BC001 - Structural Basis of the Interaction of a *Trypanosoma cruzi* Surface Molecule Implicated in Oral Infection with Host Cells and Gastric Mucin

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Host cell invasion and dissemination within the host are hallmarks of virulence for many pathogenic microorganisms. As concerns *Trypanosoma cruzi* that causes Chagas disease, the insect vector-derived metacyclic trypomastigotes (MT) initiate infection by invading host cells, and later blood trypomastigotes disseminate to diverse organs and tissues. Studies with MT generated in vitro and tissue culture-derived trypomastigotes (TCT), as counterparts of insect-borne and bloodstream parasites, have implicated members of the gp85/trans-sialidase superfamily, MT gp82 and TCT Tc85-11, in cell invasion and interaction with host factors. Here we analyzed the gp82 structure/function characteristics and compared them with those previously reported for Tc85-11. One of the gp82 sequences identified as a cell binding site consisted of an alpha-helix, which connects the N-terminal beta-propeller domain to the C-terminal beta-sandwich domain where the second binding site is nested. In the gp82 structure model, both sites were exposed at the surface. Unlike gp82, the Tc85-11 cell adhesion sites are located in the N-terminal beta-propeller region. The gp82 sequence corresponding to the epitope for a monoclonal antibody that inhibits MT entry into target cells was exposed on the surface, upstream and contiguous to the alpha-helix. Located downstream and close to the alpha-helix was the gp82 gastric mucin binding site, which plays a central role in oral T. cruzi infection. The sequences equivalent to Tc85-11 laminin-binding sites, which have been associated with the parasite ability to overcome extracellular matrices and basal laminae, was poorly conserved in gp82, compatible with its reduced capacity to bind laminin. Our study indicates that gp82 is structurally suited for MT to initiate infection by the oral route, whereas Tc85-11, with its affinity for laminin, would facilitate the parasite dissemination through diverse organs and tissues.**Supported by:**:CNpq - FAPESP

BC002 - *Giardia lamblia* infection: study of parasite adhesion to intestinal mucin and mucolytic activity as a mechanism of infection

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Giardia lamblia, a flagellated parasite protozoan, attaches to enterocytes from the small intestine in order to survive and multiply, thus causing the disease known as Giardiasis. As a mechanism of defense, the inner wall of the small intestine is covered by a mucus layer, composed mostly by a highly O-glycosylated (50-80% w/w) mucin glycoprotein (MUC 2). It is widely known that most intestinal pathogens possess mechanisms of trespassing the MUC 2 layer. This study investigates the ability of G. lamblia to degrade this mucus layer, allowing the protozoan to adhere to enterocytes. Intestinal mucus has been obtained from Balb/C mice-extracted small intestines by scraping the luminal part with a glass microscope slide into a 4M guanidine-HCl solution. After ultracentrifugation and dialysis, the material was checked for the presence of glycoproteins, glycosaminoglycans and proteoglycans by dot-blottings, stained with PAS-Schiff and Toluidine Blue. A degradation assay by live trophozoites was performed with the purified mouse intestinal mucus (MIM), using bovine submaxilary mucin (BSM) and porcine gastric mucin (PGM), as controls. After PAS-Schiff staining it was found that the G. lamblia trophozoites degrade considerably more MIM compared to BSM and PGM. This result was corroborated in transwell assays where the number of trophozoites that trespassed the MIM-coated insert was greater than with BSM or PGM-coated inserts. The data suggest that degradation of the mucus layer may be a mechanism exploited by G. lamblia trophozoites to penetrate the secreted and cellular mucus barrier. More assays will be performed to better elucidate the mucolytic capacity of G. lamblia. **Supported by:**:Capes e Fapesp

BC003 - The role of SUMOylation in the cell biology of *Giardia lamblia*

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The ubiquitin family of proteins constitutes important signaling pathway in eukaryotic cells. In the last years a new group of proteins with distinct role was described, the Small Ubiquitin Modifiers (SUMOs). Despite the ubiquitin function in degradation of proteins and apoptosis, SUMOs are correlated with stabilization and transport of proteins and DNA repair. While in superior eukaryotes four isoforms of SUMO were described with particular characteristics, in unicellular organisms just one SUMO isoform is found. *Giardia lamblia* is a unicellular protozoan parasite that colonizes the small intestine of mammals (including humans) causing diarrhea. The cell biology of this organism is very unique and the existence of SUMO pathway was suggested (unpublished results). In this study, the role of SUMOylation on the cell biology of G. lamblia was investigated. Immunofluorescence using anti--SUMO antibodies revealed the presence of SUMO--associated proteins in the cytosol, membrane periphery and the median body of trophozoites. SUMO labeling co--localizes with acetylated tubulin in the median body and with a VSP, but not with peripheral vesicles. In immunoblots of total extracts of trophozoites the anti--SUMO reacted with numerous bands but mainly with a protein with ~ 70 kDa protein. Immunoprecipitation confirmed that the 70 kDa protein is sumoylated tubulin as expected by the 10-20 kDa increase in protein mass. Finally, when trophozoites were incubated with HeLa cells, the cellular localization of SUMOs was altered suggesting a participation of SUMO--associated proteins in the establishment of G. lamblia infection.**Supported by:**:CNPQ, FAPESP

BC004 - Exploring the ultrastructure and function of the cytostome-cytopharinx complex of *Trypanosoma cruzi* epimastigotes

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The cytostome is an aperture at the plasma membrane close to the flagellar pocket (FP) followed by a profound invagination called cytopharinx. This complex is the major portal for endocytosis in *T. cruzi* epimastigotes. Data from literature has shown that this region presents a prominent glycocalix continuous to the preoral ridge (POR), the membrane domain between the cytostome and the FP, and an uncharacterized set of microtubules (MTs) and vesicles along the cytopharinx. Little is known about its detailed structure and the correlation with the endocytic function. In this work we focused on the 3D structure of the cytostome-cytopharinx complex trying to describe how endocytic cargo passes through and leaves it. Epimastigotes were submitted to endocytosis of transferrin-gold particles with short incubations times. The material was processed to electron microscopy and serial semi-thin sections were observed by electron tomography. The tomograms were aligned and the structures of interest were segmented using appropriated software. We observed seven MTs supporting the opening of cytostome and the cytopharinx. These MTs could be separated in two sets: a quartet coming from the FP that also sustains the POR and a triplet coming from the subpellicular microtubules. The MTs arrangement around the cytopharinx leaves a free side, where aligned vesicles were seen. As the invagination becomes deeper and thinner, two MTs finish, one from the quartet and one from the triplet. Moreover, the MTs continue even beyond the end of the cytopharinx and accompany vesicles and tubules that seem to be originated from the cytopharinx. We also used horseradish peroxidase as a tracer to follow the cytopharinx in FIB-SEM preparations. We measured the cytopharinx length in 25 whole cells and found out that the length is independent of cell cycle or differentiation stage. Together these data provides a new vision about the structure and dynamics of endocytic pathway of epimastigotes. **Supported by:**:Capes; CNPq

BC005 - Loss of endocytic activity during *Trypanosoma cruzi* metacyclogenesis

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Trypanosomatids require essential exogenous nutrients and growth factors in order to survive and divide. *Trypanosoma cruzi* epimastigotes acquire them by endocytosis via the cytostome and the flagellar pocket, both localized at the parasite's anterior region. The cytostome is an opening at the plasma membrane surface that deepens in a membrane invagination called cytopharinx. This structure is responsible for 85% of endocytic activity in epimastigotes, delivering cargo to reservosomes. T. cruzi loses its endocytic ability somewhere during the transformation into trypomastigotes (metacyclogenesis), as these infective forms are not able to uptake exogenous cargo and do not present the cytostome-cytopharinx complex. Between epimastigotes and trypomastigotes, three subsequent types of *T.cruzi* intermediate forms were described: Ia, where the kinetoplast is close to the elongated nucleus, Ib where the kinetoplast is located side by side with nucleus, and Ic that presents the kinetoplast posterior to the nucleus. The latter is the form that just precedes the complete morphological transformation into trypomastigotes (Ferreira et al., 2008). We have analyzed changes in the ultrastructure of endocytic apparatus and the endocytic activity along in vitro metacyclogenesis. After four hours in TAU3AAG, we have obtained 30% of intermediate forms; using transmission electron microscopy we observed that Ic intermediate forms still present a cytostome. Interestingly, this invagination maintained its typical morphology, even after the migration backwards, accompanying the kinetoplast. Moreover, the endocytic apparatus proved to be functional in Ic intermediate forms, as they were able to uptake transferrin-gold offered after metacyclogenesis and store in typical reservosomes. Our data indicate that the disassembly and loss of function of the endocytic machinery is a very late event in metacyclogenesis. **Supported by:**:CAPES

BC006 - Immunolocalization of 75kDa serine oligopeptidase in *Trypanosoma cruzi* bloodstream trypomastigotes

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The protozoan *Trypanosoma cruzi* is a causative agent of Chagas' disease, an endemic neglected illness in Latin America, responsible for considerable human mortality and morbidity. Two hosts are involved in *T. cruzi* life cycle (invertebrate and vertebrate), being two parasite forms observed in mammals: intracellular amastigotes and bloodstream trypomastigotes that can infect all cells, disseminating the disease. The current chemotherapy presents variable efficacy, especially in the chronic phase, besides others disadvantages, what incites the research for new medicines. In this context, the identification of molecules associated to infectivity and virulence is essential for future studies about drugs intervention. An extracellular 75kDa serine oligopeptidase was previously purified from epimasigotes supernatant with $(NH_4)SO_4$ precipitation followed by affinity chromatography. The rabbit anti-*T. cruzi* serine peptidase antiserum was also produced and the localization in the insect form investigated by fluorescence and electron microscopy, showing the labeling mainly in reservosomes, plasma membrane and flagellar pocket. Here, our goal is identify the presence and localization of serine oligopeptidase in bloodstream trypomastigotes. Our fluorescence microscopy evidences demonstrated the labeling with the antiserum in bloodstream stages, essentially in the central and posterior region of the parasites close to flagellar pocket, presenting similar pattern to the epimastigotes. The presence of serine oligopeptidase in both *T. cruzi* forms suggests a conserved role for the parasite cell biology, however the exact participation of this enzyme must be evaluated by molecular and biochemistry assays. **Supported by::**CNPq, FAPERJ e FIOCRUZ

BC007 - *Trypanosoma cruzi* factors required for metacyclic trypomastigote migration through the gastric mucin layer

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Studies on oral T. cruzi infection in mice have shown that metacyclic trypomastigotes (MT) invade the gastric mucosal epithelium. To reach the target epithelial cells, MT have to traverse the mucus barrier, whose main macromolecular component is the gastric mucin (GM). Selective MT binding to GM, mediated by the surface molecule gp82, apparently directs the parasites to their target cells. Here we performed experiments to demonstrate that MT migration through a GM layer is in fact gp82-mediated. MT migration through transwell filters coated with GM mixed with J18, the recombinant protein containing the full length gp82 sequence fused to GST, was negligible. Filters coated with GM mixed with GST allowed efficient MT translocation, similarly to filters coated with GM alone. Transwell filters coated with GM mixed with the synthetic peptide P7 or P7* were also tested. In filters coated with GM mixed with peptide P7, containing the GM-binding site of gp82, parasite migration was almost completely abolished, whereas peptide P7*, with the same composition as p7 but with a scrambled sequence, had no effect. We also addressed the question whether MT secreted mucinolytic activitiy that could contribute for migration. Purified MT were maintained in PBS for 18 horas, a condition that preserves the infective capacity. After centrifugation, the supernatant (MT secretion) was collected and its mucinolytic activity was tested. GM was incubated with MT secretion for 4 h, and then analyzed by staining SDS-PAGE gel with Schiff reagent, or by Western blotting using anti-GM antibodies. Both procedures revealed GM degradation. To determine whether proteases contributed to mucinolytic activity, GM was incubated with MT secretion in absence or in the presence of diverse protease inhibitors. PMSF, a serine protease inhibitor, reduced GM degradation. Assays to determine the involvement of proteases in MT migration through a GM layer are under way. **Supported by:**:FAPESP e CNPq

BC008 - The Diverse and Dynamic Nature of Leishmania Parasitophorous Vacuoles Studied by Multidimensional Imaging

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An important area in the cell biology of intracellular parasitism is the customization of parasitophorous vacuoles (PVs) by prokaryotic or eukaryotic intracellular microorganisms. We were curious to compare PV biogenesis in primary mouse bone marrow-derived macrophages exposed to carefully prepared amastigotes of either *Leishmania major* or *L. amazonensis*. While tight-fitting PVs are housing one or two *L. major* amastigotes, giant PVs are housing many *L. amazonensis* amastigotes. In this study, using multidimensional imaging of live cells, we compare and characterize the PV biogenesis/remodeling of macrophages i) hosting amastigotes of either *L. major* or *L. amazonensis* and ii) loaded with Lysotracker, a lysosomotropic fluorescent probe. Three dynamic features of *Leishmania* amastigote-hosting PVs are documented: they range from i) entry of Lysotracker transients within tight-fitting, fission-prone *L. major* amastigote-housing PVs; ii) the decrease in the number of macrophage acidic vesicles during the *L. major* PV fission or *L. amazonensis* PV enlargement; to iii) the *L. amazonensis* PV remodeling after homotypic fusion. The high content information of multidimensional images allowed updating our understanding of the *Leishmania* species-specific differences in PV biogenesis/remodeling and could be useful for the study of other intracellular microorganisms. **Supported by:**:FAPESP

BC009 - Intracardiac injection of Dm28c *Trypanosoma cruzi* provides a model of infection-associated myocarditis and heart fibrosis that depends critically on cooperative activation of the kallikrein/kinin system and the endothelin pathway

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In this study, we tested the hypothesis (Andrade *et al*., 2012; Scharfstein and Andrade, 2011) that kinins released intramyocardiacally by Dm28c trypomastigotes, acting cooperatively with cardiac endothelins, infect heart tissues through the activation of bradykinin (BKRs) and endothelin receptors (ETRs). Guided by high-resolution echocardiography, we injected tissue-culture derived trypomastigotes (TCTs) in the left ventricle of C57BL/6 $B_2R^{+/+}$(wt), $B_2R^{-/-}$ or Balb/c (naïve). One hour before parasite inoculation, wt mice were treated with a single dose (systemic) of (i) HOE-140 (BK_2R antagonist) (ii) B_1R antagonist (iii) Bosentan (ET_AR/ET_BR antagonist). The functional parameters analyzed p.i.were intracardiac oedema (2 hp.i.); parasite load and mRNA levels of chemokines/cytokine in heart tissues (qPCR); myocarditis and fibrosis at 30 d p.i. As predicted, Dm28c TCTs evoked an early-phase intracardiac oedema in wt mice. In contrast, plasma extravasation was virtually abolished in the heart of $B_2R^{-/-}$ mice, or in infected-$B_2R^{+/+}$ mice that were either pretreated with a single dose of BKR antagonists or with Bosentan. Strikingly, our results showed that parasite load in the heart tissue (3 dp.i.) was markedly reduced by both GPCR antagonists. Collectively, these findings suggest that kinins generated intracardiacally may potentiate trypomastigote invasion of cardiovascular cells through the cooperative signaling of BKRs and ETRs. We then asked whether these pharmacological interventions, all of which strictly made at the onset of chagasic infection, could attenuate heart pathology in the long term. Indeed, our results (30 d p.i.) showed that myocarditis and fibrosis were virtually absent in infected mice that were pretreated with BKR or ETR antagonists whereas control mice exhibited conspicuous pathology. Additional studies are required to evaluate whether these anti-inflammatory drugs might ameliorate heart pathology during the chronic stage of Dm28c infection. **Supported by:**::CNPq, FAPERJ, Instituto Nacional de Ciência e Tecnologia de Biologia Estrutural e Bioimagem (INBEB)

BC010 - Neutrophil extracellular traps (NETs) decrease the viability of *Toxoplasma gondii*

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Toxoplasma gondii is an obligate intracellular parasite that can invade any nucleated cell from warm blooded animals. *T. gondii* is the etiologic agent of toxoplasmosis, a disease with worldwide distribution that can be a serious and deadly for unborn children and immune compromised patients. Neutrophils are essential for innate immune response, fundamental to control of infections being, in general, the first cells to be recruited at the inflammation sites. They are able to destroy pathogens either by phagocytosis or by releasing cytoplasmic granules. A new mechanism of neutrophil cell death was described: NETosis. It involves the release of neutrophil extracellular traps (NETs) rich in DNA and proteins, such as histones and elastase. These NETs can immobilize and kill bacteria, fungi and parasites, also providing a high concentration of antimicrobial molecules. Thus, the aim of our work is to evaluate if *T. gondii* is able to release NETs and to analyze the infectivity of the parasite after incubation with the supernatants rich in NETs components. For this, tachyzoites from the RH strain of *T. gondii* were incubated with supernatants of neutrophil activated by phorbol-12-myristate-13-acetate (PMA) and then these parasites interacted with LLC-MK_2 epithelial cells for 24 hours. Many parasites were seen trapped in NET filaments, maintaining their normal shape, without morphological dead signals. However, the viability of the parasites was compromising because their infectivity was decreased. According to our results, we conclude that NETs could participate in the control during the acute phase of toxoplasmosis.**Supported by:**::CNPq amd FAPERJ

BC011 - Investigation of target sequences of the secreted acid phosphatase in human malaria parasite *Plasmodium falciparum*

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A new possibility for fighting malaria might be the nutrient uptake of the parasite during the blood stage. However a variety of nutrients are phosphorylated which interfere with the uptake process and consequently the parasite needs to extracellular de-phosphorylate these nutrients prior uptake. Genomic analysis of *Plasmodium falciparum* identified an acid phosphatase and biochemical analysis of the recombinant enzyme revealed a broad substrate acceptance at an acidic pH optimum. In order to follow the localization of this protein, green fluorescent protein (GFP) chimeras were generated. Since the plasmodial SAP consists of an N-terminal signal anchor this sequence (corresponding to the encoding region of the first 26 amino acid residues) was cloned in front of GFP and analyzed for its localization within the cell. The fluorescence signal was found in the periphery of the parasite suggesting a transport of the plasmodial protein to the parasite plasma membrane (PPM). In contrast the full length protein was visualized in the food vacuole (FV) of the parasite. These results clearly indicate a transport process of the protein from the PPM towards the FV. Subsequently, mutagenic studies within the C-terminal region (corresponding to the encoding region of the last 63 amino acid residues) of the respective GFP chimeric protein suggest its necessity for protein trafficking into the FV.**Supported by:**:Deutsche Forschungsgemeinschaft

BC012 - *Trypanosoma cruzi* infectivity and Ssp-4 expression on different parasite isolates: a possible correlation.

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Trypanosoma cruzi amastigotes derived from the extracellular differentiation of tissue culture trypomastigotes (TCT) are as capable of infecting cells in vitro and in vivo as the classically described infective forms metacyclic and bloodstream trypomastigotes. The so called extracellular amastigotes (EA) display stage-specific surface components such as the antigen Ssp-4, an 84 kDa glycoprotein anchored to the membrane by a GPI anchor, which role in the parasite interaction with the host cell is still unknown. Considering that the expression of carbohydrate epitopes of Ssp-4 varies among *T. cruzi* isolates (grouped into six distinct lineages, Tc I to VI), the aim of the present study is to evaluate the expression of Ssp-4 in *T. cruzi* from different lineages and correlate it to their different infectivity towards host cells. The expression of Ssp-4 antigen was evaluated by confocal microscopy and western blotting using the monoclonal antibodies (Mabs) 2C2 and 1D9. In parallel, invasion assays was performed by the addition of EA or TCT forms to HeLa cell cultures and the number of internalized parasites was determined by microscopic countings. In contrast to TCT forms, which the isolate 863 (Tc III) presented the highest infectivity among other isolates tested, EA forms from the isolates G and 1522 (Tc I) presented higher infectivity than isolates 863 (Tc III) and 1994 (clone from TcBat). This difference in infectivity could be related to the low or absent reactivity to Mab 1D9 in 863 isolates.Since the funcion of amastigote surface components in *T. cruzi* infection is poorly characterized, this experimental work proposes a role for the glycoprotein Ssp-4 in T. cruzi amastigote which could be important in host cell invasion. **Supported by:**:FAPESP

BC013 - Cell-to-cell transfer of Leishmania amastigotes observed by multidimensional live imaging: participation of extrusomes in host cell egress and reinfection

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The last step of *Leishmania* intracellular life cycle is the egress of amastigotes from the host cell and their uptake by adjacent cells. The almost exclusive intracellular location of amastigotes was puzzling to the first histopathologists, who regarded host cell lysis with cautiousness. Taking advantage of multidimensional live imaging of macrophage cultures infected with *L. amazonensis*, we describe some features of this process.
Amastigotes are transferred from cell to cell when the donor host macrophage collapses; transference between live cells was not detected. The spacious structure in which *L. amazonensis* amastigotes grow (the large parasitophorous vacuole, PV) do not burst as parasites multiply but rather implode after several hours of PV growth and amastigote multiplication arrest.
We found that amastigotes are extruded from the collapsed host macrophage within zeiotic structures (blebs) rich in late endosome/lysosome components such as LAMP1 and Rab7 (tagged with GFP in transfected RAW macrophages). Extrusion from live host cells, but not cell-to-cell transfer, is stimulated by nocodazole. Transfer without extrusion is stimulated when macrophages are treated with streptolysin O, a pore-forming protein innocuous to amastigotes.
The extrusome is internalized by vicinal macrophages and the rescued amastigotes, carrying host lysosomal components attached to their surfaces, are able to survive and develop spacious PVs in recipient macrophages. The participation of host lysosomal components associated with amastigote surfaces in transfer of parasites was investigated; amastigotes isolated from LAMP1/LAMP2 knockout host cells were less phagocytosed by macrophage cultures when compared to amastigotes isolated from wild-type cells.
We provide evidence that amastigotes, enclosed within host cell membranes, can be transferred from cell to cell without exposure to the extracellular milieu.
Supported by::FAPESP

BC014 - *Trypanosoma cruzi* extracellular amastigotes (EAs) and host cell signaling: more pieces to the puzzle

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The parasite *Trypanosoma cruzi* uses distinct strategies to invade mammalian cells. The ability of *T. cruzi* to invade, persist and adapt in both invertebrate and vertebrate hosts is multifactorial and depends on both host and parasite fitness. The cellular communication between parasite and its host is a constant event and has evolved to be relatively benign, since killing the host is not productive to the parasite. EAs correspond to 10% of the circulating forms in mice during the acute phase of *T. cruzi* infection and are also capable of sustaining an infective cycle in the mammalian host and cells. EAs of the G strain are more infective than CL strain EAs. Unlike to previously shown for trypomastigotes, the recruitment of actin is central to the uptake of EA forms in mammalian host cells, including HeLa cells. Actin is early recruited by EAs and forms cup-like structures beneath the parasite. We studied EA invasion by evaluating parasite and host factors. On the parasite side, we characterized a classical enzyme of the isoprenoid pathway which is unexpectedly secreted only by *T. cruzi*. This enzyme modulated both HeLa cell signaling and invasion by EA in a behavior consistent to a moonlighting protein. EAs of G strain induced phosphorylation of HeLa MAPK pathway in a biphasic fashion. From the host cell point of view, we focused on the role of Protein Kinase D (PKD) and cortactin in EA uptake by HeLa cells. The recruitment of cortactin and PKD-GFP-constructions by EA in HeLa cells was assessed both in vitro (fixed cells and indirect immunofluorescence) and in vivo (live-cell imaging by time-lapsed confocal microscopy). PKD was recruited with cortactin to sites of actin-rich cup-like structures induced by EA invasion. Heat-killed parasites or epimastigotes (non-infective forms) did not recruit these proteins. These results suggest that unexpected novel roads may also be explored by *Trypanosoma cruzi* to invade cells. **Supported by:**:FAPESP, CNPq, Capes

BC015 - Evaluation of the trypanocidal activity of 1,4-naphthoquinones against *Trypanosoma brucei* strains

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Trypanosoma brucei is the etiologic agent of sleeping sickness, transmitted by flies of *Glossina* genus, known as tsé-tsé flies. Pentamidine is the main of treatment for stage I infection with *T. brucei*. High cost, toxicity and resistance problems of conventional drugs result in an urgent need to identify and develop new therapeutic alternatives. Novel drugs with less adverse effects than pentamidine need to be studied. In this work, we evaluated the trypanocidal activity of a series 2-(1-alquenyl)-3-metoxy/hidroxy-1,4-naphthoquinones against *T. brucei* parasite strains (427 and 29-13). Cytotoxic assay was performed using MTT. The compounds 1, 2, 5 and 8 showed a good activity against *T. brucei* 427 and the CI_{50} values obtained were 4.72 µM, 5.65 µM, 6.93 µM and 7.95 µM, respectively. The amines derivatives of 1,4-naphthoquinones with trypanocidal activity in *T. brucei* 427 were 1a and 2a showed CI_{50} 8.5 µM and 4.8 µM, respectively. Our results showed that some substances tested had trypanocidal activity higher with less concentration than the pentamidine (CI_{50}: 6.43 µM), indicating that these naphthoquinones are interesting compounds for further studies using these parasites as a model. Next steps will involve the testing of the cytotoxicity of these naphthoquinones derivatives in HepG2, which are human liver cells using as a drug metabolism model. **Supported by:**:FAPESP, FUNDUNESP e CAPES

BC016 - Spontaneous cystogenesis of *Toxoplasma gondii* in feline epithelial cells, in vitro.

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Toxoplasma gondii, which is an obligate intracellular protozoan parasite, belonging to the phylum Apicomplexa, has the ability to infect a wide range of hosts, including mammals and birds. Felids are the only definitive hosts for T. gondii leading to the formation of immature oocysts that are shed in their feces. The spread of oocysts in the environment is the main factor that explains the global distribution and dissemination of toxoplasmosis. Since the specificity of the sexual cycle occurs in epithelial tissue, the main aim of this study was to investigate the in vitro interaction of T. gondii and epithelial cells derived from cat kidneys (CRFK) and from rat intestines (IEC-6), in order to determine the intracellular fate of the parasite in these cells. CRFK and IEC-6 cultures were infected with T. gondii ME49 strain bradyzoites. Assays were performed at different ratios: 1:5, 1:10 and 1:20 (parasite-host cell) for periods ranging from 1 to 14 days of infection. The intracellular development of T. gondii was dependent on the source of epithelial cell and also on the parasite/host cell ratio. Cystogenesis was well established in CRFK line at the ratio 1:10 after 10 days of infection. Infected feline cells were maintained until 14 days and then were processed for fluorescence microscopy and transmission (TEM) and scanning electron microscopy (SEM). This cellular model opens up a new field of investigation into the molecular aspects of the T. gondii-feline epithelial cell interactions. The CRFK line appears as a potential cellular model for large scale cyst production in vitro, which would allow a reduction in the use of experimental animals for cyst isolation. **Supported by:**:FAPERJ, IOC/Fiocruz e Pronex.

BC017 - Effect of Cytochalasin D and Jasplakinolide on Neospora caninum actin

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Neospora caninum is an obligatory intracellular protozoan parasite that belongs to the phylum Apicomplexa and infects primarily dogs and cattle, where the neosporosis represents one of the most important causes of reproductive problems and abortion in cows. Apicomplexan organisms utilize a unique form of substrate-dependent locomotion termed gliding motility to migrate through tissues and invade their host cells. This locomotion system is conducted by the actin/myosin motor complex that is located between parasite's plasma membrane and the inner membrane. The role of actin in gliding motility and host cell invasion has been extensively investigated in *T. gondii* and *Plasmodium* spp., however there are no publications on *N. caninum*. This study aims at investigating the localization and distribution of *N. caninum* actin in tachyzoites treated or not with the actin inhibitors. The actin was visualized by an actin-specific commercial antibody (Anti-Actin Monoclonal Antibody C4 – Abcam) conjugated to AlexaFluor488 (Molecular Probes) through immunofluorescence confocal microscopy (IFA) technique and nucleus staining was made by propidium idodide (PI). The tachyzoites used in this assay were previously treated, in separate groups, by drugs which act on actin dynamics: 5 µM of Jasplakinolide (JAS), an actin polymerizing and filament-stabilizing drug, 2 µM of Cytochalasin D (CytD), that disrupts actin filaments and prevents actin polymerization; and dimethyl sulfoxide 1% (DMSO), the control. As result, the DMSO treated tachyzoites had a peripheral distribution of most of actin, unlike CytD tachyzoites whose actin was well distributed throughtout the cytoplasm and JAS tachyzoites that presented occasionally clustered actin in certain regions of *N. caninum* cytoplasm. These results show the localization of *N. caninum* actin near gliding motor complex (in control treated tachyzoites) and also confirm that CytD and JAS has effect in *N. caninum* actin. **Supported by:**::CAPES

BC018 - TcVTC4 has a role in polyphosphate synthesis in Trypanosoma cruzi

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Polyphosphate (polyP) is an anionic polymer of orthophosphate groups linked by high-energy bonds that typically accumulates in acidic, calcium-rich organelles known as acidocalcisomes. Previous work has shown that polyP has a critical role in *T. cruzi* differentiation and its survival to sharp environmental changes, including osmotic stress. Osmoregulation is essential for digenetic trypanosomatids as they encounter osmotic stress in both the insect vector and vertebrate host. PolyP synthesis in eukaryotes was unclear until recent work demonstrated that vacuolar transporter chaperone 4 (Vtc4p) is a polyP kinase in yeasts (Hothorn *et al*. Science 324, 513-516, 2009). *Vtc4p* othologs have been identified in *Trypanosoma brucei* (*TbVTC4*, Tb11.01.4040) and *T. cruzi* (*TcVTC4*, Tc00.1047053511127.100). Our studies have indicated that TbVTC4 is an acidocalcisomal short-chain polyP kinase involved in osmoregulation, and essential for virulence in mice. The T. cruzi enzyme is also localized in acidocalcisomes as demonstrated by immunofluorescence microscopy using a mutant cell line expressing a C-terminal tagged version of the gene (*TcVTC4*-GFP). The subcellular localization was confirmed by electron microscopy in *T. cruzi* epimastigotes. Recombinant TcVTC4 has been expressed in bacteria and polyP kinase activity assayed *in vitro*. Furthermore, a *TcVTC4* knockdown mutant cell line is being generated in order to evaluate the physiological role of this enzyme in *T. cruzi*. We expect TcVTC4 to be essential for osmoregulation and virulence in *T. cruzi*. Since VTC4 is absent in vertebrates, this enzyme could be a potential target for drug development and parasite control. This work was funded in part by a pre-doctoral fellowship for the American Heart Association to N.L. and NIH grant AI077538 to R.D. **Supported by:**:NIH and American Heart Association

BC019 - Distinct functions for the two homologs of Asf1 in *Trypanosoma brucei*

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Asf 1 (anti-silencing function 1) is one of the most conserved histone chaperones among all the eukaryotes. It interacts with the histone heterodimer H3/H4 by its C-terminus and with proteins that can modify these histones. For this reason Asf 1 is consequently involved in DNA replication, transcription, DNA repair and silencing/anti-silencing. Differently from most eukaryotes, trypanosomes have two distinct Asf1 homologs, A and B, and little is known about its function in these parasites. Here we generated RNAi knockdowns and overexpressors of the two forms of the protein in *Trypanosoma brucei* and confirmed that both proteins are essential for parasite growth, arresting cells in S phase of the cell cycle. In addition, we showed a specific decrease of histone H3/H4, while an increase of these histones is observed in the overexpressors. Our results also indicate that these two homologs have different cellular localization. While Asf1A is mainly nuclear Asf1B is distributed in the cell cytosol. Coimmunoprecipitation of the tagged versions of the two proteins revealed different set of proteins, with Asf1B interacting with ribosomal proteins in addition to histones. These results indicate that in T. brucei Asf1 could also act as a chaperone for ribosomal proteins in addition to histones. **Supported by:**:Fapesp

BC020 - In vitro cystogenesis of *Toxoplasma gondii*

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Conversion of T.gondii tachyzoites to the bradyzoite stage and tissue cyst formation in the life cycle of the parasite has a crucial role in the establishment of chronic toxoplasmosis. In this work we investigated the in vitro cystogenesis and behavior of the EGS strain, isolated from human amniotic fluid. We observed that tachyzoites of the EGS strain converted to intracellular cysts spontaneously in epithelial cell cultures. The peak of conversion was reached after 4 days of infection, when 30% of the infected cells contained cysts. However, the majority of infected host cells harbored parasitophorous vacuoles containing tachyzoites and bradyzoites (in a cyst-like structure). Using specific markers against bradyzoite, tachyzoite and cyst wall components, we confirmed stage conversion and distinguished immature from mature cysts. Transmission and scanning electron microscopy showed that the thickness and electron density of the cyst wall increased with the maturation of the cysts. Also, the cyst matrix tubules were shorter than those from the intravacuolar network and were immersed in granular electron dense material. We demonstrated that the EGS strain spontaneously converts to bradyzoites in tissue culture cells without artificial stress conditions, resulting in a high burden of cysts in vitro and constituting a useful tool to study this stage of the *Toxoplasma gondii* life cycle. **Supported by:**:CAPES-FAPERJ-CNPq

BC021 - PATHOGENICITY FOR MICE OF *TRYPANOSOMA CRUZI* STRAINS FROM PARANÁ (TcII) AND AMAZONAS (TcIV) IN ACUTE AND CHRONIC PHASES OF INFECTION

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The clinical diversity of human *Trypanosoma cruzi* infection has been attributed to genetic heterogeneity of parasite populations and to host's genetic background. There are few studies exploring the correlation of genetic diversity and biological characteristics of T. cruzi strains from Brazilian Amazon. Our objective was perform histopathological evaluations of mice inoculated with *T.cruzi* strains from states of Paraná (TcII) and Amazonas (TcIV) in acute and chronic phases. Were used three TcII strains (chronic patients–Paraná) and three TcIV strains (acute cases–Amazonas). For each strain, 13 Swiss mice, 21 to 28 days, were inoculated via IP with 10.000 blood trypomastigotes/animal. Parasitemia was evaluated daily from 3rd day after inoculation (dai). The animals were euthanized one day after the peak parasitemia (Pmax) in the recent acute phase (rAP), at 30° (late acute phase–lAP) and 100º (chronic phase–CP) dai. Fragments from heart, skeletal muscle, liver, spleen, brain, diaphragm, abdominal wall and large intestine were stained with HE. For each strain were obtained the pre-patent period (PPP), patent period (PP), Pmax, day of peak parasitemia (Dpmax), inflammatory process and tissue parasitism. Mice inoculated with TcII strains showed higher PP and Pmax, whereas that inoculated with TcIV strains had lower PPP, earlier Dpmax, and low levels of parasitemia. TcII displayed more organs with tissue parasitism in rAP and lAP in comparison with TcIV, which presented amastigotes nests only at rAP. No tissue parasitism was observed in CP. TcII had more organ's presenting inflammatory process than TcIV at all phases. The inflammatory process was more intense at lAP for both lineage and was observed more tissue damage for TcII.So, TcII from Paraná was considered more pathogenic to mice than TcIV from Amazonas in all stages of experimental infection, in agreement with lower severity of Chagas disease at Amazon region in relation to old endemic areas as the Paraná state. **Supported by:**:FUNDAÇÃO ARAUCÁRIA / CNPQ

BC022 - The extracellular amastigotes of *Trypanosoma cruzi* trigger specific signaling events in host cell: actin-cortactin-protein kinase D pathway

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The cellular invasion by the extracellular amastigotes (EA) of *T. cruzi* is dependent on the actin cytoskeleton dynamics of the host cells. Protein kinase D (PKD) is a family of serine/threonine kinases with three members (PKD1, 2 and 3) involved in a variety of processes such as apoptosis, Golgi morphology, immune responses and cellular migration. Regarding cellular migration, PKD phosphorylates cortactin – a key regulator of the cortical actin cytoskeleton – that disturbs cell actin dynamics and, consequently, reduces cellular migration. The aim of this study was to evaluate the role of PKD and cortactin at the actin dynamics during the cellular invasion by EA in HeLa cells. Using transiently transfected HeLa cells we could observe that only PKD1 and 2 (GFP-tagged) were recruited and colocalized with actin and cortactin at the EA invasion sites. PKD and cortactin recruitment seemed to occur in a multi-step manner as observed in kinetic assays using immunolabelled-fixed cells and live cells (time-lapse confocal microscopy). EA attachment triggered specific signaling events in HeLa cells leading to PKD phosphorylation/activation but not cortactin phosphorylation by Src as evaluated by Western blotting. Heat-killed EA, latex beads and the non-infective epimastigotes forms did not induce a notable signaling in HeLa cells and did not lead to the recruitment of the evaluated host cell molecules. Finally, these results suggest that the recruitment and activation of cortactin and PKD during host actin dynamics are triggered by a particular signaling following viable and infective parasite contact. We are currently knocking down these host cell proteins in order to evaluate their role in cellular invasion by EA. **Supported by:**:FAPESP, CNPq, CAPES

BC023 - Effect of CGS 15943 on ectonucleotidasic activity of two *Leishmania amazonensis* strains.

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Leishmaniasis is a heterogeneous group of diseases caused by parasites of the genus Leishmania. These parasites are unable to perform de novo synthesis of purines and are dependent on the salvage pathways to obtain these substances. To feed this pathway, they exhibit ecto-NTPDase and ecto-5'-nucleotidase activities that hydrolyze extracellular ATP to adenosine, that can be internalized by the parasites. Our objective was to modulate the ectonucleotidasic activity in two strains of *L. amazonensis* (PH8 and LTB) by CGS15943 (non-specific antagonist of adenosine receptors in mammals) treatment. For this, metacyclic and non metacyclic promastigotes were purified by gradient centrifugation in Ficoll® and Percoll®, respectively. To try to modulate the ectonucleotidasic activity, parasites were treated with CGS15943 24 h before analysis. The hydrolysis of ATP, ADP and AMP was measured by Pi released using malachite green. We found an increase of metacyclogenesis by CGS15943 treatment in both strains, as previously shown in our laboratory for PH8 strain. Moreover, CGS-treated parasites were smaller, highly mobile and exhibited long flagellum, characteristics of metacyclic promastigotes. Ectonucleotidasic activity was similar in control and CGS15943-treated promastigotes. The hydrolysis of ATP, ADP and AMP was higher in metacyclics than in non metacyclics, only when promastigotes were obtained of control cultures of PH8 and LTB strains. CGS15943-treatment prevented the increase of ectonucleotidasic activity in metacyclic when compared with non metacyclic promastigotes, also for both strains. We conclude that CGS15943 induces metacyclogenesis and prevent the increase of ectonucleotidasic activity in metacyclic promastigotes of PH8 and LTB *L. amazonensis* strains. **Supported by:**:CAPES, CNPq, FAPEMIG

BC024 - Exposure of phosphatidylserine by *Toxoplasma gondii* induces lipid bodies in macrophages

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Lipid bodies (LB) are organelles rich in lipids present in many cell types. LB can be modulated by specific signals such as the interaction with apoptotic cells and parasites. Apoptotic cells and some protozoan parasites such as *Toxoplasma gondii* expose phosphatidylserine (PS) at their plasma membrane; this exposure has been involved in the evasion mechanism of parasites. *Toxoplasma gondii* needs host cell lipids for its replication. Thus, the exposure of PS by the parasite might be involved in the induction of LB in macrophages. To test this hypothesis LB of macrophages were quantified after interaction with *T. gondii* (RH and Me-49 strains, virulent and less virulent, respectively). Tachyzoites of both *T. gondii* strains were added separately to macrophages for 1h, cells were washed and cultured for 24 h with fetal bovine serum, LB were stained with Nile Red or Red Oil, and their presence scored under a microscope. Noninfected macrophages cultured for 24h were used as negative control. Infection by *T. gondii* induced LB with the RH inducing slightly more LB than the ME-49 strain. Some macrophages had many LB, however, were not infected by *T. gondii*. This datum led us to believe that cytokine(s), on a paracrine signaling pathway, may be participating in the induction of LB. Knowing that TGF-beta and IL-10 are cytokines released by macrophages after contact with cells that expose PS, neutralizing antibodies for these cytokines were used during the interaction of macrophages with *T. gondii* and LB induction investigated. In both cases the use of antibodies decreased the amount of LB. Although, neutralizing antibodies decreased the presence of LB in *T. gondii* infected macrophage, noninfected macrophages in the presence of neutralizing antibodies presented less LB. Thus, other T. gondii factor(s) may also be contributing to the induction of LB. The experiments suggest the involvement of PS in the induction of LB. **Supported by:**:FAPERJ, CNPq, CAPES

BC025 - The role of Nod/Rip2 pathway in autophagy induction in response to *Trypanosoma cruzi* infection

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Chaga's disease, caused by *Trypanosoma cruzi* protozoan, is the most serious parasitic disease in Latin America. The control of infection by host is dependent on effective immune response which is initiated by pattern recognition receptors such as Nod-like receptors (NLRs). NLRs are responsible to recognize pathogens molecules and activate the innate immune response. Little is known about the role of NLRs in immunity against T. cruzi. We have demonstrated that the NLR Nod1 accounts for host resistance against T. cruzi. Although, the mechanisms that operate in this response were not determined. It was reported that Nod1 and Nod2 are capable to induce autophagy in response to bacterial infection. Thus, we aim to investigate the role of Nod/Rip2 pathway to induce autophagy in response to T. cruzi infection. Macrophages from C57BL/6 (WT), Nod1-/- or Rip2-/- were transduced with GFP-LC3 and infected with T. cruzi. We observed that infected macrophages from WT mice presented higher number of LC3 puncta when compared with uninfected cells. In addition, we found that T. cruzi infected macrophages from Nod1-/- and Rip2-/- presented less LC3 puncta when compared with WT cells. To confirm these data we evaluated the autophagy by determining the LC3-I lipidation to LC3-II form by western blot in macrophages at 12, 24 and 48h after infection. We observed the presence of the LC3-II form in response to T. cruzi in macrophages from WT, Nod1-/- and Rip2-/- in all the times evaluated. To evaluate the role of autophagy to control T. cruzi multiplication we inhibited autophagy by using shRNA against Atg16 in macrophages. We found that macrophages silenced for Atg16 presented higher number of amastigotes compared with macrophages transduced with control shRNA. These results suggest that T. cruzi infection trigger autophagy in macrophages by a pathway dependent on Nod1 and Rip2 proteins and that autophagy effectively contributes to restriction of T. cruzi replication in macrophages. **Supported by:**:FAPESP

BC026 - T. vaginalis exposes phosphatidylserine (PS) and inhibits the Nitric Oxide (NO) production in activated IEC-6

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T. vaginalis a parasite of the uro-genital tract in humans and provokes an important infection which is not fully understood. PS is a phospholipid and in normal cells is located in the inner moiety of the cell plasma membrane. The PS exposure is a sign of apoptotis, a programmed cell death. The apoptotic mimicry has been described in other protozoa as a scape mechanism against the action of effector cells. In a previous work, we have shown that PS exposure by *Toxoplasma gondii* induced the TGF-β1 secretion by infected activated macrophages, leading to iNOS degradation, inhibition of NO production and consequent persistence of these cells infection. Our group has been shown that IEC-6 activation is able to produce NO through interferon γ cultivation. It was analyzed the behavior of T. vaginalis on IEC-6 activaded by interferon gamma and featured the PS exposure. In addition, the ultrastructure of this interaction was analyzed by scanning electron microscopy (SEM). By flow cytometry it was found that 37.06% of T. vaginalis population exhibited PS on their surface. The modulation of T. vaginalis infection in IEC-6 cells through the NO dosage, showed the reduction of this production by activated IEC-6 cells. The results by using SEM showed several vesicles which were secreted by IEC-6 after interaction. These results suggest that T. vaginalis is able to control the IEC-6 activation and probably induce apoptosis in this host cells.**Supported by:**:FAPERJ

BC027 - Participation of PKR in Modulation of macrophage infection by the protozoan parasite *Toxoplasma gondii*

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The evolution of *Toxoplasma gondii* infection depends of the balance between microbicidal and suppressor macrophage functions. Double-stranded RNA (dsRNA)-activated protein kinase R (PKR), a classic antiviral protein, is able to regulate a number of signaling pathways and macrophage functions. PKR is a key component of the cell antiviral response that acts by modulating the activity of a number of cellular proteins. In this work, we investigated the role of PKR during protozoan parasite *T. gondii* infection. For this, we utilized trhee types of modificated macrophages, RAW 264.7 cells expressing either PKR K296R (RAW-DN-PKR cells), an empty vector (RAW-bla cells) and the wild type. Cells were infected cell ratio of 3:1 to evaluate tachyzoites proliferation inside macrophages.for 1 h, 24h and 48h at 37°C with tachyzoites of *T.gondii*: We observed that *T.gondii* infection led to PKR activation and increased PKR expression levels. *T. gondii* infection was strongly reduced in RAW-DN-PKR cells after 48 hours of infection. The RAW-bla cells have the reduced expression of protein kinase R (PKR) at a ratio of 3 parasites/cell. These results suggest that PKR expression is very important for *T. gondii* infection control in macrophages.**Supported by:**:FAPERJ

BC028 - Functional characterization of giant protein in *Trypanosoma brucei*

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Trypanosoma brucei is a unicellular protozoa of great medical relevance since it is the etiological agent of sleeping sickness (African trypanosomiasis). Over the past decades, this organism has been used as experimental model for cellular, biochemical and molecular studies considering its particular structures as nucleus, cytoskeleton and the presence of unique organelles as kinetoplast and flagellum. In all genus of the Trypanosomatidae family, giant proteins were described as a novel class of high molecular mass phosphoproteins (1000-4000kDa) which, besides its structural function, might play a role in the organization and regulation of cytoskeleton and its constituents. However, factors that interact with these proteins in both the cytoskeleton and the host are still unknown and little is known about the giant proteins of the pathogenic trypanosomatids and their functions. Based on what has been described, this project aims to determine the functional role of *T. brucei* giant protein together with the biochemical and immunocytochemical characterization of this protein. The cell fraction containing the cytoskeleton proteins, including the giant protein, were resolved by SDS-PAGE. Then the slice relative to this protein was cut from the gel and injected in Balb/c mice to produce specific antibody. The polyclonal serum recognized the giant protein and immunofluorescence preliminary observations suggest this protein is localized along the flagellum and in the flagellar pocket. Moreover, mass spectrometry will be performed in order to determine the partial sequence, which will be used to identify the respective gene in *T. brucei* genome, what allow us to investigate the function of specific catalytic domains. Further, immunoprecipitation and pull-down assays will be performed in order to define which putative partners interact with the giant protein. Furthermore RNAi knockdown combined with confocal and electron microscopy will validate the giant protein function. **Supported by:**:FAPESP

BC029 - 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, which induces an anti-inflammatory response during phagocytosis of apoptotic cells. The interaction *Toxoplasma gondii* with activated murine peritoneal macrophages inhibits nitric oxide (NO) production, allowing the persistence of the parasite in macrophages. Our group showed that the mechanism used by *T. gondii* to inhibit the production of NO in activated macrophages is similar to *Leishmania sp.* and involves the exposure of PS by parasites. Malaria infection is characterized by both 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 PS exposure by *P. chabaudi chabaudi* come eritrocytes and verify the possibility of parasites inhibit the nitric oxide production during interaction by activated mouse peritoneal macrophages. The results show that 90 % of P. chabaudi chabaudi population presents PS exposure and the NO production inhibition occurs after 24 hours of interaction with activated macrophages. In addition, the observation of interactions showed that *P. chabaudi chabaudi* was capable to activate events similar to autophagy in macrophages. These results confirm that PS exposure can be considered the common escape mechanism of parasitic protozoa. **Supported by:**:FAPERJ

BC030 - Characterization of actin in trypanosomatids

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The trypanosomatids are organisms of medical importance because they cause many neglected diseases, including Chagas disease, sleeping sickness and leishmaniasis, caused by Trypanosoma cruzi, *Trypanosoma brucei* and Leishmania respectively (Vickerman, 1994). The cytoskeleton trypanosomatids differs from most eukaryotic cells because it did not contain transcellular filaments and is composed almost entirely of microtubules and associated proteins to microtubules. Actin is a cytoskeletal protein present in eukaryotes, however, this protein in trypanosomes is only in the monomeric form, and presents in the cortical regions and in the flagellar pocket (Sahasrabuddhe, et al., 2004, Kapoor et al, 2010). The goal of this project is to identify proteins that interacting with actin in the trypanosomatid cell body. Trypanosomatid actin was amplified by PCR using a genomic library of Leishmania major, and then cloned in fusion with GST at the N-terminus using pGEX4T vector. The GST-actin recombinant protein was purified by affinity chromatography using glutathione-sepharose system, and GST was cleaved with thrombin releasing actin. The actin was used for rabbit immunization to obtain a polyclonal actin antibody, that were purified by protein A sepharose. Immunofluorescence in Leishmania major using the purified antibody revealed the distribution of actin in the flagelar pocket area as described (Sahasrabuddhe, et al, 2004, Kapoor et al, 2010). In Trypanosoma cruzi, actin staining was observed in the flagellar pocket and also seen along the flagellum. Recombinant GST-actin and antibodies will be used to perform pulldown and co-immunoprecipitation assays to observe the interaction of actin with other proteins in the cell body of trypanosomatids. **Supported by:**:satander/fapesp

BC031 - Study of the effects of compounds coordinated with the metal core (metalocomplexes) on epimastigote of Trypanosoma cruzi

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Trypanosoma cruzi is the ethiologic agent of Chagas Disease. The epimastigote form of *Trypanosoma cruzi* is present in the midgut of triatominae insect vector. In previous studies, the family of compounds known as metalocomplexes has demonstrated biological activity acting as antifungi,,antivirus and bactericida. Complex iron (III), cobalt (II), copper (II) and zinc (II) exhibited activity against Staphylococcus aureus. Because they are metal compounds, it allowed locate its site of action in the target cell by electron microscopy. In the present study, the activity of new compounds against epimastigote of T. cruzi was tested in a period of up to 96 hours. The growth parasite curve was analyzed as well the parasite morphology after treatment. We show that metalocomplexes of iron (III) have antiproliferative effects on the epimastigote form of T. cruzi, with IC50 10 µM. More experiments are necessary with other forms of T. cruzi, as tripomastigote and amastigote forms. **Supported by:**:FAPERJ

BC032 - Endosymbiosis in trypanosomatids: Model for the study of cell division control and co-evolution

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Recently, our group described that a bacterium that maintains a symbiotic relationship with some protozoa, as *Angomonas deanei* and *Strigomonas culicis*, divides in coordination with the protozoa structures, so each daughter cell harbors only one bacterium. To ensure these events, a tight control is required. Also, in both protozoa species the bacterium is usually observed close to the host cell nucleus and is the first structure to divide. In order to better understand the mechanisms involved in the coordinated division, protozoa were treated with inhibitors that block cell cycle in different phases and the effects were evaluated by microscopy techniques, as well as viability assays. Here we show that the treatment with cycloheximide, an eukaryotic protein synthesis inhibitor, induced cell proliferation arrest and blocked the symbiont envelope segregation, suggesting that the bacterium somehow depends on host protein factors to conclude division. Treatment with aphidicolin, an inhibitor of eukaryote DNA polymerase I, arrested protozoan proliferation but did not block the symbiont replication, generating unusual cell patterns, with more than one unsegregated symbiont. Interestingly, *A. deanei* reverses this status and after 24h the pattern of one symbiont per cell is prevalent again. In *S. culicis* aphidicolin generated filamentous bacterium and the effect was irreversible. On the other hand, after treatment with camptothecin, a topoisomerase I inhibitor, filamentous bacterium were observed in both species and the host was not able to reverse this uncontrolled situation. Genome dataset revealed loss of genes in the division and cell wall cluster (dcw) of the symbiont, and conservation of housekeeping genes. Taken together, these results support that eukaryote division controls are differentially involved in the coordinated division of the bacterium suggesting different co-evolution processes between symbionts with their respective hosts. **Supported by:**:CNPq, FAPERJ AND FAPESP

BC033 - CD14 GENOTYPICAL AND PHENOTYPICAL VARIANTS IN CHRONIC CHAGAS DISEASE

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Chagas' disease (CD) shows great clinical variability, with crucial role for inflammatory response in development of cardiac disease. The innate response, highly variable among individuals, is important for parasite control and establishment of adaptative response. Receptor CD14 can act as a signaling adjuvant on innate immunity. Therefore, we aimed to investigate the role of CD14 genotypic and phenotypic variants in the chronic phase of CD. We investigated -260C/T polymorphism, by RFLP. Oral swabs samples were collected from 64 Indeterminate patients (I), 53 Non-Dilated Cardiac (C), 68 Dilated Cardiac (DC) and 63 healthy individuals (N). The allele -260T, associated with high transcriptional activity, may affect monocyte activation ability. Moreover, we evaluated CD14+ and CD14- subsets functional activity on an in vitro infection assay using 10 trypo-CFSE/cell. CD14- cells from monocyte-lineage may be an immature dendritic cell (CD14-CD11c+) with less inflammatory profile. PBMC were obtained from peripheral blood from I (6), DC (6) and N (4) as a source of adherent cells. First, allele -260T carrier (genotype -260T+) was associated with I group, suggesting a two-fold protective role (p=0.05; OR: 2.24; CI: 0.97-5.14). CD14 was less expressed in DC than N, while I group had higher frequency of CD14+ than N. CD14+ were significantly more infected than CD14-, and also expressed higher levels of all markers evaluated: CD11b, CD80, CD86, IL-10, IL-12 and TNF-alpha. After in vitro infection, CD11b was more expressed on CD14- cells from N than I. In vitro infection caused decrease of CD86 in CD14- from N and DC, only. Therefore, our study suggests that CD14 polymorphism may be relevant for clinical course. Moreover, CD14- cells, possibly an immature phenotype, are diminished on I, suggesting a better innate response. Additional work is required for better determining the role of innate immune response in the clinical outcome of CD. Financed by: WHO/TDR; NIH; CNPq; CAPES. **Supported by:**:CAPES

BC034 - THE NATURAL CYSTEINE PEPTIDASE INHIBITOR OF THE AFRICAN TRYPANOSOME PLAYS A ROLE IN BRAIN ENDOTHELIAL ACTIVATION AND IN PARASITE VIRULENCE

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Natural peptidase inhibitors are among important mechanisms of proteolysis control. Inhibitors of cysteine peptidases (ICP) are tight binding inhibitors belonging to the chagasin-family, which inactivate family C1 cysteine peptidases, such as cathepsin L-like enzymes. We previously generated ICP null mutants in *Trypanosoma b. brucei* bloodstream forms, revealing that it regulates the activity of parasite cathepsin L (*Tb*CATL) and surface coat exchange during bloodstream to procyclic differentiation. ICP null mutants displayed higher *Tb*CATL activity, enhanced intracellular proteolysis and higher parasitemia in mice. In human trypanosomiasis, *T. b. rhodesiense* penetrates the central nervous system by unknown mechanisms, leading to meningoencephalitis. Studies using an *in vitro* model of the human blood brain barrier (BBB) composed of brain microvascular endothelial cells (BMECs) suggest that *Tb*CATL is required for efficient transmigration of *T. rhodesiense* through BBB. To further investigate the role of parasite cysteine peptidases and ICP in the dynamics of *T. rhodesiense*-BBB interactions, we generated *T. rhodesiense* lines lacking ICP (Δ*icp*). Those lines have 50% increase in cysteine peptidase activity and traverse BMEC monolayers more efficiently than WT. FACS analysis of BMECs showed that ICP null mutants induce increased surface expression of VCAM-1 and E-selectin but drastically reduce surface ICAM-1. Consistently, human isolated neutrophils adhere at higher numbers to BMECs previously exposed to Δ*icp*. Surprisingly, BALB/c mice infected with Δ*icp* display lower blood parasitemia and delayed death, as compared to those infected with WT parasites. Immunohistochemistry of brain sections showed higher amounts of microglia at day 6, suggesting that infection affects the central nervous system before parasite penetration. We provide, for the first time, genetic evidence that *T. rhodesiense* modulates brain endothelial cells.**Supported by:**:FAPERJ e CNPq

BC035 - Characterization of the biochemical and cellular properties of the high molecular weight protein in Leishmania

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Over the past decades, the trypanosomatids represent an excellent model for cell biology suitable for investigation analysis of basic questions about the cytoskeleton and its evolution in higher eukaryotes. In the Trypanosomatidae family, it was demonstrated the existence of a new class of high molecular mass phosphoproteins (1000-4000kDa) in the cytoskeleton, besides having a structural role, have been implicated in the regulation and organization of the cytoskeleton. These proteins are partially located in the flagellar pocket, but they differ for each genera analyzed, and that may be involved with the way of life of these parasites. However, elements that interact with these proteins in the cytoskeleton are still unknown and little is known about the giant proteins of the pathogenic trypanosomatids and their functions. In Leishmania major, we identified a giant protein, which comprises 1500 kDa in the cytoskeletal insoluble fraction. The gel slice relative to this protein was injected in BALB/c mice to produce specific antibody. The antibody was purified by immunoaffinity and by western blotts recognizes the Leishmania giant protein in the cytoskeletal fraction as well in the isolated flagella. Immunofluorescence assay revealed this protein in the flagellar pocket and along the flagella of the *L. major* and L. tarentolae. Therefore, using antibodies and pull-down techniques we will identify proteins in the cytoskeleton that interact with giant protein. Also, a partial amino acid sequence of Leishmania giant protein is determined by MS mass spectrometry and will be analyzed in the database (www.tritrypdb.org) to know the nature of this protein. **Supported by:**:FAPESP

BC036 - PREINFECTION EXERCISE TRAINING ATTENUATES CARDIOMYOCYTES MORPHOFUNCTIONAL REMODELING IN A MURINE MODEL OF *TRYPANOSOMA CRUZI* INFECTION

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Aim: To investigate the effect of preinfection treadmill training on the morphology and function of cardiomyocytes in *Trypanosoma cruzi* infection. Methods and Results: Four-month-old male Wistar rats weighting 369.72±18.02g were randomized into 4 groups: sedentary no infected (SN=14), sedentary infected (SI=14), trained no infected (TN=14) and trained infected (TI=14). Infected animals were inoculated with T. cruzi Y strain (300,000 trypomastigotes/50g). After nine weeks, the animals were euthanized and the right atrium (RA) and left ventricle (LV) were removed for morphological, biochemical and isolated-cardiomyocyte contractile function evaluation (ethical approval, UFV 30/2009). The SI animals' presented marked inflammatory infiltrate, collagen content, reduction of cardiomyocytes volume and sarcomere length in the RA and LV. In addition, malondialdeyde and protein carbonils levels and the activities of catalase and superoxide dismutase were significantly increased in the RA and LV compared to SN and TN animals. The RA and LV cardiomyocytes from SI animals showed significant reduction in cell shortening and maximal rate of contraction and relaxation compared to SN and TN animals. In TI animals, preinfection treadmill training reduced significantly the myocardial inflammation, collagen content and cardiomyocytes atrophy induced by *Trypanosoma cruzi* in RA and LV. In this group the activity of catalase was maintained and superoxide dismutase was increased. At the same time, there was a significant reduction of the tissue levels of malondialdeyde and protein carbonils. Moreover, TI animals presented normal cardiomyocyte contractile pattern, similar to the SN animals. Conclusion: Preinfection treadmill training reduces cardiomyocytes morphofunctional changes induced by *Trypanosoma cruzi* infection, an effect potentially mediated by an increase in antioxidant enzymes activity with subsequent inhibition of oxidative cell damage. **Supported by:**:FAPEMIG, CAPES

BC037 - THE PHOSPHORYLATED FORM OF THE RNA POL II OF *TRYPANOSOMA CRUZI* IS THE FORM TRANSCRIPTIONALLY ACTIVE

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RNA polymerase II is a large, multisubunit enzyme, which catalyzes the transcription of protein coding genes in eukaryotic cells. Transcription initiation and elongation, splicing reactions, and termination are regulated by differential phosphorylation of C-terminus (CTD) of the largest subunit of RNA Pol II (Rpb1) in highly conserved heptapeptide repeats. It has been shown that in *T. brucei* the largest subunit is also phosphorylated despite the lack of the typical heptapeptide repeats, but the function of these phosphorylations are unknown in this class of parasite that display a divergent transcriptional regulation. Here we studied the relationship between the possible phosphorylation of *T. cruzi* Rpb1 (DM28c strain) with the transcription events. Western blots using anti-CTD showed that, likewise in other eukaryotes, Rpb1 of *T. cruzi* is found at least two forms, one phosphorylated and other dephosphorylated. The phosphorylated form is tightly associated to the chromatin. Addition of the transcription inhibitor proflavine, genotoxic agents (hydrogen peroxide methyl methanesulfonate), or heat shock led to a markedly decrease of the phosphorylated form with dissociation of the enzyme from the chromatin. Immunofluorescence analysis, however, indicated that RNA Pol II remained concentrated in a nuclear spot, known to contain spliced leader RNA genes, with a marked decrease in RNA transcription, as seen by bromo-deoxyuridine triphosphate labeling. These results lead us to conclude that both forms of RNA Pol II remains associated to SL RNA DNA, but only the phosphorylated form is strongly attached to the chromatin and transcriptionally active.**Supported by:**:Fapesp

BC038 - Study of the behavior of Bacteroides spp. in the interaction with Trichomonas vaginalis

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The *Bacteroides* genus is composed by gram-negative bacillus obligate anaerobes, bile-resistant and non-spore –forming bacteria. The genus species are commonly found on the mucous membranes, including the female genital tract. *Bacteroides fragilis* is considered, clinically, the most important in its genus, once it is the most commonly bacterium isolated from endogenous infectious processes, being usually associated to gastrointestinal, respiratory and female genital tracts infections. The anaerobic infections are usually polymicrobial and endogenous. *Trichomonas vaginalis*, is an anaerobic facultative flagellate parasite with worldwide distribution which is trichomoniasis the etiologic agent, the most common non-viral sexually transmitted disease in humans. In women, the infection is associated to complications like cervical cancer, infertility, atypical inflammatory pelvic disease and HIV progression. As for the infection persistence, this parasite possesses a phagocytosis mechanism of microorganisms, but this process is not completely elucidated. In this study we use *T. vaginalis* trophozoites and the strain ATCC 25285 of *B. fragilis*. We performed parasite-bacterium interaction in 1:10, 1:50 and 1:100 proportions in periods between 6 and 48 hours, and viability tests have been applied. The samples have been fixed and processed as usual for scanning electron microscopy (SEM) and transmission electron microscopy (TEM). These data have been analyzed to rival the pathogen growth *in vitro* in the presence and absence of *B. fragilis*. Preliminary results showed that in the smaller parasite-bacterium interaction proportions there are no alterations in the parasite proliferation or in its ultrastructure, indicating evolution of the infection in a regular form even in the presence of *B. fragilis*.**Supported by:**:FAPERJ

BC039 - Morphology and taxonomy of ciliates (Alveolata, Ciliophora) from an eutrophized water reservoir in Belém, PA.

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The northern region of Brazil contains a huge diversity of animals and plants, but also of unicellular eukaryotes, which were object of relatively few descriptive studies so far. Among those, the ciliates (Phylum Ciliophora) display the widest range of morphological diversification and are important functional elements in the microbial food webs. In the present study, we investigated the diversity of ciliates present in an eutrophized artificial reservoir, located on the Guamá campus of the Federal University of Pará. Samples of water with suspension debris were collected between november of 2011 and may of 2012, using glass bottles (500ml) with a lid. The ciliates were first observed *in vivo* under a stereomicroscope, in order to identify morphotypes and preliminarily estimate their relative abundance using an ACFOR criterion. Proper identification and morphological characterization of the ciliates was performed after bright-field and phase-contrast observations (100x – 1.000x), protargol-impregnation and transmission electron-microscopy preparations. As result, we found 23 species among the genera *Brachonella*, *Caenomorpha*, *Chilodonella*, *Cristigera*, *Euplotes*, *Loxodes*, *Metopus*, *Paramecium*, *Plagiopyla, Saprodinium*, *Spirostomum*, *Tetmemena*, *Urocentrum* and *Urosoma*, representing a total of eight ciliate classes. Among the studied species, *Cristigera hammeri* and *Urocentrum turbo* were the most abundant. Remarkably, most of the organisms found in the study area are characteristic of polysaprobic/microaerophylic environments, which is consistent with the water characteristics therein.

BC040 - On the Ultrastructural Organization of *Trypanosoma cruzi* Using Cryopreparation Methods and Electron Tomography

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The structural organization of *Trypanosoma cruzi* has been intensely investigated by different microscopy techniques. At the electron microscopy level, bi-dimensional analysis of thin sections of chemically fixed cells has been one of the most commonly used techniques, despite the known potential of generating artifacts during chemical fixation and the subsequent steps of sample preparation. In contrast, more sophisticated and elaborate techniques, such as cryofixation followed by freeze substitution that are known to preserve the samples in a more close-to-native state, have not been widely applied to *T. cruzi*. In addition, the 3D characterization of such cells has been carried out mostly using 3D reconstruction from serial sections, currently considered a low resolution technique when compared to electron tomography. In this work, we re-visited the 3D ultrastructure of *T. cruzi* using a combination of two approaches: (1) analysis of both conventionally processed and cryofixed and freeze substituted cells and (2) 3D reconstruction of large volumes by serial electron tomography. The analysis of high-pressure frozen and freeze substituted parasites showed novel characteristics in a number of intracellular structures, both in their structure and content. Organelles generally showed a smooth and regular morphology in some cases presenting a characteristic electron dense content. Ribosomes and new microtubule sets showed an unexpected localization in the cell body. The improved preservation and imaging in 3D of *T. cruzi* cells using cryopreparation techniques has revealed some novel aspects of the ultrastructural organization of this parasite.**Supported by:**:CNPq, FAPERJ, FINEP and CAPES

BC041 - Interaction of Phytomonas serpens with Oncopeltus fasciatus hemocytes

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Phytomonas spp. are the etiological agents of important plant diseases in Central and South America. These parasites are transmitted to plants through the bite of an infected phytophagous hemipteran. Despite the economic importance of the *Phytomonas* parasites, many aspects of their life cycle are mostly unknown. Here, we described the interaction of the tomato parasite *P. serpens* with the hemocytes of the hemipteran *Oncopeltus fasciatus*, using a model of systemic infection, where the parasites were inoculated into the insect haemocel. The hemocytes responded to the infection by trapping the parasites in nodular structures and by phagocytizing the parasites. Using fluorescence microscopy and transmission electron microscopy of the infected hemocytes we observed that the parasites were localized inside vacuolar structures, and that these vacuoles appear to fuse with lysosomes. Moreover, despite cellular immune response towards the parasites, *P. serpens* multiplied in the hemolymph and reached the *O. fasciatus* salivary glands. The present study reports for the first time the interaction of *Phytomonas* parasites with insect hemocytes. Ongoing experiments in our laboratory aim at further studying the molecular aspects that underline these interactions, in order to understand the role of the insect immune responses towards *Phytomonas* parasites and how these parasites circumvent these events to complete their life cycle. **Supported by:**:CNPq, FAPERJ, CAPES, INCT-Entomologia Molecular

BC042 - Molecular mechanisms involved in netosis induction by *Leishmania amazonensis*.

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Neutrophil extracellular traps (NETs) are web-like structures composed by chromatin associated with cytoplasmic and granules proteins, released during netosis, a novel neutrophil death mechanism which depends on reactive oxygen species (ROS) production by the NADPH oxidase enzyme. *Leishmania amazonensis* promastigotes (La) induce NET release and are caught and killed by these structures. Here, we aim to elucidate the signaling pathways behind NET release after La stimulation. Hence, neutrophils, isolated from healthy donors, were pretreated with inhibitors of protein kinase C (PKC; Bisindolilmaleimide - BIS I and RO-31), phosphoinositide 3-kinase (PI3K; LY-294002, Wortmannin, AS-605240), ERK (PD98059) or calcium chelators (BAPTA and EGTA) and then activated by PMA, a classic activator of NETs, or La. NETs were measured as DNA in the culture supernatants. ROS generation was measured with specific probes by flow citometry. Our results showed that RO-31 treatment decreased NET release by neutrophils stimulated with PMA (78%) and with La (32%), and BIS I inhibited 50% La-induced NET. Also, LY-294002 (17%), Wortmannin (54%) and AS-605240 (50%) decreased NETs release by neutrophils stimulated with La. LY-294002 and Wortmannin decreased 36% netosis induced by PMA. AS-605240 completely abrogated ROS generation induced by both inducers. PD98059 decreased netosis stimulated with PMA (42%) and La (45%). Furthermore, immunoblotting showed ERK phosphorylation 15 minutes after neutrophil-parasite interaction and ERK inhibition diminished ROS generation by neutrophils stimulated with PMA (90%) or La (53%). BAPTA pretreatment decreased 40% NET release, whereas EGTA inhibited 79% netosis stimulated by La. In totum, we demonstrated that La induced netosis occurs with the participation of PKC, ERK, PI3K and calcium. Additionally, PI3K and ERK activation are upstream ROS production on netosis signaling pathway. Thanks to Hemotherapy Service of HUCFF, UFRJ. **Supported by:**:CAPES, CNPq, FAPERJ

BC043 - LAMP-1 AND LAMP-2 DIFFERENTIAL ROLE DURING T. CRUZI HOST CELL INVASION AND INTRACELLULAR MULTPLICATION

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Lysosomes are essential for T. cruzi invasion. Recently, we have shown that LAMP proteins 1 and 2, the major lysosomal membrane proteins, are key factors during T. cruzi infection in host cells. Deficiency in both LAMP-1 and 2 leads to reduced parasite cell invasion, but also augments parasite intracellular multiplication. Similar in structure, LAMP proteins are also highly sialilated and sialic acid plays an important role facilitating parasite cell invasion, helping in the formation of a tight parasitophorous vacuole. However, this proteins only have 37% sequence homology. Therefore not only sialic acid, but also intrinsic characteristics of the protein could be involved in its role during infection. Invasion assays using LAMP-1 or 2 knockout cells had shown that LAMP-1 seemed to be more important during parasite cell entry, resembling the LAMP-1/2 KO cells, while LAMP-2 presented an intermediate invasion level between the latter and WT cells. To evaluate whether sialic acid modifications in LAMP were still important in this process, we performed morphometrical analysis of T. cruzi parasitophorous vacuole derived from WT and LAMP-1/2 knockout cells. No differences were found, suggesting that LAMP silaic acid is, apparently, not important for host cell invasion. We then evaluated whether deficiency in both or each isoform of LAMP interfered with lysosomal exocytosis, a critical event for parasite internalization. However, no differences in lysosomal exocytic ability were found among the different cell types. We have also evaluated parasite intracellular development and have shown that LAMP-2 is more important to T. cruzi intracellular multiplication. All these results together confirm a specific role for each LAMP protein during parasite infection. We are now performing other experiments to determine how each LAMP participates during T. cruzi invasion and intracellular multiplication. **Supported by:**:CNPq and FAPEMIG

BC044 - 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-β1 by infected macrophages leading the degradation of inducible nitric oxide (NO) synthase, NO production inhibition and, consequently, persistence in these cells. Here, PS^+ and PS^- subpopulations 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 subpopulations, but lower when compared to the total population. Growth in macrophages was higher for the total *T. gondii* population, intermediate for the PS^+ subpopulation and lower for the PS^- subpopulation. Production of NO by macrophages was inhibited after infection with PS^+ subpopulation and the total population of tachyzoites. However, PS^- subpopulation was not able to inhibit NO production. PS^+ subpopulation invaded macrophages by active penetration as demonstrated by predominance of tight-fitting vacuoles, but the PS^- subpopulation entered macrophages by phagocytosis, as seen by prevalence of loose-fitting vacuoles containing these tachyzoites. The entrance mechanism of both subpopulations were confirmed in a non-professional cell line where only the PS^+ parasites 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 infection. **Supported by:**:FAPERJ CNPQ

BC045 - EFFECT OF LOW–INTENSITY LASER IRRADIATION ON EXPERIMENTAL CUTANEOUS LEISHMANIOSIS WOUND HEALING DYNAMICS

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Text: Cutaneous leishmaniasis (CL) is a common health problem in Brazil and causes typical and difficult-to-heal wound. Conventional treatment has many limitations such as side effects, drug resistance and the need for long term medication. Given these difficulties new and alternative treatments should be pursued. Here we analyzed the effect of Low–Intensity Laser irradiation on the healing of wounds caused by experimental CL. Methods/Results: 20 BALB/c male mice were inoculated with $5x10^6$ promastigotes of MAB-6 isolate of *Leishmania (Leishmania) amazonensis* in their left footpad. Approximately seven weeks after infection, half of the mice with lesions were treated with a laser of 660 nm for five consecutive days, with 3.0 J/cm2. During treatment, their feet were measured daily and macroscopical alterations were recorded. Both treated and control mice were sacrificed having their footpads removed and processed for histology, histochemistry (Sirius Red) for collagen analysis and immunohistochemistry for macrophage (MP-23) analysis. Treated foot reduced in size (4.8 ± 0.3 mm) compared to untreated ones (5.8 ± 0.7 mm). Mice submitted to laser therapy had a decrease in edema and an increase in reepithelization areas. There was a significant decrease in lesion size and wound number per animal. However, untreated lesions had higher collagen fiber expression than treated ones. MP-23 macrophage expression was also higher in untreated lesions. Conclusion: Our results show that 660nm laser irradiation is effective in minimizing local detrimental effects of leishmania infection. Laser irradiation is likely to affect wound healing dynamics, producing marked alterations in both inflammatory and proliferative phases of tissue repairing process. Patients with CL could benefit from laser, accelerating the healing process and reducing side effects of conventional treatment. **Supported by:**:CNPq/FAPEG

BC046 - Effect of arylimidamides and dicationicamidine on functional activity of murine peritoneal macrophages and cardiac cells infected by *Trypanosoma cruzi* in vitro

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Chagas disease is a neglected disease affecting more than 10 million people in Latin America, lacking an effective medicine since the available drugs present limited activity and high side effects. In this vein, was investigated the effect of three aromatic amidines (DB569, DB766 and DB889) on the functional activation of peritoneal macrophages (PM) and cardiac cells (CC) before in vitro infection with bloodstream trypomastigotes (BT) of *T.cruzi*(Y strain). The cultures were pre-treated for 24 h with IC50 values of each amidine previously determined against BT, rinsed to remove all compounds and infected for up to 48h of infection (10:1 parasite:cell ratio). The exposure of uninfected CC toamidinesleads to an impairment of the parasitism, exhibiting 46±4%, 41±8% and52±9%of infectionreduction,when DB569, DB889 and DB766 were used, respectively. These levels reached almost the same rates as those found when the compounds were added after infection. Similar findings were noticed when PM were employed as host cells, demonstrating that these amidines induce a “memory effect” as observed in CC, possibly due to (i) maintenance of active metabolites into the cytoplasm of the host cells and/or (ii) ability of the compounds to exert an activation profile in the mammalian cells,impairing*T.cruzi*survival as previously reported for Apicomplexa parasites. In this context, to explore the later possibility, the nitric oxide (NO) production was measured. DB569 and DB889 increased release of nitrite in the supernatants of CC as well as PM infected or not by T. cruzi, as much as IFN-g and TNF-a addition. Increased nitrite levels in the uninfected treated host cells suggest that amidines may be regulators of NO production, acting on proliferation control of the intracellular parasites. Our present data demonstrate the interplay effect of amidine as trypanocidal agent as well as mammalian cells activator, contributing for their excellent activity as microbicidal candidates.**Supported by:**:CNPQ

BC047 - A new role for Galectin-3 during intracellular parasite multiplication.

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Galectin-3 is a protein from the beta-galactoside-binding family and it is involved in several biological events, including protein trafficking, endocytosis pathways and cytoskeleton rearrangement. We have already demonstrated that extracellular amastigotes (EA) from *Trypanosoma cruzi* were associated with a galectin-3 containing structure at times related to the vacuole lysys in mouse embryonic fibroblasts (MEFs). The aim of this study was to evaluate the presence of galectin-3 during intracellular traffic and its importance during invasion and multiplication of *T. cruzi* EA. For the invasion (2 hours) and multiplication (24, 48, 72 and 96 hours) assays, we collected peritoneal macrophage from wild type (WT) or galectin-3 knockout C57Bl/6 and infected them with a ratio of 1:10 cells to EA, then 100 of total cells were counted for invasion and 100 infected cells for multiplication. Using kinects assays we analyze the presence of galectin-3 during intracellular traffic of both EA and trypomastigotes forms. We also wanted to demonstrate that recruitment of galectin-3 was dependent on the host cell and not from the parasite, so we conduct the same experiment using fixed parasites with 4% formaldehyde instead. Those experiments are being counted for events of colocalization between galectin-3 and actin marked with a monoclonal and faloidina-TRITC, respectively. Until now, there was no significant difference between invasion on cells galectin-3 knockout or WT, but multiplication increased significantly on knockout cells at 72 and 96 hours after the invasion, suggesting a new role from galectin-3 in the multiplication rate. Financial Support: FAPEMIG, CAPES and CNPq. **Supported by:**:FAPEMIG / CAPES / CNPq

BC048 - Rho-actin signaling pathway is linked with cellular events such as proliferation and endocytosis in Trypanosoma cruzi.

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Rho family GTPases play critical roles in phagocytosis, intracellular transport, adhesion and morphology, performing these functions via the actin cytoskeleton. The etiologic agent of Chagas disease has orthologs for Rho, actin, and several actin-binding proteins. In the current work, we carry out assays to link Rho phenotypes with actin cytoskeleton. *T. cruzi* clone Dm 28c stably transfected to overexpress actin (Actin-GFP) or the dominant negative Rho (GFP-RhoT20N) both in fusion with GFP, were used to analyse proliferation in axenic medium, endocytosis with fluorescent tracers and infectivity. The assays were performed in the presence or absence of actin microfilaments disrupting drugs such as cytochalasin D (induce depolymerization of F-actin) and jasplakinolide (induce actin polymerization). After incubation with drugs, the deficit in proliferation observed for parasites overexpressing actin was similar to that observed for parasites expressing the dominant negative Rho, suggesting that actin and rho are members of a similar signaling pathway. The receptor-mediated and fluid phase endocytosis were impaired by the adition of jasplakinolide, except for parasites overexpressing actin, whose tracer uptake was increased up to 3 times compared to control. Actin-GFP and GFP-RhoT20N shows an arrest of ~35% in endocytosis under normal conditions compared to control (Dm28c-GFP). However, GFP-Actin recovered the endocytic potential in the presence of jasplakinolide. Trypomastigote suspensions of Dm28c GFP, Actin-GFP and GFP-T20N grown in Vero pCDNA3.1 were used in assays of in vitro infection. Similar levels of infected cells and amastigogenesis was observed, except at 36 h p.i., whose infection was increased by GFP-RhoT20N trypomastigotes. The suggested increase in GFP-RhoT20N infection may be related to low matrix adhesion and greater parasites mobility, as shown previously by the group. These results provide a better understanding in cell physiology of *T. cruzi*. **Supported by:**:FAPERJ, IFRJ-Prociência

BC049 - The O2 consumption of the Angomonas deanei endosymbiont

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Some trypanosomatid protozoa, as *Angomonas deanei*, harbour a symbiotic bacterium. This relationship is characterized by a mutualism, in which both partners co-evolve, thus constituting an excellent model to study the origin of organelles and the cellular evolution. Some host alterations can be noticed due to the presence of the symbiont, as morphological modifications and intense metabolic exchanges. The symbiont contains enzymes and metabolic precursor that complete essential biosynthetic routes in the trypanosomatid, as the urea cycle and hemin production. Conversely, the symbiont obtain part of the required ATP molecules from the host, as also described to other obligatory intracellular bacteria, The presence of the symbiont influences the trypanosomatid energetic metabolism, since the wild strain of *A. deanei* shows an increased O_2 consumption when compared to the aposymbiotic strain. As a first step, we investigated the *A. deanei* symbiont genome in order to identify genesequences that encode subunits of all complexes of the oxidative phosphorylation. Our results showed that the complex I, IV and FoF1 ATP synthase are present, however some important subunits are absent in such complexes. In order to characterize the symbiont contribution to host energetic metabolism, we tested the effect of different inhibitors and an ionophore on isolated bacteria of *A. deanei*. It was not observed inhibition of oxygen consumption when oligomycin (FoF1 ATP synthase inhibitor) is used in the respiration buffer. The FCCP, a proton ionophore, did not increase the O_2 consumption, which means that the synthesis of ATP is not coupled to a proton gradient. The cyanide, a potent inhibitor of complex IV, completely abolishes O_2 consumption in symbiont. Taken together, our data suggest that the symbionts are able to respire once isolated from the host protozoan, thus presenting an active respiratory chain. Supported: CAPES, FAPERJ, CNPQ, INCTEN. **Supported by:**:CAPES, FAPERJ, CNPQ, INCTEN

BC050 - Experimental infection of pigs with RH and ME-49 strains of *Toxoplasma gondii*: hematological, serum biochemistry and biological tests

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Toxoplasma gondii causes Toxoplasmosis, a worldwide disease that infects one third of the human population. Medium sized animal experimentation is necessary for the development of new therapeutic approaches to human diseases and pigs are a good model due to physiological similarities. Here hematological parameters, parasite circulation and tissue dissemination in a T. gondii pig infection model was monitored. Castrated female minipigs BR-1 were infected intramuscularly with tachyzoites (RH strain), or by the oral route with cysts (ME-49 strain). For the first 14 days, three times a week, blood was analyzed by classical hematology and serum biochemistry, and buffy coat cells were inoculated in mice to determine tachyzoite circulation. Hematology and serum biochemistry continued twice a week for the next 14 days, and weekly after 30 days of infection. After 90 days pigs were euthanized and tissues were histopathologically processed and pepsin digested for mice inoculation; later MAT serology was done in mice. No alterations were observed in the erythrocytes series nor platelets values, but an increased ($p<0.05$) in band neutrophils were detected 7 days after infection in the ME-49 group, probably due to ileitis. Mice serology reveled tachyzoites of the ME-49 and RH strain in peripheral blood between 2 - 9 and 9 - 11 days post infection, respectively. Tachyzoites were observed in blood smears outside and inside neutrophils and monocytes. Alanine aminotransferase was high at days 21 and 32 post infection in pigs infected with the RH strain, possibly due to hepatic injury caused by parasitic replication. After 90 days, mice serology confirmed ME-49 cysts in heart, ileum and mesenteric lymph node. Infected pigs presented similar human outcome with relative low pathogenicity and no death. Furthermore, it was possible to monitor physiological changes and the infection indicating that the model minipig BR-1 ME-49 is suitable to monitor experimental toxoplasmosis. **Supported by:**:UENF, FAPERJ, CAPES, CNPQ

BC051 - Effect of Posaconazole on three-dimensional cardiomyocytes culture during *Trypanosoma cruzi* infection with emphasis in extracellular matrix proteins and gap junctions.

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Infection by Trypanosoma cruzi (T. cruzi) has cardiomyopathy as the most common manifestation with progressive deposition of fibrous tissue and rhythm disorders. Recent findings from our group demonstrated that three-dimensional (3D) culture of cardiomyocytes infected with T. cruzi reproduces features such as fibrosis and hypertrophy (Garzoni et al., 2008). The treatment of this pathology is performed with nifurtimox and benznidazole, however, these drugs have major side effects and low efficacy in the chronic phase. In this context is extreme important the study of therapeutic strategies that allow patients to better conditions. An excellent candidate is the compound posaconazole (Schering-Plough Research Institute), a inhibitor of sterol synthesis. It has potent anti- T. cruzi activity in vitro and in vivo and improves cardiac function in infected and treated animals (Romanha et al., 2010). The purpose of this study is to evaluate the effect of posaconazole in the reversion of cardiac damage through the analysis of proteins involved in fibrosis and intercellular communication. To analyze the action of this compound, cardiomyocytes of fetal Swiss webster mice were obtained and the 3D cultures were infected with trypomastigotes forms of T. cruzi (Y strain) at 20:1 parasite: cardiomyocyte ratio. The treatment of the cultures with a concentration of 5 nM posaconazole started after 144 hours of interaction parasites - cardiac spheroids and the analyses were performed at 96 hours post treatment. Immunofluorescence and western blot were utilized for the analysis of fibronectin, laminin and the Cx43 expression. Our results showed that Posaconazole was able to reduce the expression of extracellular matrix proteins as laminin and fibronectin and recover the cx43 organization, suggesting that this compound besides presents a potent trypanocida activity is able to reduce proteins involved on fibrosis genesis and to contribute for the electrical function improvement. **Supported by:**:Instituto Oswaldo Cruz, CnPQ

BC052 - Role of angiogenesis in chagasic cardiomyopathy in the experimental model of acute Chagas disease

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In chagasic cardiomyopathy microvascular changes lead to tissue ischemia and cell death triggering the inflammatory process and cardiac remodeling. In situations of ischemia and inflammation the activation of mechanisms that regulate angiogenesis mainly through the induction of vascular endothelial growth factor (VEGF), which also induces increased vascular permeability. Diseases that activate tissue metabolism and cell growth such as cancer and chronic inflammatory diseases such as rheumatoid arthritis, angiogenesis, in an attempt to improve the perfusion, contributes to the progression and maintenance of tissue damage. Our data demonstrate that T. cruzi acute infection in mice induces: i) the decrease in functional capillary density and vascular reactivity, increased leukocyte rolling and adhesion and increased vascular permeability, by intravital microscopy ii) structural changes in the coronary microcirculation by exacerbated vascular growth resulting in changes of cardiac tissue architecture and iii) increased expression of VEGF and its receptor Flk-1 in the heart. Our results suggest a harmful effect of exacerbated angiogenesis which contributes to the maintenance of inflammation and cardiac remodeling in chagasic cardiomyopathy. In this context, we will begin anti VEGF therapy by treatment of infected animals for the control of exacerbated vascular growth, decreased vascular permeability and reduction of inflammation. We believe that in future the VEGF signaling pathway may be a promising chemotherapeutic target for use in combination therapy with trypanocidal drugs aimed at the improvement of patients with Chagas disease.**Supported by:**:IOC, POM Fiocruz, FAPERJ, CNPq

BC053 - *Trypanosoma cruzi* heparin-binding proteins present serine proteinase activity

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Heparin-binding proteins (HBPs) play a key role in *Trypanosoma cruzi*-host cell interactions. HBPs recognize heparan sulfate (HS) at the host cell surface and are able to induce the cytoadherence and invasion of this parasite. Herein, we analyzed the biochemical properties of the HBPs and also evaluated the expression and subcellular localization of HBPs in *T. cruzi* trypomastigotes. A flow cytometry analysis revealed that HBPs are highly expressed at the surface of trypomastigotes, and their peculiar localization mainly at the flagellar membrane, which is known as an important signaling domain, may enhance their binding to HS and elicit the parasite invasion. The plasmon surface resonance results demonstrated the stability of HBPs and their affinity to HS and heparin. Additionally, gelatinolytic activities of 70 kDa, 65.8 kDa and 59 kDa HBPs over a broad pH range (5.5-8.0) were revealed using a zymography assay. These proteolytic activities were sensitive to serine proteinase inhibitors, such as aprotinin and phenylmethylsulfonyl fluoride, suggesting that HBPs have the properties of trypsin-like proteinases.

BC054 - Interaction of *Trypanosoma cruzi* infective forms and monocytes produces microvesicles with parasite proteins that increase infection of host cells

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Trypanosoma cruzi, the causative agent of Chagas disease, has evolved several mechanisms to survive the hostile environments encountered during its life cycle. As part of these mechanisms, our group demonstrated that microvesicles (MVs) production during the interaction of metacyclic forms and host cells protect the parasite from complement lysis enhancing the parasite infectivity and promoting a higher infection in mice (Cestari et al., 2012). Different strains of *T. cruzi* share this feature and, although all forms of parasite induce MVs formation, THP-1 interaction with metacyclic and cell derived trypomastigotes showed higher number of MVs induction. Proteomic analysis of purified MVs has shown that both cells, parasite and monocyte, contribute with membranes for the formation of these structures. However, trypomastigote membrane proteins can be observed in higher number than proteins from other stages of the parasite, among them 85 kDa surface glycoprotein and transialidase. These vesicles also present a high percentage of exposed phosphatidylserine (59%). Further analysis using NBD-PE fluorescent lipids showed that MVs from monocytes and all stages of parasite can fuse, with more fusion between MVs of trypomastigotes interaction. In vitro transwell assays showed that MVs produced exclusively during the interaction of the infective forms and monocyte in the upper chamber increased significantly (~90%) the infectivity of parasites into Vero cells in the lower chamber. The same was not observed with non infective parasite stage. MVs are internalized by Vero cells in a process dependent on actin polymerization, as demonstrated with Cythocalasin D and Wortmannin treatment. Based on the differences presented between MVs from parasite stages, we are interested in dissect the MVs pathway in infection as well as understand the signals of these structures in the communication between parasite and host cell.**Supported by:**:Capes

BC055 - Biological behavior of Leishmania (Viannia) panamensis in Mesocricetus auratus.

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In order to standardize an experimental model to study biological parameters of *L*. (*V*.) *panamensis* infection, hamsters were inoculated subcutaneously with 10^7 promastigotes in the culture stationary phase. The parasite was isolated from a human cutaneous lesion in Panama and it was characterized by molecular techniques. The evolution of experimental infection was evaluated weekly by the measure of the hind footpads swelling. At 30th and 60th day PI, biopsies from the skin inoculation site were collected for histopathological studies and determination of the parasite load by limiting dilution. Increase in the lesion size was observed until 56th day PI followed by a regression. The parasite load in the skin was higher at 30th PI ($4.63x10^8$ parasites/g of tissue) than at 60th PI ($1.29x10^7$). Concerning to the histopathological changes, an intense mononuclear inflammatory infiltrate characterized by the presence macrophages with mild to moderate parasitism and lymphocytes was present in the dermis at 30th PI. Granulomatous reaction with the presence of giant cells was also observed. At 60th PI, the inflammatory process decreased and it was characterized by moderate mononuclear infiltrate, well defined epithelioid granulomas with the presence of giant cells and lamellar bodies without evidence of parasites. The results showed that *Mesocricetus auratus* is susceptible to *Leishmania* (*Viannia*) *panamensis* infection and could be used as experimental model. More studies are necessary in order to characterize the humoral and cellular immune response in this murine model. **Supported by:** LIM-50 HC-FMUSP.

BC056 - CELLULAR RESPONSE OF SKIN FIBROBLAST TO LEISHMANIA (LEISHMANIA) AMAZONENSIS INFECTION

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The first moments of infection by protozoa of Leishmania genus occur in the host skin, which is the primary organ involved in infection. Studies point to the possibility that fibroblasts can represent a target cell in Leishmania infection due to their abundant presence at the sites of parasite inoculation. In this work we characterized the by light and electron microscopy and cytometry flow the interaction and the cellular response of skin fibroblasts (SF) during the infection with *L. (L.) amazonensis*. The SF was obtained from primary cultures of C57BL/6 mice embryos and infected with L. (L.) amazonensis promastigotes forms. The analysis by light and electron microscopy revealed that *L. (L.) amazonensis* promastigotes adheres to primary cultures of skin fibroblasts but without a preferential orientation, adhered both by the posterior region from the promastigote cell body as well as by the flagellum. After the first steps of infection, the promastigotes initially localized in narrow parasitophorous vacuoles, convert to amastigotes, being later on localized in larger vacuoles. Evaluation of the cellular response of SF showed that these cells were able to respond in the first two hours of infection, with the production of inflammatory mediators such as IL-12, IFN-gamma, iNOS and more increases expression of MHC II. However, after the establishment of infection the parasites were able to modulate this response, increasing the production of IL-4, IL-10 and TGF-beta, reducing the expression of MHC II and NO production, making of SF an environment refuge to the survival of the parasite.

BC057 - Oxidative stress and histopathology in canine visceral leishmaniasis: imbalance of trace elements and antioxidant enzymes

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Leishmania activates macrophages to synthesize large quantities of reactive oxygen species and nitric oxide, which have cytotoxic effects. To protect against oxidative damage, organisms have developed a variety of defenses that include metal sequestering proteins, no enzymatic antioxidants and specialized antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase in which copper, zinc, iron and selenium are required for activity. The aim of this study was to investigate trace elements, oxidative stress and pathology in dogs naturally infected with L. infantum. Thirty dogs with clinical status defined as symptomatic and asymptomatic were used. Serum levels of trace elements and nitric oxide were measured by atomic absorption spectrometry and the Greiss reaction, respectively. Antioxidant enzymes were measured by spectrophotometry and lipid peroxidation by the TBARs method. Microscopic and morphometric analyses were used for histological studies of liver, spleen and lymph nodes, and tissue iron deposition. We observed decreasing in serum iron, zinc and selenium levels and in catalase and glutathione peroxidase activities. In contrast, the copper level and superoxide dismutase activity increased. These results were associated with oxidative stress as evaluated by higher levels of nitric oxide and malonyldialdehyde, and the inference was corroborated by the increased iron deposition in tissues and histopathologic injury in symptomatic dogs. We concluded that an imbalance of trace elements, especially iron and copper, can affect antioxidant cytoprotective systems, enzymes that are important in generating oxidative stress, and enhance lipid peroxidation and histological alterations in CVL. **Supported by:**:FAPEMIG APQ00068-08; APQ-01355-09

BC058 - Susceptibility and worse histopathology in Canine Visceral Leishmaniasis: iron implication

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Canine Visceral Leishmaniasis (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. This fact is associated to abnormal collagen synthesis, parasite-host interactions and Nitric Oxide (NO) synthesis. Some works have been demonstrated correlations between iron increases, NO production and collagen deposition. Based on this fact, our aim was to investigate iron levels and its correlation with NO synthesis; 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 9 asymptomatic (ASD); 19 symptomatic (SD) and 9 control (C) dogs. Serum of all and samples from livers and spleens for histological studies were collected. Graphite Furnace Atomic Absorption Spectrometric was used for iron serical detection. Histological studies were done in tissue using Prussia Blue for iron, Gomori ammoniacal silver-staining for collagen deposition and immunohistochemistry to detect iNOS expression. NO detection was done by Griess technique. We found a higher deposition of iron in livers and spleens tissues of SD than ASD and C group. However, a lower detection of iron serum levels was observed in SD group than the other groups. Moreover, besides NO levels were higher in SD and ASD than C dogs, NO levels were higher in ASD than SD. The same was found to INOS expression. In addition, higher colagenesis deposition and parasite load was observed in SD than ASD and C. In according to these results, we have seen an inverse correlation between iron and INOS expression and activity and direct correlation between iron and collagen deposition in CVL. So, we could consider that there is a correlation among iron levels, INOS fisiology, pathology and clinical disease in CVL. **Supported by:**:FAPEMIG - APQ00068-08 APQ-01355-09 and CNPq - 473601/2009-5

BC059 - *TRYPANOSOMA CRUZI* CONGENITAL TRANSMISSION IN EXPERIMENTAL INFECTION

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Introduction: *Trypanosoma cruzi* is an intracellular obligatory parasite. The parasite life cycle is heteroxenic involving mammalian and Reduvidae Family hosts. The principal forms of transmission are contamination during infected vector blood meal, blood transfusion, congenital and orally. *T. cruzi* is subdivided in several groups, G and CL strains are examples of *T. cruzi* I and IV subdivisions. CL strain induces higher parasitemia in acute phase while G strain induces low or none parasitemia in the same phase. Thus, the influence of parasitemia in development of gestation was evaluated. Methodology: C57BL/6 female mice were orally infected by trypomastigotes from G or CL strains. After *T. cruzi* infection females were put together with male in order to procreate. From this day on the vaginal plug was observed every day, as a copula confirmation. The offspring were analyzed from 28º day after birth, considering the presence of parasite. Results: Vaginal plug was observed in every female and gestational confirmation of others females was observed by abdominal growth. From those, 75% consisted of female mice infected with CL strain that did not sustained gestation. Others females just developed gestation in chronic phase. In G strain group, 25% of female did not sustained gestation and others developed gestation in acute phase. Embryo implantation took place in the acute phase of infection in females of G strain, whereas in CL strain infected females embryo implantation occurred only in chronic phase. Conclusion: These results suggest that, in the case of CL strain infected female, higher parasitemia in acute phase interfere in development of gestation. Acute phase caused by infection of G strain, otherwise, was not sufficient to impair the development of gestation.**Supported by:**:FAPEMIG, CAPES e CNPq