSOMA CRUZI INVADES HOST CELLS THROUGH THE ACTIVATION OF ENDOTHELIN AND BRADYKININ RECEPTORS: A CONVERGING PATHWAY LEADING TO CHAGASIC VASCULOPATHY**

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Independent studies in experimental models of *Trypanosoma cruzi* appointed different roles for endothelin-1 (ET-1) and bradykinin (BK) in the immunopathogenesis of Chagas disease. Considering that ET-1 is upregulated in chagasic plasma patients and that *T. cruzi* trypomastigotes induces plasma leakage via the activation $B_2R$/TLR2/CXCR2 pathway, we addressed the hypothesis that pathogenic outcome is influenced by functional interplay between endothelin receptors ($ET_AR$ and $ET_BR$) and bradykinin receptors ($B_2R$). Intravascular microscopy assays demonstrated that $ET_AR$ antagonist (BQ-123), $ET_BR$ antagonist (BQ-788) and $B_2R$ antagonist (HOE-140) reduced leukocyte accumulation in hamster cheek pouch topically exposed to Dm28c trypomastigotes and the inflammatory edema was blocked in infected BALB/c paw. Acting synergistically, $ET_AR$ and $ET_BR$ antagonists reduced parasite invasion of human smooth muscle cells (HSMCs) and mouse cardiomyocytes to the same extent as HOE-140. Exogenous ET-1 potentiated *T. cruzi* uptake by HSMCs via ETRs/$B_2R$ whereas the RNA interference of $ET_AR$ and $ET_BR$ genes conversely reduced infection indexes. Parasite internalization via the $ET_AR/ET_BR/B_2R$-dependent axis was reduced in HSMCs pretreated with MβCD, a cholesterol depleting drug, or in thapsigargin or verapamil treated target cells. Our findings suggest that *T. cruzi* may take advantage of kininogen and ET-1 leakage to extravascular sites of infection to infect cardiomyocytes via cross-talk between ETRs/BKRs.
Supported by:CNPq, Pronex, INBEB, FAPERJ

### BC029 - **MODULATION OF AN ECTO-3'-NUCLEOTIDASE ACTIVITY FROM PROCYCLIC PROMASTIGOTES FORMS OF LEISHMANIA AMAZONENSIS BY NUCLEOTIDES**

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*Leishmania amazonensis* parasites are intracellular protozoa and the etiological agent of cutaneous and diffuse cutaneous leishmaniasis. The flagellated metacyclic promastigote forms are transmitted to vertebrate hosts by sandfly bites. The promastigotes develop into aflagellated amastigotes in the vertebrate host and multiply by binary division, inside macrophages. Membrane interactions between parasites and hosts are crucial for its survival, from both physiological and immunological viewpoints. Cytoplasmic membranes contain enzymes whose active sites face the external medium rather than the cytoplasm. The activities of these enzymes, referred to as ecto-enzymes, can be measured using intact living cells. *Leishmania* species have membrane bound 3'-nucleotidases on their extracellular surface that hydrolyze extracellular 3'-nucleotides or nucleic acids to form nucleosides. The hydrolysis of 3'AMP generates, as final products, inorganic phosphate, an essential nutrient for cells, and adenosine, that plays important role in immunological events. Since trypanosomatids are unable to synthesize purines *de novo* and the plasma membrane of *Leishmania* is permeable to nucleosides, this enzyme plays a role in the acquisition of purines, It's known that variations in ecto-3'-nucleotidase activity can modulate the interaction between *Leishmania* and macrophages *in vitro*. In this context, the objective of the present study was to investigate the modulation of ecto-3'-nucleotidase activity from *L. amazonensis* by several nucleotides and the products of its hydrolysis. Both adenosine and 3'AMP increased macrophage infection by parasites. The nucleotides 5'AMP and ADP, that inhibited ecto-3'-nucleotidase activity, were able to attenuate the increase of interaction promoted by 3'AMP. The determination of compounds that are capable of inhibiting the ecto-3'-nucleotidase activity is very important, since this enzyme does not occur in mammals, making it a potential therapeutic target.
Supported by:CNPq, FAPERJ, UFRJ/PIBIC

### BC030 - **ESCAPE MECHANISM OF PLASMODIUM CHABAUDI CHABAUDI DURING INTERACTION WITH ACTIVATED MURINE MACROPHAGES**

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Exposure of phosphatidylserine (PS) by cells indicates apoptosis. This exposure is essential for signaling the release of transforming growth factor-beta1 (TGF-b1) by macrophages, inducing an anti-inflammatory response during phagocytosis of apoptotic cells. The interaction *Toxoplasma gondii* with activated murine macrophages inhibits nitric oxide (NO) production, allowing the persistence of the parasite in the macrophage. Our group showed that the mechanism used by *T. gondii* to inhibit the production of NO in activated macrophages is similar to Leishmania because it involves the exposure of PS by the parasites. Malaria infection is characterized by major activation and suppression of the immune system during different phases of the disease. The immune response to the intraerythrocytic stages of malarial parasites has been best characterized in the rodent model *Plasmodium chabaudi chabaudi*. In this work we characterized the possible exposure of PS by *P. chabaudi chabaudi* merozoites obtained from infected erythrocytes and verify the production of NO by activated mouse macrophages after the interaction with this parasite form. The results show that 90 % of the merozoite population exposed PS. NO production by activated macrophages was inhibited after 24 h of interaction. In addition, macrophages infected by merozites presented morphological changes similar to apoptosis. These results suggest that PS exposure can be considered a common escape mechanism of parasitic protozoa.
Supported by:FAPERJ/CNPQ

## BC031 - **TRYPANOSOMA CRUZI HEPARIN-BINDING PROTEINS MEDIATE ADHERENCE OF EPIMASTIGOTES TO SULFATED GLYCOSAMINOGLYCANS IN THE MIDGUT CELLS OF RHODNIUS PROLIXUS**

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Heparin-binding proteins (HBPs) have been reported as potential candidate for therapeutic intervention against parasitic infection. In *Trypanosoma cruzi*, the etiological agent of Chagas diseases, proteins with property to bind heparin have been demonstrated in both infective forms, trypomastigotes and amastigotes, and are involved in the recognition and invasion of mammalian cells. Herein, we evaluated the potential biological function of these proteins from epimastigote forms of *T. cruzi* in the parasite-vector interaction.

HBPs of epimastigotes were isolated using Triton X-114 extraction followed by heparin chromatography. This strategy yielded a protein fraction containing two major proteins with molecular masses of 65.8 kDa and 59 kDa, which were recognized by biotin-conjugated sulfated glycosaminoglycans (GAGs) in Western blot assay. The real-time interaction of HBP with immobilized heparin was explored by the surface plasmon resonance (SPR) biosensor technique. The resultant sensorgrams demonstrated stable receptor-ligand binding based on the association and dissociation values. Additionally, preincubation of epimastigotes with GAGs lead to an inhibition of parasite binding to immobilized heparin. Competition assays were also performed to evaluate the role of HBP-GAG interaction in the recognition and adhesion of epimastigotes to the midgut epithelial cells of *Rhodnius prolixus*. Preincubation of the epithelium cells with HBPs yielded a 3.8-fold inhibition in the adhesion of epimastigotes. Also, the pretreatment of epimastigotes with heparin, heparan sulfate and chondroitin sulfate inhibited significantly the parasites adhesion to midgut epithelium cells, which were confirmed by scanning electron microscopy.

We provide evidence that proteins with property to bind heparin are detected on *T. cruzi* epimastigotes surface and demonstrate their key role in the recognition of sulfated GAGs at the surface of midgut epithelial cells of the insect vector.

## BC032 - **IDENTIFICATION, EXPRESSION AND CELLULAR LOCALIZATION OF THE A SUBUNIT OF THE ADAPTER COMPLEX 2 (AP-2A) IN TRYPANOSOMA CRUZI**

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Heterotetrameric Adaptor Complexes (AP) are involved in the recruitment of clathrin and its binding to membrane receptors to form endocytic and exocytic vesicles. In eukaryotic cells, endocytic clathrin-coated vesicles are formed at the plasma membrane with the help of AP-2 complex. This adaptor complex contains two major (α and β-2), one mean (μ-2) and one small (σ-2) subunits. There is little information on the expression and characterization of AP complex proteins in the protozoan *Trypanosoma cruzi* and investigation on the presence of AP-2 subunits could help to know the proteins involved in the endocytic pathway in this parasite. This study aimed the identification, cloning and expression of the gene corresponding to the AP-2α subunit in *T. cruzi*. Search at the GenBank database allowed the identification of a gene sequence coding for a AP-2α homologue in *Trypanosoma cruzi* (Tc00.1047053511391.140), encoding a protein of 108.3 kDa. The gen sequence was amplified by PCR, cloned and expressed in *E. coli* using the Gateway platform (Invitrogen). The recombinant protein was purified and inoculated into mice to obtain a polyclonal antiserum. Analysis by Western blot of whole cell lysates of *T. cruzi* epimastigotes demonstrated a cross-reaction with a polypeptide of about 100 kDa, consistent with the molecular mass of the AP-2α subunit. The cellular localization of AP-2α was performed by immunofluorescence using laser confocal microscopy (Leica SP5), showing a strong reaction at the bottom of the flagellar pocket of epimastigote forms, a place known to present clathrin-mediated endocytic activity. Further studies are underway to identify and locate the other three AP-2 sub-units.
Supported by:CNPq

BC033 - **CHARACTERIZATION OF HIP-1 (HUNTINGTIN-INTERACTING PROTEIN) IN TRYPANOSOMA CRUZI, A PROTEIN INVOLVED IN THE ASSEMBLY OF CLATHRIN-COATED VESICLES.**

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HIP-1 (Huntingtin Interacting Protein-1) is a protein that regulates clathrin assembly through direct binding to the regulatory region of the clathrin light chain. It is known that clathrin-coated vesicles bud off at the flagellar pocket membrane (endocytic vesicles) and at the Trans-Golgi-Network (exocytic vesicles) of *Trypanosoma cruzi* epimastigotes. Therefore, HIP-1 could play a role in regulating clathrin assembly in this parasite. Thus, the aim of our study was to characterize a possible HIP-1 protein in *T. cruzi* and determine its cellular localization. Search at the TritrypDB database allowed the identification of a gene (Tc00.1047053508199.50) coding for a protein sequence of about 95, 4 kDa with homology to HIP-1. The gene sequence was amplified by PCR, cloned and expressed in *E. coli* using the Gateway platform (Invitrogen). The recombinant protein was purified and inoculated into mice to obtain a polyclonal antiserum. Analysis by Western blot with whole cell lysates of *T. cruzi* Dm28c epimastigotes demonstrated a cross-reaction with a polypeptide of about 90 kDa, consistent with molecular mass of HIP-1 protein. Immunofluorescence analysis by using confocal laser microscopy showed a cellular localization in large round spots lateral to the kinetoplast of epimastigote forms, consistent with the localization of the Golgi apparatus. Further studies are underway to perform immunolocalization by transmission electron microscopy, to confirm the results obtained by immunofluorescence.

Supported by:CNPq

BC034 - **CANINE VISCERAL LEISHMANIASIS:PARASITE LOAD AND CLINICAL MANIFESTATION IN DOGS NATURALLY AND EXPERIMENTALLY INFECTED.**

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Canine Visceral Leishmaniasis (CVL) is caused by the protozoan parasite *Leishmania infantum chagasi*. The symptoms of this disease include progressive loss of body weight, cutaneous, ocular, and musculoskeletal signs, renal and liver disease, peripheral lymphadenomegaly, hepatosplenomegaly, and epistaxis. The development of an experimental model that reproduces the natural disease has a great value to testing new vaccines and effectiveness of drugs. The aim of this study is to compare naturally infected dogs (NID) and experimentally infected dogs (EID) in relation of parasite load and clinical manifestation of this disease. The dogs were divided in two groups: eight dogs naturally infected that lived in an endemic area and eight beagles that were infected with $10^7$ promastigotes of *Leishmania chagasi* with salivary gland sonicate of *Lutzomyia longipalpis* and followed for 735 days. All the dogs had positive serology and characteristic clinical signs of visceral leishmaniasis. The immunohistochemical technique was used for detection and quantification of parasites in the skin and by PCR was possible detect parasites in the spleen and liver of dogs. The animals were scored on a scale from 0 to 10 by observing the severity of clinical signs. Splenomegaly, hepatomegaly, lymphadenopathy and weight loss were the clinical findings more commonly observed in both groups. The clinical score for the NID dogs were 4.8 and for the EID were 5.25. The PCR detected parasite in the spleen of 75% of dogs in both groups and 75% and 62.5% of NID and EID liver, respectively. All dogs were positive by immunohistochemical technique in the skin, but EID group showed significantly more parasites than NID group ($p>0.05$). We conclude that beagles can be used as an experimental model of CVL, however more studies are necessary to adequate the conditions to observe a less severe disease.

Supported by:CNPQ,FAPESB,FIOCRUZ

BC035 - **SEPTINS 4 AND 14 INVOLVEMENT DURING CELL INVASION BY TRIPANOSSOMATIDES AND APICOMPLEX PARASITES**

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The septins are proteins that express a characteristic nucleotide GTP (guanosine triphosphate active) binding-domain and other two variable domains (N and C-terminal). These proteins were first characterized in yeast and, subsequently, in several eukaryotic organisms, except plants. Thus, the septins are associated with many biological events, such as: cell migration, cytokinesis, cell's cycle regulation, exocytosis, apoptosis, among others. Such events can be explained due to the principal features of the septins: the ability to promote interaction between them and other proteins as well as the ability to form filaments. Therefore, the functions of septins became much more extensive than initially believed, being of utmost importance to conduct further research on these proteins. It is noteworthy that in humans were found 14 different genes for septins divided into groups. Thus, this study aims to analyze the involvement of septins 4 and 14 (both in the wild type and in the mutant forms) during *Trypanosoma cruzi*, *Toxoplasma gondii* and *Leishmania amazonensis* cell invasion into Hela cells transfected with the wild type and mutated plasmids. These parasites have the ability to promote actin polymerization during cell invasion. Also, we will use septin inhibitor during invasion assays. Moreover, we will use siRNA for both septins. The dosage of such plasmids was standardized by using the concentration of 10 µg in order to have 50% efficiency in transfection. Accordingly, the experiments are in progress and the involvement of septins in cell invasion by these pathogens is the first time, described in the literature.
Supported by:CNPq e FAPEMIG

BC036 - **DIFFERENT SECRETED PHOSPHATASE ACTIVITY IN PROMASTIGOTES OF LEISHMANIA AMAZONENSIS**

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Leishmania presented intense acid phosphatase activities located on the external surface of plasma membrane and secreted to the extracellular milieu through the flagellar pocket. The secretory acid phosphatase, which are the most abundant secretory protein of Leishmania is a virulence factor, plays a role in vertebrate infection and survival within sandfly. During our previous studies on the role of PLA2 in the secretory activity of L amazonensis we detected distinct phosphatase activities based on their ultrastructural localization and in the affinity to substrates and susceptibility to inhibitors. In the present study we have characterized the phosphatase activities secreted by L. amazonensis.
Parasites were grown for 72h in Schneider medium. Viable parasites assessed by viability tests were analyzed by ultrastructural cytochemistry and biochemical assays. Secreted phosphatase activity showed to be affected by different pH (pH range tested 6.5-8.5). Two different activities, one acid and the other alkaline, were observed. The secreted acid phosphatase activity changed according the substrate. A stronger activity is observed in the presence of b-glycerophosphate compared to that obtained with p-nitrophenylphosphate (p-NPP) in the same conditions of incubation. Both of them were inhibited by sodium tartrate. Cytochemical analysis showed the presence of acid phosphatase activities on the parasite surface (cell body and flagellum) and in intracellular compartments of exocytic/endocytic pathways which intensity varied according to the substrate used. Based on the results obtained we identified: the presence of different acid phosphatases in the intact parasite and secretion of two different acid phosphatases by Leishmania amazonensis. The presence of different phosphatase may be useful in the harsh environment in which Leishmania survives.
Supported by:CNPq, CAPES, FAPERJ

BC037 - **MACROPHAGE INFECTION BY LEISHMANIA CHAGASI IS ENHANCED BY LYSOPHOSPHATIDYLCHOLINE.**

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The saliva of Lutzomyia longipalpis sandflies contains elements that directly affect the host infection by Leishmania chagasi. The lysophospholipid lysophosphatidylcholine (LPC) has long been recognized as a molecule that can interfere in cellular functions. The aim of this study is to investigate the role of phospholipids on L. chagasi infection. Sonicated salivary gland lipids from L. longipalpis were analyzed by thin layer chromatography. The analysis showed the presence of phospholipids and free fatty acids, suggesting a phospholipase action under phospholipids, producing lysophospholipids. Therefore, we evaluated the effect of commercial LPC on mouse macrophage infection by L. chagasi in vitro. The parasite burden was significantly higher in macrophages treated with 20 μM LPC when compared with untreated macrophages. Then, the effect of LPC on cytokine production was evaluated. 20μM LPC stimulated the production of TNFα, IL1β and IL-12 by uninfected macrophage. Surprisingly, the LPC-stimulated production of those cytokine was reverted in the presence of L.chagasi. On the other hand, the production of IL-10 was reduced by LPC and this effect was unaffected by L. chagasi. The synthesis of chemokine MCP-1 was stimulated by LPC, as evaluated by RT-PCR. Pre-treatment of macrophages with antibodies against G2A, a possible LPC receptor, diminished the LPC effect on macrophage infection by L. chagasi. The results showed that the pro-inflammatory effect of LPC on macrophages is down regulated by the parasite infection. This suggests that LPC from saliva of sandfly could modulates the immune response of macrophages favoring the L.chagasi infection via inhibition of LPC-induced pro-inflammatory and stimulating chemokine production, possibly by a specific receptor. Taken together, this data suggest that early events in host cells induced by L. longipalpis saliva lipids components can represent an important mechanism in the establishment of Leishmania infection.
Supported by:CNPq, FIOCRUZ, FAPESB.

BC038 - **AFAP1 AND AFAP1L1 PARTICIPATION ON TRYPANOSOMATIDAE AND APICOMPLEXA INTRACELLULAR TRAFFIC**

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AFAP1 and AFAP1L1 are recently characterized members of the actin-filament associated protein (AFAP) family. It have been demonstrated that AFAP1 has a strong affinity for actin-filaments and c-Src (which promotes changes in adhesion and invasion abilities in addition to the capacity of assemble, disassemble and remodel the actin cytoskeleton). Meanwhile, the AFAP1L1 may prefer cortactin as a binding partner, despite its ability to associate with actin. In order to verify the role of these proteins during the intracellular traffic of Trypanosomatidae and Apicomplexa protozoan, peritoneal macrophages and HeLa cells are being submitted to invasion by *Trypanosoma cruzi*, *Leishmania amazonensis*, *Toxoplasma gondii* and *Neospora caninum* and stained with anti AFAP1 and AFAP1L1 antibodies, alternatively HeLa cells are being transfected with plasmids encoding AFAP1 and AFAP1L1 in fusion with GFP. For the kinetic studies of *L. amazonensis* cell invasion, 2x10^5 peritoneal macrophages were plated, allowed to adhere overnight and incubated with 4x10^6 parasites. After 15 minutes the plates were washed with PBS and kept with DMEM+10%FCS. Invasion times of 15min, 30min, 1h, 3h, 6h and 12h were fixed and kept for posterior analysis in PGN. AFAP1 and AFAP1L1 were marked with their respective antibodies for one hour, followed by a secondary antibody; DAPI and phalloidin. Our preliminary results with *L. amazonensis* showed the recruitment of AFAP1 to the entry site and co-localizing with actin staining during the first time points. *T. cruzi*, *T. gondii* and *N. caninum* kinetic assays are being conducted in non-transfected HeLa cells (for indirect immunofluorescence reaction) or transfected with AFAP1 and AFAP1L1 encoding plasmids. Experiments are being conducted and the implications of AFAP1 and AFAP1L1 on the intracellular traffic are being gradually disclosed and shown for the first time in the scientific literature.
Supported by:CAPES, CNPq e FAPEMIG

BC039 - **GALECTIN-3, A TRYPANOSOMA CRUZI PHAGOLYSOSOME LYSIS MARKER**

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Cell invasion by the intracellular protozoan Trypanosoma cruzi requires interactions with host molecules, and its replication requires escape from the parasitophorous vacuole into the host cell cytosol. Galectin-3, a member of β-galactosidase-binding lectin family, is involved in numerous extracellular and intracellular functions. This study investigated the role of galectin-3 during the invasion and intracellular trafficking of T. cruzi extracellular amastigotes (EA). Exogenous galectin-3 did not significantly interfere with EA entry into host cells, and a lack of galectin-3 expression mainly affected the replication phase of EA and not lysosomal-marker acquisition. Endogenous galectin-3 accumulated around the pathogen in a lectin-dependent manner during EA intracellular trafficking and vacuole membrane disruption. Therefore, galectin-3-containing structures might be used as vacuole lysis markers not only for bacteria but also for other intracellular pathogens, such as T. cruzi.
Supported by:CAPES; CNPq; FAPEMIG; FAPESP

BC040 - **VIABILITY OF GIARDIA CYSTS: ASSESSMENT BY DYE PERMEABILITY ASSAY AND BIOASSAY IN MICE**

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*Giardia* sp occurs frequently in our environment and this protozoan is present in many mammals, including humans. *Giardia* presents two forms: cyst and trophozoite, which colonizes the small intestine, causing giardiasis. Infection can be spread from person to person indirectly by water, by fecal-oral contamination with cysts or occasionally by food. The aim of this study was to evaluate the viability and infectivity of *Giardia* cysts by the inclusion/exclusion of fluorogenic vital dye 4 ',6-diamidino-2-phenylindole (DAPI) and by bioassay in two Swiss mice as a confirmatory test. One animal was infected with 103 cysts obtained from a fecal sample frozen for a year and another animal with a sample recently obtained. The same samples were diluted in distilled water and analyzed by direct immunofluorescence using the commercial kit Merifluor. Simultaneously, a confirmatory test of feasibility with DAPI was done with visualization of morphological characters. The observation was made in an epifluorescent microscope equipped with UV filter excitation of 350 nm and 450 nm emission for DAPI. The cysts were considered viable and DAPI positive (DAPI +) when presented distinct fluoresce blue under UV filter and non-viable and DAPI negative (DAPI -) in the absence of fluorescence. The analysis of Giardia cysts in mice stool was performed on alternate days for 30 days after inoculation using the technique of Faust et al. Animal infected with *Giardia* cysts from frozen sample showed negative results in fecal material. The animal inoculated with recently obtained sample eliminated cysts 7 days after inoculation. Regarding inclusion/exclusion of DAPI, we observed that frozen sample showed cysts without fluorescence (DAPI -) and recent sample showed cysts with strong fluorescence in blue (DAPI +). The observed results lead us to consider that the technique of inclusion/exclusion of DAPI can be used as an indicator of viability of *Giardia* sp cysts.
Supported by:CNPQ/FUNDAÇÃO ARAUCÁRIA/PROAP-CAPES

BC041 - **ANTI-TOXOPLASMA GONDII EFFECTS OF NEW PHENYLSEMICARBAZIDE AND PHENYLTHIOSEMICARBAZIDE IN VITRO**

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Agent of toxoplasmosis, T. gondii currently affects about 20-30% of immunocompetent human population and is fatal in immunodeficient individuals. The discovery of less-toxic and more-efficacious parasite-specific drugs becomes very important. Our research group has synthesized new phenylsemicarbazides (PSC) and phenylthiosemicarbazides (PTSC), since these compounds has great biological activities. Nine new compounds belong to semicarbazides class were synthesized from substituted at arylhydrazone position, containing the polyhidroxilated and polymethoxylated rings, and described by IR, 1H, 13C NMR and HR-MS. Vero cells infected with tachyzoites were incubated with test compounds in concentrations of 0.01, 0.1, 0.5 and 1 mM for 24 h (37˚C). The infected cultures were processed for light microscope. After incubation with the compounds, the infection and number of intracellular tachyzoites decreased. These compounds were more effective than standard drugs (Hydroxyurea and sulfadiazine). At the 0.5 and 1mM, only the compounds DMT-HB-PSC did not showed citotoxicity. Considering all compounds, THB-PSC had the most efficient action on reduced 50% of infection and 43% intracellular parasites at the 0,01mM. These new compounds have a promissory action against intracellular Toxoplasma. The difference in biological action of the compounds is a result of the variation of the type of radical present in each compound.

Supported by:FAPERJ

BC042 - **IN VITRO INFECTIVITY ASSAYS AND IMMUNOCYTOCHEMICAL LOCALIZATION OF NTPDASE 1 IN TRYPANOSOMA CRUZI USING PURIFIED SPECIFIC ANTIBODIES**

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T. cruzi is the etiological agent of Chagas' disease a major public health problem endemic in Central and South American countries. Parasites' NTPDases are enzymes that can hydrolyze ecto-localized tri and diphosphate nucleotides controlling the purinergic signaling in hosts. Classical NTPDase activity was previously demonstrated in T. cruzi surface and a gene coding an NTPDase-1 was isolated, cloned and expressed in bacterial system. This protein has been demonstrated to be a virulence factor in T. cruzi acting as facilitator of infection both in vitro and in vivo. Recombinant NTPDase-1 was used to produce and purify specific anti-NTPDase-1 antibodies. In this work, the main objective was to evaluate the biological role of NTPDase-1 in the early stages of infection in mammalian cells and to localize the protein by immunocytochemistry using the specific antibodies and immunogold electron microscopy. In order to achieve these goals the specific antibodies were used to block epimastigotes NTPDase-1 and the adhesion index were measured in the 30, 60 and 120 min after infections at 37°C. Our results showed a reduction of 700% in the rate of adherence in 30 min, 35% of reduction in 60 min and 11% of reduction in 120 min. The results of electron microscopy confirmed the localization of the protein in the parasite surface and showed for the first time the internal localization. The next steps of this work are the evaluation of trypomastigotes adhesion and infection during the early steps of in vitro infection and to compare the levels of membrane and internal localizations of NTPDase-1 in trypomastigotes and amastigotes.

Supported by:FAPEMIG, FINEP, CNPq, CAPES

BC043 - **ANTILEISHMANIAL ACTIVITY OF SUB-2, A SEMI-SYNTHETIC MOLECULE**

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Leishmaniasis, zoonotic protozoan diseases caused by Leishmania spp., are still considered a major health problem. The number of effective drugs available against the disease is extremely limited. Bioassay-guided approach has been used in our laboratory in the search for bioactive agents of plant origin. We have already tested 346 compounds obtained from different plant families against L. amazonensis promastigotes. Semi-synthetic sub-2, was also assayed against amastigotes forms. In this work we evaluate sub-2 citotoxicity against macrophages J774 culture and the leishmanicidal activity (against promastigote and amastigote forms), using the MTT colorimetric assay. To investigate the sub-2 effects on intracellular L. amazonensis, the MTT colorimetric assay was performed according to the Mosmann (1983). To obtain intracellular amastigotes, J774 macrophages were plated in coverslips in 24 wells and allowed to adhere for 24hs at 37°C in 5% CO2. Adherent macrophages were infected with metacyclic promastigotes forms at a macrophage to parasite ratio of 1:10 at 37°C for 2hs. Then, the parasites which have not penetrated the macrophages were removed and the infected cultures were incubated for 24hs in RPMI medium. Sub-2 was added at different concentrations (3.0 to 0.19µg/mL) after 24hs post infection and each concentration was screened in triplicate. The coverslips were Giemsa stained and microscopically analyzed. Sub-2 showed potent activity against L. amazonensis promastigote forms (IC90 = 1.8 µg/mL) when compared to positive control pentamidine (IC90=6.5 µg/mL). Sub-2 does not show to be cytotoxy to J774 macrophages, presenting selectivity index of 80. Sub-2 activity against intracellular amastigotes showed to be specific against parasites (Phagocyte index = 21.47%). Preliminary in vitro assays suggested that sub-2 is a promising compound against L. amazonensis infection. The molecule sub-2 might be interesting as lead in the search for new antiparasital drugs.

BC044 - **EFFECTS OF N-GLYCOSYLATION INHIBITORS ON CELL PROLIFERATION, ULTRASTRUCTURE AND GLYCOPROTEIN COMPOSITION OF SYMBIONT CONTAINING TRYPANOSOMATIDS**

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Some trypanosomatids establish an obligatory relationship with a symbiotic bacterium. This symbiont maintains a close association with the protozoa endoplasmic reticulum (ER), reinforcing the idea that metabolic changes occurs between these associated partners. Thus, symbiont containing trypanosomatids constitute an excellent model to study the origin of organelles and the eukaryotic cell evolution. Previous studies showed that the symbiont influences the carbohydrate composition and the surface charge of the host trypanosomatid. Oligosaccharides are molecules able to mediate specific processes, as cell adhesion and recognition. During the N-glycosylation, oligosaccharides are transferred to the side-chain $NH_2$ group of an asparagine residue producing glycoproteins in the ER. This mechanism is directly related to the correct folding of newly synthesized proteins. The tunicamycin (TM) and the 1-deoxynojirimycin (DNJ) are N-acetylglucosamine transferase and glycosydase II inhibitors, respectively, which affect different stages of type N glycosylation, blocking the protein exit from the ER. In this work, we investigated how glycosylation inhibitors disturb the symbiotic relationship in *Crithidia deanei* and *Blastocrithidia culicis*. For this purpose, we performed assays in order to check the effects of TM and DNJ on protozoa proliferation, ultrastructure and glycoprotein composition. Our results showed that both inhibitors affect cell proliferation in a dose dependent manner. Furthermore, analysis by transmission electron microscopy showed an enlargement of the ER, as well as, the disorganization of the Golgi cisternae. Biochemical analysis revealed that the host cell glycoprotein composition was modified after treatment with both inhibitors. Taken together, these results will contribute to the characterization of the glycosylation process in trypanosomatids and offer new clues to the better understanting of symbiosis in such protozoa.
Supported by:CNPq e FAPERJ

BC045 - **DNA REPLICATION CONTROL ON TRYPANOSOMES**

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During the S phase of the cell cycle, DNA must be replicated precisely once. The start points of DNA replication (called origins of replication) are established by the formation of the Pre-Replication Complex (PRC). The PRC is composed in metazoan by ORC1-6, Cdc6, Cdt1 and Mcm2-7, and is assembled at origins of replication at G1 phase, being activated only at the S phase allowing DNA replication. After DNA replication initiation the PRC needs to be disassembled preventing re-firing of the same origin of replication. In trypanosomes Orc1/Cdc6 and Mcm2-7 form the PRC. Our lab have already showed that Orc1/Cdc6 does not seem to be involved in DNA replication control once it is in the nucleus, bound to the DNA, during the entire cell cycle. Then we are investigating the possible role of the Mcm2-7 complex in DNA replication control in trypanosomes. For that purpose, antibodies against *Trypanosoma cruzi* Mcm 3/4 and 7 subunits are being raised. We performed immunofluorescence assay using antibody against Plasmodium falciparum Mcm4 that is able to recognize Trypanosome brucei Mcm4. The TbMcm4 is located at the nucleus of some cells with one nucleus, one kinetoplast, and one flagellum (1N1K1F - G1 phase); and one nucleus, two kinetoplast and two flagella (1N2K2F - S and G2 phase). But some of these cells did not show TbMcm4 at the nucleus. From this data is possible to suppose that cells in beginning of G1 phase do not have Mcm4 at the nucleus. As cells progress to the end of G1 phase, TbMcm4 is recruited to the PRC and can be found at the nucleus until the beginning of S phase. During S phase, TbMcm4 is displaced from the nucleus, and therefore cells in G2 phase do not show Mcm4 at the nucleus. We are now labeling early/late S cells by Edu incorporation in order to demonstrate this hypothesis. These recent data from our lab strong evidence that DNA replication control in trypanosomes may rely on the Mcm2-7 complex.

Supported by:FAPESP

BC046 - **CHARACTERIZATION OF SEQUENTIAL SURVIVAL RESPONSES CAUSED BY SINGLE AMINO ACID DEPRIVATION IN TRYPANOSOMA BRUCEI**

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Developmental events in the life cycle of T. brucei, the sleeping sickness parasite, comprise integrated changes in cell morphology, metabolism and gene expression. The signaling mechanisms that control these changes are however, poorly understood. It has been shown that differentiation occurs upon environmental changes that involve alterations in amino acids. To further understand these signaling mechanisms, here we studied the effect of single amino acid deprivation on procyclic forms of T. brucei. Cells were incubated in media lacking proline, leucine or glutamic acid. After 30 min of amino acid deprivation, the parasites showed a rapid increase in the phosphorylation of the alpha subunit of the eukaryotic initiation factor 2 (eIF2alpha). This factor is phosphorylated by specific protein kinases that are activated in stress conditions, resulting in translation inhibition. After 24 hours, the level of eIF2alpha phosphorylation returns to normal, but the level of eIF5A, a putative translation elongation factor, required for translation under stress conditions, is increased. Cells maintained in the absence of leucine and glutamic acid, but not of proline, for 24 hours showed increased levels of short chain polyphosphate levels. In all cases, cellular division is arrested after 48 hours of the amino acid deprivation, although the procyclic cells remained fully motile and viable for several days. The level of eIF5A then decreases and cells become thinner and elongated, as stationary cells. Cell cycle analysis revealed that growth arrested cells contained cells in both G1 and G2 phase of cell cycle, but not cells in S phase. These results indicated that multiple events take place under nutritional stress, causing translation and cell cycle arrest, which result in morphological modifications of the parasite. Apparently, the parasite sequentially utilizes specific signals to compensate the lack of a single amino acid in order to survive.

Supported by:Fapesp/Cnpq

## BC047 - **UPTAKE OF AROMATIC AMIDINES BY DIFFERENT EVOLUTIVE FORMS OF T. CRUZI**

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Trypanosoma cruzi is the etiological agent of Chagas disease, an important neglected illness affecting about 12–14 million people in endemic areas of Latin America. The chemotherapy of Chagas disease is quite unsatisfactory mainly due to its poor efficacy especially during the later chronic phase and the considerable well-known side effects. Several aromatic amidines like pentamidine (PT) and furamidine and some of their related compounds are minor groove binders that target AT-rich sequences presenting excellent anti-trypanosomal activity. However, the mechanism of action of these aromatic compounds has not been fully elucidated. While transporters, such as P1, P2, HAPT1 and LAPT1, have been identified as the routes of entry for amidines, including PT and berenil, into African trypanosomes, Leishmania spp. and Plasmodium, no studies have been conducted in T. cruzi. As the transport of amidines is a fundamental step of their drug action and is likely to contribute to their selectivity, the knowledge of their mechanisms of entrance in T. cruzi is largely desirable. Presently, epimastigotes and trypomastigotes forms of T. cruzi were treated with five structurally related amidines (DB709, DB75, DB766, DB1831 and DB613A), in the presence or not of 10 mM of adenosine and inosine, two well-known P1 and P2 transport inhibitors. After 15min, 1h, 4h and 24 h the protection rate (PR) was determined based in the percentage of decrease in compound efficacy in the presence of each inhibitor. The preliminary data showed that for epimastigotes, adenosine protected about 50% the rates of parasite death after 4 h of DB766 and DB613A treatment, suggesting that those compounds are transported by a P2 like transporter. As no effect was observed in the presence of adenosine or inosine, DB709, DB75, and DB1831 may be uptake by other yet unknown cellular mechanisms. For trypomastigotes it is likely that internalization of DB709 and DB613A occurs mainly by a P1 transport, while DB75 is transported via P2. On the other side DB1831 was affected by both inhibitors, indicating that the transport could be mediated by P1 and P2 transporters. Further studies are been undertaken to confirm these data and further evaluate the internalization kinetics of each compound by T. cruzi parasites.
Supported by:FAPERJ

## BC048 - **PATHOLOGICAL CHANGES BETWEEN AMINOGUANIDIN TREATED AND UNTREATED CHICKENS INFECTED WITH PLASMODIUM GALLINACEUM**

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Malaria is a disease that infects a large number of people in the world and can lead to death. Its pathology is associated with several factors, among them different species of parasite and different forms during the evolutive cycle. Plasmodium falciparum specie causes cerebral malaria being this phenomenon associated with erythrocytes adhesion in cerebral capillaries and production of inflammatory mediators, such as nitric oxide (NO). In this context, the avian model with P. gallinaceum, represent a powerful strategy to characterize mechanisms related to cerebral malaria. The aim of the present study was to determine alterations in the histopathology of infected birds treated or not with aminoguanidin (NO synthase inhibitor). Chickens were infected with P. gallinaceum, and parasitemia and clinical manifestations determined each other day. Organs were collected from chickens with different parasitemias and histology performed after hematoxylin-eosin staining. Aminoguanidin treated chickens had lower mortality and presented higher parasitemia peak in comparison to untreated birds. Spleen of chickens with higher parasitemia presented lymphocyte depression that increased in aminoguanidin treated birds and was moderate in untreated ones. Liver of untreated chickens presented a riche inflammatory infiltrate, composed of mononuclear cells and rare heterophils, with perivascular location and necrosis due to coagulation. Both organs showed inflammatory infiltrate with less malaria pigment in chickens treated with aminoguanidin, but in chickens that chronified no clear differences were found. Brains had vascular estase with a concentration of leukocytes in treated and untreated chickens. Cells in the vasculature presented malaric pigment. Treatment with aminoguanidin demonstrated involvement of the nitrergic system in the pathogenesis of avian malaria, but the role of NO is not clear yet.
Supported by:CAPES, CNPq, FAPERJ, FAPESPA, UENF, UFPA

### BC049 - **HEMATOLOGICAL AND SERUM BIOCHEMISTRY CHARACTERIZATION OF CHICKENS INFECTED WITH PLASMODIUM GALLINACEUM TREATED WITH AMINOGUANIDIN**

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Nitric oxide (NO) is involved in malaria infection. To better understand the role of NO in chickens infected with Plasmodium gallinaceum, hematological and serum biochemistry changes were characterized after treatment with aminoguanidin, a drug that decreases NO production. Blood analyses were carried out on alternative days. Treated and nontreated chickens presented similar hemolytic anemia that was not different between both groups. Treated chickens had statistically lower mortality; chickens from both groups became chronic with low parasitemia and no clinical signs or anemia. Young erythrocytes were observed in chickens with high parasitemia. P. gallinaceum infected young and mature erythrocytes. Leucopenia was observed in both groups and chronified chickens recovered the normal count. Monocytes presented more vacuoles and increased area of cytoplasm with the infection. During the infection, lymphocytes became larger presenting irregular stained nuclei, plasma cells and toxic heterophils were more frequently observed and eosinophils disappeared. During high parasitemia monocyte number increased in the untreated group, probably because of higher inflammation due to tissue deposit of hemozoin. Lymphocytes and heterophils counts were not different between both groups during the infection. Serum biochemistry showed that Aspartate aminotransferase and alanine aminotransferase increased in both groups confirming hepatic damage. Acid fosfatase increased with infection correlating with cholestasis due to hepatomegalia observed in high parasitemia chickens. Urea and uric acid were higher in untreated chickens, indicating kidney failure. P. gallinaceum infection caused drastic alteration of chicken hematology and serum biochemistry, but with less mortality in chickens treated with aminoguanidin possibly by less inflammation and renal damage.
Supported by:CAPES, CNPq, FAPERJ, FAPESPA, UENF, UFPA

### BC050 - **VARIATION IN THE EXPOSURE OF PHOSPHATIDYLSERINE BY TOXOPLASMA GONDII STRAINS OF DIFFERENT VIRULENCE.**

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*Toxoplasma gondii* is considered an extreme example of a generalist parasite because it infects virtually all mammal and bird species. Furthermore, this parasite escapes the immune system by using different strategies. One of the evasion mechanism of *T. gondii* against activated macrophages is based on the exposure of phosphatidylserine (PS) that induces secretion of TGF-b1, resulting in a decreased of nitric oxide (NO) production. Aiming to correlate the virulence of different *T. gondii* strains with the exposure of PS, this phospholipid was assayed by flow cytometry on tachyzoites of the RH (virulent), VEG and ME-49 (both less virulent) strains. RH was obtained from mice peritoneal lavage, VEG and ME-49 from cell culture. Mouse peritoneal macrophages were activated with interferon-gamma and lipopolysaccharide and infected with these tachyzoites for 2h. NO production was determined after 24 and 48h and was effectively inhibited by RH that persisted in iNOS negative macrophages. ME-49 strain infected macrophages produced intermediate levels of NO after 24h and showed similar production to non-infected macrophages after 48h, no parasites were detected inside macrophages at this period. VEG tachyzoites exposed PS in a higher proportion in relation to the ME-49 strain, however, in a lower proportion in relation to the RH strain. These results suggest that the virulence of *T. gondii* strains may be correlated to PS exposure.
Supported by:CAPES, CNPq, FAPERJ e UENF

BC051 - **ENTAMOEBA HISTOLYTICA-TRYPANOSOMA CRUZI INTERACTION: IDENTIFICATION OF POTENTIAL ANTI-*T.CRUZI*. COMPONENTS.**

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Amebiasis is caused by the etiologic agent *Entamoeba histolytica*. Chagas disease is caused by the flagellate protozoan *Trypanosoma cruzi*. To analyze the possible interactions between *T. cruzi* and *E. histolytica* that could occur in the gastrointestinal tract of the host, considering the cases of co-infection, we performed *in vitro* invasion assays of trypomastigotes from *T. cruzi* (G strain) into trophozoites of *E. histolytica* in a kinetics of infection, followed by immunofluorescence and analysis on the confocal microscope. Preliminary results showed that *T. cruzi* is internalized by *E. histolytica*. However, we founded that *T. cruzi* is degraded in the vacuole formed after its invasion. A very relevant characteristic about the strain of *E. histolytica* studied (HM1) is the high amount of enzymes, vacuoles and vesicles, which is closely related to its pathogenicity. Also, we verified the activity of crude extract of *E. histolytica* on trypomastigotes of *T. cruzi* from G and CL strains. For this, we used MTT assay , where *T. cruzi* trypomastigotes from G and CL strains were incubated with different concentrations of the crude extract . The MTT assay and light microcopy counting of parasites, showed high activity of crude extracts of *E. histolytica* on *T. cruzi* from both strains. Thus, it showed to be a very encouraging result, after all, we observed by the same tests that the toxicity to mammalian cells is much lower. In this context,on further studies we propose to identify the active components present in the crude extract. On this sense, we intend, after isolating the active components, to produce the recombinant forms and to apply them in order to treat experimental *T. cruzi* infection.
Supported by:fapemig

BC052 - **THROMBOCYTE ALTERATION IN CHICKENS INFECTED WITH PLASMODIUM GALINACEUM**

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Avian malaria was the first model used to study this disease. Thrombocyte, functional homolog to mammalian platelets, lowers during chicken infection with P. juxtanucleare, however, nothing is known about this cell in P. gallinaceum infection. This study characterized changes in morphology and number of thrombocytes from infected chickens treated with aminoguanidin, which inhibits nitric oxide production, a known mediator involved in malaria. Blood was collected from chickens treated or not with aminoguanidin. Thrombocyte were counted, analyzed in Giemsa stained smears, and cultured for 1 and 24h after cell separation over a Percoll cushion. Aminoguanidin treatment increased by two fold the survival of infected chickens. Thrombocytes counts lower with infection and treated chickens had higher values than untreated, showing that aminoguanidin may prevent coagulation damages in infected chickens. Thrombocytes were found infected and also became larger in chickens with higher parasitemia. After culture, atypical thrombocytes (long or two nuclei) became evident in infected chickens. These cells were confirmed as thrombocytes after immunofluorescence with anti-chicken thrombocyte (mAb 11C3). Normal, pyknotic, long or two nucleus thrombocytes were scored in uninfected or infected and untreated or treated chickens with different parasitemias ranges. After 1h of culture, thrombocytes from uninfected and treated chickens were less pyknotic, a two fold increase in atypical thrombocytes were observed in chickens with high parasitemia that were treated. After 24h of culture, the number of pyknotic thrombocytes from infected chickens decreased and a significant increase in two nucleus thrombocytes was detected independently of the treatment. Taken together, infection promotes the emergence of atypical thrombocytes in treated or untreated chickens. Aminoguanidin decreased the number of pyknotic thrombocytes, which may be related to increased survival of treated infected chickens.
Supported by:CAPES, CNPq, FAPERJ, FAPESPA, UENF, UFPA

### BC053 - **CHARACTERIZATION OF THE SERINE PEPTIDASE INHIBITOR (ISP2) OF *TRYPANOSOMA CRUZI***

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The fine control of peptidase activity is achieved in different ways. Protein peptidase inhibitors are central players. Ecotin is a macromolecular inhibitor of clan PA, S1A family of serine peptidases that is found in the periplasm of several bacteria species. They strongly inactivate trypsin, chymotrypsin, cathepsin G, neutrophil elastase (NE), and also inhibit members of the coagulation cascade. Three genes presenting similarity to ecotins were identified in the genome of *Leishmania*, and have been designated Inhibitors of Serine Peptidases (ISPs). *L. major* ISP2 has been implicated in the modulation of parasite phagocytosis by murine macrophages and as an important factor for parasite survival. In *T. cruzi*, a single copy ecotin-like gene similar to *L. major* ISP2 was found. The genome of *T. cruzi* apparently lacks genes encoding clan PA serine peptidases, raising the possibility that ISP2 could function to modulate the activity of host enzymes. The ISP2 gene of *T. cruzi* Dm28c was cloned into pQE-30 and expressed as a fusion protein. Soluble recombinant ISP2 was functional, as assessed by the inhibition of trypsin, chymotrypsin and neutrophil elastase and cathepsin G activity. Recombinant ISP2 significantly reduced host cell invasion by *T. cruzi*, suggesting that host serine peptidases might play a role in parasite invasion. To further investigate a possible role of *T. cruzi* ISP2 in the host-parasite interaction, we have generated parasites overexpressing ISP2. Epimastigotes ISP2-over expressors contain higher levels of ISP2 when compared to WT parasites or parasites transfected with the vector alone. ISP2-over expressors were submitted to metacyclogenesis and trypomastigotes were used to infect LLC-MK2 cells to generate tissue culture trypomastigotes. Tryposmastigotes overexpressing ISP2 were less infective to smooth muscle and endothelial cell lines, indicating that ISP2 modulates parasite infectivity. The generation of ISP2-deficient mutant cell lines is ongoing.
Supported by:Faperj

### BC054 - **ANALYSIS OF THE ACTIVITY AND IMMUNOLOCALIZATION OF THE ENZYME CONSTITUTIVE NITRIC OXIDE SYNTHESIS (CNOS) IN PROMASTIGOTES FORMS OF THE LEISHMANIA (VIANNIA) BRAZILIENSIS AT DIFFERENT GROWTH PHASES IN VITRO**

FURTADO, R.R.*; RODRIGUES, A.P.D.; DE FARIAS, L.H.S.; HAGE, A.A.P.; DA SILVA, R.R.P.; DA COSTA, J.P.; SILVA, E.O.
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American Tegumentary Leishmaniasis (ATL) is a parasitic disease, widely spread in most countries of Latin America and caused by different species of the genus *Leishmania*. This protozoan is an obligate intracellular parasite that developed mechanisms to subvert the microbicidal activity of macrophages, such as regulation of superoxide and nitric oxide (NO) production. During *Leishmania* infection, the nitric oxide plays a crucial role in the killing of parasites in vitro and in vivo. In this work, we analyzed the constitutive Oxido Nitric Synthase (cNOS) expression and NO production by *Leishmania (Viannia) braziliensis*, during logarithmic (LOG) and stationary phase (STAT). *normal">Leishmania* cNOS was identified in promastigotes using indirect immunofluorescence assay by confocal microscopy and immunolocalization by Transmission Electron Microscopy (TEM). These results showed that promastigotes of *L. braziliensis* are able to express cNOS in both growth phases. Immunolocalization by TEM allowed observing localization of cNOS in the cytoplasm. For detection of NADPH-diaphorasic activity, promastigotes were incubated with NADPH and demonstrated that promastigotes in log phase showed a more intense reaction when compared to STAT growth phase. The production of NO was measured in the supernatants of promastigotes cultures as nitrite form by adding Griess reagent. To confirm the enzyme activity, nitrite measure showed that this parasite is able to produce NO and that *Leishmania* promastigotes in LOG phase has a higher production of NO when compared to parasites in STAT phase. In conclusion, a correlation between the expression of cNOS and NO production by *L. braziliensis* suggest a possible virulence factor, which is able to regulate the mechanism of the NO production by the host cell, conferring parasites resistance to NO damages.
Supported by:CAPES, CNPq, UFPa

BC055 - **PHOSPHATIDYLSERINE EXPOSURE BY TOXOPLASMA GONDII IS FUNDAMENTAL TO BALANCE THE HOST'S IMMUNE RESPONSE**

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Phosphatidylserine (PS) exposure by cells indicates apoptosis, but has also been related to evasion mechanisms of parasites, a concept known as apoptotic mimicry. Toxoplasma gondii mimics apoptotic cells by exposuring PS, inducing secretion of TGF-beta1 by infected activated macrophages leading to degradation of inducible nitric oxide (NO) synthase, NO production inhibition and consequently persistence in these cells. Here PS+ and PS- subpopulation of tachyzoites were separated and the entrance mechanism, growth and NO inhibition in murine macrophages, mice survival and pathology were analyzed. Infection index in macrophages was similar for both PS subpopulations but lower when compared to the total T. gondii population. Growth in macrophages was higher for the total T. gondii population, intermediate for the PS+ and lower for the PS- subpopulation. Production of NO by macrophages was inhibited after infection with the PS+ subpopulation and the total populations of tachyzoites. However, the PS- subpopulation was not able to inhibit NO production. PS+ subpopulation invaded macrophages by active penetration as demonstrated by tight-fitting vacuoles, but the PS- subpopulation entered macrophages by phagocytosis as seen by loose-fitting vacuoles containing these tachyzoites. The entrance mechanism of both subpopulations were confirmed in a non-professional cell line where only the PS+ tachyzoites were found inside these cells in tight-fitting vacuoles. Both subpopulations of T. gondii killed mice faster than the total population. Clear signs of inflammation and no tachyzoites were seen in the peritoneal cavity of mice infected with the PS- subpopulation. Moreover, mice infected with the PS+ subpopulation had no sign of inflammation and the parasite burden was intense. These results show that PS+ and PS- subpopulations of T. gondii are necessary for a successful toxoplasma infection.
Supported by:CNPQ, CAPES and FAPERJ

BC056 - **LIPIDS DROPLETS IDENTIFICATION IN LEISHMANIA (VIANNIA) BRAZILIENSIS PROMASTIGOTES CAUSED OF MUCOSAL LEISHMANIASIS**

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The American Tegumentary Leishmaniasis (ATL) is an infectious disease world wide spread, caused by a protozoan of genus Leishmania. Leishmania (Viannia) braziliensis is the main etiologic agent of ATL. The strain and cellular immune response of the infected host can develop the cutaneous or mucocutaneous forms of the diseases. The latter is the most severe type of clinical manifestations. Lipid droplets are emerging as highly dynamic organelles that play crucial roles in mammalian cellular energy homeostasis and lipid metabolism. Little is known about the relation between this specie of Leishmania and the lipid droplets and it is important to a better understanding of a possible virulence factor that could be a therapeutic target. The present work analyzed the presence of lipid droplets in L. (V.) braziliensis promastigotes in different days of the grown, early (7 days) and later (10 days) stationary phase. In the present study we could observe using sudan black B dye and light microscopy that promastigote forms cultivated until the later stationary phase showed more lipid droplets distributed in the cytoplasm than cells cultivated in early stationary phase. The same result was observed by routine electron microscope and confirmed with osmium-imidazole cytochemistry analysis. The largest distribution of lipid droplets in late stationary phase promastigotes was also observed using the fluorescent BODIPY 493/503 labeling. The presence of lipids reserves can be related with parasite energetic necessities during the stationary growth period when the parasite presents the higher capacity of infection. The identification of this structure is important to understand the role of lipid reserves in Leishmania parasites. Moreover, those data are essential for future identification of possible targets for action of therapeutic drugs.
Supported by:PROCAD, CAPES, UFPA

## BC057 - **IDENTIFICATION OF GLYCERALDEHYDE 3-PHOSPHATE DEHYDROGENASE AS A DNA BINDING PROTEIN IN TRYPANOSOMA**

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Recent studies have established that the glycolytic protein, glyceraldehyde 3-phosphate dehydrogenase (GAPDH), is a multifunctional protein with defined functions in numerous subcellular processes. New roles for GAPDH include transcriptional control, nuclear membrane fusion, recognition of fraudulently incorporated nucleotides in DNA, and its mandatory participation in the maintenance of telomere structure. Using fractionated extract in order to separate soluble proteins from DNA binding proteins we demonstrated that GAPDH is found at DNA bound fraction from Trypanosoma brucei. In *Trypanosoma cruzi*, GAPDH is only found in DNA bound fraction when cells are treated with leptomycin B, which blocks nucleus – cytoplasm transport. Preliminary data using T. cruzi recombinant GAPDH protein in electrophoretic mobility shift assay showed that it binds double strand telomeric DNA, but not single strand telomeric DNA. These data suggest that the role of GAPDH is more than energy production even in T. cruzi and T. brucei, which are early divergent eukaryotes.
Supported by:Fapesp

## BC058 - **LEISHMANIA (VIANNIA) BRAZILIENSIS: STUDY OF VIRULENCE FACTORS AND THE INTERACTIONS WITH HOST CELLS IN CUTANEOUS AND MUCOCUTANEOUS LEISHMANIASIS STRAINS IN AMAZON REGION.**

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Parasite of genus Leishmania is an obligate intracellular organism that multiplies, mainly, inside mammals host macrophages. This parasite is able to cause different clinical manifestations among them localized cutaneous leishmaniasis (LCL) and mucocutaneous leishmaniasis (MCL). The objective of this work is to compare virulence factors between strains of Leishmania (Viannia) braziliensis obtained from patients with different clinical manifestations. Among these virulent factors we can highlight the phospholipid phosphatidylserine (PS) present on cells plasmatic membrane composition, as well as on parasites, and the surface sugars that are the main component of lipophosphoglycan (LPG), which have a key role in the attachment of parasite to the host cell. Analyses performed by flow cytometry demonstrated a significant difference on exposure of PS between strains. According to analysis conducted, the LCL strain exposed a greater quantity of PS when compared to MCL strain. The importance of PS during Leishmania-host cell interaction was evaluated calculating the endocytic index (EI). After 1h of Leishmania-host cell interaction, we observed that annexin V-bound LCL promastigotes with 7 days of culture presented a no significant decrease of EI when compared to promastigotes without annexin V treatment. Meanwhile, the LCL strain with 10 days of culture showed a significant decrease of EI, when promastigotes were treated with annexin V. However, after 24h of interaction with LCL strain, it was observed a significant decrease of EI, during 7 and 10 days of growth. During interaction of MCL strain and host cells (1h of interaction), there were no significant reduction of EI both in 7 days and 10 days of culture. In other hand, it was observed in MCL a significant decrease of EI only with promastigotes with 10 days of growth. For analysis of surface sugars, agglutination tests were performed using specific lectins. Differences were detected in agglutination pattern related to sugar concentration when compared on different days of culture, which was not detected in the strain of MCL. However, the latter presented the sugar acetyl-galactosamine, which was not detected in the LCL strain. Thus, this study demonstrated the importance of identifying virulence factors present on the surface of the parasites once that the identification of these factors provides a better understanding of the mechanism of interaction of Leishmania with the host cell.
Supported by:FAPESPA, CNPq

BC059 - **POSSIBLE INVOLVEMENT OF AUTOPHAGY IN THE INHIBITION OF TOXOPLASMA GONDII PROLIFERATION IN INTESTINAL EPITHELIAL CELLS IEC-6**

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Autophagy is a homeostatic process whereby cytosol or intracellular organelles are sequestered by a double membrane structure termed autophagosome for subsequent delivery to lysosome and degradation. Autophagy has been implicated in the intracellular destruction of Toxoplasma gondii by primed macrophages (MØ) following gamma interferon (IFN-γ) activation. In IFN-γ-activated MØ, the p47 GTPases traffic to the T. gondii parasitophorous vacuole, followed by vacuolar disruption, parasite killing and clearance of the dead parasites. Thus, in macrophages it is relatively well established that autophagy is involved in parasite elimination and killing. It has been shown that intestinal ephitelial cells (IEC-6) activated with IFN-γ inhibits T. gondii replications by a mechanism that involves the low availability of iron. We analyzed the possible involvement of autophagy in the inhibition of T. gondii replication in IEC-6 activated with IFN-γ. IEC-6 were activated with IFN-γ for 2, 24 and 48h and infected with T. gondii. Autophagic vesicles, cell nucleus and T. gondii were fluorescently localized in activated IEC-6 that was infected for 3, 6, 12 and 24h. Activated IEC-6 infected for 2, 24 and 48h inhibited the proliferation of T. gondii. Activated IEC-6 infected for 3, 6, 12 and 24h showed more autophagic vesicles than infected cells that were not activated. Furthermore, activated IEC-6 presented an increased in the number of parasites in autophagic vesicles. The increase of autophagic vesicles in activated cells indicated that autophagy may be involved in inhibiting the replication of T. gondii in IEC-6.
Supported by:CAPES, FAPERJ and CNPq.

BC060 - **PROSTAGLANDIN F2-ALPHA SYNTHASE IN LEISHMANIA V. BRAZILIENSIS**

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It has been recently described that Prostaglandin (PG) production is not restricted to mammals. Trypanosomatids also synthesize several metabolites of arachidonic acid, but the biological role of PGs in these protozoan parasites and their influence in the infection are not elucidated. Proteome comparative analysis between isolates of Leishmania V. braziliensis from mucosal and cutaneous sites of patients with concomitant mucosal (LbrM) and cutaneous lesions (LbrC) revealed a differential pattern of expression of Prostaglandin f2-alpha synthase (PGF2S). In addition, histological analysis from cutaneous tissue suggests that the LbrC isolates induce a stronger inflammatory response than LbrM isolates in BALB/c mice. In silico analysis using the TritrypDB and TDR Target Database showed that PGF2S has been identified in the secretome of L. V. braziliensis, in the exosome of L. major and that it has a high potential as a drug target. We have quantified the PGE2 synthesized in L. braziliensis promastigotes using enzymatic immunoassay, and observed no significant differences of PGE2 levels between clinical isolates, but a significant difference between procyclic and metacyclic promastigotes. Next, we will evaluate PGF2-alpha levels. Based on recently published results suggesting a positive correlation between the presence of a single stranded RNA virus (LRV) in Leishmania V. guyanensis and the metastatic behaviour of the strain, we searched for the virus in LbrM and LbrC isolates. We were unable to detect the virus in the isolates by RTPCR in the cutaneous or mucosal L.V. braziliensis strains tested. Primers and experimental conditions used allowed detection of the virus in L.V. guyanensis. We are currently analyzing the in vitro infection profile of the isolates, producing LbrPGF2S polyclonal antibodies and quantifying the levels of cytokines released (IL-4, IL-10, IL-2, IFN-λ end IL-12) after infection in vivo. The possible correlation between PGF2S levels of expression and the profile of parasite infection and inflammatory response will be discussed.
Supported by:FAPESP, CNPq

## BC061 - *TRYPANOSOMA CRUZI* RESPONSE TO STEROL BIOSYNTHESIS INHIBITORS: MORPHOPHYSIOLOGICAL ALTERATIONS AND CELL DEATH

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*Trypanosoma cruzi* is the protozoan that causes Chagas Disease, illness that affects about 10 million people in the American continent. Unlike mammals that synthesize cholesterol, T. cruzi produces mainly ergosterol, which is characteristic of fungi. Due to this, the route of synthesis of this lipid represents a potential chemotherapy target of Chagas disease. In the present work we present a detailed comparative study of growth inhibition, ultrastructural analysis and physiological changes that lead to cell death of T. cruzi Dm28c epimastigotes in response to the sterol biosynthesis inhibitors (SBIs) ketoconazole and lovastatin in function of exposure time and concentration of drugs. First we calculated the doses of drugs capable of inhibiting the growth of epimastigote cultures by 50% after 3 days of exposure (IC50: 32μM for Ketoconazole and 50μM for Lovastatin), or kill all cells in 24 hours (LD100: 120μM for ketoconazole and 100μM for Lovastatin). At the IC50 dose, we observed alterations in mitochondria not yet demonstrated in response to SBIs, exemplified by intense proliferation of the inner mitochondrial membrane. In addition, the electron microscopy experiments show an significant increase of reservosomes, supported by acridine orange staining. These results point to the importance of mitochondria and reservosomes in the sterols biosynthesis of T. cruzi. The exposure of T. cruzi epimastigotes to the IC50 of both drugs culminates in cell lysis after 5 to 6 days of treatment with the presence of autophagic vacuoles. In contrast, treatment with high doses of SBIs (DL100) results in a fast cell death through an unknown mechanism independent of endogenous sterols levels. The cells die with classical necrotic phenotype and flow cytometry experiments shows a time dependent citosolic calcium overload, mitochondrial depolarization, permeabilization of reservosomes (acridine orange analysis) and cell lysis, with absence of classical apoptotic markers (phosphatidylserine exposure and internucleosomal DNA degradation). As far as we know, this is the first demonstration of reservosome lysis during T. cruzi cell death, reinforcing that the response to LD100 doses of SBIs represent an interesting model for analysis of programmed cell death in this parasite.
Supported by:CNPq, CAPES, Fundação Araucária and FIOCRUZ

## BC062 - HISTOPATHOLOGICAL AND IMMUNOLOGICAL ASPECTS OF IRON INCREASE IN CANINE VISCERAL LEISHMANIASIS

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Canine Visceral Leishmaniosis (CVL) is a zoonotic infection that leads to chronic and systemic disease and affects many organs and tissue. Infections and immune system can be influenced by the trace elements as iron (Fe). This are associated to abnormal collagen synthesis, parasite-host interactions and Nitric oxide (NO) synthesis Some works have been demonstrated correlations between iron increase (Fe) and NO production and collagen deposition. Based on this fact, our aim was to investigate Fe levels and its correlation with NO; collagen deposition, parasitological and clinical aspects in dogs with VL. We used thirty-seven mongrel dogs naturally infected with L. infantum. These were divided into tree groups: Group 1 - nine asymptomatic (ASD), group 2 - 19 symptomatic (SD) and group 3 - nine controls (C) dogs. Sera of all and samples from livers and spleens for histological studies were collected. Graphite Furnace Atomic Absorption Spectrometricy was used for Fe serical detection.. Histological studies were done in tissue using Prussia Blue for Fe and Gomori ammoniacal silver-staining for collagen deposition. NO detection was done by Griess technique. We found a higher deposition of Fe in livers and spleens tissues of SD than ASD and C group. However, a lower detection of Fe serum levels was observed in SD group than the other groups. Moreover, besides NO levels were higher in infected dogs than C, NO levels were higher in ASD than in SD. In addition, higher collagen deposition and parasite load was observed in SD than ASD and C. Thus, we have seen an inverse correlation between Fe and NO levels and direct correlation between Fe and collagen deposition in CVL. In the literature, higher intracellular Fe levels downregulate NO production and upregulate the collagen deposition. In according to this, we could consider that there is a correlation among Fe levels, NO production, pathology and clinical disease progression in CVL.
Supported by:FAPEMIG APQ-00068-08, UFMG

## BC063 - **HISTOLOGYCAL, PARASITOLOGYCAL AND IMMUNOLOGYCAL PROFILE INDUCED BY LEISHMANIA MAJOR INFECTION IN A MODEL OF SPONGE IMPLANTATION**

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A model of monocytic inflammation using a subcutaneous implantation of sponge is able to produce a chronic inflammatory reaction. Our aim was investigate inflammatory response, parasite load and tissue repair in a sponge implantation model with Leishmania major infection in resistant C57BL/6 and susceptible BALB/c mice. This model was carried out by implantation of sponge under the dorsal skin of Balb/c or C57BL/6 mice. Mice were then infected with L. major and sacrificed 21 and 30 days after infection. Sponge was collected for histopathological, parasitological and immunological analysis. After 21 and 30 days, both strains of mice demonstrated an intense chronic inflammatory reaction with a diffuse cellular exudate containing macrophages, lymphocytes, and some neutrophils and eosinophils. Macrophages loaded with amastigotes of Leishmania could be observed in all animals. New blood vessel formation, edema and loose collagen tissue with discrete fibroblast proliferation were formed as granulation tissue. C57BL/6 mice presented with a resistant immunological phenotype characterized by higher expression levels of IFN-gamma compared with Balb/c. It was confirmed by histological results demonstrating the presence of granuloma formation in these animals. C57BL/6 mice presented large numbers of parasite associated with inflammatory macrophages. We have found that IL-4 expression was higher in Balb/c infected mice than C57BL/6. Thus, we are investigating a possible correlation between the collagen deposition and endothelial cell proliferation, which is essential to the angiogenesis process. As a partial conclusion we have that in spite of C57BL/6 had high expression of IFN-gamma, it does not resolve the infection during the periods analyzed.
Supported by:Capes, CNPq

## BC064 - **EPIMASTIGOTES OF *TRYPANOSOMA CRUZI* DOES NOT REQUIRE RESERVOSOMES TO GROW.**

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mso-ansi-language:EN-US">Reservosomes are electrondense organelles with lipids inclusions present at the posterior region of epimastigote forms in *Trypanosoma cruzi*. These organelles are storage vesicles containing proteins and hydrolytic enzymes such as cysteine proteases. Reservosomes are formed by the continuous fluid phase endocytosis. Upon cell starvation, reservosomes are consumed, indicating that they are a source of nutrients for survival under stress conditions. Here we show that parasites can grow normally in synthetic medium such as SDM-79 containing putrescine and that these parasites do not accumulate reservosomes. The cells are not labeled by immunofluorescence using a monoclonal antibody that recognizes the reservosomes. Cells present reduced levels of cysteine protease activity and antibodies to cruzipain, a major *T. cruzi* cysteine protease, labeled small vesicles scattered in the cytosol. These results indicate that epimastigote proliferation is not dependent on the presence of reservosomes and nutrient accumulation.
Supported by:Fapesp

BC065 - **IDENTIFICATION OF X-PROLYL DIPEPTIDYL AMINOPEPTIDASE: A PUTATIVE SERINE PROTEASE FROM *TRYPANOSOMA CRUZI***

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The X-prolyl dipeptidyl aminopeptidases (X-PDAP, S15 family) belong to serine proteases with α/β hidrolase fold grouped in the clan of serine peptidases (SC clan). These specialized peptidases cleave polypeptide chain at proline. We previously showed that the POP of *Trypanosoma cruzi* (POPTc80), another member of SC clan (POP, S9A family) is involved in the process of the parasite entry into mammalian cells. In this study, we present the cloning, expression and cellular localization of X-PDAP of *T. cruzi* (X-PDAPTc), a potential drug targets for Chagas' disease chemotherapy. The recombinant protein was expressed inactive in *E. coli* BL21 (DE3) and purified from the insoluble fraction for production of antibodies. The molecular mass of X-PDAPTc estimated by reducing SDS-PAGE was about 75 kDa as predicted in the database. Immunocytolocalization using mouse anti-XPDAPTc antiserum revealed that the enzyme is restricted to intracellular structures in epimastigote forms of the parasite. It is worth to notice that the distribution of X-PDAP is restricting to bacteria, protozoa and archea. This feature turns X-PDAPTc a very interesting target in development of selective drugs to Chagas' disease.

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