BC.01 - *Trypanosoma cruzi* eIF2α IS PHOSPHORYLATED DURING NUTRITIONAL STRESS REQUIRED FOR DIFFERENTIATION INTO THE INFECTIVE FORMS.

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Unlike other eukaryotes, in trypanosomatids regulation of gene expression is essentially post-transcriptional. Nevertheless, mechanisms of regulation of translation initiation have not been described in these parasites, although they express typical initiation factors and also three eIF2α kinases. The transformation of *Trypanosoma cruzi* epimastigotes into infective metacyclic trypomastigotes (metacyclogenesis), occurs naturally in the last portion of the digestive tract of triatomine insect vector, when nutrients are scarce. Metacyclogenesis can be reproduced *in vitro* when epimastigotes are submitted to a nutrient poor medium that mimics the composition of triatomine urine (TAU) followed by incubation in TAU supplemented with amino acids and glucose (TAU3AAG). Here we investigated whether the phosphorylation of the alpha subunit of eIF2, which causes translational arrest in response to amino acid starvation in other eukaryotes, is involved in this differentiation process. Trypanosomatids' eIF2α diverge from other eukaryotes in having a threonine residue (T^{169}) in place of the typical serine 51 that is phosphorylated in all other eukaryotes. We generated antibodies that recognize the phosphorylated form of *T. cruzi* eIF2α specifically at Thr^{169}. The specificity of this antibody was determined by showing that recognition was abolished after treatment with active λ-phosphatase and the antibodies did not react with the eIF2α protein of trypanosomes containing a Thr^{169}Ala mutation. When exponentially growing epimastigotes were incubated in TAU medium, an increase in Tc-eIF2α phosphorylation was observed along with a simultaneous decrease in polysomes and in total protein synthesis. Upon transfer to TAU3AAG medium, there was a decrease in eIF2α phosphorylation concomitant with partial recovery of translation. These results indicate that nutritional stress in trypanosomatids result in phosphorylation of Tc-eIF2α and translational arrest. Furthermore, our analysis also indicated that translational inhibition mediated by eIF2α phosphorylation is involved in the generation of infective forms of *T. cruzi*. Supported by FAPESP and CNPq

BC.02 - DIFFERENTIAL EXPRESSION AND CELLULAR ADDRESSING OF GP82 AND GP90 SURFACE PROTEINS DURING *TRYPANOSOMA CRUZI* METACYCLOGENESIS

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Trypanosoma cruzi GP82 and GP90 are GPI anchored glycoproteins, members of the *trans*-sialidase-like family, expressed on the surface of metacyclic trypomastigote. GP82 is an adhesin involved in mammalian cell invasion while GP90 seems to act in a negative manner. Previous studies demonstrated that GP82 and GP90 mRNAs are stabilized and associated to polyssomes at the metacyclic trypomastigote stage. Metacyclogenesis involves the differentiation of replicating non-infective epimastigotes into non-replicating metacyclic trypomastigotes. This process is characterized by the presence of intermediate forms accompanied by several morphological changes and structural alterations. In this work we analyzed the expression of GP82 and GP90 genes during *in vitro* metacyclogenesis, giving special attention to intermediate forms. Epimastigotes were incubated in TAU medium and the following time points were analyzed: stationary epimastigotes before and after 2 h stress, epimastigotes and intermediate forms attached to culture flask 24 h and 48 h after inoculum in TAU3AAG. We observed increased levels of GP82 and GP90 mRNAs by real-time PCR in epimastigotes after 2 h of stress and also in epimastigotes and intermediate forms attached to culture flask. Protein levels were determined by western blot. While GP90 seems to increase continuously after 2 h stress, GP82 peaks in attached cells at 48 h. Immunofluorescence analysis of intermediate forms showed that GP90 localizes in the flagellar pocket (FP) and plasma membrane (PM), indicating that it is being produced and exported from Golgi to FP and then to PM. In contrast, GP82 localizes in reservosomes and PM, being easily detected at 48 h in those organelles. This indicates that GP82 proceed in a different way passing through reservosomes before reaching the PM. It is possible that GP82 undergoes some processing step in reservosomes before going to the PM. Further characterization of GP82 and GP90 processing during metacyclogenesis is underway in our laboratory. Supported by FAPESP and CNPq.

BC.03 - HeLa CELL INVASION BY AMASTIGOTES: REMARKABLE DIFFERENCES BETWEEN *Trypanosoma cruzi* AND *Leishmania L. amazonensis*

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Trypanosoma cruzi amastigotes of the G strain promptly invade mammalian cells in culture. During the invasion of HeLa cells, actin-rich surface protrusions originally named 'cups' are formed. *Leishmania (L.) amazonensis* axenic amastigotes also invade HeLa cells, but with a much lower efficiency. The aim of this study was to examine the membrane protrusions formed during the invasion of HeLa cells by amastigotes of the two parasites. HeLa cells were infected for 30 min-1h with *T. cruzi* or for 2-4h with *L. L. amazonensis* amastigotes and: 1: imaged live on a spinning disk system with HeLa cells transfected with GPI-YFP and LAMP1-RFP or; 2: processed for field emission scanning electron microscopy. Alternatively, samples on coverslips were lysed with 1% Triton X-100 in the presence of 10 μg/ml of taxol and phalloidin to stabilize microtubules and actin microfilaments. Samples were fixed in aldehydes and post-fixed with OsO_4 and tannic acid. Contrast was also applied using rotary shadowing with Pt. *T. cruzi* amastigotes promptly invade HeLa cells and by live microscopy, cups are detectable around 10 min. after parasites were added to the cells. By contrast, *Leishmania* parasites do not cause significant alterations on the markers even after 1h. By SEM *T. cruzi* EA readily attach to and deform the tips of surface microvilli whereas *Leishmania* amastigotesare rarely seen in contact with the surface projections. TX-100 extracted material reveal parasites surrounded by meshwork of filaments at the early stages of *T. cruzi* invasion whereas *Leishmania* parasites are only detectable inside the cells at 2 and 24 h post infection. These results reveal that amastigotes of the two species engage distinct mechanisms to colonize HeLa cells. Supported by FAPESP, CNPq and CAPES.

BC.04 - THE PROTEIN KINASE D IS RECRUITED TO INVASION SITES OF *TRYPANOSOMA CRUZI* EXTRACELLULAR AMASTIGOTES (EA)

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Trypanosoma cruzi life cycle comprises distinct developmental stages. Extracellular Amastigotes (EA) are generated by the extracellular differentiation of trypomastigotes and are dependent on host actin filaments polymerization to invade cultured cells. Signaling events surrounding these processes are poorly understood. The protein kinase D (PKD) family comprises three different but closely related serine-threonine kinases, PKD1, PKD2, and PKD3, all of which have a highly conserved N-terminal regulatory domain containing two cysteine-rich diacylglycerol (DAG) binding domains and an autoinhibitory pleckstrin homology (PH) domain. PKD not only is a direct DAG target but also lies downstream of PKCs in a novel signal transduction pathway implicated in the regulation of multiple fundamental biological processes such as cell shape, adhesion, and migration. At the leading edge of migrating cells active PKD co-localizes with F-actin, Arp3 and cortactin. Cortactin has emerged as a key signaling protein in cellular processes such as endocytosis and tumor invasion. The ability of cortactin to interact with and alter the cortical actin network is central to its role in regulating these processes. We attempted to evaluate the structural requirements of PKD in the EA uptake of HeLa cells. HeLa cells were transfected with cortactin and PKD GFP-vectors, infected with EA and examined for the acquisition of these markers. Wild type PKD1 and 2, but not PKD3 are recruited to sites of actin remodeling and EA invasion, which also contain cortactin. Both PKD1 pleckstrin homology-deleted and PKD1-kinase-dead were not recruited to EA invasion sites. PKD1 lacking N-terminal domain was recruited to and colocalized with actin and cortactin. These results together with previous literature data suggest that EA internalization could be regulated by different processes from those which regulate other actin related events like lamellipodia formation. Support: FAPESP, CAPES

BC.05 - INDUCTION OF PROINFLAMMATORY CYTOKINES AND NITRIC OXIDE BY *TRYPANOSOMA CRUZI* IN RENAL CELLS

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Chagas disease, caused by the protozoan parasite *Trypanosoma cruzi*, is tipically associated with cardiac involvement. In the murine model of *T. cruzi* infection, myocarditis may develop during the acute phase, but prior to the cardiac alteration the infected animals present renal inflammatory infiltration causing acute kidney injury (AKY) due to an ischemia/reperfusion lesion. In an attempt to understand the genesis of AKI in *T. cruzi* infection, the various aspects of parasite-renal cell interactions were examined in this study by using three cell lines (HMC, MDCK and LLC-PK1). The susceptibility to infection of these cells was low, even after 72 h interaction with trypomastigotes. HMC cells were the most resistant and LLC-PK1 cells the most susceptible to *T. cruzi*, about 13% of the latter being infected and supporting robust intracellular parasite multiplication. Upon interaction with *T. cruzi*, some cell line-dependent alterations in the cellular integrity and in the levels of inflammatory mediators, such as nitric oxide (NO), tumor factor necrosis-alpha (TNF-α) and interferon-gamma (INF-σ), were observed. Mesangial HMC cells, but not the other cell types, had their viability diminished and NO release augmented upon 72h contact with the parasites. NO production was induced in HMC and MDCK cells upon 3 h or 72 h incubation with *T. cruzi* secreted factors, and this NO-inducing property was abolished by heating. The recombinant *T. cruzi* trans-sialidase, an enzyme that is secreted during the acute infection in mice and humans, displayed a temperature-sensitive NO-inducing capacity toward HMC and MDCK cells. Increased levels of TNF-α and INF-σ were detected in HMC cell cultures at 72 h post-infection. Our results suggest that ischemia/reperfusion lesions in acute *T. cruzi* infection do not result from cellular parasitism; they are apparently associated with the alterations in renal cells and generation of inflammatory mediators. Supported by FAPERJ; CNPq and FIOCRUZ/RJ

BC.06 – DOES PHOSPHATIDYLSERINE MODULATE *Trypanosoma cruzi* ENTRY'S PROCESS INTO PERITONEAL MACROPHAGES?

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Trypanosoma cruzi, the etiological agent of Chagas disease, is an intracellular parasite that as others intracellular pathogens developed evasion mechanisms, enabling the establishment of infection. It has been shown that virus and parasitic protozoa, including *Leishmania amazonensis* and *Toxoplasma gondii*, are capable of mimicking mammalian apoptotic cell death by the exposure of phosphatidylserine (PS). This process, called "apoptotic mimicry", justify the occurrence of apoptotic features in a unicellular pathogen. Seabra *et al* (2006) demonstrated that the exposure of PS by a population of *T.Cruzi* trypomastigotes, but not epimastigotes or intracellular amastigotes, leads to the deactivation of macrophages through a TGF-β signaling pathway. Here, we investigated if the presence of PS modulates trypomastigotes' entry process into macrophages. In order to quantify the percentage of PS^+ trypomastigotes, we used flow cytometry and a low percentage was found. Then, we separated PS^- and PS^+ trypomastigotes, using an Annexin V microbead kit, which allowed us to interact separately these subpopulations with macrophages for 1, 24 and 48 hours, always comparing with total population (PS^- and PS^+). The subpopulations' separation through this Kit showed a consonance with the percentage of PS^+ trypomastigotes found through flow cytometry, validating this method. We could observe that the PS^+ parasite's internalization was drastic diminished (higher than 90%) within 24 and 48 hours of interaction. The *T. cruzi*'s adhesion index was not altered. Besides that, PS^- parasites showed an infection's progression of 60% higher when compared with total population within 48 hours. By field emission scanning electron microscopy, we observed that PS^- parasites enter in macrophages preferentially by anterior region, but further quantifications will be done to confirm this data. Together, our findings suggest the participation of PS in trypomastigotes' entry process into peritoneal macrophages, indicating cooperation between PS^- and PS^+ parasites in the establishment of the infection. Supported by CNPq, CAPES and Faperj.

BC.07 - MURINE INFECTION WITH *TRYPANOSOMA CRUZI* LIKE ISOLATED FROM BATS: THE FIRST FIVE DAYS

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Bats are important reservoirs of trypanosomes of the subgenus *Schizotrypanum* including *Trypanosoma cruzi* and other trypanosomatids. This study aims to evaluate the kinetics of infection within the first 120 hours in mice inoculated with trypanosomes isolated from bat species *Artibeus planirostris* (EM437), *Phyllostomus hastatus* (EM239 and EM245) and *Phyllostomus discolor* (EM465). The isolates were genetically characterized by multiplex-PCR with primers D72/D75/RG3 and PCR-RFLP analysis of the mitochondrial cytochrome oxidase subunit II gene (COII-RFLP). To study the kinetics of infection, groups of 13 non-isogenic mice per isolate were inoculated intradermally in the psoas muscle with 2 x 10ϖ culture trypomastigotes/mL. Mice were euthanized at 2, 4, 8, 12, 24, 36, 48, 60, 72, 84, 96, 108 and 120 hours after infection and their paws were removed for histopathological studies by HE stain. In multiplex-PCR and COII-RFLP all isolates showed amplification products of 250 bp and 263 bp, respectively, which are compatible with *T. cruzi* I. In all animals inoculated with four isolates, the histopathological study of the psoas muscle showed the presence of intense inflammatory infiltrates characterized by polymorphonuclear, neutrophils and eosinophils in the first 48 hours. After 60 hours the infiltrate was composed primarily of the eosinophils. Unlike what is observed in infections with *T. cruzi* isolated from human infections, where there are high numbers of amastigotes within macrophages, infection by these isolates found only one to six amastigotes forms within scarce macrophages from 36 hours to 120 hours. This can tell us: 1) that these isolates are not virulent and pathogenic, 2) that the forms of amastigotes observed early in infection did not correspond to *T. cruzi* but other trypanosomatids the subgenus *Schizotrypanum*. Supported by CAPES. CNPq, FAPEMIG and FUNEPU.

BC.08 - BIOLOGICAL BEHAVIOR OF A STRAIN OF Trypanosoma cruzi TcV GROUP ISOLATED FROM A HUMAN CONGENITAL TRANSMISSION

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Data about biological behavior of *Trypanosoma cruzi* TcV group is scarce in the literature, however, suggest its association with congenital transmission in some endemic regions for Chagas disease. In this study, we analyzed the in vitro and in vivo behavior of TcV strain 3048, isolated from a Brazilian child infected by congenital transmission. Balb/C females mice were infected with 1 x 10^5 trypomastigotes by the intraperitoneal route. The parasitemia was evaluated during 40 days using the microhematocrit and blood fresh examination. After 4 months of infection (chronic phase), the animals were killed and 16 tissue/organs were collected to histopathological analysis. The metacyclogenesis was performed by cultivation in TAU (Artificial Urine Triatomine) medium and the infectivity for cells was assessed by exposition of MK2 cells to trypomastigotes. During the acute phase the parasitemia was subpatent and was detectable in 100% of animals only by the microhematocrit. No mortality was observed among animals in both acute and chronic phases. No intracellular amastigotes nests were detected by hematoxylin-eosin staining, althought, different degrees of inflammatory foci (IF) with mononuclear cells and macrophages were seen: moderate/intense IF in spleen (66,6%), smooth muscle (55.6% gastroesophageal and 44.5% gastroduodenal junctions), lungs (44.4%) and uterus (22.2%); mild/moderate IF in liver (88,9%), skeletal muscle and diaphragm (55.5%); mild IF in heart (88.9%). Metacyclogenesis rate after 7 days in TAU medium was 9% (90% broad forms).The rate of infected MK2 cells was 74.3% and 74.6% after 3 and 72 hours respectively, the number of amastigotes/cell (72 h exposure) was 1.6. The strain 3048 (TcV), despite the high in vitro infectivity for Mk2 cells, presented a low blood and tissue parasitism in Balb/C mice, with greater impairment of smooth muscle and organs of the mononuclear phagocytic system as liver and spleen, but in a few cases in uterus.
Supported by Capes, CNPq Universal 2008; FUNEPU and FAPEMIG.

BC.09 - CONGENITAL TRANSMISSION OF EXPERIMENTAL CHAGAS DISEASE INDUCED BY *Trypanosoma cruzi* STRAIN GROUP TcV

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The factors involved in congenital transmission of Chagas disease are not completely understood and include multifactorial mechanisms related to the host and the *Trypanosoma cruzi* strain. In this study, we evaluate the congenital transmission in mice infected with strain 3048 (TcV), isolated from a child infected via the same pathway. A total of twenty female BALB/c mice were intraperitonially infected with 1 x 10^5 trypomastigotes. Ten of these females were mated during the acute phase of the infection and another ten in the chronic phase. The microhematocrit technique was performed to evaluate the parasitemia during pregnancy. Congenital infection was diagnosed either by hemoculture in LIT (Liver Infusion Tryptose) and by PCR (polymerase chain reaction) using 121 and 122 primers. A half of the offspring from each litter was submitted to euthanasia after birth when heart, liver and spleen were obtained focusing the PCR tests. The other half of the offspring provided sources of blood for hemoculture and to perform PCR at 30 and 60 days of life. In the acute phase, the maternal mortality rate was 40% (4/10) and no female reached pregnancy. In the chronic phase, this rate was reduced to 10% (1/10), in which all females reached pregnancy, the mean litter size per offspring was 8.67 and the mortality rate was 6.41% (5/78). Parasitemia levels presented a subpatent profile and were detectable only by the microhematocrit method during pregnancy. Hemoculture tests were negative for all offspring. The congenital transmission rate detected by PCR were 43.84% (32/73), in which 45.45% (15/33) corresponded to tissue samples obtained from newborn offspring and 42.50% (17/40) to blood samples obtained after 30 days of life. The hemoculture test showed itself ineffective in the diagnosis of congenital transmission. Strain 3048 (TcV) was able to vertically transmit Chagas disease, even in the presence of low maternal parasitaemia levels.
Supported by Capes, CNPq Universal 2008; FUNEPU and FAPEMIG

BC.10 - DIFFERENT CHANGES IN THE NUMBER OF CIRCULATING LEUKOCYTES IN PERIPHERAL BLOOD OF MICE INFECTED WITH BLOOD OR METACYCLIC TRYPOMASTIGOTES OF *TRYPANOSOMA CRUZI*

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Although it was shown that metacyclic and blood trypomastigotes are completely functional in relation to parasite-host interaction and invasion of the target cell, they differ in the molecules present on the surface. Based on this, the aim of this study was to investigate changes in peripheral blood leukocytes before and after infection with metacyclic (MT) or blood (BT) forms of Be-78 *T. cruzi* strain. Animals of the MT group showed an increase in total leukocyte at 28 days after infection, and this increase was maintained until 42 days after infection. However animals of the BT group showed an increase of these cells only in 42 days after infection. Moreover, animals in the MT group presented an increase in the number of eosinophils throughout infection, this increase was not observed in BT group. Regarding monocytes, animals in the BT group showed an early increase of these cells on the seventh day after infection, but this increase was not maintained throughout the infection, returning to baseline already on the fourteenth day after infection. The values of lymphocytes were found elevated after the twenty-first day after infection in group MT, but in group BT there was an early increase on the seventh day, declining after this day. Increased again only in the forty-second day after infection. Taking together, these results demonstrate that the source of inoculum can influence the course of Chagas' disease, because infection with metacyclic forms it takes change early in a greater number of cells which could explain the lower parasitemia of this group compared to the animals infected by blood forms.
Supported by FAPEMIG (PPM, Redes Toxifar e Bioterismo), CNPq and UFOP.

BC.11 - B1 CELLS ARE TARGETS OF INFECTION BY *TRYPANOSOMA CRUZI* IN VITRO AND IN VIVO

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T. cruzi has the ability to infect different mammalian cell types in vitro and in vivo, but infectivity may vary considerably depending on the parasite strain and the host cell type. Trypomastigotes of CL strain used in this study efficiently enter cultured epithelial cells, as well fibroblasts, and replicate intracellularly as amastigotes. Mouse peritoneal macrophages are also invaded but the number of intracellular parasites decreases with time. Here we examined the ability of CL strain trypomastigotes to invade and develop in C57BL/6 mouse B1 cells, which predominate in the peritoneal cavity and can be distinguished from conventional B cells. Of all cell types examined to date, B1 cells (CD23+CD11b+CD19+), which transform in a novel type of mononuclear phagocytes in culture while maintaining lymphoid characteristics, were the most susceptible to *T. cruzi* infection. Incubation of B1 cells with trypomastigotes for 1 h, at 1:1 parasite:cell ratio, resulted in about 60% infected cells, whereas under the same condition the percentage of infected HeLa cells was <1%. B1 cells also supported intense parasite multiplication and trypomastigotes were released into medium by 96 h. To determine whether B1 cells were targets for *T. cruzi* in vivo, C57BL/6 mice were separated in two groups. The control group received PBS intraperitoneally and the other group received heat-inactivated *Propionibacterium acnes*, which induces an increase in macrophages, immature dendritic cells and B1 cells. Twenty four hours later, the animals were challenged with *T. cruzi* trypomastigotes labeled with fluorescent dye CFSE. Cells were collected from the peritoneal cavity of control and experimental mice 2 hours after challenge and analyzed by flow cytometry. The number of B1 cells infected with *T. cruzi*, i.e., cells containing labeled parasites was about 6 fold higher in cells from mice inoculated with *P. acnes* than in cells derived from control mice. Work supported by FAPESP and CNPq.

BC.12 - FAK SIGNALING PATHWAY IS INVOLVED IN *T.CRUZI*-CARDIOMYOCYTE INTERACTION

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Trypanosoma cruzi invasion is mediated by receptor-ligand recognition between the surface of both parasite and target cell. *Different signaling pathways may be activated during mammalian cells invasion, including protein tyrosine kinases activation that can be important in the regulation of parasite entry.* In this work, we demonstrate the participation of protein tyrosine kinases, especially focal adhesion kinase (FAK) and SRC, during T. cruzi invasion in cardiomyocytes. Treatment of cardiomyocytes with genistein, a protein tyrosine kinase inhibitor, PP1, a potent SRC-family protein inhibitor and PF573228, a potent FAK inhibitor, reduced in a dose-dependent manner the invasion of T. cruzi. Furthermore, T. cruzi entry was accompanied by changes in c-SRC expression and FAK phosphorylation levels. Enhancement of FAK activation takes place during initial stage of T. cruzi-cardiomyocyte interaction (30 and 60 min) with concomitant 2-fold increase in the level of c-SRC expression. Dephosphorylation of FAK also coincided with lower level of c-SRC expression after 2h of interaction, suggesting that FAK/c-SRC promotes an integrated signaling that coordinates parasite entry. These data provide novel insight into signaling pathway involved in T. cruzi uptake in cardiomyocytes. Another approach of this study was to evaluate the effect of T. cruzi infection on focal adhesion proteins, since structural changes, including breakdown of myofibrillar (Pereira et al., 1993), besides downregulation of α-cardiac-actin mRNA, were evidenced in cardiomyocytes infected by T. cruzi (Pereira et al., 2000). Our results demonstrated that the localization of focal adhesion proteins remained unaltered in 24h-infected cardiomyocytes. In contrast, biochemical assays demonstrated a decline in focal adhesion proteins expression after 72h of infection. Our data demonstrate that T. cruzi infection disturbs the structural integrity in cardiomyocytes in vitro, which can result in loss of cardiac tension, suggesting that structural changes can contribute to the cardiac dysfunction evidenced in Chagas' disease. Supported by FIOCRUZ, CAPES and FAPERJ.

BC.13 - LIMP-1 IS IMPORTANT FOR *TRYPANOSOMA CRUZI* EXTRACELLULAR AMASTIGOTE INTERNALIZATION INTO MEF CELLS

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The parasitophorous vacuole membrane formed during cell infection by *Trypanosoma cruzi*, the intracellular protozoan that causes Chagas' disease in humans, may display endocytic pathway markers such as Associated and Integrated lysosomal membrane glycoproteins, LAMPs (LAMP-1 and LAMP-2) and LIMPs (LIMP-1 and LIMP-2), respectively. Previous studies have suggested that extracellular amastigotes (EA) cell invasion, but not the multiplication of parasites within cells, could be differentially modulated in LAMP knockout mammalian cells suggesting that LAMPs might play a role in internalization processes, although the exact function has not yet been elucidated. Our study aimed to evaluate the role of LAMP and LIMP proteins during the invasion by EA of *T. cruzi* (G strain) using mouse embryonic fibroblast cell lines (MEFs) derived from LAMP-1, LAMP-2, LIMP-1 and LIMP-2 single knockout and LAMP-1 and 2 double knockout mice. Our results indicate that the lack of LIMP-1 decreases cell invasion by the parasite, while in LAMP-2 or LIMP-2 knockout cells we observed an increased level of parasite internalization. We propose that lysosomal glycoproteins can modulate events related to cell internalization processes.
Supported by CNPq and FAPESP.

BC.14 - HOST-PATHOGEN INTERPLAY: HOW INHIBITORS CAN AFFECT BOTH PARTNERS AND MODULATE INFECTIVITY

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Trypanosoma cruzi amastigotes which are generated by the extracellular differentiation of trypomastigotes are referred to as Extracellular Amastigotes (EAs) and are able to invade mammalian cells. EAs of the G strain promptly aggregate actin filaments by attaching to dorsal microvilli of HeLa cells and, as a result, cup-like structures are formed underneath the parasites. EAs is therefore dependent on host actin filament polymerization to invade cells. However, detailed signaling events surrounding these processes are still obscure. In the present study, we aimed to evaluate the effect of signaling pathways' inhibitors on both parasite and host cells. Interacting partners were pre-treated with PP2 and DASATINIB (Src kinase pathway inhibitors); wortmannin, 3-methyladenine and LY294002 (conventional phosphatidylinositol kinase inhibitors); rapamycin and ATM/ATR inhibitor (phosphatidylinositol-related kinase inhibitors) and a protein kinase D inhibitor. Control cells and parasites were left untreated. HeLa or Vero cells were then infected with EAs. The treatment of host cells with Src inhibitors decreased EA entry, thus suggesting that EA may need Fyn-Src kinase pathway to invade cells. By contrast, after treatment with conventional phosphatidylinositol kinase inhibitors, parasites acquired LAMP-1 (Lysosomal Associated Membrane Protein-1) markers faster than the controls and remained longer in LAMP-1 positive vacuoles. The inhibition of the phosphatidylinositol-related kinase pathways in host cells led to the enhancement of parasite entry. Here, we propose a signaling pathway model of EA entry into host cells different from that already described for trypomastigotes (TCTs).
Supported by: FAPESP.

BC.15 – CHOLESTEROL DEPLETION IN PRIMARY MURINE CARDIOMYOCYTES DIMINISHES *TRYPANOSOMA CRUZI* ENTRY AND ALTERS LYSOSOMAL FUSION FOR THE FORMATION OF THE PARASITOPHOROUS VACUOLE

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Trypanosoma cruzi, the etiological agent of Chagas´disease, can invade several types of non-professional phagocytic cells including myocytes and others. Invasion occurs when parasites attach to and stimulate host cells, producing intracellular calcium signaling events that culminate with lysosome recruitment and fusion with the host cell plasma membrane for the formation of the parasitophorous vacuole. Several factors influence *T. cruzi* entry in host cells: *membrane rafts*, sphingolipid and cholesterol enriched plasma membrane domains, interferes in parasite invasion of fibroblasts and macrophages. Recently we have also shown that both membrane rafts and cholesterol are important for parasite invasion in murine cardiomyocytes. However this mechanism of *T. cruzi* host cell invasion through rafts is still under investigation. Since lysosomes are crucial for parasite stable host cell infection, we decided to study the effect of cholesterol depletion in the recruitment of these organelles during *T. cruzi* entry. We verified that lysosomal fusion during parasite invasion diminished when cholesterol was depleted by incubation with MβCD. Treatment with HγCD, a cyclodextrin with less affinity for cholesterol, did not show any difference in relation to control cells. We also studied the effect of host PI-3 kinase inhibition in cholesterol depleted cells, since this enzyme apparently regulates lysosomal fusion during parasite invasion. No significant differences have been observed. We then investigated if cholesterol depletion deregulates lysosomal exocytosis. Our results show that cholesterol depletion alone leads to lysosomal exocytosis, suggesting that these membrane domains might regulate lysosomal fusion events. We have also tested the effects of cholesterol depletion in host cell actin distribution and observed a more organized cortical cytoskeleton pattern in cells with less cholesterol. Altogether, our results suggest that cholesterol depletion alters host cell lysosomal fusion with the plasma membrane interfering with the process of *T. cruzi* host cell invasion. Supported by Capes/INCT, CNPq and FAPEMIG

BC-16 - ANALYSIS OF BIOLOGICAL BEHAVIOR AND CONGENITAL TRANSMISSION IN EXPERIMENTAL CHAGAS' DISEASE INDUCED BY *Trypanosoma cruzi* STRAIN GROUP TcI

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Nowadays congenital Chagas' disease has become a public health problem in several countries because of migration of infected people from endemic countries. We evaluate the biological behavior and congenital transmission in mice with strain AQ1-7 (TcI), isolated from *Triatoma sordida*. We used 20 female Balb/C, half for analyzing the biological behavior and the remainder for mating in the chronic phase. The inoculum used was $1x10^5$ trypomastigotes by the intraperitoneal route. Parasitemia was monitored by microhematocrit and blood fresh examination. The rate of infectivity was analyzed in MK2 cells. The histological analysis of 16 organs, obtained in the chronic phase, was performed with hematoxylin-eosin. The females were mated after 30 days of infection and congenital transmission was diagnosed by hemoculture in LIT medium and PCR (primers 121 and 122). Half of the offspring was killed at birth and heart, liver and spleen were obtained to perform PCR. The other half were killed 60 days after birth and blood was collected for hemoculture and PCR. Parasitemia presented subpatent and experimental infection was confirmed only by hemoculture. The histological analysis revealed inflammation of mild to moderate and the parasite has been found in the skeletal muscle in only one animal. The infectivity in MK2 cells was 69.7% after 3 hours of infection, reaching 100% after 72 hours. During mating occurred the death of a female (1/10) and one was not pregnant (1/9). The average number of offspring per female was 5.33 (43 offspring in total). Hemoculture resulted negative for all offsprings. However, the blood PCR was positive in 33.33% (8/24) and PCR tissue in 89.47% (17/19), representing an overall rate of congenital transmission of 58.14% (25/43). The strain AQ1-7 (TcI) showed a high rate of congenital transmission, suggesting the importance of this genotype be better studied in the context of vertical transmission. Supported by Capes, CNPq Universal 2008; FUNEPU and FAPEMIG.

BC.17 - AMASTIN AS A VIRULENCE FACTOR OF *TRYPANOSOMA CRUZI*

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Trypanosoma cruzi extracellular amastigote (EA) from G strain (*T. cruzi* I) shows high infectivity towards host cells *in vitro* when compared to the traditionally more infectious CL strain (*T. cruzi* IV). We observed in a microarray data that amastin, an amastigote stage-specific surface protein, was 21 times more expressed in EA from CL strain. In order to study the putative involvement of amastin in cell cycle, we cloned, expressed and purified the less hydrophobic region of amastin in fusion with GST to developed polyclonal antibodies in rabbits. Immunolocalization of amastin in *T. cruzi* amastigotes confirmed its location on the parasite surface. HeLa cells pre-incubated with 5 μg/ml of the recombinant protein showed a decreased in cell invasion by EA. Parasites from G strain were transfected with pTREX-Amastin-GFP and pTREX-GFP: a significant decrease in cell invasion of EA that over-expressed amastin was observed when compared to the controls. However, the number of trypomastigotes released into the supernatant of infected HeLa cells 96h and 120h after the invasion was higher in cells infected with the parasites that over-expressed amastin. In susceptible mouse strains (A/J) EA overexpressing amastin were precociously observed in liver and spleen nests whereas parasitemia was never detected. These results show that amastin plays a key role in the course of intracellular *T. cruzi* infection both *in vitro* as well as *in vivo.*

Financial support: CNPq, CAPES and FAPESP.

BC.18 - CELL SURFACE MODULATION PROTECTS Y STRAIN TRYPOMASTIGOTES OF *TRYPANOSOMA CRUZI* FROM ANTI-α-GAL ANTIBODY LYSIS

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Trypanosoma cruzi trypomastigotes present a negative charge mainly attributed to the presence of sialic acids that is incorporated mostly into mucin-like molecules and that prevent the parasite killing by complement and Chagasic anti-α-Gal antibodies (anti-α-Gal). We previously showed that tissue culture trypomastigotes from Y strain and CL-Brener clone have distinct capacities to modulate the composition of their surfaces in the presence of cationized ferritin (CF) and Concanavalin A (Con A). In the present study we analyzed whether those cell surface modifications could inhibit the anti-α-Gal mediated lysis. Trypomastigotes were incubated for 10 min at 37°C in the presence of CF and Con A (both at 10μg/ml concentration). Then 8μl of anti-α-Gal (20μg/ml) was added, and the mixture was further incubated for 30 minutes at 37°C. As the anti-α-Gal induces both agglutination and lysis, we measure the number of motile and free (non-agglutinated) trypomastigotes in a hemocytometer. Control Y strain trypomastigotes were more susceptible to lysis by anti-α-Gal compared to CL-Brener parasites. Incubation with CF and Con A, protected Y parasites from lysis by anti-α-Gal while did not cause significant changes in CL-Brener trypomastigotes. Flow cytometry analysis of the intensity of fluorescence of Y and CL-Brener parasites after anti-α-Gal antibody labeling showed that Y parasites presented two different cell populations and that after CF and Con A incubation, the pattern of fluorescence of Y parasites changed and the most fluorescent population is lost. No changes were observed in CL-Brener parasites submitted to the same procedures. The data suggested that some anti-α-Gal binding sites in Y parasites were shed under the influence of CF and Con A and that the decrease in the amount and/or redistribution of those binding sites protect the Y parasites from the anti-α-Gal induced lysis.

Supported by: CNPq, CAPES, FAPERJ, Pronex

BC.19 - DELETION OF ALPHA-2,3-SIALILTRASFERASE IV DECREASES PARASITEMIA AND MORTALITY OF MICE INFECTED WITH *TRYPANOSOMA CRUZI*

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Chagas' disease is the leading cause of death from heart problems in endemic areas in Latin America. The most severe manifestation of the disease caused by the protozoan *Trypanosoma cruzi* is a chronic cardiomyopathy, which is mainly caused by an intense inflammatory response triggered by the parasite's persistence in heart tissue. Interaction between selectins and cell surface sialyl Lewis^{x} ligands promote tethering and rolling of leukocyte on vascular endothelium, an important step for lymphocyte homing to secondary lymphoid organs and for leukocytes recruitment to injured non-lymphoid tissues. The aim of this study was to investigate the impact of deletion of the enzyme alpha-2,3- sialiltrasferase IV (ST3Gal IV), witch catalyses the final glycosylation step in sialyl Lewis^{x} synthesis, in the infection by *T. cruzi* (Y strain). ST3Gal-IV deficient (ST3Gal IV KO) or wild type mice (WT) were infected i.p. with trypanomastigotes ($1x10^{4}$). Our results demonstrate that, despite the decrease of pro-inflammatory cytokines, such as IL-6, TNF-alpha and INF-gamma in intra-peritoneal lavage, 6 h after infection, ST3Gal-IV knockout mice infected with *T. cruzi* had a significant reduction of parasitemia and mortality, when compared with wild type mice. Consistent with these observations, $CD8^{+}$ T cells isolated from infected ST3Gal-IV KO mice showed increased cytotoxic activity. These results open a new perspective for the role of ST3Gal-IV in the development of adaptive immunity during infection by *T. cruzi*.

BC.20 - RHO-ACTIN SIGNALING *PATHWAY* IS LINKED WITH CELLULAR EVENTS SUCH AS ADHESION AND ENDOCYTOSIS IN *TRYPANOSOMA CRUZI*

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Rho family GTPases play critical roles in motility, phagocytosis, intracellular transport, adhesion and morphology, performing these functions via the actin cytoskeleton. The etiologic agent of Chagas disease, *Trypanosoma cruzi*, has orthologs for Rho, actin, and several actin-binding proteins. Knowing how the Rho orthologue acts on the actin cytoskeleton may provide a better understanding of cellular physiology of the parasite, providing new therapeutic targets. Previous experiments performed by our group showed that evolutionarily conserved Rho retains its function on cell-substrate adhesion. Previous assays showed that constitutively active Rho potentiated the event, while the inactive Rho mutant limited adhesion. In the current work, we carry out assays to link the Rho phenotypes with actin cytoskeleton. Parasites overexpressing actin showed high levels of adhesion on substrate under *in vitro* conditions. Similar results were also observed in an *ex vivo* model, incubating epimastigotes on the epithelial tissue of insects *Rhodnius prolixus* dissected. In summary, these results allow us to suggest that Rho activation and actin levels themselves leads to an enhancement of adhesion in parasites. Moreover, having prior knowledge of the damages caused by the expression of inactive Rho mutant on proliferation and receptor-mediated endocytosis, we carry out electron microscopy analysis to investigate the morphology of these parasites, was identified a raised accumulation of reservossomes in epimastigotes expressing inactive Rho mutant. These morphological changes in endocytic organelles may explain the effects already observed in endocytosis and proliferation after inactive Rho mutant expression. A better understanding of how environmental stimuli act on the regulatory proteins of GTPases, their effectors, and how they transmit stimuli to the actin cytoskeleton modulating the phenotypes observed depends on the identification of protein-protein interactions of the *pathway*. Currently, a strategy of yeast two-hybrid system is underway to identify the molecular partners of Rho-actin signaling *pathway in T. cruzi.* This work was supported by FAPERJ, CNPQ, and IFRJ-PROCIÊNCIA

BC.21 - TARGETING THE KDNA: PROMISSORY CHEMOTHERAPIES APPROACHES AGAINST TRYPANOSOMATIDS

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The mitochondrial DNA of trypanosomatids protozoa, termed kinetoplast DNA (kDNA), is a complex network, composed of topologically interlocked DNA circles, which are of two types: maxicircles and minicircles. The maxicircles, which encode ribosomal RNAs and several mitochondrial proteins, are similar in structure and genetic function to the mitochondrial DNA of other eukaryotes. A most remarkable feature of maxicircles is that most of their transcripts undergo RNA editing to form a functional mRNA, a process involving the insertion or deletion of uridine residues. The minicircles encode small guide RNA´s that control the specificity of editing. Aiming to arrest crucial steps of RNA editing in trypanosomatids that could represent an efficient chemotherapeutic approach to combat the diseases caused by these parasites, based on literature and bioinformatics analyses, presently we are searching for potential conserved sequences in the mitochondrial genome of trypanosomatids involved in the RNA anchors coding, which could finally target maxicircle sequences that should be edited. These selected sequences were found to be conserved in *Trypanosoma cruzi*, *T. brucei* and *Leishmania spp*. Next, the sequences were synthesized and assayed, through biophysical interaction studies, on eight different aromatic compounds (aromatic diamidines (AD) – including DB75 and DB569 - and arylimidamides (AIA) - including DB766, DB709, DB613, DB1831 and DB1852. Although AD and AIA present high activity and selectivity against a wide range of organisms, including *T. brucei and T. cruzi*, their mechanism of action is still poorly known. Also, literature data show morphological and biophysical evidences that many ADs are strong DNA binders triggering striking alterations on the mitochondria and KDNA of these parasite, but still very little is known regarding AIA effects. Thus, using Circular Dichroism (CD) and Tm studies we evaluated the mode of interaction and level of affinity of these aromatic compounds upon the RNAg anchor sequence. Our data show that all compounds display a typical CD spectrum of minor groove binders, with some of them leading to a considerable perturbation on the DNA molecules. Interestingly, although some of them, like DB1831 and DB766, do not bind strongly to the DNA sequence, they do present a considerable trypanocidal activity against *T. cruzi* suggesting that only their direct interaction and/or perturbation of the KDNA topology and sequence *per se* may not be the only cause of the parasite death and that other secondary effects could participate in the parasite killing processes. Supported by: Fiocruz/PAPES V, FAPERJ, CNPq, CPDD, NIH and the Gates Foundation

BC.22 - ENDOTHELIN AND BRADYKININ RECEPTORS ACTING INTERDEPENDENTLY, INCREASE VASCULAR PERMEABILITY AND DRIVE THE UPTAKE OF *TRYPANOSOMA CRUZI* BY CARDIOVASCULAR CELLS

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Infection-associated vasculopathy in Chagas' disease was ascribed to upregulated expression of endothelin-1 in parasitized myocardial tissues. Another evidence linking *T. cruzi* function to microvascular derangement came from research on pro-inflammatory proteolytic responses evoked by trypomastigotes. Using the Dm28 strain as a model, we have previously shown that tissue culture trypomastigotes (TCT) evoke neutrophil-dependent plasma leakage through mechanisms involving cooperation between TLR2, CXCR2 and bradykinin B2 receptors (BK2R). Here we examined the possibility that the endothelin pathway integrates the above mentioned activation axis. Using the hamster cheek pouch as a model, control experiments showed that topically applied TCT induced the accumulation (15-60 min) of rhodamine-labeled leukocytes in microvascular beds. These effects were markedly reduced either by BQ-788 (ETBR antagonist) or BQ-123 (ETAR antagonist), however, these ETR antagonists did not interfere with the parasite competence to evoke plasma leakage. Notwithstanding this, measurements of footpad swelling made 3 h p.i. Balb/c mice indicated that the inflammatory edema was drastically reduced by the ETR antagonists (BQ-123 or BQ-788) or by HOE-140 (BK2R antagonist). We then asked if TCT were able to invade mammalian cells *in vitro* through the signaling of ETRs. First, we found that BQ-123 or BQ-788, respectively inhibited extent of infection of CHO cells overexpressing ETARs or ETBRs. Invasion assays with mouse cardiomyocytes or human smooth muscle cells revealed that parasite-uptake was partially, albeit significantly inhibited by BQ-123, BQ-788 or HOE-140, whereas HUVECs were protected by BQ-788 or HOE-140, but not by BQ-123. Interestingly, host cell invasion was not further reduced by the combined addition of ETR antagonists and HOE-140. These results indicate that kinin-releasing strains of *T. cruzi* elicit the activation of BK2R and ETAR/ETBR in interdependent manner. Further studies may clarify if the ETR/BK2R pathway jointly contributes to infection-associated vasculopathy in Chagas' disease. Supported by CNPq and FAPERJ

BC.23 – TGF-BETA EXERTS DIFFERENTIAL EFFECT ON EXTRACELLULAR MATRIX REMODELING OF TRYPANOSOMA CRUZI- INFECTED CULTURES.

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Extracellular matrix (ECM) components are involved in the pathogenesis of the chronic phase of Chagas' disease due to its progressive accumulation. TGF-beta, a cytokine related to ECM stimulation, is implicated with chagasic fibrosis. The fact that cardiac cells present low responsiveness to TGF-beta stimulus *in vitro* opened the question whether TGF-beta treatment alters ECM remodeling after infection of different cell types with *T. cruzi*. Confocal laser scanning microscopy revealed enhancement of FN signal in cardiomyocytes (CMs) stimulated with doses higher than 10ng/ml of TGF-beta as previously demonstrated. Curiously, addition of only 1ng/ml of TGF-beta resulted in augmented FN fibril formation in skeletal myoblasts (L6E9 cells) and cardiac fibroblasts. In contrast, *T. cruzi* infection (Y strain) reduced fibrillar FN matrix in CM and L6E9, while uninfected cells in *T. cruzi*-infected culture presented FN staining similar to control. Remarkably, FN disorganization in highly infected cells was visualized even after addition of 10-15 ng/ml doses of TGF-beta. In contrast, *T cruzi* infection seems not to alter FN distribution in cardiac fibroblasts. Western blot assay revealed a raise of 2.39 and 3.35 folds in FN levels in L6E9 stimulated with 1 ng/ml and 10 ng/ml of TGF-beta, respectively. In CMs, only a 15ng/ml dose of TGF-beta induced a raise of 2 fold in FN expression, suggesting that CM are less responsive to TGF-beta treatment than skeletal mioblasts and cardiac fibroblasts concerning FN expression. This differential response can be due to distinct intracellular signaling mechanisms, since skeletal myoblasts presents phosphorylated Smad 2 levels 3 folds higher than cardiomyocytes. This way, our data raises new perspectives to evaluate alterations in TGF-beta signaling and the mechanisms that result in ECM reduction in *T. cruzi* infected cells, including cytoskeleton alterations and receptors modulation. Supported by CNPq, FAPERJ, FIOCRUZ, PAPES V.

BC.24 - *TRYPANOSOMA CRUZI*: *IN VITRO* AND *IN VIVO* EFFECT OF CARRAGEENAN

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Carrangeenans (CAR) are heterogeneous mixtures of sulfated polygalactans obtained from the cell wall of *Chondrus crispus*, a red alga found on rocky areas of the Atlantic coast of Europe and North America. CAR inhibit the replication of viruses (dengue, herpes simplex and hepatitis A), bacteria such as *Helicobacter pylor*i (Girond et al. 1991; Utt 1997; Carlucci et al. 1999; Adams et al., 2005; Buck et al, 2008) and also the binding of growth factors, such as transforming growth factor-1 (TGF-1), fibroblast growth factor-2 (FGF-2) and platelet-derived growth factor (PDGF) to cells, modulating cell invasion and proliferation. However, only a few studies were done investigating CAR effect on others pathogenic protozoa, including *Trypanosoma cruzi*. At present, the only accepted drugs for treatment of Chagas disease are nifurtimox and benznidazole. This controversy is primarily due to the undesirable side effects that frequently force the abandonment of treatment and poor indices of apparent cure from the disease (Janin and Villa 2007; Soeiro and De Castro 2009). In the present work the objective was verify the effects of CAR on *T. cruzi* evaluating both *in vitro* and *in vivo* systems. In conclusion we observed that CAR administration in Swiss mice before parasite inoculation provoked inhibition of the multiplication of circulating parasites, suggesting an antiparasite response produced by CAR.
Supported by FIOCRUZ and FAPERJ

BC.25 - COMPARATIVE ANALYSIS OF *TRYPANOSOMA* OF SUBGENUS *SCHIZOTRYPANUM* FROM BATS AND HUMAN

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Within the genus *Trypanosoma*, species of the subgenus *Schizotrypanum* can invade and develop in mammalian cells. Here we analysed *T. dionisii* from bats, which does not infect humans, *T. cruzi* strain Y from Chagasic patient and an isolate of a new lineage of *T. cruzi* associated with bats (TcBat). Metacyclic trypomastigotes of Y strain and TcBat efficiently infected human epithelial cells in medium containing serum whereas infection by *T. dionisii* was effective in PBS containing Ca^{2+}/Mg^{2+}, in absence of glucose or aminoacids. The ability of Y strain and TcBat metacyclic forms to enter host cells was mediated by the surface molecule gp82 whereas *T. dionisii* apparently relied on mucin-like molecules. Expression of members of gp82 family was detected in Y strain and TcBat but not in *T. dionisii* by monoclonal antibody (mAb) 3F6 and anti-gp82 polyclonal antibodies. Analysis of genomic organization of gp82 gene family, and the chromosomal mapping of gp82 genes, showed distinct profiles in these parasites. Profiles of surface mucin-like molecules were also different in Y strain, TcBat and *T. dionisii.* Unlike the double bands of 35 and 50 kDa detected by mAb 2B10 in immunoblots in different *T. cruzi* strains examined to date, TcBat metacylic trypomastigotes exhibited bands of approximately 90, 55 and 30 KDa. When administered orally into mice, Y strain metacyclid forms produced high parasitemias, compatible with the gastric mucin- binding and epithelial cell invasion properties of gp82. Although expressing gp82 at high levels, TcBat metacyclic forms were poorly infective in mice by the oral route, what may be due to their relative susceptibility to complement-mediated lysis. In contrast to Y strain, TcBat metacyclic forms were lysed upon incubation with undiluted normal human serum as source of complement. *T. dionisii* metacyclic forms, which are highly susceptible to complement-mediated lyis, failed to infect mice. Supported by FAPESP and CNPq.

BC.27 - IDENTIFICATION AND CLASSIFICATION OF NC-RNAS IN *TRYPANOSOMA CRUZI*: A MULTISTEP APPROACH

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Non-coding RNAs (ncRNAs) prediction has become a vast field of research and several classes of ncRNAs with different regulatory, catalytic and structural functions have been discovered. Few years ago, some kinetoplastid genomes have been finalized, and a recent study to predict ncRNAs in *Leishmania braziliensis* and *Trypanosoma brucei* has been published. Similarly, we propose to predict and classify ncRNAs for the complete genome of *Trypanosoma cruzi*. For this purpose, we used eQRNA, an algorithm for comparative analysis of biological sequences that performs probabilistic inference on genomic alignments. The entire genomes of *T. brucei* and *T. cruzi* were used to generate the initial alignments submitted to eQRNA, and 4195 ncRNA candidate sequences equal to or longer than 30 nucleotides were found. The candidate sequences were used for blastx search (e-value = 10e-05) against *T. cruzi* annotated proteins. 2816 candidates matched protein-coding sequences and the remaining 1382 candidates were submitted to a pipeline that included search against 25 different ncRNA databases, ab initio RNA tools and structural analysis. 1301 candidates had no evidence to be classified as ncRNAs and 49 candidates are tRNAs or rRNAs. Twenty-nine candidates presented similarity with ncRNAs from several databases. Our next goal is to identify putative regulatory ncRNAs that may be directed to UTR elements by matching the 29 ncRNAs to a catalog of 5' and 3' UTR sequences of *T. cruzi* transcripts retrieved from dbEST. *In silico* approaches concerning energy parameters will be employed to test the validity of these findings.
Supported by CAPES, Faperj, Fapemig

BC.28 - THE DIFFERENTIAL ROLE OF LYSOSOMAL PROTEINS LAMP-1 AND LAMP-2 IN HOST CELL INVASION BY *T. CRUZI* TISSUE CULTURE TRYPOMASTIGOTES

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Many pathogens have developed mechanisms to prevent fusion with lysosomes during cell invasion. For *Trypanosoma cruzi* (etiologic agent of Chagas disease), however, lysosomes are essential in this process, participating in the formation of the parasitophorus vacuole and in the anchoring of parasites inside host cells. Lysosomal anchorage of *T. cruzi* inside host cells is probably a consequence of the interaction of parasite surface with lysosomal integral membrane proteins. Among the lysosomal integral membrane proteins, the main ones are LAMP-1 and 2 (Lysosomal Associated Membrane Protein-1 and 2), belonging to groups lgpA and lgpB, respectively. Recently we have demonstrated that lack of this two proteins have a profound effect on host cell infection by *T. cruzi*. Invasion assays studies of *T. cruzi* trypomastigotes in fibroblasts derived from mice knocked out for these two proteins (LAMP-1/2 KO cells) showed a reduction of 50% in parasite cell entry when compared to wild type (WT) fibroblasts. On the other hand, they also revealed that parasite intracellular development is higher in LAMP-1/ 2 KO when compared to WT cells. Whether the phenotypes observed are a consequence of the absence of both isoforms concomitantly is still uncertain. Despite being both highly sialilated proteins and presenting similar structure and biochemical properties, LAMP-1 and 2 are distinct proteins that have diverged during evolution. Intraspecies comparison between the two groups, A and B, show that despite their high similarity they are still less related to each other than are, for example, mouse and mammalian proteins from the same group. Therefore, more studies are needed to reveal the unique role of each isoform of LAMP in the interaction of the parasite with the host cell. In order to investigate this issue we decided to study whether these proteins have functional differences in the interaction process of the cell with the parasite. We then performed the same invasion assays described above, now using LAMP-1 or 2 single Knock out cells (LAMP-1 KO or LAMP-2 KO). Preliminary results indicate a differential role for each LAMP isoform. Cells lacking LAMP1 (LAMP-1 KO cells), at first, seem to better reproduce the results obtained with LAMP-1/2 KO cells, showing a reduction in *T. cruzi* host cell entry as compared to WT cells. On the other hand, cells lacking LAMP-2 (LAMP-2 KO cells) showed parasite invasion rates more similar to WT cells. Thus, these results suggest the existence of a differential role of these proteins in the invasion of this parasite in the host cell. We are now investigating the effect of the absence of each LAMP isoform in the intracellular development of *T. cruzi*, aiming to determine whether the isoforms also have different roles in the intracellular multiplication of the parasite. Supported by CNPq and FAPEMIG

BC.29 – DISORGANIZATION OF TGF-BETA RECEPTOR TYPE II COSTAMERIC DISTRIBUTION IN CARDIOMYOCYTES AFFECTS TGF-BETA RESPONSE: ROLE OF *TRYPANOSOMA CRUZI* INFECTION

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Transforming growth factor beta (TGF-beta) family cytokines have been found to regulate growth, differentiation, immune response and fibrosis. TGF-beta is involved in Chagas disease establishment and progression, participating in *T. cruzi* host cell invasion, intracellular parasite cycle, regulation of immune response and heart remodeling. However, TGF-beta receptors in the host cell have been poorly studied. Since previous reports demonstrated that *T. cruzi* can modulate host cell receptors, we were interested to analyze the TGF-beta receptor type II (TbetaRII) expression and distribution during *T. cruzi* – cardiomyocyte interaction. TbetaRII staining by indirect immunofluorescence revealed an unexpected striated organization of in cardiomyocytes, which was enhanced (38%) after TGF-beta treatment. Double labeling with anti-vinculin and anti-TbetaRII antibodies showed a co-localization of TbetaRII with costameres of vinculin by confocal microscopy. The association of TbetaRII with the cytoskeleton was also demonstrated by cytochalasin D treatment, which resulted in a decrease of 45.3% in the ratio of cardiomyocytes presenting TbetaRII striations. This association of TbetaRII with the cytoskeleton may be involved in triggering TGF-beta signaling, since western blot analysis showed that cytochalasin D significantly inhibited Smad 2 phosphorylation and fibronectin stimulation after TGF-beta treatment in cardiomyocytes. *T. cruzi* infection elicited a decrease of 79.8% in the frequency of cardiomyocytes presenting TbetaRII striations. The treatment of *T. cruzi*-infected cultures with TGF-beta did not provoke any significant alteration in the frequency of TbetaRII striations, still showing low TbetaRII striation percentage, in contrast with the raise observed in control cultures. Together, these results suggest that the co-localization of TbetaRII with costameres are important to activate TGF-beta signaling cascade, and *T. cruzi* derived cytoskeleton disorganization could result in altered or low TGF-beta response in infected cardiomyocytes. **Supported by:** FIOCRUZ, FAPERJ, PAPES V and CNPq

BC.30 - INFLUENCE OF ASF1 AND THE ACETYLATION OF HISTONE H4 IN THE DNA DOUBLE STRAND BREAK IN *TRYPANOSOMA*

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Histone post-translational modifications are involved in replication, transcription, chromatin assembly and DNA repair. We have previously identified acetylations at the lysine residues 4, 10 and 14 of the histone H4 of *Trypanosoma cruzi.* K10 and K14 were also shown to increase after gamma irradiation (Nardelli et al, 2009, Chromosoma, 118:487). To understand how these modifications are associated with the DNA repair process, we overexpressed the histone H4 with the lysine 4, 10 and 14 replaced by arginine. Parasites containing mutated K10 and K14 were more sensitive to irradiation. Resistance to irradiation can be increased by overexpressing TcRad51, a protein that is required for DNA repair by homologous recombination (HR). In this overexpressor, K10 and K14, but not K4 modifications are already increased in non-irradiated cells. Upon irradiation, immunofluorescence labeling with specific antibodies for K10 and K14 modifications were found to colocalize with TcRAD51 in the nucleus. We also investigated the effect of overexpression of the histone chaperone Anti-silencing factor 1 (Asf1) in *Trypanosoma brucei.* Asf1 stabilizes the heterodimer H3/H4 and forms a complex with the histone acetyltransferase 1 (Hat1) that acetylates histone H4 at position K5 and K12 just after the synthesis. These modifications seem to correspond to K4 and K10 of *Trypanosoma* histone H4. Overexpressors of Asf1 are more sensible to DNA damage induced by gamma irradiation. Taken together, these results suggest that histone H4 acetylations at K10 and K14 residues participate in repair mechanism of double strand breaks in *Trypanosoma.* Supported by FAPESP and CNPq

BC.31 - EXPRESSION AND CHARACTERIZATION OF CLATHRIN HEAVY CHAIN IN *TRYPANOSOMA CRUZI*

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Clathrin-coated vesicles mediate cellular endocytosis of nutrients and molecules that are involved in a variety of biological processes. Basic components of the vesicle coat are clathrin heavy chain (Chc) and clathrin light chain molecules. Previous ultrastructural studies in *Trypanosoma cruzi* epimastigote and trypomastigote forms have indicated the presence of coated pits and vesicles at the flagellar pocket region, which were morphologically similar to clathrin-coated vesicles. Thus, in the present study we have identified, cloned and expressed the gene corresponding to the clathrin heavy chain of *T. cruzi*. Thereafter, we have produced a mouse polyclonal antibody and expression of the protein in epimastigote and trypomastigote forms was confirmed by Western blot. Cellular localization of clathrin in epimastigote forms was performed by immunofluorescence using a Leica SP5 confocal laser microscope, showing a location close to the flagellar pocket region. Further studies are underway to demonstrate the subcellular localization of clathrin heavy chain in epimastigotes and trypomastigotes by immunocytochemistry (transmission electron microscopy). Supported by CNPq and Fiocruz.

BC.32 - EXPRESSION AND CHARACTERIZATION OF CLATHRIN LIGHT CHAIN IN *TRYPANOSOMA CRUZI*

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The molecules involved in transport of nutrients from the cell plasma membrane into the cytoplasm (endocytosis) are poorly known in *Trypanosoma cruzi*. Coated vesicles have been already shown in the cytoplasm of *T. cruzi* epimastigotes and trypomastigotes, suggesting that this parasite is able to perform clathrin-mediated endocytosis. The clathrin protein is formed by the assembly of three heavy chains and three light chains. A previous study has indicated the expression of clathrin heavy chain in *T. cruzi*, but nothing is known about the light chain. Therefore, aim of this work is to express and characterize the subcellular localization of the clathrin light chain in *T. cruzi*. For this, we have identified the gene in the genome of this parasite, which was amplified and cloned into bacterial vectors for the expression of recombinant proteins. Specific antibodies were produced in mice and these antibodies were used to evaluate the gene expression, using immunoassays, immunofluorescence and immunocytochemistry. Our data indicate the expression of the clathrin light chain in *T. cruzi*, as demonstrated by Western blot, cellular localization by immunofluorescence using confocal laser microscopy, and subcellular localization by transmission electron microscopy by using immunocytochemistry. Characterization of the expression of clathrin light chain in *T. cruzi* helps to shed some light on the processes of endocytosis performed by this parasite, which are essential for obtaining nutrients and survival of the parasite inside the hosts.Supported by CNPq.

BC.33 - TRANSFERRIN RECYCLING IN EPIMASTIGOTES FROM *TRYPANOSOMA CRUZI*

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Eukaryotic cells internalize a wide range of molecules from the extracellular medium. One of the most extensively studied endocytic system has been the process of transferrin uptake. In mammal cells, transferring is recycled back to the cell surface coupled to its receptor. *Trypanosoma cruzi* has a characteristic organelle called reservosome, considered the final organelle of the endocytic pathway. Reservosomes are acidic compartments where endocytic cargo is stored and eventually degraded. It also concentrates proteolytic enzymes, such as cruzipain and serine carboxypeptidase, and has been considered as a late endosome or a lysosome like organelle. In 2000, Mendonça *et al* a localized TcRab11, a homologue of the molecular marker of recycling endosomes in mammal cells, at the reservosomes. In order to investigate the reservosome role as a recycling compartment, we used FITC-transferrin or gold-labeled transferrin as tracers. After 30 min of incubation with the tracer, when it is concentrated inside reservosomes, parasites was washed and incubated in fresh medium. We collected the supernatants after different times and quantified the tracer in a microplate reader. The amount of FITC-transferrin in the supernatant increased with time, decreasing in cells concomitantly. We did not detect the presence of gold labeled transferrin in the supernatants. It is possible that gold coupling impairs transferrin from going on the recycling route. Considering that we are assaying recycling from reservosomes, it is possible that transferrin arrives in the supernatant total or partially degraded. To investigate this possibility, we are performing the whole assays in the presence or absence of the inhibitors of the main reservosome proteases. Intact FITC-transferrin or its fragments were detected in the supernatant by western blot using anti-FITC antibodies. We are using the same strategy in electron microscopy assays to follow the exocytic pathway of transferrin and/or transferrin fragments from reservosomes towards cell exterior. Supported by: CAPES, FAPERJ, CNPq

BC.34 - VARIATIONS IN THE FORMS OF RNA POL II IN THE PARASITE *TRYPANOSOMA CRUZI*

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RNA polymerase II is a large, multisubunit enzyme which catalyzes the transcription of protein coding genes in eukaryotic cells. The largest subunit of RNAP II (Rpb1) contains a highly conserved heptapeptide repeat at its C-terminus (CTD) which is absent in trypanosomes. The CTD is modified by phosphorylation. The enzyme switches between the phosphorylated state, referred to as RNAP IIo and the unphosphorylated state, referred to as RNAP IIa. These variations are control switching from transcription initiation to transcription elongation, transcription termination, mRNA capping, RNA splicing and RNA polyadenylation. It has been shown recently that in *T. brucei* CTD is modified by phosphorylation, despite the lack of the heptapeptide repeats. In the present work, we subjected *T. cruzi* parasite cultures to a series of different stimulus in order to analyze the variations of the phosphorylated state of Rpb1. Our results showed that during exponential growth there was a predominance of the Rpb1 IIo form in strains Y, DM28c and CL Brener. Stationary phase was marked by an increase of the levels of Rpb1 IIa form in the same strains. Addition of proflavine (a transcription inhibitor) led to a slightly decrease in the Rpb1 IIo form and a marked increase in the Rpb1 IIa form in DM28c and CL Brener strains. When these same strains were subjected to heat shock, soon after the first 30 minutes at 42ºC the IIo form of Rpb1 decreased markedly while IIa form increased substantially. During metacyclogenesis using DM28c strain, both Rpb1 IIo and IIa forms remained equal throughout the process. Our results lead us to conclude that RNA pol II state in *T. cruzi* varies switching between two forms, one possibly phosphorylated and other unphosphorylated.
Supported by Fapesp and CNPq.

BC.35 - BIODEMES, ISOENZYMES AND MOLECULAR GENOTYPING OF *TRYPANOSOMA CRUZI* STRAINS FROM SANTA CATARINA STATE (2005) ACCORDING WITH NEW CONSENSUSFOR MOLECULAR NOMECLATURE (2009).

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In 2005 an outbreak of acute Chagas disease occurred in Santa Catarina State-Br. Twenty four persons became infected by the ingestion of sugar cane juice accidentally contaminated with this parasite in the locality of Navegantes, with three fatal cases. We received 09 strains isolated from the human cases, identified as SC94, SC95, SC96, SC97, SC98, SC99, SC100, SC101, SC102, and 2 cultures isolated in the same area from one marsupial (*Didelphis aurita*) SC90, and from a Triatomine naturally infected *(T. tibiamaculata)* SC93. *Biodemes* characterization was performed after 2 to 5 passages in mice to establish the patterns of parasitemia, mortality rates, histopathological alterations, virulence and pathogenicity. The tissue lesions in the different groups, revealed early lesions in the myocardium and skeletal muscles characteristics of the *Type II Biodeme* . In six cases the presence of late alterations together with increasing of parasitemic levels suggested double infection with *Types II / III Biodemes* corresponding to Taxas *T.cruzi II* and *T. cruzi I (1999).* For isoenzymic characterization the enzymes ASAT, ALAT, PGM and GPI were used. With the Biological methods, the strains were identified as from the *Biodeme Type II* but the isoenzymic patterns were variable, suggesting the mixture of two types of zymodemes Z1 and Z2. The findings of the present investigation are in accordance with the molecular characterization established in the 2009 Consensus, which was afterwards performed by analyzing three polymorphic genes (Cytochrome Oxidase –COII, spliced leader intergenic region - SL-IR, and 24Sα rRNA genes and six microsatellite loci (SCLE10, SCLE11, MCLF10, TcAAAT6, TcTAC15 and TcTAT20). The molecular characterization confirms that the majority of the isolates were classified into T. cruzi II lineage, but three isolates were composed by populations mixture : Tc I + Tc II or TcII + Tc VI. Supported by: FAPESB, FAPEMIG, CNPq and CAPES

BC.36 - A ROLE OF TRANSLATION INITIATION FACTOR 2 PHOSPHORYLATION IN THE DIFFERENTIATION OF *TRYPANOSOMA CRUZI*

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Control of translation initiation has an important role in the adaptation to nutrient stress in many eukaryotes. One of the key regulatory steps in protein synthesis occurs through the phosphorylation of the alpha subunit of the translation initiation factor 2 (eIF2α) by a family of proteins kinases activated under stress conditions. The phosphorylation of the serine 51 in mammalian eIF2α inhibits the GDP/GTP exchange in eiF2 preventing the formation of new t-RNA/GTP/eiF2 complexes, which are required to the start the scanning of mRNAs. In trypanosomatids eIF2α has an extra N-terminus domain and alignments with several eukaryote eIF2α revealed that Ser 51 corresponds to the Thr 169 and that an additional serine at position 43 could also be phosphorylated. Here we show that specific antibodies against a peptide containing phosphorylated Thr 169 and antibodies to mammalian Ser 51 recognizes in western blot a protein of 50 kDa corresponding to eIF2α of *Trypanosoma cruzi* epimastigotes. The reactivity of these antibodies increases upon several nutritional stress, which decrease protein synthesis and are able to induce differentiation into metacyclic forms of the parasites. *T. cruzi* expressing eIF2a mutants (T169A and S43A) were generated and used to confirm the lack of reactivity in these cases. We are currently investigating whether parasites over expressing these mutants are able to differentiate after nutritional stress.
Supported by Fapesp and CNPq.

BC.37 - TOR-like 1 kinase is involved in the control of osmotic stress response in *Trypanosoma brucei*

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Target of rapamycin (TOR) kinases are highly conserved protein kinases that integrate signals from nutrients and growth factors to coordinate cell growth and cell cycle progression. Two TOR kinases were described with the function of controlling cell growth in the protozoan parasite *Trypanosoma brucei,* the causative agent of African trypanosomiasis. However, two additional sequences were described as encoding putative kinases with high domain similarity to TOR kinases in *T. brucei*: TbTOR-like 1 and TbTOR-like 2. Here we studied the TOR-like 1 protein, which contains an unusual PDZ domain, not found in any other TOR kinase. PDZ domains are though to be involved in protein-protein interactions, mediating binding of a class of submembranous proteins to membrane receptors and ion channels. We previously found that ablation of TbTOR-like 1 by RNAi causes a progressive inhibition of cell proliferation with parasites accumulating in S/G2 phase of the cell cycle and showing increased cell size. Moreover, RNAi cells presented an increase in the acidocalcisomes size and higher levels of polyphosphate and pyrophosphate content. Now we show that TbTOR-like 1 localizes to unique cytosolic granules. After hyperosmotic stress, the localization of the protein shifts to the cell periphery, differently from other organelle markers. We also found that cultivating *T. brucei* under hyperosmotic conditions reduced parasite growth and increased the levels of polyphosphate within acidocalcisomes. Importantly, TbTOR-like 1 ablation resulted in an increment of polyphosphate early after induction and before growth arrest. These results suggest that TbTOR-like 1 kinase participates in the control of osmotic stress response as a consequence of changes in the acidocalcisomes and polyphosphate content in *T. brucei*. The data establishes a link between the control of cell growth by TOR kinases and the polyphosphate metabolism.
Supported by: FAPESP, CNPq and Ellison Medical Foundation.

BC.38 - The subcellular localization of phosphatidylinositol-related kinase TOR1 and TOR 2 (Target Of Rapamycin 1 and 2) in *Trypanosoma cruzi* is distinct from that in *Trypanosoma brucei*

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Target of rapamycin (TOR) is a serine/threonine (phosphatidylinositol-related) kinase that couples nutrient availability to activation of processes including metabolism, transcription and translation that lead to cell growth. TOR participates in the maintenance of energy and amino acid homeostasis. Upon nutrient starvation or energy depletion, TOR activity is inhibited, triggering processes such as autophagy aimed to maintain cellular viability and overcome unfavorable conditions. TOR acts as two functionally and structurally distinct complexes, TORC1 and TORC2. This dual function of TOR confers the capability of governing spatial and temporal cell growth separately. TORC1 controls temporal aspects of cell growth through processes such as ribosome biogenesis, transcription, translation and repression of autophagy, while TORC2 controls spatial aspects of cell growth by actin cytoskeleton remodeling. The overall TOR functions may be conserved in early-branching eukaryote. However, several new findings have emerged that differ from those described for other eukaryotes. Localization of signaling molecules is key in regulating their function and specificity. *Trypanosoma brucei* TOR, TbTOR1, has been shown to be predominantly nuclear, and TbTOR2 associated with the endoplasmic reticulum (ER) and mitochondria. Surprisingly, the subcellular localization of *Trypanosoma cruzi* TOR1 and TOR2 (TcTOR1 and TcTOR2) is completely distinct from that previously observed in *Trypanosoma brucei*. ***Rabbit and mouse serum raised against specific TcTOR1 and TcTOR2 peptides coupled to KLH were IgG-purified*** by using ***biochemical affinity column.*** **T. cruzi** ***was fixed and imaged by*** three-dimensional (3D) microscopy. Unlike TbTOR1, TcTOR1 is excluded from nucleus, concentrated at the posterior portion in punctuated compartments which coincide with reservosomes. TcTOR2, unlike TbTOR2, is excluded from ER and mitochondria and is dispersed in the cytoplasm, concentrating around TcTOR1 location. This unusual localization of TOR proteins may shed new lights to TOR function in metabolism, differentiation and invasion process of *Trypanosoma cruzi*, which are the current challenges of our group.Supported by: FAPESP

BC.39 - IDENTIFICATION AND ALIGNMENT OF THE GENES CORRESPONDING TO THE FOUR SUBUNITS OF THE ADAPTOR COMPLEX 1 IN *TRYPANOSOMA CRUZI*

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Adaptor complexes are formed by heterotetrameric subunits and play a role in both recruiting clathrin and binding to membrane receptors to make endocytic vesicles. In eukaryotic cells the AP1 complex is involved in the traffic of vesicles that carry acid hydrolases between the Trans Golgi Network and the lysosomes. The AP1 adaptor complex is formed by two large (γ and β1), a medium (μ1) and a small (σ1) subunits. Genes encoding the AP1 proteins have been already described as part of the genome of *Trypanosoma cruzi*. Aim of this work is to further characterize and align the genes corresponding to the four subunits of the AP1 adaptor complex in *Trypanosoma cruzi* and other pathogenic trypanosomatids. Furthermore, the genes identified in T. cruzi were compared with corresponding genes found in human (*H. sapiens*), fruit fly (*D. melanogaster*), rooster (*G. gallus*) and fish (*D. rerio*), in order to identify the similarity and conservation rates. A genomic data bank search allowed the identification of all genes encoding the four subunits of the AP1 adapter complex in *T. cruzi*. A comparison between these genes and those of other higher eukaryotic organisms showed a low identity (between 30 to 50%), as expected for the evolutive divergence of this protozoan parasite. On the other hand, a higher identity (between 50 and 70%) was observed when *T. cruzi* genes were compared with those of other trypanosomatids (*L. brasiliensis, L. major, L. infantum and T. brucei*), with the highest identity between *T. cruzi* and *T. brucei*. These results indicate that due to its important role in the cell metabolism the AP1 complex adaptor has been conserved in the eukaryonts. Studies are underway to obtain antibodies against the *T. cruzi* AP1 subunits in order to perform subcellular localization of the proteins.

This work has been supported by CNPq and Fiocruz.

BC.40 - SEARCHING FOR THE CONTROL OF NUCLEAR DNA REPLICATION IN TRYPANOSOMES

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Chromosomal replication initiates with the assembly of the prereplication complex (pre-RC) at replication origins. In eukaryotes, the pre-RC is composed of ORC complex containing six proteins, Orc1-Orc6, two proteins named Cdc6 and Cdt1, and the minichromosome mainenance (MCM) complex, which is composed of Mcm2 to Mcm7 proteins and presents helicase activity, essential for DNA replication. As long as the pre-RC is organized on the chromatin, origins become licensed to replicate. Since ORC, Cdc6, and Cdt1 are required for loading MCM onto DNA, but are not required for the continued MCM-DNA interaction, the downregulation of their expression and/or activity at the end of G1 represents, in eukaryotes, an effective way to block DNA replication. Trypanosomes do not contain the complex ORC, Cdc6 or Cdt1. Instead, they contain a protein homologous to Orc1 and Cdc6, named Orc1/Cdc6 that are a component of pre-RC. Orc1/Cdc6, however, does not seem to be involved at DNA replication control, since it is bound to DNA during the entire cell cycle. Therefore, we asked if Mcm proteins could be involved in this control in trypanosomes. In this work, we searched for sequences in trypanosomes databases and we found 10 genes for *T. cruzi* and 8 genes for *T. brucei* annotated as Mcms. By alignment analysis we identified a putathive Mcm7 gene. *T. cruzi* Mcm7 was then cloned and expressed by a prokaryote. The recombinat protein rTcMcm7 was used to immunize mouses. The obtained antibody was able to recognize the *T. cruzi* recombinant Mcm7 protein as well as the putative *T. brucei* recombinant Mcm7, expressed by Sf9 cells using the bacullovirus system. The anti-rTcMcm7 serum will be used in western blotting and immunofluorescence assays in order to analyze the expression and localization of Mcm7 during the cell cycle of trypanosomes.
Supported by FAPESP

BC.41 - DETERMINATION AND EVALUATION OF TRACE ELEMENTES OF DOGS NATURALLY INFECTED WITH *Leishmania (Leishmania) infantum*

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Canine Visceral Leishmaniosis is a chronic and systematic disease involving many organs. The trace elements cupper (Cu), iron (Fe) are related to the immunological effectiveness. Cu alterations are associated to anemia, abnormal collagen synthesis and Fe is related of parasite-host interactions, Nitric oxide (NO) synthesis and fibrogenesis. Several works have related Cu and Fe with diseases, but this isn´t well defined in dogs, and neither is a possible correlation with CVL. Graphite Furnace Atomic Absorption Spectrometry was used for trace elements detection. Morfometric studies were done for Fe (Prussia Blue) and Cu (Timm`s) in tissue samples. NO detection was done by Griess technique. The results obtained for variables pyrolysis and atomization temperatures were: 1460 - 2090 ^{o}C for Cu and 1530 - 2500 ^{o}C for Fe. We found matrix effect for Fe but not for Cu. Permanent modifiers using Ruthenium for Cu and without modifier for Fe. Canine sera were collected and distribute: 9 from uninfected dogs, 9 from symptomatic (SD) and 4 from asymptomatic (ASD). Statistical difference shown higher levels of Cu and Fe serumin ASD than SD and controls group. In contrast, morfometrical analysis showed higher deposition of Fe in all tissues samples of SD than AS and control groups. In parallel, we observed higher levels of NO in serum of AS than SD and control groups. However, no correlation was found between Fe serum and NO serum levels. Chronic infection is correlated to Fe deposition in tissues, NO disturb and worse prognostic. In this study we have found this Fe deposition mainly in SD dogs in parallel to lower levels of Fe serum. However, Fe and Cu serum levels were not concluded yet and even with NO serum levels correlations. Thus, weare improving our results to make correlations to the progression of the CVL. Supported: FAPEMIG nº14138

BC.42 - THE ROLE OF ADENOSINE ON *Leishmania (Leishmania) amazonensis* METACYCLOGENESIS

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Metacyclogenesis is an important stage in the life cycle of parasitic trypanosomatids in which infective forms of the parasite are generated. However, little is known about the conditions in which these forms are generated. In this work, we investigated the effects of adenosine in the in vitro development of infective metacyclic promastigotes of *L. amazonensis*. Parasites were cultured in the presence of CGS15943, a potent adenosine receptor antagonist. CGS15943 treated cultures showed a significant increase in the number of metacyclic promastigotes (10X) if compared to the control culture, as assessed by morphology, motility and isolation in density gradient. This increased metacyclogenesis also correlated with a significant increase in survival (15X) to complement-mediated lysis and a low adherence to a sand fly gut by CGS15943 treated parasites. To investigate possible changes on parasite infectivity, peritoneal macrophages were inoculated with promastigotes obtained from control or CGS15943 treated cultures and the amount of infected cells was highly increased in the group inoculated with treated parasites. Thereafter, C57BL6 mice were inoculated on the ear with a low dose ($1x10^3$) of control or treated promastigotes. Both lesion size and tissue parasitism were significant increased in the group inoculated with parasites obtained from the treated culture. Interestingly, metacyclogenesis induction was completely reversed in *L. amazonensis* cultured in the presence of CGS15943 plus adenosine. Corroborating these results, we found increased levels of metacyclogenesis in parasites incubated with dipyridamole, a specific adenosine transport inhibitor. Moreover, ours results indicate that the effects observed for *L. amazonensis* was conserved for other species of trypanosomatids like *L. brasiliensis*, *L. major*, *L. chagasi* and *T. cruzi*. In conclusion, our data suggest that acquisition and/or metabolism of adenosine is important to control the differentiation of *L. amazonensis* promastigotes into infective forms. Supported by CNPq, CAPES, FAPEMIG and Rede Mineira de Bioterismo.

BC.43 - THE POSSIBLE RELATIONSHIP BETWEEN ECTO-ENZYMES AND DRUG TRANSPORT IN RESISTANT *LEISHMANIA*

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Ecto-nucleotidases are surface membrane-bound enzymes able to hydrolyze extracellular nucleotides. Some functions are suggested for these proteins: cell adhesion, purine acquisition, protection against cytotoxic effects of extracellular ATP and, recently, MDR phenomenon. P-glycoprotein (P-gp) is involved in the removal of drugs, most of them positively charged, from the cytoplasm. P-gp is also associated with movement of ATP, an anion, from the cytoplasm to the extracellular space. The central question of this study is to establish the relationship between ecto-enzyme activities and a possible mechanism of drug transport in *Leishmania amazonensis. L. amazonensis* promastigotes were selected by gradual increasing concentrations of the vinblastine from 10 µM to 100µM, and the cells were maintained continuously under drug pressure. The cells were used to determine Mg^{+2} dependent ecto-ATPase activity by measuring ^{32}Pi release from the substrate $\gamma^{32}Pi$ ATP. In addition, ecto-phosphatase, ecto-5'- and ecto-3'-nucleotidases were assessed by colorimetric method by the complex formation of Pi (Fiske and Subbarow, 1925). Our results show that ecto-ATPase activity from resistant *Leishmania* presented a higher activity rate compared with wild-type *Leishmania* (8,15 ± 0,74 and 19,94 ± 0,6 nmolPi x h x 10^7cels respectively). This increase is progressive with increasing drug concentrations. In addition, other ecto-enzyme activities did not presented this increment with drug pressure. We compared the ecto-ATPase activity in cells resistant to another drug. In cells resistant to chalcone the ecto-ATPasic activity is about three times higher than in with type cells. This initial result suggests a possible relationship between resistance and ecto-ATPase activity and this can contribute to elucidate the mechanism of drug transport. Supported by: CNPq, FAPERJ, IOC/FIOCRUZ.

BC.44 - HISTOPATHOLOGICAL AND PARASITOLOGICAL STUDY OF THE GASTROINTESTINAL TRACT OF DOGS NATURALLY INFECTED WITH *LEISHMANIA (LEISHMANIA) INFANTUM*

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The aim of this study was to provide a systematic pathological and parasitological overview of the gastrointestinal tract (GIT) of dogs including the stomach, duodenum, jejunum, ileum, cecum and colon, naturally infected by *Leishmania*. An epidemiological survey of 20 mongrel dogs with visceral leishmaniasis was carried out by the municipality of Belo Horizonte, MG, Brazil. The infected animals had an increased number of macrophages, plasma cells and lymphocytes. Parasite distribution in the GIT was evident in all intestinal segments and layers of the intestinal wall (mucosal, muscular and submucosal) irrespective of the clinical status of the animals. However, the parasite load was statistically higher in the cecum and colon than in other segments of the GIT. The high parasite burden evident throughout the GIT mucosa without marked pathological alterations led us to consider whether *Leishmania* gains an advantage from the intestinal immunoregulatory response (immunological tolerance). Sponsors (FAPEMIG Processo 15548/2009) ; CNPq (Processo 473601/2009-5), UFMG.

BC.45 - CYTOPHATIC EFFECTS OF *TRITRICHOMONAS FOETUS* ON BOVINE OVIDUCT CELLS-COMPARISON WITH *TRICHOMONAS VAGINALIS*

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Tritrichomonas foetus is an extracellular parasite of the reproductive tract in cattle. In order to investigate the cytophatic effects of *T. foetus* in deeper parts of the reproductive tract, a bovine primary oviduct epithelial cell system (BOECs) was developed. Reproductive tracts were obtained from cows and the effect of co-incubation of *T. foetus* and *T. vaginalis* with BOECs was analyzed by scanning, transmission and fluorescence microscopy. Viability tests were performed using colorimetric methods, *TUNEL* (*Terminal deoxynucleotidyltransferase-mediated dUTP nick-end labeling*), JC-1 and annexin-V. The results reported here demonstrate that: (1) the *in vitro* oviduct epithelium has been shown to be useful in interaction experiments with *T. foetus* and *T. vaginalis*; (2) *T. foetus* adheres to the BOECs as single separate cells, and posteriorly the cells aggregate in large clusters; (3) *T. foetus* provoked a severe damage to BOECs leaving imprints in the epithelial cells, wide intercellular spaces, and provoking large lesions in the epithelium; (4) no species-specific host-parasite interactions was observed; (5) fresh *T. vaginalis* is more aggressive to BOECs than *T. foetus*, but long-term *T. vaginalis* has similar effects to *T. foetus*. Here we show that both *T. vaginalis* and *T. foetus* are able to adhere and damage bovine oviduct cells and lead to cell death showing no species- specificity. Thus, both parasites are likely to be important in mediating infertility, since oviduct is the natural passage of early stage of embryo development. Our findings raise the possibility that trichomonads are able to attack the oviduct and thus could contribute to infertility in cows. Further studies *in vivo* are in course to elucidate this proposal.
Supported by AUSU, CNPq, FAPERJ, and PRONEX.

BC.46 - ROLE OF THE CALCIUM INDEPENDENT PHOSPHOLIPASE A2 IN DISTRIBUTION INTRA / EXTRACELLULAR ACID PHOSPHATASE ACTIVITY IN *LEISHMANIA AMAZONENSIS*

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Compartments of the endocytic and exocytic pathways in protozoa of the Trypanosomatidae family present structural and functional characteristics distinct from those described for the same compartments in mammalian cells. The dynamics of these compartments is of fundamental importance in processes such as secretion, removal and exposure of molecules on the surface of the parasite thus modulating process like adhesion, virulence, and defense against the host immune system. Although the structure and composition of many compartments of the endo/exocytic pathway in *Leishmania* have been elucidated, very little is known about mechanisms controlling their recognition and fusion. Several proteins, such as phospholipase A2 (PLA2), are involved in the process of fusion of membrane bound compartments in different cells. In this study, we investigated the effect of Bromoenol lactone (BEL), a specific and irreversible inhibitor of a calcium independent phospholipase A2 (iPLA2), on the intra and extracellular activity of different acid phosphatases in *Leishmania amazonensis*. Parasites were grown for 72h in Schneider medium, incubated for 1 h with 2.5 μM of BEL and analyzed by ultrastrucutral cytochemistry and biochemical assays. The enzymatic activity detected in the cell culture medium was reduced by 43% after BEL treatment with an accumulation in the cell as demonstrated by the total extract analysis of the parasite. Cytochemical analysis of BEL treated parasites showed a significant reduction in the electrondense labeling of the flagellar pocket membrane with an accumulation in the multivesicullar tubules, in the Golgi and in different vesicular and tubular compartments near the flagellar pocket region. Another important finding on effect of BEL was the significant reduction of the number of membrane bound vesicles within the flagellar pocket. The results suggest that iPLA2 is involved in the control fusion of different compartments of the exocytic pathway in *Leishmania*. Supported by CNPq, CAPES, FAPERJ

BC.47 - CELL DAMAGE BY *LEISHMANIA AMAZONENSIS* LEISHPORIN: AN ATOMIC FORCE MICROSCOPY STUDY

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Leishporin is a pore-forming cytolysin produced by species of the Genus *Leishmania*. Because it is optimally active at acidic pH (5,5) and at 37 °C, we have postulated that it may act in the mammalian host, being involved in phagolysosome and plasma membrane rupture, crucial steps for parasite survival and infection recrudescence. In previous works, we showed that leishporin does not need proteins or carbohydrates as receptors to lyse cells. We found that lipids from target membranes are sufficient for cytolysin binding and membrane rupture. In the present work we studied the damage caused on target membranes by leishporin using the Atomic Force Microscopy tapping-mode technique. As membrane models, we used erythrocytes and DPPC-liposomes both highly susceptible to leishporin activity. After hemolytic or lipsomes-lysis assays we analyzed the damage caused by parasite extracts in both membrane surfaces. The images obtained showed pore-like forms in both models. The circular structures measured about 25-200 nm of diameter and presented 4-8 nm of depth, the latter being sufficient to cross lipid bilayer. All observed structures are certainly enough to permeate the used membranes models and to lead to lysis. This work provided for the first time a visual evidence of leishporin activity. Support: OMS – CNPq – FAPEMIG – CAPES.

BC.48 - ROLE OF HEME-OXYGENASE 1 (HO-1) IN RESPONSE TO *LEISHMANIA CHAGASI* INFECTION WITHIN MURINE MACROPHAGES

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Visceral leishmaniasis (VL) is commonly associated with hematologic manifestations. In this scenario, mechanisms related to hemolysis, released of heme and activity of the enzyme heme oxygenase 1 (HO-1) may be involved in the immunopathogenesis of VL. However whether HO-1 influences *Leishmania (L.) chagasi* infection, etiologic agent of VL in Brazil, is poorly comprehended. Here, we evaluate the role of HO-1 in *L. chagasi* infection of murine peritoneal macrophages from C57BL/6 mice stimulated with thioglycolate and infected in presence of CoProtoporphyrinIX (CoPPIX) the HO-1 inducer, besides one of the products of HO-1 activity (Biliverdin). *L. chagasi* infection induced HO-1 production compared to uninfected macrophages. The percentage of infected macrophages and quantity of amastigotes by 100 macrophages was increased with CoPPIX treatment to infected macrophage. Beyond this, we observed that bone marrow derived macrophages knockout to HO-1 gene have a significant low parasite load when infected by *L. chagasi* than their wild type counterparts. Furthermore, upregulation of HO-1 by CoPP resulted in inhibition of TNF- α, IL-1β, IL-6, MCP-1, PGE-2 and Nitrite levels upon LPS stimulation and simultaneously induced a higher IL-10/TNF-α ratio in peritoneal macrophages contributing to the anti inflammatory pathway that favors *L. chagasi* replication. Our findings are the first evidence that HO-1 is induced during *L. chagasi* infection and favors an increased parasite load, suggesting an anti inflammatory mechanism of HO-1 in VL, an important hemolytic parasite disease. Supported by: CNPq

BC.49 - CANINE VISCERAL LEISHMANIASIS LIKE A FIBROTIC DISEASE MODEL

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The aim of this work was study the extracellular matrix alterations in liver, spleen, cervical lymph nodes, lungs and kidneys in symptomatic dogs naturally infected with *Leishmania (Leishmania) chagasi* correlating with clinical and anatomical pathological aspects. This study was carried out with 30 dogs, divided at two groups: six not infected animals (group control) and twenty four symptomatic infected animals. All them was mongrel dogs with undefined age, obtained from the municipality of Santa Luzia/MG and Ribeirão das Neves/MG municipalities. The group denominated symptomatic was composed by animals with classical clinical signals of the disease as skin lesions (alopecia, eczemas and ulcers), loss weight and lymphopathy. Paraffined sections of the tissues were stained by Hematoxylin-Eosin (HE); Gomori's ammoniacal silver staining for reticular fibers and strepto-avidin peroxidase Immunohistochemical method for tissue *Leishmania* amastigotes detection. The tissue images were transferred to a computer video screen by means of the software KS300 and relayed to a computer-assisted image analysis system (Kontron Elektronic/Carl Zeiss, Germany) for morphometrical analysis. A significant increasing of collagen deposition in all organs was found when compared to the controls. Positive correlation between the parasite load and collagen deposition was found in all organs expect the lungs of infected animals. The organs that showed higher colagenogenesis were livers, kidneys and lungs. Our results have indicated that canine visceral leishmaniasis is a fibrotic disease model.
Supported by CNPq.

BC.50 - IDENTIFICATION OF *TRITRICHOMONAS FOETUS* PSEUDOCYSTS IN FRESH PREPUTIAL SECRETION SAMPLES FROM BULLS

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Tritrichomonas foetus is a serious veterinary pathogen that causes bovine trichomonosis, a sexually transmitted disease that eventually leads to abortion and infertility. *T. foetus* has a simple life cycle that consists of only a trophozoitic form. During unfavorable environmental conditions, the trophozoites, which are polar and flagellated, can adopt a spherical shape and internalize their flagella. These rounded organisms are known as pseudocysts. Although it is currently assumed that *T. foetus* pseudocyst formation is reversible and that it represents a response to stressful conditions, there are no reports showing the presence of this form *in vivo*. For this reason, the aim of this study was to verify whether *T. foetus* pseudocysts are encountered in naturally infected bulls. Towards this goal, fresh preputial samples obtained from seven mature bulls that were naturally infected with *T. foetus* were analyzed using complementary techniques, such as video microscopy, fluorescence microscopy, scanning and transmission electron microscopy. The analyses revealed that approximately 55% of the parasites were in pseudocyst form in each preputial sample, whereas approximately 25% of *T. foetus* displayed pear-shaped bodies. Previous research demonstrated that *in vitro T. foetus* pseudocysts are able to divide by a budding process. Here, this division mode was observed in approximately 20% of fresh *T. foetus* obtained from preputial bovine samples. Thus, this study shows that in infected bulls, pseudocysts are present and occur more frequently than the pear-shaped parasites. Because *T. foetus* pseudocysts are capable of generating multinucleated organisms that release single organisms when environmental conditions become favorable, we hypothesize that *T. foetus* might be more likely transferred to a new host as a pseudocyst than as a piriform cell, which could therefore contribute to a more efficient infection of the new host. Supported by CNPq, FAPERJ, PRONEX, AUSU.

BC.51 - ULTRASTRUCTURAL ALTERATIONS INDUCED BY $\Delta^{24(25)}$-STEROL METHYLTRANSFERASE INHIBITORS ON *TRICHOMONAS VAGINALIS*

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Trichomonas vaginalis is an important human parasite that causes trichomoniasis, a cosmopolitan sexually transmitted disease. Currently, the treatment of choice for *T. vaginalis* infections is metronidazole. However, the increase in metronidazole-resistant trichomoniasis and the common and undesirable side effects of this drug make the search for alternative chemotherapeutic approaches a top priority for the management of this disease. In the present work, the antiproliferative and ultrastructural effects of sterol biosynthesis inhibitors against *T. vaginalis* were investigated. It was found that 22,26-azasterol and 24(*R,S)*,25-epiminolanosterol, known inhibitors of $\Delta^{24(25)}$-sterol methyltransferase, exhibited antiproliferative effects on *T. vaginalis* trophozoites cultured *in vitro*. Morphological analyses showed that azasterols induced changes in the ultrastructure of *T. vaginalis*. The most significant alterations were (1) membrane blebbing and disruption, (2) cell wrinkling and (3) the formation of cell clusters. In addition, autophagic vacuoles, Golgi duplication arrest, an abnormal Golgi enlargement and damaged hydrogenosomes were also observed. Nonspecific cytotoxicity assays using the cultured mammalian cell lines MDCK showed no effect of the azasterols on the viability and proliferation of these cells at a concentration that significantly inhibited the proliferation of *T. vaginalis*, indicating a selective antiparasitic action. Taken together, these results suggest that azasterols could be important compounds in the development of novel chemotherapeutic approaches against *T. vaginalis*.
Supported by CNPq, PRONEX, FAPERJ, AUSU.

BC.52 - Identification of Lipid Rafts-like in *Tritrichomonas foetus*

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Tritrichomonas foetus is a parasite that causes bovine trichomoniasis, a sexually transmitted disease that eventually leads to abortion and infertility. *T. foetus* is an extracellular parasite that adheres to epithelial cells, however, the cellular mechanism by which colonizes mucosal surfaces is not well defined. The involvement of lipid rafts has been described on parasite adhesion to host cells. However, these membrane domains have not been reported in *Trichomonas* yet. Thus, the purpose of this study was to identify rafts-like domains in *T. foetus*. For this, *T. foetus* was cultured in serum-free medium. In some trials, rafts were disrupted by Methyl-β-cyclodextrin (MBCD) or filipin for 30min. To determine whether *T. foetus* cells possess rafts-like microdomains and to distinguish them from more-fluid-phase membrane regions, cells were stained with either the order-preferring lipid analog DiIC16 or the non-order-preferring lipid analog FAST-DiI with or without chemical previous disruption of rafts . The cells were then fixed and observed in the fluorescence microscope. In addition, cells were stained with cholera toxin B subunit FITC conjugated (FITC-CTX-B), another marker of membrane rafts. Both lipid analogs and FITC-CTX-B were found in the plasma membrane and in some intracellular organelles of *T. foetus*. Treatment with MBCD or filipin abolished staining in the plasma membrane by $DiIC_{16}$. In addition, disrupted treatment resulted in alteration of the $DiIC_{16}$ staining pattern. Similar results were observed after FITC-CTX-B labeling. As expected, the raft-disrupting agents did not alter the FAST-DiI staining. Taken together, these data authenticate the colocalization of DiIC16 and FITC-CTX-B with cholesterol-rich membrane regions and suggest the existence of rafts-like domains in *T. foetus*. We verified that these raft-like microdomains had no effect on adhesion of the parasites to MDCK monolayer. However, the lipids rafts might be necessary for other events concerning cytotoxicity exerted by *T. foetus*.
Supported by CNPq, FAPERJ, PRONEX, AUSU

BC.53 - CYTOTOXIC EFFECTS EXERTED BY *TRITRICHOMONAS FOETUS* PSEUDOCYSTS

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Tritrichomonas foetus is a parasite of cattle and other animals that has a simple life cycle consisting only of a trophozoitic form. Under unfavorable environmental conditions, the trophozoites, which are polar and flagellated, can adopt a spherical shape and internalize their flagella. These rounded organisms are known as pseudocysts. It is currently believed that this form is reversible and that its formation represents a defense mechanism against stress conditions. However, there are still several open questions about pseudocyst biology, such as if it is cytotoxic. Consequently, the aim of the present study is to assess whether pseudocysts exert cytotoxic effects in interaction with epithelial cells and compare their behavior to the behavior of the pear-shaped parasites. To clarify these questions, a long-term grown and a fresh *T. foetus* isolates were used and interaction trials of both parasite forms with MDCK (an epithelial kidney canine cell) were carried out. These interactions were analyzed using complementary techniques, such as light and electron microscopy. Cytotoxicity assays were performed using the MTT (Methylthiazolyldiphenyl-tetrazolium bromide) viability staining method. This work demonstrates that both *T. foetus* isolates were able to exert cytotoxic effects in host cells. However, the fresh isolate provoked a higher damage in MDCK cells when compared with the long-term *T. foetus* strain. In both isolates, pseudocysts were more cytotoxic when in contact with host cells as compared to the flagellated pear-shaped parasites. Our results suggest that the *T. foetus* pseudocyst might be a more aggressive and infective form.
Supported by AUSU, CNPq, FAPERJ, and PRONEX.

BC.54 - MORPHOLOGICAL CHANGES IN THE CYST WALL OF THE PARASITE *GIARDIA LAMBLIA* DURING EXCYSTATION PROCESS

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Differentiation from one life cycle stage into another is an elegant adaptation by which many parasites ensure their transmission and survival. The protozoan *Giardia lamblia* is a major cause of water-borne diarrheal disease. This parasite exhibits two forms in its life cycle that includes trophozoite and cyst. The cyst presents a lower metabolic rate than trophozoites and it can survive in water for weeks. Encystation and excystation are crucial processes for establishment and maintenance of *Giardia* infection. The cyst wall of the parasite is known to be composed by carbohydrates and proteins and it provides the resistance of the cyst. Few studies were performed using the excystation as a model. Therefore, the aim of this study is to analyze the ultrastructural modifications of the cyst wall during the excystation process. Trophozoites of *G. lamblia* were grown in TYI medium and induced to encystation and excystation *in vitro*. The encystation was verified with immunofluorescence assay using the monoclonal antibody anti-CWP. The excystation process was analyzed using complementary techniques, such as scanning and transmission electron microscopy. A field emission scanning electron microscopy (FESEM) was used to compare the mature cysts and the beginning of excystation process. The *in vitro* encystation was performed successfully, because a positive staining was observed using the anti-CWP antibody. The following alterations in cell morphology during the excystation were seen: (1) a change in the cyst shape and (2) the presence of new electron-dense vesicles close to the cyst wall. In addition, the fibrillar composition of the cyst wall was better analyzed with the use of FESEM. In mature cysts, the microfibrils of the cyst wall were compacted although during of the excystation process this tight arrangement was lost. Our results suggest that the modifications of cyst wall fibrils are necessary for the development of excystation process.
Supported by AUSU, CNPq, FAPERJ, and PRONEX.

BC.55 – THE BACTERIUM ENDOSYMBIONT OF *CRITHIDIA DEANEI* UNDERGOES COORDINATED DIVISION WITH THE HOST CELL STRUCTURES

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In trypanosomatids, cell division involves morphological changes and requires coordinated replication and segregation of single copy organelles as the nucleus, kinetoplast, flagellum and basal body. Some trypanosomatids also present a symbiotic bacterium in their cytoplasm that co-evolves through a mutualistic relationship with the host protozoan. Previous studies have reported that this symbiont divides in synchrony with the host structures in such way that each daughter cell carries only one bacterium. Thus, symbiont-bearing trypanosomatids constitutes an interesting model to understand the relationship between cell cycle and organelle division processes. In the present work, we used light and electron microscopy techniques to describe the morphological events that occur during *Crithidia deanei* cell cycle, in particular the chronological division of the symbiont relative to other trypanosomatid structures. Immunofluorescence assays showed that the symbiont can present different shapes and positions during the protozoan cell cycle. Furthermore, we obtained clear evidences that the endosymbiont divides before the basal body and kinetoplast segregation and that the nucleus is the last organelle to divide, just before cytokinesis. The basal body position was not seen in coincidence with that of the symbiont during all *C. deanei* cell cycle, indicating that this microtubule-organizing structure is not directly involved in the bacterium division and segregation.These results are based on counts of a thousand cells, where structures of interest were labeled with specific antibodies and DNA-binding compounds, as DAPI. Preliminarily assays using inhibitors to DNA polymerase and to eukaryotic protein synthesis blocked the nucleus and kinetoplast segregation, as well as the symbiont duplication, suggesting that bacterium division is coordinated with other trypanosomatid structures. Supported by CNPq, CAPES and FAPERJ.

BC.56 - EFFECT OF THE MAIN CONSTITUENTS OF ESSENTIAL OILS FROM *SYZYGIUM AROMATICUM* L., *THYMUS VULGARIS* L. AND *CYMBOPOGON CITRATUS* (DC) STAPF., ALONE OR COMBINED, ON THE TRYPANOSOMATID PROTOZOAN *CRITHIDIA FASCICULATA*

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Essential oil is a complex mixture of secondary metabolites produced by plants. Those oils, or their compounds, have a broad spectrum of pharmacological effects including effects against some parasites that cause human parasitoses. For example, it has been demonstrated that some essential oil have activity against *Trypanosoma cruzi*, *Leishmania amazonensis* and *L.chagasi*. In this work we analyzed the combination of eugenol, thymol and citral, which are the major compounds of the essential oil of *Syzygium aromaticum* L. (clove), *Thymus vulgaris* L. (thyme) and *Cympobogon citratus* (DC) Stapf (lemon grass),respectively, on the growth of *C. fasciculata*. Initially the components were added separately at different concentrations to cultures of *C. fasciculata* in the exponential growth phase and then used to calculate the IC_{50} (dose that inhibited the growth of the culture at 50%) and IC_{90} for each component after 24 hours of treatment. Thymol gave the best results when given alone, with IC_{50} = 32.5 µg/ml and IC_{90} = 62.5 µg/ml, followed by citral (IC_{50} = 76.28 µg/ml, IC_{90} = 146.05 µg/ml) and eugenol (IC_{50} = 93.75 µg/ml, IC_{90} = 300 µg/ml). When we applied the combination of the constituents the best result was obtained with the combination of the three components, resulting in the decrease of IC_{50} dose to 16.66 µg/ml of thymol, 39.12 µg/ml of citral and 48.08 µg/ml of eugenol. Treatment with benznidazole at concentrations ranging 20-500 µg/ml did not affect culture growth. Analysis by SEM demonstrated that treatment with constituents has led to a rounding of the protozoa body. Our data showed that the combination of constituents of essential oils led to an increase in inhibitory activity on growth of *C. fasciculata*, suggesting that this procedure can be effective when applied against pathogenic trypanosomatids. Supported by CNPq.

BC.57 - LIPID BODY INDUCTION IN MACROPHAGES BY *CRITHIDIA DEANEI* AND *TOXOPLASMA GONDII*

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Lipid bodies (LB) are organelles rich in lipid present in many cell types modulated by specific signals such as interaction with parasites and apoptotic cells. Apoptotic cells and some parasites expose phosphatidylserine (PS) at their plasma membrane. This phospholipid might be involved in the induction of LB. To test this hypothesis LB in macrophages were analyzed after the interaction with *Crithidia deanei* and *Toxoplasma gondii* (RH and ME-49, virulent and less virulent strains respectively). *C. deanei* presented an exponential growth until day 4, followed by a sharp decline at day 6, and a gradual decrease up to day 21; no morphological change during the growth curve was detected. *C. deanei* cultured for 3 and 8 days and tachyzoites of both *T. gondii* strains interacted with macrophages for 1h and the LB induction was assayed by Nile red staining after 24h. Macrophages cultured with fetal bovine serum (FBS) were used as negative control due to the low induction of LB. *C. deanei* cultured for 8 days induced more LB than the ones cultured for 3 days. The number of necrotic cells in axenic culture increases along culture time and may be inducing LB in macrophages during the interactions with *C. deanei*. However, purified necrotic *C. deanei* was unable to induce LB in macrophages. The exposure of PS in *C. deanei* revealed a 4 fold increase of the PS positive population in cells from 8 days culture compared to those from 3 days. Both *T. gondii* strains were able to induce LB in macrophages similarly. These results indicate that both protozoa induce LB in macrophages. The hypothesis that PS exposure by these protozoa may be responsible for the induction of LB in macrophages is being further investigated.
Supported by CAPES, CNPq, FAPERJ and UENF

BC.58 - CELL CYCLE STUDY OF *BLASTOCRITHIDIA CULICIS*, AN ENDOSYMBIONT-HARBORING TRYPANOSOMATID

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As other member of the Trypanosomatidae family, *Blastocrithidia culicis* presents single-copy structures, as the nucleus, the kinetoplast and the flagellum. This species also contains a symbiotic bacterium, which establishes an obligatory relationship with the host protozoan. Investigation of the cell cycle in symbiont-harboring trypanosomatids suggests that the bacterium divides in coordination with other host cell structures, as the nucleus and kinetoplast. In this work we used optical and electron microscopy methods to study the *B. culicis* cell cycle. The immunofluorescence approach showed that the symbiont presents different shapes, sizes and positions in the host as the cell cycle proceeds. Thus, at the beginning of the cell cycle, the bacterium is located in the posterior region of the protozoan cell body and presents an elongated rod-shape form. Later on, the symbiont lies down over the protozoan nucleus and duplicates. Thereupon both symbionts acquire a constricted shape, remaining in the posterior end of the host cell body. During cytokinesis the cleavage furrow position ensures that each daughter cell will inherit a single endosymbiont. The rod shape bacterium measures about 1.5µm, while the constricted or dividing form may vary in size from 2.5µm to 4µm. Both shapes present approximately 0.5µm in diameter. The symbiont format was also studied by transmission electron microscopy and analysis by scanning electron microscopy was useful to characterize the protozoan cytokinesis. Preliminary tests using aphidicolin, a DNA polymerase inhibitor promoted cell proliferation arrest in *Blastocrithidia culicis*. Thus, our next step is to check if the bacterium division proceeds, even if nuclear DNA duplication is blocked.
Supported by CNPq e FAPERJ

BC.59 - THE ENERGETIC METABOLISM IN *CRITHIDIA DEANEI*, AN ENDOSYMBIONT-HARBORING TRYPANOSOMATID

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In trypanosomatids two organelles are involved in ATP production: the mitochondrion and glycosomes, an special type of peroxisomes Some monoxenous trypanosomatids harbor an intracellular bacterium which co-evolves with the host protozoan through a mutualist relationship, thus constituting an excellent model to study organelle origin and cellular evolution. The presence of the endosymbiont is associated to morphological alterations in the host protozoan and an intense metabolic exchange occurs between both partners. Conversely, the symbiont is capable of obtaining part of the required energetic molecules from the host glycosomes. It is well established that endosymbiont-bearing strains presents a lower generation time and a higher metabolic capacity than the aposymbiotic cells. Our previous results showed that the symbiont-containing strain of *Crithidia deanei* presents a higher O_2 consumption when compared with endosymbiont-free cells. In this work, 3-D reconstruction revealed that glycosomes are usually around the endosymbiont. This proximity suggests the occurrence of metabolic exchanges between this organelle and the symbiotic bacterium. The mitochondrial metabolism of the symbiont-bearing strain was investigated by using inhibitors of the respiratory chain. The obtained results showed: no effect by oligomycin (0.5 – 16 µg/mL), an inhibitor of FoF1ATP synthase; stimulus of the O_2 uptake up to 30% after using 2.5 µM carbonyl cyanide p-trifluoromethoxyphenylhydrazone (FCCP), a proton ionophore that uncouples O_2 consumption from ATP synthesis; and 71.5 % decrease of O_2 comsuption after treatment with 1.4 mM cyanide, a complex IV inhibitor. After cell fractioning, isolated mitochondria presented higher rates of O_2 consumption when compared to symbionts. Our data showed that the O_2 uptake rates are similar when assays are performed in Warren's medium or in Krebs-Ringer solution. Our next goal is to investigate the O_2 consumption by the aposymbiotic strain and to study the endosymbiont influence on the host respiration. Supported by: FAPERJ

BC.60 - MORPHOLOGICAL ASPECTS OF *TOXOPLASMA GONDII*-FELINE ENTEROCYTES INTERACTION *IN VITRO*

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Primary cultures of the feline intestinal epithelial cell (FIEC) have significant importance for the study of the normal development and differentiation of intestinal epithelium. Many mechanisms involved in the pathogenesis of chronic enteropathies or host-pathogen interactions in feline intestine have not been elucidated so far. This cellular model potentially applies to the investigation of the infection process provoked by enteropathogenous, in particular to the protozoan, *Toxoplasma gondii*, a coccidian which has the feline as its definitive host, and which maintains its sexual life cycle in the feline gut. Primary cultures of FIECs obtained of fetus small gut of feline collected surgically were cultivated and infected after seven days with bradyzoites forms isolated of intracerebral cysts collected of C57BL6 mice. The analysis of interaction *T. gondii*-enterocytes showed that the parasite-host cell ratio could be pointed out as a decisive factor which determines the intracellular fate of bradyzoites forms. The development of the syncytial forms of *T. gondii* was observed using the 1:20 bradyzoites-host cell ratio resulting in similar forms described in *in vivo* systems. After 6 days of parasite-host cell interaction, it was possible to show by ultrastructural analysis vacuoles containing parasites inside a vacuolar matrix full of the well developed tubulovesicular membrane network like schizont of the sexual cycle of *T. gondii* as previously described in the *in vivo* system. This alternative study potentially opens up the field for investigation of the molecular aspects of this interaction that can contribute to the developing of new strategies for intervention in one of the main routes by which toxoplasmosis spreads. Supported by FAPERJ, IOC/Fiocruz and CNPq

BC.61 - *TOXOPLASMA GONDII* DECREASES THE EXPRESSION OF CADHERIN IN SKELETAL MUSCLE CELL

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Mouse primary culture of skeletal muscle cells (SkMC) was employed as a model for experimental toxoplasmosis studies. In the present study, we examined: (i) the influence of *T. gondii* infection on the myogenesis process; (ii) the parasite's role on M-cadherin expression by infected SkMC and, (iii) its correlation with the myogenesis process. The M-cadherins (M for muscle) are involved in the initial cell-cell recognition, allowing initiation of myoblasts fusion to form multinucleated myotubes. We observed that even with a relation of only 1:1 (parasite-cell host) after 24 h of interaction, the infection was of only 43% leading to inhibition of 75% on the myogenesis process. The modulation of cadherin expression during *T.gondii*-SkMC interaction was investigated. Initialy, we demonstrated the cadherin localized at the contact areas between myoblasts and myotubes during the myogenesis process by confocal microscopy. SkMC infected with *T. gondii* analyzed by immunofluorescent and immunoblotting assays after 24 h of interaction showed that a reduction of 54% in expression of cadherin protein, leading the inhibition of the cell membrane fusion process. By PCR assays we analyzed the regulation of M-cadherin (M for muscle) by mRNA levels in SkMC in the presence and absence of infection by *T. gondii.* Our data demonstrated reduction the M-cadherin mRNA expression after 3h of interaction being higher after 24 h. These data corrobore the suggestion of that the *T. gondii* is able negatively to modulate the cadherin expression, interfering molecularly with the surface of host cell, inhibiting the membranes fusion and consequently affect the myogenesis process.
Suportted by: FIOCRUZ/IOC, FAPERJ and CNPq

BC.62 - KOJIC ACID, A SECONDARY METABOLITE PRODUCED BY THE FUNGUS *Aspergillus sp*., WAS NOT ABLE TO CONTROL THE *in vitro* DEVELOPMENT OF *Toxoplasma gondii* IN MACROPHAGES.

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Toxoplasmosis is a highly prevalent infection disease caused by *Toxoplasma gondii*, which is an obligate intracellular protozoan parasite. The disease is an important issue for public health due the existence of just a few drugs that are used to treat patients. Kojic acid, a secondary metabolite from *Aspergillus sp.*, increases the microbicidal capacity of macrophages against *Leishmania amazonensis*. For this work, we have tested if this metabolite may control *T. gondii* infection *in vitro*. Thus, the progress of *T. gondii* infection in culture of macrophages treated or not with this metabolite was assayed. To this end, the J774.A1 macrophage cell line was seeded in 24-well plates on coverslips, treated with the metabolite, activated or not with lipopolysaccharide and interferon-gamma, and infected with *T. gondii*. Coverslips and supernatants were collected after 2, 24 and 48 hours of infection, to evaluate the entrance and the development of the parasite and NO production. The metabolite treatment was not able to control *in vitro* infection of *T. gondii* in resident and activated macrophages. NO production was not altered after the metabolite treatment of non-infected macrophages. Furthermore, treatment with the metabolite was not able to revert the inhibitory capacity on NO production caused by *T. gondii* infection. *T. gondii* development was similar between treated and control group during 2, 24 and 48h of interaction. Therefore, this metabolite was not able to inhibit *T. gondii* growth.
Supported by CAPES, CNPq, FAPERJ, UENF.

BC.63 - OSTRICHES MONOCYTE-DERIVED MACROPHAGES CONTROL *TOXOPLASMA GONDII* GROWTH AFTER ACTIVATION WITH LIPOPOLYSACHARIDE

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Ostriches have gain attention as a relevant economic poultry. *Toxoplasma gondii* serum positive ostriches have been reported, thus, this bird is a potential intermediate host for human toxoplasmosis. However, little is known about its immunology, including macrophage biology, and *T. gondii* cellular infection. The aim of the present study was to adapt a methodology to obtain ostriches macrophages derived from blood monocytes and to study their behavior when infected with tachyzoites of the RH strain of *T. gondii*. Blood was collected, leukocytes separated by centrifugation, and cultured over glass coverslips in 24-well plates with Dulbecco's Modified Eagle's Medium containing 10% fetal bovine serum for 6 days at 37°C in a 5% CO_2 atmosphere. One day before experiments, half of the cells were activated with 0.1 µg/mL of lipopolysaccharide. Cells were infected with *T. gondii*, washed, cultured for 24h, fixed and the culture supernatant collected. The NO production was evaluated indirectly by measuring nitrite in macrophage culture supernatants by the Griess reagent. The percentage of infected macrophages and the mean number of *T. gondii* in macrophages were scored under a light microscope. Lipopolysaccharide did not induce NO production. However, this treatment turned macrophages more microbicidal as seen by a reduction of the percentage of infected macrophages and of the mean number of *T. gondii* in macrophages after 24h of culture. In non-activated macrophages parasites grew as expected. These results indicate that macrophage of ostrich were activated by LPS *in vitro* becoming more microbicidal against *T. gondii*. However, the microbicidal mechanism was not related to NO production. More studies are in progress to better understand the relationship between ostrich macrophage and *T. gondii*. Supported by CAPES, CNPq, FAPERJ and UENF

BC.64 - KINETICS OF NITRIC OXIDE INHIBITION AND PERSISTANCE OF TWO *TOXOPLASMA GONDII* STRAINS WITH DIFFERENT VIRULENCE

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Toxoplasmosis is a worldwide disease caused by *Toxoplasma gondii*. Like others obligate intracellular parasites, *T. gondii* can inhibit the microbicidal action of the host. One of the mechanisms in infected macrophages may be related to the exposure of phosphatidylserine (PS) by tachyzoites that causes the disappearance of the enzyme iNOS, which catalyzes the production of the microbicidal agent nitric oxide (NO). To better understand this mechanism, mouse peritoneal macrophages were activated with interferon-gamma and lipopolysaccharide and infected with tachyzoites of the RH (virulent) and ME-49 (less virulent) strains. Phosphatidylserine exposure was assayed by flow cytometry. Macrophages were infected with parasites for 2h and cultured up to 96h. Infectivity and development of *T. gondii* in macrophages were analyzed by Giemsa staining. NO production was measured by Griess reagent and iNOS localized by immunofluorescence. Tachyzoites recovered from infected macrophages by mechanical disruption were inoculated in mice to determine its infectivity. Both strains exposed PS, infected macrophage and caused disappearance of iNOS after 2h of interaction in a similar way. NO production after 24 and 48h was effectively inhibited by RH that persisted in iNOS negative macrophages. ME-49 strain infected macrophages produced intermediate levels of NO after 24h and showed similar production to non-infected macrophages after 48h, no parasites were detected inside macrophages at this period. Macrophages infected with ME-49 strain for 24 and 48h expressed iNOS as non-infected cells, confirming NO production results. Recovered RH tachyzoites from infected macrophages at all time points killed infected mice, but no cysts were found in the brains of mice inoculated with recovered ME-49. Collectively, these results indicate that both strains have the ability to initially inhibit NO production by the disappearance of iNOS probably because of PS exposure; however, ME-49 is killed probably by another microbicidal mechanism and cannot persist in activated macrophages. Supported by CAPES, CNPq, FAPERJ and UENF

BC.65 - ANALYSIS OF THE INDUCTION OF NEUTROPHIL EXTRACELLULAR TRAPS (NETS) BY *Toxoplasma gondii*

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Toxoplasma gondii is an obligate intracellular parasite that can invade any nucleated cell from warm blood animals. *T. gondii* is the etiologic agent of toxoplasmosis, a disease with worldwide distribution. Toxoplasmosis can be a serious and deadly disease for unborn children and immunocompromised patients. Neutrophils are essential for innate immune response, fundamental to control the infection, being the first cells to be recruited at the inflammation sites. They kill pathogens either by phagocytosis or by releasing cytoplasmic granules. Recently, 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. We evaluated if *T. gondii* is able to induce NETs release. Human neutrophils were incubated with *T. gondii* of the RH strain harvested from 48h in vitro cell cultures at several ratios, for 30 minutes. The samples were observed by immunofluorescence (IFA) and scanning electron microscopy (SEM). The amount of DNA released was measured from the culture supernatant by the picogreen method. In IFA assays DNA was stained with DAPI to localize the NETs, the parasites were labeled with SAG-1. Many parasites were seen trapped in NET filaments, maintaining their normal shape. These results were corroborated by SEM where we visualized the ultrastructural aspects of these extracellular traps in close contact with the parasite. Analyzing the quantification of DNA, it is possible to conclude that *Toxoplasma* is able to induce NET release. We will evaluate NET toxicity to the parasites to determine whether they are dead or just immobilized by these webs. Supported by CNPq and FAPERJ

BC.66 - BRADYKININ B2 RECEPTORS ARE NOT INVOLVED IN THE INVASION PROCESS OF *TOXOPLASMA GONDII* INTO MICE MACROPHAGES

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Bradykinin B2 receptors (BK2R) are implicated in *Trypanosoma cruzi* invasion of cardiovascular cells and *Leishmania chagasi* infection of macrophages. In both systems parasite proteases mediates the release of kinins from kininogens essential for invasion. Considering that *Toxoplasma gondii* relies on serine proteases activity to invade mammalian cells, we evaluated if BK2R may be involved in invasion and development of this parasite in macrophages as well as in the modulation of nitric oxide production. To this end, mice peritoneal macrophages and two cell lines (J774-A1 and alveolar) were seeded on coverslips in 24-well plate, activated with lipopolysaccharide and interferon-gamma and cultured with Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum at 37°C, 5% CO_2 atmosphere. Macrophages were incubated for 1 hour with Hoe-140 and infected with *T. gondii* of the RH strain. Coverslips were collected at 2 and 24 hours after infection, stained with Giemsa and parasite invasion and development evaluated by direct count; nitric oxide was evaluated by the Griess reagent. HOE-140 decreased the entry of *T. gondii* in activated and resident macrophages; however, the values were not statistically significant. Moreover, the development of *T. gondii* and nitric oxide production of macrophages treated with HOE-140 was similar to control cells. These data suggest that the BK2R is not essential to the entry of *T. gondii* in the different lineages of macrophages.
Supported by CAPES, CNPq, FAPERJ and UENF

BC.67 - *TOXOPLASMA GONDII* EVADES NITRIC OXIDE DEPENDENT IMMUNITY OF INTESTINAL EPITHELIAL CELLS (IEC-6).

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Toxoplasma gondii, the agent of toxoplasmosis, is an obligate intracellular protozoan able to infect a wide range of vertebrate cells including nonprofessional phagocytes. The natural route of infection of *T. gondii* is oral. Thus, the intestinal barrier composed by a monolayer of polarized enterocytes must be crossed by *T. gondii* for dissemination into deep tissues. This transmigration required viable and actively motile parasite, but the integrity of the host cell barrier is not altered during parasite crossing as evidenced by *ex vivo* and *in vitro* experiment. Furthermore, in *T. gondii*-induced ileitis enterocytes are one of the most resistant cells of this tissue. Thus, it seems that this cell type has microbicidal mechanisms against this parasite. It has been shown that *T. gondii* partially inhibits nitric oxide (NO) production of activated macrophages, promoting its persistence in the host cell. We analyzed the ability of an intestinal epithelial cell line (IEC-6) to express iNOS and produce NO after the infection by tachyzoites of *T. gondii*. IEC-6 was cultured at 37ºC in a 5% CO_2 atmosphere over coverslips and activated with recombinant interferon-gamma for 24h. Activated IEC-6 was infected with *T. gondii* (RH and ME-49 strains) for 2h and further cultured. After 24h the cells were fixed, and iNOS and *T. gondii* immunolocalized. NO production was evaluated at the culture supernatant by the Griess reagent. Infection by *T. gondii* of both strains at 24h was able to inhibit NO production and expression of iNOS of IEC-6. However, after 48h only the RH strains maintained NO inhibition; IEC-6 infected with the ME-49 strain by this time produced similar NO levels as non-infected IEC-6. We conclude that *T. gondii* was able to inhibit NO production and the expression of iNOS also in IEC-6 similarly as described for macrophages.
Supported by CAPES, CNPq, FAPERJ, UENF.

BC.68 - A LIGHT AND ELECTRON MICROSCOPY STUDY OF TRYPANOSOMES ISOLATED FROM *LEPTODACTYLUS OCELLATUS* FROGS

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Purpose of this study was to analyze the morphology of trypanosomes isolated from frogs by light and electron microscopy. Trypanosomes were isolated from naturally infected *Leptodactylus ocellatus* frogs collected in Seropédica City, RJ, Brazil (22^0 44' 08"S and 43^0 42' 27"W). Blood samples were collected by heart puncture and macerations of heart, liver, spleen and kidney and then inoculated into culture tubes containing blood-agar medium. After incubation for four days at 22^oC, parasites from the supernatant of positive cultures were collected by centrifugation, fixed and then processed for light microscopy (Giemsa staining), scanning electron microscopy (SEM) and transmission electron microscopy (TEM). In culture, most bloodstream trypomastigotes differentiated into epimastigotes (elongated and stump forms) and amastigotes, although a few esphaeromastigotes and trypomastigotes were also observed. Coil-shaped epimastigotes were observed by scanning electron microscopy and were characterized by TEM by the presence of an oval nucleus, a rod-shaped kinetoplast and a well-developed cytostome-cytopharynx complex. Large electron-dense vesicles similar to lipid inclusions and spherical electron-dense vesicles similar to acidocacisomes could be observed randomly distributed in the cytoplasm. Some parasites presented rod-shaped bacterium-like organisms (BLO) apparently free in the cytoplasm. The BLO were surrounded by two unit membranes that were separated by an electron-dense space, and presented ribosome-like particles and electron-lucent areas distributed throughout the cytoplasm. Further studies are underway for complementary characterization of the ultrastructure of these trypanosomes and bacterium-like organisms.
Supported by CNPq and FAPERJ

BC.69 - COMPARATIVE MORPHOLOGICAL STUDIES OF TWO CLOSE TRYPANOSOMATIDAE SPECIES: *TRYPANOSOMA CRUZI* AND *TRYPANOSOMA DIONISII*

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Trypanosoma cruzi is the protozoan that causes Chagas disease. It divides into the insect vector gut or into cytosol of an infected mammalian cell. *Trypanosoma dionisii* was isolated from a bat and is phylogenetically close to *T. cruzi.* Both present similar morphological stages and are able to infect mammalian cells in culture. Here we compared their cellular 3D ultrastructure obtained from serial sections of epimastigote forms. Ultrathin serial sections were obtained from Epon embedded parasites and were photographed under a transmission electron microscope. 3D models were generated using Reconstruct and Blender modelling softwares. Both species have an interconnected dense chromatin in the nucleus leaving an internal space for a centrally located nucleolus. They also present the kinetoplast accommodated within a separated branch from the tubular and single mitochondrion. Their cytostoma progresses from the parasite surface towards the posterior end contouring the kinetoplast and the nucleus. As the cell cycle progresses from G1 to G2 phase their cytostoma retract. As major differences we found that *T. dionissi* presents larger multivesicular structures in the posterior region that could be related to *T. cruzi* reservosomes. Also,*T. dionisii* mitochondrion is smaller and the flagellar pocket is larger than *T. cruzi* related structures. We propose that the similarities reflect the conserved features of cell division and life cycle, while the differences would be a consequence of differences in the parasite metabolism.
Supported by CNPq and FAPESP

BC.70 - PURIFICATION OF ANTI-NTPDASE 1 RECOMBINANT ANTIBODIES AND IMMUNOLOCALIZATION IN *TRYPANOSOMA CRUZI*

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T. cruzi is the etiological agent of Chagas disease an important tropical neglected disease that affects people mainly in South America. Parasites NTPDases are enzymes that can hydrolyze ecto localized tri and di-phosphate nucleotides controlling the purinergic signaling in hosts. Classical NTPDase activity was previously demonstrated in *T. cruzi* surface and a gene coding an NTPDase was isolated, cloned and expressed in bacterial system. This NTPDase-1 protein has been demonstrated to be a new virulence *T. cruzi* molecule. The main goal of this work was to purify specific anti-NTPDase-1 antibodies and use this purified antibodies to investigate the localization of NTPDase in live parasites. To achieve these goals the recombinant purified T. cruzi NTPDase-1 was immobilized on Sepharose 4 Fast Flow (GE). This resin was used to purify specific anti-NTPDase-1 antibodies. The purified antibodies, polyclonal antiserum and commercial anti-CD39 policlonal anti-serum were used in western blot analysis with epimastigotes and trypomastigotes showing higher specificity to the purified antibodies. The purified antibodies were used to analyze the immunolocalization of NTPDase1 by confocal technique. Our results showed specific points of fluorescence at surface e of all morphological T. cruzi forms. Electronic microscopy using purified antibodies will be the next step in this work in order to elucidate the real localization of this protein in the parasite.
Supported by FAPEMIG.

BC.71 - GENE EXPRESSION PROFILES OF HUMAN MACROPHAGES INFECTED WITH *LEISHMANIA BRAZILIENSIS* IN VITRO.

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The protozoan parasite *Leishmania braziliensis* has a high degree of intra-species genetic and phenotypic polymorphism, which is accompanied by a spectrum of clinical presentations in the infected human host, including: localized cutaneous leishmaniasis (CL), mucosal leishmaniasis (ML) and the more recently described disseminated leishmaniasis (DL). Our hypotheses are (1) that these parasites interfere with the gene expression of infected cells in a manner that is beneficial to their infectivity, and (2) that strains of *L. braziliensis* drawn from patients with either CL, ML or DL lead to different gene expression profiles in the infected macrophages. Employing DNA micro-array we compared the global gene expression profiles in human monocyte derived macrophages (MDM), obtained from healthy donors and infected in parallel with one *L. braziliensis* isolated from a CL, one from a ML and one from a DL case of the same endemic region in Northeastern Brazil. We also assessed how infected MDM compared with non-infected cells. Overall, *L. brazilienis* caused the repression of the majority of the genes that presented significant changes of their expression levels in infected MDM as compared to non-infected cells. Immune and non-immune response genes were affected. Among the three isolates tested, the two drawn from metastatic disease cases (i.e. ML and DL) induced more similar gene expression patterns in the MDM. These suggest that these parasites may increase their chance of survival by down regulating host cell genes during the infection process, and that strains associated with different forms of disease elicit somewhat diverse behaviors in host cells, which may be related to the different clinical outcomes of the disease.
Supported by National Institutes of Health

BC.72 - NEW ASPECTS ABOUT INTERACTION BETWEEN *GIARDIA LAMBLIA* AND INTESTINAL CELLS

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Our group previously showed studies about interaction between intestinal cells and *Giardia lamblia*. This is a parasitic protozoa that causes diarrheal and other intestinal diseases, being the mechanisms of the pathogenesis poorly understood. We presented that adhesion of parasites caused reduction in transepithelial electrical resistance (TER), concomitant with ultrastructural changes in intestinal cells. Our current results show that both samples (control and interaction) remain viable after 24 hours, although the TER has been reduced in approximately 40%. These data confirm the previous suggestion that ions probably pass through the paracellular region (junctional area). Here we also demonstrate that there was no change in expression of junctional proteins (tight and adherens junction proteins); nevertheless, the cellular distribution of these proteins in Caco-2 was significantly altered, as observed under laser scanning confocal microscope. 3D reconstructions allowed a fine analysis of the rearrangement of junctional proteins in epithelial monolayers after parasite adhesion. This study provides a deep review of the data published so far in the literature regarding the interaction *in vitro* between intestinal cells and *Giardia*.

Supported by CAPES, CNPq, Pronex/FAPERJ and FAPERJ.

BC.73 - *Plasmodium chabaudi* EXPOSE PHOSPHATIDYLSERINE AS ESCAPE MECHANISM OF IMUNE SYSTEM

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Has been shown that exposure of phosphatidylserine in outside of the plasma membrane in parasitic protozoa such as *Leishmania amazonensis*, *Toxoplasma gondii* and *Trypanosoma cruzi*, is important in the escape mechanism used by these protozoan parasites. In this paper we analyze the existence this inhibition mechanism of immune activity in *Plasmodium chabaudi*, a parasite that causes malaria in rodents. For this, the *P. chabaudi* was maintained by intraperitoneal passages in mice. During 24 hours, the blood of mice was collected and made himself a count of the forms of the parasite contained in temporal kinetics, making it possible to choose the way you would use for the study. After analysis by flow cytometry of parasites, it was observed that 90 % of parasite population exposed phosphatidylserine on the outside of membrane. Images obtained by scanning electron microscopy of the interaction of parasites with peritoneal macrophages of mice revealed that the form studied is able to penetrate the macrophages and modulate the microbicidal action of same. With these results, we conclude that exposure of phosphatidylserine may be a common escape mechanism between protozoan parasites that interact during their biological cycles with effector cells (macrophages).

Supported by FAPERJ

BC.74 - ANALYSIS OF THE INTERACTION OF MURINE MACROPHAGES WITH SUBPOPULATION OF TACHYZOITES OF *Toxoplasma gondii* THAT EXPOSE OR NOT PHOSPHATIDYLSERINE

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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. Toxoplasmosis is a worldwide disease caused by *Toxoplasma gondii*. The active invasion of *T. gondii* 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* and involves the exposure of PS. In this work the *T. gondii* tachyzoite population was separated into PS positive and PS negative subsets (by annexin V conjugated to magnetic beads) and *in vitro* interactions with murine macrophages were performed for the analysis of the penetration mechanism and survival of the parasite. Analysis by flow cytometry confirmed the efficiency of the isolation procedure of *T. gondii* PS subpopulations. Nitrite measurements in culture medium after the interaction showed a significant decrease in NO production after interaction of macrophages with PS positive subset of *T. gondii* compared to the PS negative. Scanning electron microscopy showed that the PS positive subpopulation invaded macrophages by active penetration, but the PS negative subset entered these cells by macropinocytosis. Treatment of cells with dynasore (inhibitor of macropinocytosis) showed that the invasion of the PS negative *T. gondii* subset in macrophages was inhibited with increasing concentration of this compound confirming the result. *In vivo* experiments shows that survival of mice infected with subsets of T. gondii were lower when compared to mice infected with both parasite total population simultaneously. These results suggest that the PS positive and PS negative population of *T. gondii* invade macrophages by different mechanisms and the growth of the parasite depends on both populations.
Supported by: FAPERJ, CNPq, CAPES.