BC001 - **DOWN REGULATION OF HOST CELL SIGNALING AND LYSOSOME EXOCYTOSIS BY *TRYPANOSOMA CRUZI* ADHESIN GP90 THAT FUNCTIONS AS CELL INVASION INHIBITOR**

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Metacyclic trypomastigotes of *T. cruzi* strains, such as the G strain, which express high levels of gp90, the surface molecule that negatively modulates host cell invasion, are poorly infective. Gp90 lacks several properties exhibited by gp82, the metacyclic stage-specific surface molecule that, upon binding to human epithelial HeLa cells, induces an increase in cytosolic Ca^{2+} concentration and promotes lysosome exocytosis, events required for parasite internalization. Binding of gp90 to human epithelial HeLa cells does not trigger Ca^{2+} signaling and inhibits lysosome exocytosis. The down regulatory effect of gp90 on metacyclic trypomastigote entry into HeLa cells can be reverted if the invasion assay is performed in PBS++, a solution without macromolecules or aminoacids, suggesting that full nutrient medium favor the parasite-host cell interaction mediated by gp90 over that mediated by gp82. Compatible with this assumption, the signaling pathways activated during parasite interaction with HeLa cells in PBS++ were found to involve protein kinase C (PKC), phosphatidylinositol 3 kinase (PI3P) and mammalian target of rapamycin (mTOR), kinases previouyly shown to be induced by gp82. When cell invasion assays were performed in PBS++ in the presence of gp90, parasite internalization decreased with the increasing doses of gp90. During short term (1 h) incubation of HeLa cells in PBS++, lysosomes were mobilized from the perinuclear region to the cell periphery. In the presence of gp90, lysosome mobilization in was prevented. This inhibitory effect of gp90 on lysosome mobilization is similar to that previously observed when HeLa cells were treated with PKC, PI3K or mTOR inhibitors.Taken together, our data suggest that gp90 adhesion to host cells down regulates the signaling cascades that are activated by gp82. **Supported by:**CNPq/FAPESP

BC002 - **MEMBRANE CHOLESTEROL REGULATES THE EXCYTOSIS OF A PERIPHERAL POOL OF LYSOSOMES IN HOST CELLS, USED BY *T. CRUZI* DURING INVASION**

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We have previously shown that cholesterol removal from host cell plasma membrane decreases *T. cruzi* cell invasion by depleting lysosomes from cell periphery. However the mechanism by which cholesterol induced lysosomal exocytosis was not clear. In the present work we have investigated the role of cholesterol in controlling mechanical properties of cells and triggering lysosomal exocytosis, as well as the influence of these parameters in *T. cruzi* cell invasion. We observed, using tether extraction with optical tweezers (OT) and defocusing microscopy (DM), that cells become stiffer when treated with MβCD, a drug that sequesters cholesterol from cell membranes. These changes in membrane dynamics involved not only the actin cytoskeleton rearrangement, but also its polymerization *de novo* and stress fiber formation through Rho activation. Additionally, we have shown that changes in cellular mechanics is involved with lysosome exocytosis triggered by cholesterol removal. Exocytosis triggered by cholesterol removal led to the secretion of a specific pool of lysosomes in comparison to the pool recruited by actin depolymerizing drugs, such as latrunculin-A. This specific pool of peripheral lysosomes corresponds to the pool used by *T. cruzi* to enter host cells. These data strongly support the existence of at least two different pools of lysosomes with different exocytosis dynamics and regulation. **Supported by:**FAPEMIG / CNPq / INCT-FCx

BC003 - **AN ENDOSSOMAL EIF2A KINASE IS ACTIVATED BY PHOSPHORYLATION CAUSING TRANSLATION ARREST IN RESPONSE TO HEME DEPRIVATION IN *TRYPANOSOMA CRUZI***

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Trypanosoma cruzi faces different environmental conditions during its life cycle, such as starvation and alterations in nutrient availability. One of the major signaling responses that occur by nutrient deprivation is through the phosphorylation of the alpha-subunit of translation initiation factor 2 (eIF2a), which causes translation arrest and is required for epimastigote differentiation in metacyclic-trypomastigotes (Tonelli et al. 2011, *PlosOne* 6:e27904). Three eIF2a putative kinases are present in *T. cruzi*, TceIF2-K1, K2 and K3 that could be involved in this signaling response. Here, we characterized the TceIF2-K2, a protein that presents a topology similar to PERK, a kinase that signals translation arrest when unfolded proteins accumulate in the ER of most eukaryotes. We found that TceIF2-K2 is not present in the ER. Instead, it is found in endosomal organelles called reservosomes in the epimastigote form. Upon differentiation, in nutrient poor medium, reservosomes are consumed and the enzyme disperses as small vesicles throughout the cytosol, becoming phosphorylated and probably inhibiting translation in trypomastigotes. Growth arrest of epimastigotes and TceIF2-K2 phosphorylation can also be observed medium containing limiting amounts of heme, which is found to accumulate in the reservosomes. Indeed, the presence of heme inhibits both TceIF2-K2 and eIF2a phosphorylation *in vitro*. These results indicate that heme in the reservosomes maintain the kinase in low activated state allowing enough translation for growing. TceIF2-K2 is activated in heme poor conditions arresting protein synthesis in the metacyclic-trypomastigotes and trypomastigotes. Remarkably, a double TceIF2-K2 knockout grows much slowly than wild type epimastigotes and becomes insensitive to heme. However, low concentrations of ascorbate fully rescue the growing phenotype, indicating that TceIF2-K2 is additionally required in the control of heme induced ROS production, required for cell growth of *T. cruzi* (Nogueira *et al.* (2011) *PLoS One* 6: e25935). **Supported by:**FAPESP

BC004 - **INFLUENCE OF NEUTROPHIL ELASTASE IN THE INFECTION OF MURINE MACROPHAGES BY *LEISHMANIA DONOVANI***

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Leishmania species cause a wide spectrum of diseases that range from self-healing cutaneous lesions to visceral leishmaniasis. The development of the clinical symptoms depends on a complex combination of parasite factors and host responses. The genomes of *L. major*, *L. braziliensis* and *L. infantum* were recently compared, revealing that only a few genes are species-specific and suggesting that gene expression regulation and/or specific interactions can be determinant for pathogenesis. In *L. major*, we described three ecotin-like genes (ISP1, ISP2 and ISP3) and found that ISP2 inhibits the activity of neutrophil elastase (NE) present in murine macrophages preventing the activation of Toll -like receptor 4 (TLR4). *L. major* mutant lines lacking ISP2 are partially eliminated in infected macrophages within 24 hours. The search for the ISP2 gene in the *L. donovani* genome revealed mutations in the open reading frame (ORF), suggesting that this species might not express a functional serine peptidase inhibitor. We sought to investigate the putative role of ISPs in *L. donovani* by using a strain isolated from a Sudanese patient as a model. The ISP2 gene was identified by Southern Blot using the *L. major* ISP2 ORF as a probe. However, ISP2 expression was not detected in lysates of stationary phase promastigotes by Western blot, suggesting that *L. donovani* is incapable of controlling neutrophil elastase activity. The use of a synthetic NE inhibitor during *in vitro* infections of macrophages from C57B6 mice did not affect parasite uptake but decreased intracellular survival. Likewise, *L. donovani* survived poorly in macrophages from elastase knock-out mice, suggesting that NE activity plays a role in infection. To further address the role of NE, we transfected *L. donovani* with the ISP2 gene from *L. major*. ISP2 overexpression was confirmed by Western blot in cloned parasite lines and the role of ISP2 in macrophage infections is currently under investigation. **Supported by:**CNPq

BC005 - **A COMMANDMENT FROM THE TRYPANOSOME SURFACE - (THOUGH SHALT) *EAT ME*.**

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Epithelial cell invasion by the protozoan parasite Trypanosoma cruzi is enhanced by the presence of an enzyme expressed on its cell surface during the trypomastigote life cycle stage. The enzyme, trans-sialidase (TS), is a member of one of the largest gene families expressed by the parasite and the role of its activity in mediating epithelial cell entry has not hitherto been fully understood. Here we show that the T. cruzi TS generates an eat me signal which is capable of enabling epithelial cell entry. We have utilized purified, recombinant, active (TcTS) and inactive (TcTS2V0) TS coated onto beads to challenge an epithelial cell line. We find that TS activity acts upon G protein coupled receptors present at the epithelial cell synapse with the coated bead, thereby enhancing cell entry. Further we provide evidence that TS proteins (active, inactive and GP82), bind glycans, mediate the formation of distinct synaptic domains and promote macropinocytotic uptake of microparticles into a perinuclear compartment in a manner which may emulate entosis. **Supported by:**Wellcome Trust

BC006 - **INTRACARDIAC EDEMA PROPAGATED VIA THE MAST CELL- KALLIKREIN-KININ SYSTEM FUELS *TRYPANOSOMA CRUZI* INFECTION, CONTRIBUTING TO CHRONIC MYOCARDITIS AND HEART FIBROSIS**

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In a recent article (Scharfstein et al., *Frontiers Immunol*, 2013) we suggested that extracellular trypomastigotes released from disrupted "pseudocysts" evoke intermittent flares of trans-endothelium plasma-leakage in the chronic chagasic heart through the activation of the kallikrein-kinin system (KKS). Here we tested the hypothesis that kinins, i.e., bradykinin (BK) and des-Arg-BK, released in inflammatory exudates, may fuel T. cruzi invasion of cardiovascular cells through the signaling of bradykinin (B2R or B1R) receptors. Guided by echocardiography, Dm28c TCTs were injected in the left ventricle of C57BL/6 (WT), $B2R^{-/-}$ mice, mast cell deficient (B6-KitW-sh/W-sh), or WT mice pretreated with a single dose of HOE-140 (B2R antagonist), B9858 (B1R antagonist), or FXII inhibitor (blocker of contact phase/KKS activation). We then measured (i) intracardiac leakage of TRITC-dextran (detected by confocal microscopy) (ii) parasite DNA in heart tissues (qPCR at 3 d.p.i) (iii) myocarditis and fibrosis at 30 d.pi. Controls showed that TCTs evoked intracardiac edema in WT mice, but not in $B2R^{-/-}$ mice, nor in WT mice pretreated with the above mentioned drugs. We then injected TCTs in the heart of mast cell deficient W-sh or WT mice pretreated with cromoglycate (a mast cell stabilizer) and found that *T. cruzi* DNA was drastically reduced in both cases. These results linked parasite infectivity to intracardiac activation of the mast cell/KKS pathway. We then checked whether these early pharmacological interventions had long-term therapeutic effects. Histopathological analysis (30 d.pi.) showed that myocarditis and collagen deposition were drastically reduced in WT mice pretreated with HOE-140, B9858, bosentan or FXIIa inhibitor. Our study suggests that drugs that restore the integrity of endothelium barrier function in the chagasic heart may limit parasite infectivity (via the mast cell/KKS pathway), hence sparing the myocardium from excessive inflammation and fibrosis. **Supported by:**CNPQ, FAPERJ, PRONEX, INBEB e FAPESP

BC007 - **DISTINCT ACTIVATION OF HELA CELL KINASES DURING INVASION BY *TRYPANOSOMA CRUZI* EXTRACELLULAR AMASTIGOTES**

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Cellular invasion by *Trypanosoma cruzi* extracellular amastigotes (EAs) is very distinct from the classic infective forms, metacyclic and bloodstream trypomastigotes. EAs activate several host cell proteins that lead to actin cytoskeleton mobilization. The recruitment of actin is central to the uptake of EA forms by mammalian host cells. Signaling pathways that control this process are still poorly characterized. Phosphatidylinositol-3 kinase (PI3-k)/Akt and Src family kinases (SFKs) pathways control several cellular processes and may also regulate host cell invasion by intracellular pathogens. HeLa cell treatment with Wortmannin, a PI3-k inhibitor, reduced EAs internalization. On the other hand the role of SFKs in *T. cruzi*-host cell interaction has never been studied. We aimed to characterize the role of both (PI3-k)/Akt and Src signaling pathways during host cell invasion by EAs. We analyzed protein phosphorylation of HeLa cell proteins previously incubated with EAs, using a phospho-kinase array kit® in which cell extracts were incubated with a nitrocellulose membrane containing blotted antibodies. EAs increased the phosphorylation of Akt activation sites including the Phosphoinositide-Dependent Kinase-1(PDK1) substrate residue, but not the phosphorylation of SFKs. The treatment of HeLa cells with Akt inhibitors reduced EAs internalization similarly to those groups treated with PI3-k inhibitors (Wortmannin and LY-294,002). However, host cell treatment with PP2, a specific SFKs inhibitor, did not interfere with EAs internalization. Together these results suggest the involvement of PI3-k/Akt but not SFKs pathway during EAs cellular invasion. **Supported by:**CNPq; FAPESP

BC008 - **CAMPTOTHECIN AS A TOOL TO STUDY *STRIGOMONAS CULICIS* CELL BIOLOGY**

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Strigomonas culicis, a monoxenic protozoan of the Trypanosomatidae famíly, presents single copy structures, as the nucleus, the kinetoplast and the endosymbiotic bacterium, which maintains a mutualistic relationship with the host protozoan. During *S. culicis* cell cycle, the symbiont divides in coordination with host structures, so that each daughter cell harbors only one bacterium. Camptothecin is a topoisomerase I inhibitor that promotes cell cycle arrest and ultrastructural alterations in trypanosomatids. In this work, we investigated the effects of camptothecin on *S. culicis* proliferation, ultrastructure and on symbiont division. For these purposes, cells were treated with different concentrations of camptothecin for 72 hours. After every 12 hours, cells were collected for counting in a Neubauer chamber and for analysis using fluorescence or transmission electron microscopy. Our results showed that camptothecin inhibited cell proliferation and caused ultrastructural modifications, such as the unpacking of nuclear heterochromatin, mitochondrial swelling and accumulation of lipid bodies. After using DAPI and Nile Red as markers in fluorescence microscopy assays, we observed an increased number of lipid bodies and the presence of elongated symbionts in treated cells. Most protozoa contained one nucleus and one kinetoplast indicating that the parasite cell cycle was arrested. However, the presence of filamentous symbionts suggested that duplication and segregation of the bacterium DNA was not blocked, but only its cytokinesis. This result reinforces the idea that a perfect synchrony of division between the bacterium and the host protozoan host is necessary for the maintenance of endosymbiosis in trypanosomatids. **Supported by:**FAPERJ e CNPq

BC009 - **ENDOSYMBIOSIS IN TRYPANOSOMATIDS: A METABOLIC INTERDEPENDENCE BETWEEN THE BACTERIUM AND THE HOST PROTOZOAN IS SUGGESTED BY ULTRASTRUCTURAL AND GENOMIC ANALYSES**

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Co-evolution between primitive organisms associated by symbiotic relationship can offer valuable information about the origin of organelles and the evolution of of the eukaryotic cell. Some monexenic protozoa from Trypanosomatid family maintain a mutualistic relationship with a bacterium. We performed structural analysis using high pressure freezing and freeze substitution associated to the electron tomography technique in order to study the symbiont ultrastructure and its association with protozoan organelles. Such analyses were combined with genomic data obtained from the bacterium and its host. Our results showed a close association of the symbiont with the host nucleus, indicating that the protozoan controls bacterium division and during this process the nucleus serves as a topological reference to the symbiont segregation. During the protozoan cytokinesis, the bacterium maintains its position close to the nucleus ensuring its inheritance to each daughter cell. Our genomic data showed that the symbiont presents a reduced content, indicating a massive gene loss as those from the division and cell wall cluster, which codify for proteins of the bacterial division ring and peptidoglycan layer synthesis and septum formation. Such structures are not detected in the symbiont envelope after using classical optical and electron microscopy techniques. In this work, the use of electron tomography revealed a detailed ultrastructure of the symbiont envelope that presents a reduced cell wall and lacks the Z ring. Another interesting aspect of this symbiotic relationship is the intense metabolic exchange between the associated partners, as the symbiont obtainment of phosphatidylcholine from the host Trypanosomatid. According to this idea the high-resolution microscopy showed that the endoplasmic reticulum presents an intimate relationship with the bacterium, with some contact points observed between these structures. Taken together, our results assume that an ultrastructural and metabolic association between the symbiont and host structures is essential to maintain the endosymbiosis in Trypanosomatids. **Supported by:**CNPq, FAPERJ, INBEB

BC010 - **PERIPHERAL LOCATION OF HOST CELL LYSOSOMES IS INFLUENCED BY NUTRIENTS AND INCREASES THE INTERNALIZATION OF *TRYPANOSOMA CRUZI* METACYCLIC TRIPOMASTIGOTES**

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Efficient *T. cruzi* invasion of host cells requires the activation of signaling pathways that results in intracellular Ca^{2+}-dependent depolymerization of cortical actin cytoskeleton and mobilization of lysosomes from the perinuclear region to the cell periphery, culminating in exocytosis that contributes to parasitophorous vacuole biogenesis. In this study we investigated how the metacyclic trypomastigote (MT) internalization was affected by intracellular location of lysosomes. When HeLa cells were incubated with MT for 1h in PBS^{++}, a solution without macromolecules, glucose or amino acid, the parasite internalization increased as compared with samples incubated in full-nutrient DMEM medium. By fluorescence microscopy, using anti-LAMP2 antibodies, lysosomes were visualized scattered toward the cell periphery upon 1 h incubation of HeLa cells in PBS^{++}. For up to 60 min incubation of HeLa cells in PBS^{++}, the mammalian target of rapamycin (mTOR), a kinase activated during MT invasion, was highly phosphorylated. Longer incubation in PBS^{++} (> 1 h) markedly reduced mTOR phosphorylation levels. Compatible with this, preincubation of HeLa cells in PBS^{++} for 1 h or longer, before addition of MT, decreased parasite entry. Perinuclear lysosomes predominated in cells incubated for 2 h in PBS^{++}. Supplementation of PBS^{++} with amino acids or serum reduced lysosome mobilization and, accordingly, decreased MT invasion. To identify which amino acids retain lysosomes to the perinuclear region, the 20 amino acids present in DMEM were added individually to PBS^{++}. L-glutamine and phenylalanine were found to affect lysosome mobilization and to significantly inhibit MT invasion. Our results indicated a close association of lysosome relocation to the cell periphery under short term nutrient depletion (up to 1 h) and MT internalization, events that are influenced by nutrient availability. **Supported by:**FAPESP - CNPq

BC011 - **MECHANISMS OF HOST CELL INVASION BY METAYCLIC FORMS OF *TRYPANOSOMA CRUZI* FROM GENETIC GROUP TCI GROUP DERIVE FROM CHAGASIC PATIENTS**

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T. cruzi genetic group TcI is the predominant agent of Chagas disease in countries North of the Amazon and in the Amazon region, where outbreaks of acute Chagas disease by oral infection have been reported. Studies with metacyclic trypomastigote (MT) forms have shown that TcI strains from the sylvatic transmission cycle are poorly infective in vitro and in vivo, and that this characteristic is associated with the expression of MT-specific gp90, a surface molecule that negatively regulates host cell invasion. We investigated the mechanisms of cell invasion in vitro and the infectivity by oral route of TcI strains isolated from Chagasic patients in Brazilian Amazon, Venezuela, Guatemala and Northeast Brasil. MT of the referred strains expressed high gp90 levels and were poorly infective in mice by the oral route. Differently from MT of *T. cruzi* strains from genetic group VI that efficiently infect mice *per os*, and efficiently migrate through the gastric mucin-coated transwell filter, the TcI strains analyzed here displayed reduced capacity to overcome the gastric mucin barrier. Invasion of human epithelial HeLa cells by MT of TcI strains, which was very low in nutrient-full DMEM medium, increased several fold upon parasite-cell interaction in PBS^{++}, a solution without macromolecule, glucose or amino acid. In PBS^{++}, apparently the gp90-mediated MT host cell interaction that predominated in DMEM switches to that mediated by gp82, the MT surface molecule that promotes parasite internalization. Accordingly, MT invasion of HeLa cells in PBS^{++} was inhibited either by the recombinant gp82 or the monoclonal antibody 3F6 directed to gp82. Also compatible with gp82-dependent MT invasion that is associated with activation of the mammalian target of rapamycin (mTOR), phosphatidylinositol 3-kinase (PI3K) and protein kinase C (PKC), treatment of HeLa cells with inhibitors of these kinases significantly inhibited MT invasion of TcI strain in PBS^{++}. **Supported by:**FAPESP CNPq

BC012 - **ACTIVATION OF APOPTOSIS CASCADE BY PAC-1 ON PROMASTIGOTES OF *LEISHMANIA* SPP.**

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Programmed cell death by apoptosis has been described in different species of unicellular eukaryotes, including obligate parasitic trypanosomatids. However, the molecular events underlying this type of death in unicellular organisms are not fully understood. In animals, caspases are the major regulators of apoptotic death. Procaspase-3, the inactive precursor of caspase-3, is extremely resistant to activation. This is due to an intrinsic tripeptidic motif DDD that, acting as a safety-catch, prevents accidental procaspase activation. Putt and collaborators (Nature Chem. BioL. 2006) reported the property of PAC-1 to transform procaspase-3 into active caspase-3. Metacaspases were proposed as the putative functional equivalent of caspases in plants, fungi and parasitic protozoa. This had not been clearly demonstrated yet. Now we show that the procaspase-3 regulatory motif is conserved in all the studied species of metacaspases of *Leishmania*; the same molecule (PAC-1) capable of activating procaspase-3 is also able to induce cell growth arrest and apoptotic features in promastigotes of *Leishmania (L) amazonensis*. PAC-1 induces a dose-dependent inhibition of cell growth along with inhibition of mitochondrial transmembrane potential and induction of PS exposure. These two phenotypes are characteristic of apoptotic death; together with cell cycle arrest they can be interpreted as resulting from metacaspase activation. The physicochemical nature of metacaspase activation is still unknown and the prerequisite of proteolytic maturation for its activity is controversia. PAC-1 can be a useful tool to address those issues. Furthermore, the absence of metacaspases in mammals warrants the development of strategies for the use of PAC-1 as an anti-parasitic drug.**Supported by:**CNPq AND FAPESP

BC013 - **EVALUATION OF HOST CELL MODIFICATIONS UNDER CYST FORMATION**

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The conversion of *Toxoplasma gondii* from the tachyzoite to the bradyzoite form leads to the persistence of infection in intermediate hosts, including humans. In the process, the reminiscent parasitophorous vacuole (PV) converts to an intracellular cyst presenting a cyst wall; this stage can persist for long periods in the host. The modifications of host cell organelles are poorly understood until now. Previous works have already shown that *T. gondii* can recruit host cell organelles and cytoskeleton filaments to the vicinity of the PV along the infection. Our aim was to investigate the reorientation of host cell organelles during spontaneous *in vitro* cystogenesis. The epithelial cell line LLC-MK_2 infected with the cystogenic strain EGS was used as experimental model. The distribution of host cytoskeleton was evaluated by electron and fluorescence microcopy and compared to uninfected cells. Intermediate filaments of cytokeratin were observed concentrated around the cysts by electron microscopy, however, using the anti pan cytokeratin staining, it was not possible to observe this phenomenon in the fluorescence mycroscopy. Fluorescence analysis showed that the distribution of actin filaments was unaltered upon cyst formation. Furthermore, microtubules were seen surrounding the cysts, forming a cage around it both by TEM and IFA. The presence of microtubules around the cysts may be indicative that endosomal compartments are able to reach the cyst wall vicinity and in an IFA assay it was possible to observe lysosomes recruited to the border of the cysts. Besides, several profiles of endoplasmic reticulum were seen closely related to the cyst wall membrane, although mitochondria were seen not associated as shown previously for PV. These preliminary results indicate that formation of *T. gondii* cysts in EGS strain modifies the inner organization of host cells likely to obtain nutrients or to control host cell responses not yet determined till now.**Supported by:**CNPq, CAPES and FAPERJ

BC014 - **CELLULAR IMMUNE RESPONSE IN THE SKIN OF *LEISHMANIA* (*LEISHMANIA*) *INFANTUM CHAGASI* NATURALLY INFECTED DOGS IS CORRELATED WITH THE TISSUE PARASITISM AND CLINICAL SIGNS**

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Thirty-eight dogs naturally infected by *Leishmania (Leishmania) infantum chagasi* were randomly selected from Araçatuba region of São Paulo state (Brazil), an endemic area for visceral leishmaniasis. The subjects were divided into two groups: the first comprising 24 symptomatic dogs and the second consisting of 14 asymptomatic dogs. Correlations of clinical characterization with skin histological pattern, cellular immunity and parasitism were investigated using immunohistochemical techniques. Regarding to the number of *Leishmania* amastigotes/mm^2 in the skin, there was not significant difference between the groups (p=0.1584); density of skin parasites showed a moderate positive correlation with anti-*Leishmania* antibody titers (p=0.042). Histological features in the skin were similar in both groups and were generally characterized by an inflammatory infiltrate, either diffuse or focal, in the dermis, mainly consisted of mononuclear cells (macrophages, lymphocytes and plasma cells), varying from mild to intense. Concerning characterization of the cutaneous cellular immune response, only $iNOS^+$ cells density (cells/mm^2) was significantly higher in the dermis of the symptomatic dogs compared to the asymptomatic ones (p=0.0368). Moderate positive correlation between the cutaneous parasites density and the macrophages density (p=0.031), $CD4^+$ T cells (p=0.015) and $CD8^+$ T cells (p=0.023) were observed. Furthermore, density of $iNOS^+$ cells in relation to $CD3^+$ T cells (p=0.005), $CD4^+$ T cells (p=0.001) and $CD8^+$ T cells (p=0.0001) were found positively correlated at a moderate level. Taking together, the results showed that the skin histopathological lesions as well as the cutaneous cellular immune response were associated with the skin parasitism which was higher in symptomatic dogs.**Supported by:**CNPq and FAPESP 2004/07965-2

BC015 - **A NOVEL GOLGI MARKER FOR *TRYPANOSOMA CRUZI*: IDENTIFICATION AND SUBCELLULAR LOCALIZATION OF TCHIP, A PUTATIVE ZDHHC PALMITOYL TRANSFERASE**

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Palmitoylation is a post-translational modification that contributes to determining protein localization and function. It has been described in trypanosomatid protozoa, but the enzyme zDHHC palmitoyl transferase has not been identified in *Trypanosoma cruzi*, the etiological agent of Chagas disease. In this study we identify and show the subcellular localization of TcHIP (Tc00.1047053508199.50), a putative *T. cruzi* zDHHC palmitoyl transferase. Analysis of the deduced protein sequence indicates that it contains ankyrin repeats (Ank and Ank2) and the zDHHC conserved domain, typical of zDHHC palmitoyl transferases. A TcHIP polyclonal antiserum obtained from mice immunized with the purified recombinant protein was used to study the presence and subcellular localization of the native enzyme. By western blots this antiserum recognized a protein of about 95 kDa, consistent with the predicted molecular mass of TcHIP (95.4 kDa), in whole extracts of *T. cruzi* epimastigotes, metacyclic trypomastigotes and intracellular amastigotes. Immunolocalization by confocal microscopy showed TcHIP labeling at the Golgi complex, co-localizing with the *T. cruzi* Golgi marker TcRab7-GFP. Transfectant *T. cruzi* epimastigotes containing a construct encoding TcHIP fused to proteins A and C (TcHIP/AC) were obtained. In western blotting experiments, the TcHIP polyclonal antiserum recognized both native and TcHIP/AC proteins in extracts of the transfectants. Confocal microscopy showed co-localization of native TcHIP with TcHIP/AC. These findings demonstrate the presence of a putative zDHHC palmitoyl transferase (TcHIP) containing ankyrin and zDHHC domains in different developmental forms of *T. cruzi*, and its association with the Golgi complex. **Supported by:**CNPq, CAPES, FIOCRUZ

BC016 - **PARASITOLOGICAL AND HISTOPATHOLOGICAL EVALUATION OF MICE INOCULATED WITH *TRYPANOSOMA CRUZI* I, II AND IV AND TREATED WITH BENZNIDAZOLE**

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Chagas disease (CD) has been recognized as an emerging anthropozoonosis in the Brazilian Amazon, outbreaks of acute CD are common in this region and etiological treatment must be initiated fast. Studies from specific chemotherapy with benznidazole (BZ) at this region have great relevance due to the different Discrete Typing Units (DTUs) of *Trypanosoma cruzi* infecting humans. The aim of this study was performed parasitological and histopathological analysis of mice inoculated with *T. cruzi* I, II and IV, and BZ-treated in the acute phase of infection. Groups of 26 Swiss mice with 21 to 28 days were inoculated with $1x10^4$ blood or $2x10^6$ metacyclic trypomastigotes/animal: TcI (6) and TcIV (8) – Amazonas State, TcII (4) - Paraná. Thirteen mice were treated orally with BZ (TBZ) and the other 13 constituted the untreated control group (NT). Parasitological, molecular and histopathological parameters were assessed. The animals were sacrificed one day after the peak parasitemia (recent acute phase - rAP), at day 30 (late acute phase- lAP) and 100^{th} (chronic phase - CP) day after inoculation. Fragments were obtained from the heart, skeletal muscle, liver, spleen, intestine and brain, stained with HE. The number of tissues with histopathological changes were compared between TBZ and NT groups and among DTUs. Infectivity, mortality and cure rates didn't vary among DTUs. Mices inoculated with TcI showed subpatent parasitemia and TcII with a parasitemia approximately 10 folds higher than TcIV. TcII was considered the most pathogenic and TcIV the least. BZ treatment significantly reduced the most parasitological parameters, tissue parasitism in rAP and lAP and inflammatory process in all stages of infection and for all DTUs. Treatment with BZ during acute phase promoted benefits for mice inoculated with all DTUs studied and at different stages of infection, however, the number of significant reductions varied according to the parameter measured, with the DTU and phase of infection. **Supported by:**CAPES/CNPQ

BC017 - ***LEISHMANIA AMAZONENSIS*** **SURVIVAL INSIDE ARTIFICIAL UNILAMELLAR VESICLES**

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Leishmania are heteroxen parasites that infect mammal macrophages. In the amastigote form, *Leishmania* inhabits inside host cell parasitophorous vacuoles (PV) that are compartments rich in lysosomal enzymes, presenting low pH and protein membrane markers of late endocytic compartments, indicating they derive from host endocytic pathway. *Leishmania amazonensis* induces large vacuoles containing a high number of intracellular amastigotes, whereas *L. major* generates small vacuoles with single parasite. These data indicate that vacuole shapes depend on parasite species and survival strategies vary according to PV biogenesis. In the present study, we describe a novel in vitro model that aims to reconstitute parasite intracellular life-stage, in order to future test whether parasite survival depends on delivery of nutrients contained in endocytic vesicles to PV by fusion events that probably differ according to parasite species. Initially, we produced free-parasite giant unilamellar vesicles ranged in size from 10 to 60 µM constituted by EPC and PE-PEG phospholipids, using the droplet-transfer technique. Then, *L. amazonensis* promastigotes were encapsulated inside these vesicles containing intravacuolar culture medium composed of RPMI plus dextran at pH 7.2 or 5.5. Parasite survival inside vacuoles was evaluated during four days by observing parasite shape and movement at optical phase microscopy. Parasites incubated at 24°C survived for four days inside vesicles containing cukture medium at pH 7.2 and two days at pH 5.5. To mimic intracellular microenvironment, we plan to perform encapsulation assays at 34°C pH 5.5, to parasites transform into amastigote intracellular life-stage. Furthermore, we plan to encapsulate parasites inside charged vesicles to perform assays of fusion between vacuoles and small vesicles containing different type of nutrients to evaluate parasite survival and multiplication inside these artificial vacuoles.**Supported by:**CAPES-COFECUB

BC018 - **EFECCTS OF FARNESOL, AN INTERMEDIATE OF MEVALONATE PATHWAY, ON *LEISHMANIA AMAZONENSIS* PROMASTIGOTES**

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Farnesol (FOH), an intermediate of mevalonate pathway, is known by the essential role it plays in the processes of cell growth and differentiation. Several studies have also shown that FOH acts as an inducer of apoptosis in different cell types. The objective of this study is to evaluate the FOH effects on *Leishmania amazonensis* promastigotes. Our results showed that this compound inhibits the growth of promastigotes after 24, 48 and 72 h of incubation (IC_{50} of 46, 34 and 36 µM, respectively). The analysis of the membrane integrity of the treated parasites showed that only cells incubated with 80µM FOH for 72 h presented labeling for PI, indicating the occurrence of necrotic process. Similarly, we observed that only in cells treated with the highest concentration of FOH (92 µM) occurred mitochondrial membrane depolarization. The effect of different concentrations of FOH on the cell division of *L. amazonensis* promastigotes was evaluated using cells labeled with carboxyfluorescein diacetate succinimidyl ester (CFSE). The analysis were performed just after marking (time 0) and 24h, 48h and 72h later. We observed that the treatment with FOH (74µM) promotes retention of CFSE by the cells, indicating that FOH does interfere with the cell division of the parasite. We also evaluated the interference of FOH on the cell cycle of *L. amazonensis*. For this, the cells were incubated with different concentrations of FOH for 72 h. After this period, cells were labeled with PI and analyzed by flow cytometry. The treatment of the cells with 15µM and 30µM of FOH increased the percentage of cells in Go/G1 phase and reduced the percentage of cells in G2, suggesting a decrease in mitosis. Taken together, these results indicate that FOH, an endogenous metabolite of *Leishmania,* interferes with parasite cell cycle, suggesting a possible feedback regulation. **Supported by:**IOC

BC019 - **EFFECT OF KINASE, ACTIN, MYOSIN AND DYNAMIN INHIBITORS IN HOST CELL EGRESS BY *TOXOPLASMA GONDII***

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Toxoplasma gondii is a parasite that still has medical and veterinary importance, but its egress from host cell remains poorly understood. This step of the protozoan cell cycle is usually studied upon triggering during calcium ionophore treatment. In this work we employed kinase, cytoskeleton and dynamin inhibitors in order to examine their role in egress. Although parasite egress was only slightly impaired by treatment with the PI3K and PKC inhibitors of respectively, wortmannin and staurosporine, the addition of the tyrosine kinase-specific inhibitor genistein efficiently blocked the exit of parasites by more than 50%. IPA-3, a non-ATP-competitive inhibitor of p21-activated kinases, which plays a role in actin cytoskeleton remodeling inhibited egress of *T. gondii* by only 15%. The myosin motor inhibitor blebbistatin and the actin polymerization inhibitor cytochalasin D blocked the egress of *T. gondii*. Nevertheless, dynasore, which is known to block the GTPase activity of dynamin, had little or no effect on *T. gondii* egress. The effect of cytochalasin D and dynasore were also verified by confocal microscopy.**Supported by:**CAPES, CNPq, FAPERJ

BC020 - **HISTONE H4 ACETYLATIONS AFFECTS *TRYPANOSOMA CRUZI* CHROMATIN ORGANIZATION**

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Trypanosoma cruzi, the agent of Chagas disease presents a complex life cycle involving insect Reduviidae and the mammalian hosts. The infective forms are the trypomastigotes (metacyclic and bloodstream), while epimastigotes and amastigotes proliferate by binary fission. During the passage through its hosts, the parasite undergoes severe morphological and biochemical changes, which culminates in a complete reorganization of the nucleus. The *T. cruzi* chromatin is also formed by DNA wrapped around one of two octamers consisting of copies of each histone (H2A, H2B, H3 and H4), plus H1 histone. When compared with other eukaryotic histones *T. cruzi* sequences are highly divergent, especially when concerning the sites of post-translational modifications (PTMs), known to act as important regulators of several nuclear functions. In this study, we generated parasites expressing the wild type or mutated forms of histone H4 with the lysine 4, 10 and 14 residues replaced by arginine. All modified histones are incorporated in the chromatin, but only H4K10R and H4K14R mutation affected growth. These effects were not caused by changes in overall transcription or increased damage in DNA, known to depend on the K10 and K14 modifications. However, changes in chromatin compaction were found by using differential histone extraction by increased salt concentrations and by variations in the susceptibility to *Micrococcus* nuclease, without large changes in the nucleus structure. These results indicate that acetylation of lysine residues at histone H4 are required for optimal chromatin structure formation and organization. **Supported by:**FAPESP

BC021 - **MUCOLYTIC ACTIVITY AS A MECHANISM OF *GIARDIA LAMBLIA* INFECTION**

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Giardia lamblia is a protozoan parasite and is one of the most common causes of diarrhea and intestinal upset worldwide. It has a simple life cycle with motile trophozoites (*Glt*) colonizing the duodenum of a variety of vertebrates, causing the symptoms of the disease. In order to make contact with the epithelium, *Glt* must overcome a thick mucus barrier, composed mostly by mucin glycoproteins. It is known that most enteropathogens accomplish this task by a variety of mechanisms, including degradation of mucin oligosaccharides and proteolytic degradation of the mucin polymer. However, nothing is known about how *Glt* deal with it. Previously, we showed that Glt bound to purified intestinal mucus (MIM) and mucins (bovine submaxillary mucin - BSM and porcine gastric mucin - PGM) and migrated through transwells coated MIM and BSM, in contrary to PGM. Now, to determine how *Glt* overcomes this mucus barrier, we investigated the effects of secreted products on the degradation of the intestinal mucus and mucins through SDS-PAGE and PAS-Schiff stain. Our results showed that *Glt* degraded both MIM and BSM but not PGM, reinforcing the results from the migration assay. Now we examined the hypothesis that secreted products from *Glt* could degrade mucus and mucins. For this, secreted products were tested for the presence of proteolytic activity. Using a panel of synthetic substrate FRET peptides, protease activity was detected (pH range 7-8) with specificity towards Leu, Phe and Arg. These observations suggest that proteases from *Glt* may facilitate invasion of the small intestine by disrupting the mucus layer. However, a more detailed molecular mechanism of how the parasite disassembles the mucin polymer has yet to be determined. Supported by FAPESP and Capes. **Supported by:**Fapesp; Capes

BC022 - ***TRYPANOSOMA CRUZI* MEMBRANE VESICLES DISPLAY ACID AND ALKALINE PHOSPHATASE ACTIVITIES: A COMPARATIVE STUDY BETWEEN Y AND CL STRAIN**

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Secretion of virulence factors is a major mechanism by which pathogens interfere or modulate the cell response. Secreted and surface phosphates play a critical role during host cell interaction. This study characterized the ecto- and secreted phosphatase activities from Y and CL-Brener tissue culture trypomastigotes and the involvement of phosphatases during parasite-macrophage interaction. Secreted activity was evaluated in two parts: in the pellet (membrane vesicles - MVs) obtained after centrifugation at 100,00 x g for 1 h at 4^{o}C and in the supernatant of the same preparation (final supernatant) as previously described in (Aparício *et al.,* 2004). In all samples analyzed, phosphatase activities were shown to be affected by Mg^{2+} concentration and by changes in pH (range 6.5-8.5) with optimum pH 6.5 (acidic) and 8.5 (alkaline). CL-Brener parasites showed a greater Mg^{2+}-dependent ecto and secreted phosphatases (final supernatant) at pH 8.5 compared to those from Y strain. At pH 6.5 Mg^{2+}-dependent phosphatase activity of CL-Brener is about 3 times than that of Y strain and was concentrated in the final supernatant. The Mg^{2+}-independent phosphatase activity evaluated at pH 8.5 is concentrated in the MVs of CL-Brener parasites while in the Y strain the ecto-phosphatase activity is the most evident. At pH 6.5 the most intense enzyme activity is concentrated in the MVs of both Y and CL-Brener parasites although Y parasites also presented an intense ecto-phosphatase activity. The phosphatase inhibitors tartarate and fluoride inhibited the acid ecto-phosphatase activity present in the MVs of Y parasites. In CL-Brener parasites the same inhibitors were only effective in the acidic activity observed in the MVs. The acid phosphatase activity present in the MVs interfered significantly in adhesion and infection of host cells by Y and CL-Brener trypomastigotes. These results show that *T. cruzi* MVs display different phosphatase activities involved in host cell interaction.**Supported by:**CNPq, CAPES, FAPERJ, Pronex

BC023 - **THE ROLE OF SUMOYLATION OF PROTEINS IN CELL BIOLOGY OF GIARDIA LAMBLIA**

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SUMOylation is a post-translational modification that plays a role in various cellular processes, such as nuclear-cytosolic transport, transcriptional regulation, protein stability, response to stress and progression through the cell cycle. It consists of the covalent attachment of a small ubiquitin-related modifier (SUMO) to substrate proteins, a process that is biochemically analogous to, but functionally distinct from, ubiquitination. SUMOylation and deSUMOylation are highly dynamic processes occurring from yeasts to mammals. The aim of this study was to investigate the role of SUMO pathway in the cell biology of *Giardia lamblia*. Previous work demonstrated that *G. lamblia* contains a single gene that codifies a SUMO protein (glSUMO) and genes encoding putative enzymes of the SUMOylation pathway. Now we show that the single glSUMO and SUMO-associated proteins are present in the cytosol, membrane periphery and median body of trophozoites, and within vesicles of different sizes and in the axoneme in cysts. SUMOylation patterns in cysts and trophozoites, as assessed by immunobloting of whole cell extracts with anti-SUMO, revealed similar SUMO-substrate profiles. Importantly, immunobloting using the affnity-purified His_8-glSUMO reacted with both the His-tagged and anti-SUMO antibodies. Further a detailed annalysis of the sumoylation substrates were carried out by 2D SDS-PAGE and mass spectrometry. With this approach many SUMO-substrates were identified with a representative number of hits corresponding to α-tubulins. To confirm this data, colocalization assays with anti-SUMO and antibodies recognizing different tubulins (α-tubulin, acetylated and glutamylated tubulin) were carried out. As expected, the SUMO and acetylated tubulin signals appeared totally colocalized in trohozoites while acetylated and glutamylated tubulins showed spatial overlap with SUMO proteins, in cysts. The function of SUMO-tubulin in *G. lamblia* in now being investigated. **Supported by:**FAPESP

BC024 - **THE THREE-DIMENSIONAL STRUCTURE OF THE CYTOSTOME-CYTOPHARINX COMPLEX OF *TRYPANOSOMA CRUZI* EPIMASTIGOTES**

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The cytostome-cytopharinx complex is the main site of endocytosis in *Trypanosoma cruzi* epimastigotes. Despite its importance, little is known about the detailed morphology of this remarkable endocytic structure. In this work, we used advanced electron microscopy techniques - such as serial electron tomography and focused ion beam-scanning electron microscopy - to reconstruct the entire cytostome-cytopharinx complex, including the surrounding cytoskeleton and vesicles. Also, by focusing on cells that had taken up gold-labeled endocytic tracers, we produced 3D snapshots of the process of endocytosis. The cytostome cytoskeleton was composed of two microtubule sets: a triplet that started underneath the cytostome membrane; and a quartet whose microtubules originated from staggered positions underneath the flagellar pocket membrane and followed the preoral ridge before reaching the cytopharinx. These two microtubule sets accompanied the cytopharinx forming a `gutter` and leaving a microtubule-free side, where vesicles, loaded or not with endocytic tracer, were found in direct contact with the membrane. After membrane extraction, negatively stained cytoskeletons showed that the microtubules that accompanies de cytopharinx remained intact and were resistant to high-salt treatment. Cargo was unevenly distributed along the lumen of the cytopharinx, forming clusters that occupied regions of enlarged diameter. The cytopharinx was slightly longer and bent towards the anterior in the G2 phase of the cell cycle. Therefore, the cytopharinx is a dynamic structure, undergoing remodeling along the cell cycle, likely associated with the preparation for cell division. **Supported by:**Capes/CNPq/Faperj

BC025 - **THE ORIGIN OF LYSOSOMES RELATED ORGANELLES OF *TRYPANOSOMA CRUZI* TRYPOMASTIGOTES**

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Inside the invertebrate host, *Trypanosoma cruzi* epimastigotes, proliferative forms with very active endocytosis, undergoes metacyclogenesis, differentiating into non-proliferative trypomastigote forms, unable to uptake macromolecules. Epimastigotes acquire exogenous macromolecules and store inside reservosomes. Reservosomes present an acidic character, maintained by an unusual P-type H^+ATPase, and concentrate cruzipain, its natural inhibitor chagasin, as well as carboxipeptidase. Despite lacking classic lysosome markers, they were classified as lysosome-related organelles (LROs). Stereological studies carried out during metacyclogenesis showed that reservosome contents were consumed along differentiation (Soares et al. Parasitol Res. 75:522, 1989). Although unable to acquire endocytic cargo, metacyclic trypomastigotes also present cruzipain, chagasin, carboxipeptidase and H^+ATPase concentrated in acidic organelles placed between kinetoplast and nucleus, also named LROs (Sant'Anna et al., Histochem Cell Biol. 130:1187, 2008). The present study aimed to investigate the origin of trypomastigote LROs. We have preloaded Dm28c epimastigote reservosomes with uncoated fluorescents beads before inducing metacyclogenesis. The rate of metacyclogenesis did not change, compared to control, in preloaded parasites: after 48h of differentiation we have obtained 53% of trypomastigotes. Surprisingly, 25% of them contained beads between kinetoplast and nucleus, consistent with the localization of LROs. In a similar experiment, using gold-labeled transferrin as tracer for electron microscopy, we observed spherical organelles containing Tf-Au in parasites at the end of metacyclogenesis. Moreover, we have found trypomastigotes with Tf-Au filled vesicles between kinetoplast and nucleus. We are now characterizing these compartments by immunocytochemistry. Our observations are a strong indication that the LROs of metacyclic trypomastigotes originate from reservosomes of epimastigotes. **Supported by:**Cnpq, Faperj, Capes, Inbeb

BC026 - **VISUALIZATION OF ENTEROEPITHELIAL STAGES OF *TOXOPLASMA GONDII* IN THE CAT**

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Toxoplasmosis is a worldwide disease caused by the intracellular protozoan *Toxoplasma gondii*. This parasite can infect any nucleated cell of any warm-blooded animal. The transmission can be from the mother to the foetus by tachyzoites, by eating raw or undercooked meat containing tissue cysts or by ingestion of contaminated food and water with sporulated oocysts. The sexual cycle occurs only into the felids small intestine where the oocysts are formed and released with the feces to the environment. The aim of this work was to visualize the enteroepithelial stages of *Toxoplasma gondii* in cat intestinal villi by scanning electron microscopy after cold fracture. For this, cats were contaminated with tissue cysts of the VEG strain, and feces examined daily for detection of oocysts. When positive, cats were sacrificed and the ileum collected and immediately fixed in 2.5% glutaraldehyde and 1% formaldehyde. Intestines were opened and the inner surface of the ileum was gently scrapped. This scrap contained most of the villi and was further processed, being post-fixed with 1% of osmium tetroxide (OsO_4), embedded in 10% gelatin, re-fixed with 0.5% glutaraldehyde and 0.5% formaldehyde for 1 hour at 4^oC. After this, the samples were cut into small pieces and cryoprotected with 25-50% glycerol, frozen by immersion in liquid nitrogen and fractured with a razor blade. Fracture was followed by thawing and glycerol deinfiltration in water. After dehydration, critical point drying and gold sputtering cleaved faces were observed in a Quanta 250 or in a Quanta 450 FEG scanning electron microscopes. In all cleaved planes, intestinal cells were infected. Macrogamonts predominated and microgamonts, when present, were always in adjacent host cells. Some cells contained both gamonts and merozoites. Oocysts were not seen inside host cells, but were frequently observed at the surface of villi. The absence of schizonts in this sample may be a consequence of the time of infection of the cat. **Supported by:**Capes, CNPq, FAPERJ

BC027 - **INVOLVEMENT OF SSP-4 FROM *TRYPANOSOMA CRUZI* EXTRACELLULAR AMASTIGOTES IN HOST CELL INVASION**

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Trypanosoma cruzi extracellular amastigotes (EA) display the stage-specific surface component Ssp-4, an 84 kDa glycoprotein anchored to the membrane by a GPI anchor. Ssp-4 is expressed on parasite surface and is gradually released into the extracellular environment by EAs. Different epitopes from this glycoprotein are recognized by the monoclonal antibodies (mAb) 2C2 and 1D9. However, structural and functional aspects of this surface glycoprotein are still unknown.The expression of Ssp-4 antigen on EA membrane and in amastigotes secreted vesicles was evaluated by confocal and electron microscopy as well as by western blotting using the mAbs 2C2 and 1D9. EAs of *T. cruzi* strains that were more infective, such as G and 1522, also expressed higher reactivity with mAb 1D9, but not 2C2. Conversely, EAs of the less infective strains presented lower or absent reactivity with the mAb 1D9 and higher reactivity with mAb 2C2. HeLa cells invasion by EA was inhibited by treating the parasite with mAb 1D9, suggesting that the epitope recognized by this antibody is important for parasite infection in host cells. We also detected Ssp-4 in EA culture supernatant fractioned by ultracentrifugation, suggesting that this glycoprotein is secreted by amastigotes in association to vesicles. Using confocal and electron microscopy, we observed structures with similar dimensions (100-200 nm), in trails, and on parasites' surface. These structures are rich in Ssp-4, as revealed by 1D9 immunostaining, which corroborates the vesicle secretion by EA. **Supported by:**FAPESP; CNPq; CAPES

BC028 - **EXTRACELLULAR AMASTIGOTES OF *TRYPANOSOMA CRUZI* (EAS) CONTROL ACTIN DYNAMICS THROUGH CORTACTIN SPECIFIC PHOPHORYLATION DURING HOST CELL INVASION**

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Extracellular amastigotes are alternative infective forms of *Trypanosoma cruzi* and, together with bloodstream trypomastigotes, sustain the parasite cycle in mammalian hosts. Their internalization is highly dependent on host cell actin cytoskeleton but the underlying mechanisms are still poorly characterized. Cortactin is a key protein in actin dynamics, involved in diverse actin-related cellular processes and also host cell invasion by pathogens such as bacteria. Previous studies have shown that cortactin phophorylation by Erk (Extracellular-regulated kinase) or Src Family kinases (SFK) plays positive or negative role in actin dynamics, respectively. We then evaluated host cell signaling events that control cortactin during EA internalization. EA were incubated to HeLa cells and host kinase phosphorylation was evaluated by Western blotting using phospho-residue antibodies. EAs induced the phosphorylation/activation of Erk but not SFK. Cortactin phosphorylation by Erk, but not by SFKs, was also observed. Heat-killed parasites and the non-infective epimastigote forms induced different Erk phosphorylation profiles and lower cortactin phosphorylation by this kinase when compared to controls. Both groups induced detectable SFK activation and also cortactin phosphorylation by this kinase family. Finally, EA invasion was reduced in a HeLa cell line constitutively down-expressing cortactin when compared to the control groups. Taken together, these results suggest that cortactin participates in the internalization of EAs by host cell after activation of specific signaling pathways triggered only by infective or live parasites. **Supported by:**FAPESP CNPq

BC029 - ***TRICHOMONAS VAGINALIS*** **MODULATES THE NITRIC OXIDE PRODUCTION OF IEC-6 ACTIVATED BY EXPOSURE OF PHOSPHATIDYLSERINE**

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Trichomonas vaginalis is human urogenital tract parasite that causes trichomoniasis, cosmopolitan disease currently considered a sexually transmitted disease most common non-viral. Previous records estimated that about 10% of the sexually active population have owned or own this parasite. Despite various studies, little is known about the factors that lead to severe infection of trichomoniasis, and can conduct to miscarriage and sterility temporary or permanent. Thus, studies of the development mechanisms of this parasite infection are needed. Phosphatidylserine (PS) is a phospholipid in normal cells and is located on the inner face of the plasma membrane, the exposure of PS on the external face of the membrane is a sign of apoptosis and programmed cell death. The apoptotic mimicry has been described in other protozoa as an escape mechanism against the action of effector cells. It has been shown that exposure of PS of Toxoplasma gondii induces TGF-β1 secretion by activated macrophages infected, leading to degradation of iNOS, inhibition of NO production and the subsequent persistent infection in these cells. In this work, the interaction behavior of *T. vaginalis* with IEC-6 activated by interferon gamma was analyzed by optical microscopy and scanning electron microscopy and transmission. By flow cytometry, we observed that approximately 37% of the total population of these parasites exposed PS. Data obtained from the measurement of NO produced by the IEC-6 during interaction with *T. vaginalis* demonstrated reduced compared NO production by activated IEC-6 and uninfected. On the other hand, *T. vaginalis* incubated with annexin-V, which binds to PS exposed, did not modulate the production of NO on IEC-6 activated. These results suggest that *T. vaginalis* is able to modulate the production of NO on IEC-6 activated by exposure of PS. **Supported by:**FAPERJ

BC030 - **CHARACTERIZATION OF THE *TRICHOMONAS VAGINALIS* WITH *BACTEROIDES FRAGILIS* INTERACTION**

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The Bacteroides genus is composed by gram-negative, bacillus, obligate anaerobes, bile-resistant and non-spore forming bacterium. The genus species are commonly found on the mucous membranes, including the female genital tract, acting as agents of several site infections. The anaerobic infections are usually polymicrobial and endogenous. *Trichomonas vaginalis*, for its part, is an anaerobic facultative flagellate parasite with worldwide distribution which is trichomoniasis etiologic agent, the most common non-viral sexually transmitted disease in humans. As for the infection persistence, this parasite possesses as a mechanism phagocyting other microorganisms, but this process is not completely elucidated. In this study we make use of *T. vaginalis* trophozoites and the species *Bacteroides fragilis* (ATCC 25285), for *B. fragilis* is considered, clinically, the most important in its genus, once it is the most commonly bacteria isolated from endogenous infectious processes, being usually associated to gastrointestinal, respiratory and female genital tracts infections. We performed the parasite-bacteria interaction in 1:10, 1:50 and 1:100 proportions in periods between 1 and 12 hours and viability tests have been applied. These data have been analyzed to rival the pathogen growth in vitro in the presence and absence of *B. fragilis*. Preliminary results indicate that in the 1:100 proportion post-interaction analysis, ultrastructural alterations were noticeable after 6 hours. Furthermore, it was observed that after 8 hours the *T. vaginalis* viability decays, and that after 12 hours of interaction no viable trophozoites are found. These data suggest that the parasite covers *B. fragilis* in short interaction periods and that in this time there are no variations of the parasite proliferation, but as time goes by the trophozoites collapse, indicating that *B. fragilis* may produce toxic metabolites against the *T. vaginalis* activity. **Supported by:**FAPERJ

BC031 - **POSSIBLE ROLE OF THE SERINE PEPTIDASE INHIBITOR (ISP2) OF *TRYPANOSOMA CRUZI* IN MAMMALIAN CELL INFECTION.**

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Three genes similar to bacterial ecotins were first identified in the genome of *Leishmania*, and have been designated Inhibitors of Serine Peptidases (ISPs). Ecotins are found in the periplasm of several bacteria species and are macromolecular inhibitors of serine peptidases belonging to clan PA, family S1A such as trypsin, chymotrypsin, cathepsin G and neutrophil elastase (NE). In *L. major*, ISP2 has been implicated in the modulation of parasite phagocytosis by murine macrophages due to the regulation of neutrophil elastase activity and prevention of the triggering of Toll-like receptor 4. ISP2 protects the parasite from intracellular death, contributing to the initial stages of infection. *Trypanosoma cruzi* has a single copy ecotin-like gene similar to *L. major* ISP2. Similarly to Leishmania, *T. cruzi* apparently lacks genes encoding clan PA serine peptidases, raising the possibility that ISP2 could function to modulate the activity of host enzymes, influencing parasite interaction with the host cell. We cloned and expressed recombinant Histidine-tagged *T. cruzi* ISP2 that inactivates trypsin, chymotrypsin, neutrophil elastase and cathepsin G, displaying Ki values in the nanomolar range. To investigate the role of *T. cruzi* ISP2, we generated parasite lines overexpressing ISP2 and single allele ISP2 knock-out lines. Epimastigotes overexpressing ISP2 grow normally in culture and were submitted to in vitro metacyclogenesis. Trypomastigotes were used to infect LLC-MK2 cells to generate tissue culture trypomastigotes (TCT). TCTs overexpressing ISP2 secrete higher levels of the inhibitor, as observed by western blot. In vitro assays of infection using different cell lines showed a distinct infective behavior depending on the cell type analysed. ISP2-over expressors were more infective to smooth muscle and epithelial cell lines, whereas in macrophages (RAW 264.7) no differences were observed. The generation of ISP2-deficient mutant cell lines is ongoing at present. **Supported by:**FAPERJ

BC032 - **CRUZIPAIN PARTICIPATES IN *T. CRUZI* INVASION BY ACTIVATING LATENT TGF-β FROM HOST CELLS**

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Several studies indicate that *T. cruzi* infectivity requires the activity of its main lysosomal cysteine peptidase, cruzipain. It was also described that the parasitic invasion process is dependent on the activation of the host cell TGF-β pathway by *T. cruzi*. In this study we tested the hypothesis that cruzipain could be an important activator of latent TGF-β and thereby triggers the TGF-β-mediated events in the development of Chagas disease. For this, we used *in vitro* assays in order to check the ability of *T. cruzi* and cruzipain to activate latent TGF-β. We observed that both the epimastigote forms of *T. cruzi* and exogenous cruzipain were able to activate latent TGF-β. The use of a cysteine peptidase inhibitor, Z-Phe-Ala-FMK, completely inhibited this activation. The addition of cruzipain or latent TGF-β in an *in vitro* model of *T. cruzi* infection, increased the percentage of infected cells. Reciprocally, addition of Z-Phe-Ala-FMK or anti-TGF-β antibody strongly reduced the number of infected cells. The activities of cruzipain and TGF-β on the process of cell invasion seem to be functionally linked. Since TGF-β is described as an important regulatory cytokine in the development of chagasic cardiomyopathy, the impairment of its activity is desirable. Thus, our results indicate that the use of anti-cruzipain compounds could be applied for Chagas therapy, not only for their trypanocidal effect, but also for their inhibitory actions on TGF-β activity. **Supported by:**CNPq/Fiocruz/PAPES V

BC033 - **EFFECT OF *TRYPANOSOMA CRUZI* INFECTION ON CARDIAC FIBROBLASTS *IN VITRO* AND *IN VIVO***

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Cardimyopathy is an important manifestation during the Chagas disease. In this context, the aim of the present work was to investigate the mechanisms involved on generation of cardiac fibrosis induced by *Trypanosoma cruzi* (*T. cruzi*) infection. We studied *in vitro* and *in vivo* the effect of *T. cruzi* infection (Y strain) on activation of cardiac fibroblasts (CF), characterized by proliferation and extracellular matrix protein production. By fluorescence microscopy (DAPI), we evaluated in culture of CF the number of cells per field as an indirect measurement of cellular proliferation. Moreover, by western blot and immunofluorescence we analyzed, the expression of α -smooth muscle actin (α-SMA) and fibronectin (FBN) *in vitro* and *in vivo*. *In vitro* the DAPI analysis indicated that after 72 hours of infection there was no difference in the number of cells per field when we compared CF cultures controls with infected ones, suggesting that *T. cruzi* infection did not induces CF proliferation *in vitro*. Immunofluorescence and western blot analysis of α-SMA and FBN showed that there was no variation in the total expression levels of these proteins comparing non-infected and infected cultures (6h – 144h). However, by immunofluorescence we observed increase in the percentage of CF strongly labeled for both proteins and a heterogeneous staining pattern in infected cultures. In the murine model (swiss mice), we verified by western blot, that infection with *T. cruzi* (Y strain) induced increase of cardiac fibronectin and α-SMA expression. To test the role of inflammation on fibroblast activation we evaluated *in vitro* the expression of α -smooth muscle actin (α-SMA) and FBN during co-culture of CF and inflammatory cells. Our results demonstrated that macrophages and splenocytes of infected animals were able to induce increase on expression of both proteins in infected CF cultures. Our results suggest an important role of inflammation on activation of CF during *T. cruzi* infection. **Supported by:**CNPQ

BC034 - **MITOSOMES IN *GIARDIA INTESTINALIS*: NEW INSIGHTS INTO RESIDENT ENZYMES AND ITS BEHAVIOR DURING THE DIFFERENTIATION**

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Mitosomes are double-membrane bounded organelles found in some unicellular eukaryotes. Recently,these organelles were identified in the human intestinal parasite *Giardia*, which has been considered one of the earliest branching eukaryotes. The mitosomes functions in *Giardia* are poorly understood, mainly because this organelle was identified recently. Here, we performed assays to track some important enzymes found in hydrogenosomes and/or mitochondria, and thus, we describe for the first time the presence of malic enzyme in this organelle. In addition, enzymes such as heat-shock protein 70 (HSP70), chaperonin 60 (cpn60) and ferredoxin oxidoreductase (PFO) were tracked during *Giardia* differentiation. To investigate the addressed questions, the antibodies were used and detected by immunofluorescence, immunocytochemistry and Western blotting assays. A double-labeling assay was also performed using the anti-giardia malic enzyme and anti-*gia*HSP70. Furthermore, the parasites were induced to encyst *in vitro* and double-labeled using both anti-*gia*CWP1 with one of the following antibodies: anti-*gia*HSP70, anti-*gia*PFO and anti-*gia*CNP60. The labeling was revealed by confocal and transmission electron microscopy in both trophozoite and *in vitro* encysting parasites in different times (3h, 6h, 12h, 21h and mature cysts). The malic enzyme labeling was found within specific structures, which are double-membraned, spherical and co-localized with anti-*gia*HSP70. During differentiation, we observed that the fluorescence labeling using the mitosome antibodies presented here, starts to decrease at 12h of the encystation process and no fluorescence signal was identified in 21h. However, in mature cysts the labeling was observed again. In conclusion, we showed by the first time the localization of the malic enzyme in within the mitosomes of *Giardia*. Moreover, it was identified a down regulation of mitosomes enzymes during *G. intestinalis* differentiation. **Supported by:**CNPq, FAPERJ, PRONEX, AUSU

BC035 - **INVESTIGATION ON THE BEHAVIOR OF *TRICHOMONAS TENAX***

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T. tenax is a commensal of the human mouth found under conditions of poor oral hygiene or associated with periodontal diseases. There are some controversies concerning the pathogenicity of this protozoan. *T. tenax* is very similar to *Trichomonas vaginalis*, a parasite that inhabits the human genitourinary tract and known to induce damage to various mammalian cells. Currently, there is a discussion whether *T. tenax* would be a genetic variant of *T. vaginalis.* The aim of this study was to investigate the capacity of *T. tenax* to provoke damage to mammalian cells and also to compare their cytotoxicity with that of *T. vaginalis*. For this, interaction assays of both protozoa with host cells, such as oral cells collected by scraping and 3D spheroids were performed with on the ratio of 5:1 in different periods of time. The samples were examined by scanning electron microscope (SEM) and the cytotoxic effects of the protozoa were analyzed by viability assays such as the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide). Our results indicated several similarities between both parasites, such as (1) *T. tenax* and *T. vaginalis* display four anterior flagella and one recurrent flagellum; (2) both trichomonad species were able to adhere on mammalian cells and both provoked injury. Interestingly, *T. tenax* provoked damage to 3D spheroids of oral cells and also caused retraction of microvilli*.* Take together, these results indicated that *T. tenax* could be a parasite, not a commensal, and further experiments are in course to better confirm its pathogenicity.
Supported by:CNPq, FAPERJ, PRONEX and AUSU

BC036 - **COMPARISON BETWEEN THE INTRACELLULAR ACTIVITY OF SERINE AND CYSTEINE PROTEASES IN *P. FALCIPARUM* AND THE EFFECT OF SELECTIVE INHIBITORS**

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Malaria is caused by *Plasmodium* parasites and remains one of the most lethal diseases. A better understanding of the cellular and biochemical pathways of the parasite is fundamental to achieve better strategies to control the disease. The regulation of *Plasmodium* proteases through cellular signaling mechanisms is still poorly understood. Using the fluorogenic peptides Z-Phe-Arg- AMC and Z-Arg-Arg-AMC (10µM) as substrate, we performed spectrofluorometry measurements to investigate intracellular proteolysis of the serine and cysteine protease activity of *P. falciparum*. The data was expressed in % of activity (AUF/min) compared to Control (parasites with substrate only). Calcium increases in the cytosol from the ER by THG (10µM) and induced a rapid increase (89%) in intracellular proteolysis. Inhibition of calmodulin by Calmidazolium (10 µM), resulted in a increase (100%) in the hydrolysis of the fluorogenic substrate Z-Phe-Arg-AMC. Interestingly, THG and Calmidazolium did not cause an increase in Z-Arg-Arg-AMC hydrolysis rate. The specific cysteine protease inhibitor E-64 (10µM) inhibited 85% of Z-Phe-Arg- AMC hydrolysis and 65.7% of Z-Arg-Arg-AM hydrolysis. However, the serine protease inhibitor PMSF (10µM) inhibited only the proteolysis of Z-Arg-Arg-AMC (35%). Other specific inhibitors were analyzed, such as Ortho-phenanthroline (metalloproteinases inhibitor) and Pepstatin A (aspartyl proteases inhibitor). Ortho-phenanthroline (10µM) was able to inhibit only the Z-Arg-Arg-AMC hydrolysis (52,5%). Both substrates hydrolysis does not significantly inhibited by Pepstatin A (10µM). The results clearly demonstrate the predominance of cysteine proteases (nearly 73% of total activity) whereas serine proteases represent only 10% in *Plasmodium falciparum* proteolysis machinery. Our data present a selective assay for measurement and inhibition of cysteine and serine proteases by *Plasmodium falciparum* and an important modulatory effect by Ca^{2+}/Calmodulin in proteolysis.
Supported by:FAPESP

BC037 - **EFFECT OF CHLOROQUINE COMBINATION WITH CAPTOPRIL IN *PLASMODIUM CHABAUDI* PROTEOLYSIS**

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Malaria remains a major global health problem with more than 104 countries affected. The continuous development of parasite resistance to available antimalarial drugs emphasizes the importance of understanding the biochemical characteristics of proteases over parasite life cycle. This study aims to evaluate the effect of Chloroquine association with Captopril (Angiotensin-converting enzyme inhibitor) in *Plasmodium chabaudi* proteolysis and monitoring the ionic homeostasis of intracellular compartments. The parasites were isolated for characterization of intracellular hydrolysis activity after chloroquine (1, 10, 25 and 50µM) incubation (10 min) and captopril (1, 10 and 25µM). The combination of 10µM chloroquine + 10µM captopril and 25µM chloroquine + 10µM captopril were assayed in lysed and unlysed cells. The measurement was performed in fluorescence spectrofluorimeter and corresponds to intracellular hydrolysis of the fluorogenic substrates Z-FR-MCA 10µM (for cysteine-proteases) and Abz-FR-(Dnp) P-OH 10µM (for ACE). We observed inhibition of proteolytic activity (AUF/min) relative to the control (parasite without incubation) in conditions: chloroquine 1 µM, 10µM, 25µM (~ 50% of inhibition) and 50µM (33.1 %) with captopril 1µM (44.1%), 10µM (52.1%), 25µM (55%) and combination of 10µM captopril + 10µM chloroquine (81%) and 10µM captopril + 25µM chloroquine (87.2%) hydrolysis with Z-FR-MCA substrate. The combination of 10µM chloroquine + 10µM captopril promoted and inhibition of 85.8% in Abz-FR-(Dnp) P-OH substrate hydrolysis. The inhibition observed with captopril, a drug widely used for hypertension pathology support the previous data described by Bagnaresi et al., 2012 were *P. falciparum* is able to modulate kinin signaling pathways in vertebrate host and shows an additive effect with chloroquine against parasite proteolytic machinery. **Supported by:**FAPESP

BC038 - ***TRYPANOSOMA CRUZI* EVOLUTIVE FORMS BEHAVE DIFFERENTLY IN CHIMERIC PARASITOPHOROUS VACUOLES INFECTED WITH *LEISHMANIA AMAZONENSIS*.**

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Intracellular parasites such as *L.amazonensis* (long term) or *T.cruzi* (transiently) are lodged within parasitophorous vacuoles (PVs) in host cells. These structures are customized by the parasites, which develop different PVs morphologies and regulate the passage of nutrients and immune host factors by interfering in PV membrane transporters or in vesicle fusion events.We investigated the coinfection between *L.amazonensis* amastigotes and *T.cruzi* epimastigotes (EPIs) or metacyclic trypomastigotes (MTs) in macrophages *in vitro*. The intracellular coinfection involving these two pathogens generates chimeric vacuoles containing both parasites, which allow for investigate the influence of a vacuole customized for the first pathogen on survival and differentiation of the second. To determine the fusion between *L. amazonensis* and *T.cruzi* PVs,we infected RAW 264.7 macrophage-like cells with non-fluorescent *L. amazonensis* amastigotes for 48 hours.After the development of spacious “recipient” PVs,we superinfected the macrophages with GFP-labeled *T.cruzi* EPIs or MTs.The formation of chimeric vacuoles, multiplication and differentiation of *T.cruzi* were observed in live cells at different time points post-*T.cruzi* infection (2, 24 and 72 h) under epifluorescence microscope.We observed that the non-infective *T.cruzi* EPIs and infective MTs were transferred to *L.amazonensis* large vacuoles, forming chimeric PVs.Although EPIs remain motile and multiply within chimeric vacuoles, MTs presented abnormal morphology and motility.The results demonstrate that the large vacuoles of *L.amazonensis* are permissive to *T.cruzi* and spare non-infective epimastigote from destruction when chimeric PVs are formed. We provide evidence that infective MTs are not adapted to *L. amazonensis* PVs environment.We further intend to evaluate the molecular mechanisms that influence the formation of chimeric vacuoles, the viability and the infectivity of these *T. cruzi* forms following coinfection with *L. amazonensis.* **Supported by:**FAPESP, CNPq, CAPES

BC039 - **ACTIN ROLE ON TRANSFERRIN ENDOCYTOSIS BY *TRYPANOSOMA CRUZI* EPIMASTIGOTES**

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Trypanosoma cruzi epimastigote forms present high endocytic activity, differing from trypomastigote and amastigote forms. Epimastigotes have two endocytic portals (Soares, Parasitol. Res. 99:321, 2006): the flagellar pocket and the cytostome-cytopharynx complex, described as a funnel-shaped opening, the cytostome that invaginates deeply, forming the cytopharynx (Milder & Deane, J. Protozool. 16:730, 1969). Our group showed recently that specialized microtubules support the cytostome-cytopharynx, playing a crucial role on its ultrastructure. Corrêa and coworkers (Exp. Parasitol. 119:58, 2008) have shown a drastic reduction in transferrin uptake and alterations in cytopharynx structure after treatment with Cytochalasin. Nevertheless, the role of the cytoskeleton on endocytosis by *T. cruzi* is not clear. In this work we focused on actin filaments, which are poorly characterized in *T. cruzi*, and its role on endocytosis. We have used transferrin-FITC as tracer in endocytosis assays by epimastigotes pretreated with Cytochalasin D, which destabilizes actin filaments, and Latrunculin B, which binds to G-actin and hinders its polymerization. The results were quantified by fluorimetry and also observed by fluorescence microscopy. We further performed immunoelectron microscopy on ultrathin sections of parasites embedded in LR White using a polyclonal antibody anti-Tc actin produced by Cevallos et al (Exp. Parasitol. 127:249, 2011). Our results showed that Cytochalasin D and Latrunculin B were both able to decrease transferrin endocytosis by about 80 %, without affecting parasite viability. By fluorescence microscopy, we found the endocytic tracer retained at the cytostome. Using transmission electron microscopy, we observed that anti-TcActin antibody recognized thin filaments not yet described at the cytostome opening, among other structures. These data suggest that actin acts on the initial stages of the endocytosis in *T. cruzi*. **Supported by:**PIBIC/CNPq, CAPES, FAPERJ, CNPq

BC040 - **DIVERGENT BIOLOGICAL PROFILES OF *TRYPANOSOMA CRUZI* I AND II ISOLATED FROM ORAL INFECTION OUTBREAK IN SANTA CATARINA, BRAZIL**

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Studies using mixed TcI/TcII *Trypanosoma cruzi* strains isolated during an acute outbreak of Chagas disease in Santa Catarina State, Brazil, revealed a selection towards TcII during both murine and human macrophages infection *in vitro*. In the present work, we investigated if this TcII selection also occurs in an *in vivo* model. Groups of six two-months old C57BL/6 mice, were orally infected by gavage with 0,1 mL of sugar cane juice containing 10^4 metacyclic trypomastigotes of SC90 (TcI), SC95 (TcII), SC92 (TcI/TcII) and SC93 (TcI/TcII) strains. Blood parasitemia was recorded in a two-day interval by the Brener's method. SC95, SC92 and SC93 strains presented the peak of parasitemia at 19 days post-infection (dpi) (293; 128 and 49x10^3 parasites/mL of blood, respectively), whereas SC90 was subpatent. On the 10^{th} and 30^{th} dpi, PCR assays directed to the mini-exon gene were carried out using DNA extracted from whole blood (50µL), allowing detection and typing of the strains (TcI/TcII). At 30 dpi, blood from mice infected with SC92 and SC93 strains was collected for hemoculture and the parasites cloned by limiting dilution technique in LIT medium. Tissue samples from esophagus, heart, quadriceps and small and large intestines were collected from each mouse at 30 and 120 dpi for PCR assays. Although only TcII was detected by PCR in the blood after 10 and 30 dpi, mixed TcI/TcII pattern was found in 12,5% of the SC92 clones (8/64) and in 29,5% of the SC93 clones (18/61). The remaining positive cultures were all typed as TcII. The expected of 330bp band amplified from *T. cruzi* kDNA was obtained for all tissues from infected mice. Our results show that TcII strains present a higher infectivity and intracellular multiplication rate than TcI. Also, TcI parasites infecting mice by oral route are not eliminated and persist during the acute and chronic phases of disease. **Supported by:** CNPq, CAPES, FINEP and UFSC

BC041 - **INVESTIGATION OF TGF-B PATHWAY IN AN EXPERIMENTAL MODEL OF ACUTE CHAGAS DISEASE**

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Studies developed by our group in the last years have shown the involvement of TGF-b in acute and chronic Chagas heart disease, with exacerbation of plasma levels and the activation of cellular signaling pathway as peculiar aspects of patients in the advanced stages of this disease, associated with high levels of fibrosis. The regulation of development and degradation of extracellular matrix compounds is the basis of fibrotic processes and TGF-b is considered as one of the key regulators of this process. In the present study we investigated the activity of TGF-b signaling pathway, including receptors and signaling proteins in the heart of animals infected with T. cruzi during the experimental acute phase and we also assessed the serum levels of TGF-b (total and active). We observed that T. cruzi-infected animals presented increased expression of TGF-b receptors as well as increased phosphorylation of Smad2/3, JUNK, p38 and ERK during the acute phase of Chagas disease. Furthermore, we correlated these activities with cellular factors involved in the fibrotic process induced in response to TGF-b. Thus, we evaluated the expression and/or deposition of some proteins that are responsive and regulated by TGF-b. Therefore, we observed that the expression of fibronectin and CTGF was increased and that infected animals showed an increased deposition of collagen, after the 15th day of infection. Correlated with the increase of TGF-b activity in the heart, we found that serum levels of total TGF-b were significantly higher during acute infection. Taken together, our data suggest that the commitment of this organ starts around the 15th day, when we observed the presence of large inflammatory infiltrates and high parasite load, associated with increased activity of TGF-b pathway and expression of its main components. Our results once again confirm the importance of this cytokine in the development and maintenance of cardiac damage in response to T. cruzi infection. **Supported by:**CNPq / INSERM / PAPES V

BC042 - **IDENTIFICATION OF GAMMA-SECRETASE COMPLEX PROTEINS IN *LEISHMANIA* AND OVEREXPRESSION OF ITS CATALYTIC UNIT, THE PRESENILIN, IN *LEISHMANIA AMAZONENSIS***

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Previous works in our laboratory identified an aspartyl proteinase that has homology to the human presenilin, the enzyme of the gamma-secretase complex. In humans, the gamma-secretase complex cleaves type I transmembrane proteins and contains four proteins: presenilin (PS), PEN-2, nicastrin and APH-1. Although the gamma-secretase complex is very well characterized in humans, its presence and function are not established in *Leishmania*. In this work we have identified the gamma-secretase complex proteins in *Leishmania* genome database by homology approaches and evaluated the expression by reverse transcriptase polymerase chain reaction (RT-PCR). Moreover, we have induced PS overexpression in *L. amazonensis* promastigotes using pNUS plasmid and evaluated the expression of PS and PEN-2, as well as the viability and growth. Among the four proteins of the complex, we have identified only PS and PEN-2 in the *Leishmania* genome, with 31% and 35% of homology to each human protein, respectively. The PS and PEN-2 proteins of *Leishmania* are very well conserved proteins, with high degree of sequence identity among different *Leishmania* species (80% and 100%, respectively). The two forms of *L. amazonensis*, amastigote and promastigote, expressed the PS and the PEN-2 proteins, as verified by RT-PCR. The overexpression of PS (between 20 to 60-fold of wild type expression) did not affect the PEN-2 expression, as well as did not affect the viability and growth of promastigotes. In this work we have demonstrated that *Leishmania* has two proteins of gamma-secretase complex, PS and PEN-2, indicating the presence of the complex in *Leishmania*. **Supported by:**CNPq, FAPERJ e CAPES

BC043 - **THREE-DIMENSIONAL ANALYSIS OF HEMOGLOBIN UPTAKE AND HEMOZOIN NUCLEATION BY THE RODENT PARASITE *PLASMODIUM CHABAUDI***

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Malaria is a disease caused by protozoan parasites from the genus *Plasmodium.* Disease pathology is associated with the asexual replication of *Plasmodium* in the erythrocyte of the mammalian host. In the course of infection, different forms of the parasite are seen as it progresses from the ring stage to the trophozoite and then to the replicating schizont stage. During its intraerythrocytic cycle the parasite internalizes massive amounts of hemoglobin from the red blood cell cytosol. Hemoglobin digestion occurs in an acid compartment termed food vacuole, where its degradation generates free aminoacids and toxic byproducts, namely heme. It is known that free heme can generate free-radicals, causing molecular and cellular damage. In order to avoid these effects the parasite quickly sequesters this released heme by the intracellular formation of inert crystals called hemozoin. Although hemoglobin uptake and heme crystallization are physiological steps used as target for many antimalarial drugs the fine mechanisms underlying hemoglobin crystallization are still under discussion. In this work, we used scanning transmission electron microscopy (STEM), tomography to analyze the nucleation of hemozoin crystals during the intraerythrocytic stages of *Plasmodium chabaudi.* Serial STEM tomography revealed the dispersion of hemozoin crystals inside the food vacuole and their location in cytoplasm of the parasite, showing *t*hat hemozoin nucleation begins in small food vacuoles near the surface of the parasite during the early trophozoite stage*. As P. chabaudi* developments, larger food vacuoles were observed, containin*g la*rge amounts of hemozoin. Concentric structures forming myelin figures, which may be related to mechanisms of hemoglobin degradation, were also observed near the newly formed hemozoin crystals. Taking together, these results provide new insights on the mechanisms of hemozoin nucleation in rodent malaria parasites. **Supported by:**Cnpq, Faperj

BC044 - **IRON REGULATES CYSTEINE PROTEASE EXPRESSION OF *TRITRICHOMONAS FOETUS* AND MODULATES THE TRANSFORMATION OF TROPHOZOITES TO PSEUDOCYSTS**

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The protozoan *Tritrichomonas foetus* is an anaerobic parasite that infects the urogenital tract of bovines, causing bovine trichomoniasis. While bulls are asymptomatic, cows display symptoms ranging from mild infections to severe clinical manifestation including vaginitis, cervicitis, and endometritis that may result in transiente or permanent infertility and fetal loss. Iron is an essential element to support the growth and survival of *T. foetus*. The aim of the present study was to investigate the effect of iron on the ultrastructure and peptidase profile of *T. foetus.* Parasites were cultivated in iron-rich and iron-depleted medium and were analyzed by scanning and transmission electron microscopy, 1-DE zymography and the activity of proteases was quantified by in-solution assays using the fluorogenic substrate Z-Phe-Arg-MCA. In the absence of iron, trophozoites underwent transformation from their usual ellipsoid forms to a rounded form, whose flagella were internalized and unexpected concentric membranar structure appearance in the cytosol. Furthermore, iron depletion leads to a strong decrease in the activities of cysteine-proteases, verified by zymographic analysis and in-solution assays. Thus, our data indicate that iron has a role in the regulation of the morphological transformation of *T. foetus* as well as in the modulation of peptidase expression of the parasite that may play a critical role the host-parasite interaction. **Supported by:**UFSJ

BC045 - **LOCALIZATION OF PHOSPHATIDYLSERINE ON THE OUTER PLASMA MEMBRANE OF *TOXOPLASMA GONDII* BY FLUORESCENCE MICROSCOPY**

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Toxoplasmosis is caused by *Toxoplasma gondii*, an obligate intracellular protozoan that has a worldwide distribution. About one-third of the human population is seropositive for *T. gondii*. The majority of infections are asymptomatic, but severe clinical manifestations may arise in immunocompromised individuals. The success of this parasite infection depend on the various evasion mechanisms of the host immune system, such as "apoptotic mimicry". Cells undergoing apoptosis are able to translocate, through flippases, phosphatidylserine (PS) from the intracellular plasma membrane leaflet to the extracellular medium, thus, inhibiting an inflammatory response in the host. *Toxoplasma gondii* is an example of a parasite that mimics apoptotic cells by PS exposure, being capable to disable the microbicidal action of macrophages. It was demonstrated that the mechanism used by *T. gondii* is similar to that shown in infective forms of *Leishmania amazonesis* and *Trypanosoma cruzi* that also expose PS. In this study, we used immunofluorescence microscopy to visualize and locate the PS exposed on the plasma membrane of parasites by labelling with fluorescent annexin-V. As expected, about 40% of the total population of *T. gondii* exposed PS. Different labelling patterns was found: apical complex, posterior end, side and in some cases throughout the body of the parasite. These results suggest that *T. gondii* expose PS in different parts of the parasite, especially in the portions that will come in contact with the host cell during the process of active penetration. **Supported by:**FAPERJ CAPES CNPq